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RESEARCH ARTICLE

Preventative treatment of tuberous sclerosis complex with sirolimus: Phase I safety and efficacy results

Jamie K. Capal[1,*], David M. Ritter[2], David Neal Franz[2], Molly Griffith[2], Kristn Currans[3], Bridget Kent[4], E. Martina Bebin[5], Hope Northrup[6], Mary Kay Koenig[7], Tomoyuki Mizuno[8], Alexander A. Vinks[8], Stephanie L. Galandi[9], Wujuan Zhang[9], Kenneth D.R. Setchell[9], Kelly M. Kremer[2], Carlos M. Prada[10], Hansel M. Greiner[2], Katherine Holland-Bouley[2], Paul S. Horn[2] & Darcy A. Krueger[2]

[1]Department of Neurology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[2]Division of Neurology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[3]Division of Behavioral Medicine and Clinical Psychology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[4]Division of Speech-Language Pathology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[5]Department of Neurology, University of Alabama at Birmingham, Birmingham, AL, USA
[6]Division of Medical Genetics, Department of Pediatrics, McGovern Medical School at the University of Texas Health Science Center at Houston (UTHealth) and Children's Memorial Hermann Hospital, Houston, TX, USA
[7]Division of Neurology, Department of Pediatrics, McGovern Medical School at the University of Texas Health Science Center at Houston (UTHealth) and Children's Memorial Hermann Hospital, Houston, TX, USA
[8]Division of Translational and Clinical Pharmacology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[9]Division of Pathology and Laboratory Medicine, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH, USA
[10]Division of Genetics, Genomics, and Metabolism, Department of Pediatrics, Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

Correspondence
Jamie K. Capal, Department of Neurology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. Email: jamiekcapal@gmail.com

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Abstract

Objective: Tuberous sclerosis complex (TSC) results from overactivity of the mechanistic target of rapamycin (mTOR). Sirolimus and everolimus are mTOR inhibitors that treat most facets of TSC but are understudied in infants. We sought to understand the safety and potential efficacy of preventative sirolimus in infants with TSC.

Methods: We conducted a phase 1 clinical trial of sirolimus, treating five patients until 12 months of age. Enrolled infants had to be younger than 6 months of age with no history of seizures and no clinical indication for sirolimus treatment. Adverse events (AEs), tolerability, and blood concentrations of sirolimus measured by tandem mass spectrometry were tracked through 12 months of age, and clinical outcomes (seizure characteristics and developmental profiles) were tracked through 24 months of age.

Results: There were 92 AEs, with 34 possibly, probably, or definitely related to treatment. Of those, only two were grade 3 (both elevated lipids) and all AEs were resolved by the age of 24 months. During the trial, 94% of blood sirolimus trough levels were in the target range (5–15 ng/mL). Treatment was well tolerated, with less than 8% of doses held because of an AE (241 of 2941). Of the five patients, three developed seizures (but were well controlled on medications) at 24 months of age. Of the five patients, four had normal cognitive development for age. One was diagnosed with possible autism spectrum disorder.

Interpretation: These results suggest that sirolimus is both safe and well tolerated by infants with TSC in the first year of life. Additionally, the preliminary work

suggests a favorable efficacy profile compared with previous TSC cohorts not exposed to early sirolimus treatment. Results support sirolimus being studied as preventive treatment in TSC, which is now underway in a prospective phase 2 clinical trial (TSC-STEPS).

Keywords: safety; seizures; sirolimus; tuberous sclerosis complex

Introduction

Tuberous sclerosis complex (TSC) is a genetic condition with a high risk for the development of epilepsy, with 80%–90% of individuals with TSC developing epilepsy over their lifetime.[1] Approximately two-thirds develop epilepsy in the first year of life, most commonly presenting as focal seizures and/or infantile spasms.[1] Seizures are often refractory to medical treatment and can result in adverse neurodevelopmental outcomes.[1–3] Previous work from the National Institutes of Health–funded TSC Autism Centers of Excellence Network (TACERN) evaluating infants and young children with TSC found that early seizure onset, prior to 12 months of age, resulted in higher rates of developmental delay and autistic behaviors at 24 months.[2] Likewise, those without seizures by 12 months exhibited developmental progress in line with typically developing peers. These data suggest that preventing or delaying seizure onset in individuals with TSC may improve neurodevelopmental outcomes.

Vigabatrin has been well studied in TSC and is the first-line treatment for infantile spasms in this group. However, it may be less effective in treating focal seizures, which may precede or co-occur with infantile spasms.[1,4–7] Its efficacy for infantile spasms in TSC has prompted interest in treating infants preemptively with vigabatrin to prevent seizure onset and thus improve outcomes. A small case series using vigabatrin to preemptively treat epileptic abnormalities recorded on EEG in infants with TSC[8] led to a prospective, European multicenter, open-label clinical trial (EPISTOP) evaluating the effectiveness of vigabatrin in preventing seizures and subsequent neurodevelopmental outcomes.[9] Although results showed some benefit in reducing the prevalence of drug-resistant epilepsy, reduction in autism risk or intellectual disability could not be demonstrated, suggesting that vigabatrin may only be partially effective in ameliorating these effects.[10] A subsequent prospective, multicenter, placebo-controlled clinical trial in the United States, PREVeNT (U01-NS092595), demonstrated that early vigabatrin treatment reduced the likelihood of infantile spasms but had less impact on other seizure types, including focal seizures,[11] that are highly prevalent in TSC at these ages alongside infantile spasms.[12]

TSC is caused by a mutation in either *TSC1* or *TSC2*, both of which form a complex critical to cellular processes in the body. The mechanistic target of rapamycin (mTOR) signaling pathway becomes disrupted in TSC due to disruption of the MTORC1 complex, leading to dysregulated cell growth, metabolism, and division.[13] Further studies of this pathway in the early 2000s led to the repurposing of mTOR inhibitors, already in clinical development to prevent transplanted organ rejection and to treat various cancers, to correct this pathway in TSC.[14] mTOR inhibitors, everolimus and sirolimus, are now approved in the United States and elsewhere for the treatment of multiple TSC manifestations, including subependymal giant cell astrocytomas (SEGA), renal angiomyolipomas, lymphangioleiomyomatosis (LAM), and facial angiofibromas.[15–24] Everolimus is also approved to treat medically refractory epilepsy in TSC, based on robust preclinical and clinical studies.[19–31] In all instances, initiation of mTOR inhibitors is used to treat clinical manifestations once they have already developed. Knowing that TSC is a genetic condition with presenting signs as early as the prenatal period and that mTOR inhibitors not only can modify the disease but are also safe and well tolerated in older children and adults, we hypothesized that treatment with mTOR inhibitors would present a unique opportunity to prevent TSC manifestations before they develop. In the case of epilepsy and neurodevelopment, mTOR inhibitors may even be advantageous over current strategies using vigabatrin that target a particular symptom of the disease (epilepsy) rather than molecular underpinnings of the disease directly (disrupted regulation of mTOR).

Before definitive clinical trials can be conducted to test our hypothesis that mTOR inhibitors during infancy can be used as preventive treatment, it is necessary to establish a minimum level of confidence that appropriate dosing levels that are safe and well tolerated in this population can be achieved and maintained. Previously, we summarized clinical experiences using everolimus or sirolimus in TSC clinics around the world in patients younger than 2 years that suggested this approach was feasible,[32] and others have described a limited subset of participants under 6 years of age in the EXIST-1 clinical trial using everolimus to treat SEGA, with similar conclusions.[31] However, until now

there have been no prospective clinical trials evaluating mTOR inhibitor treatment during infancy. We therefore conducted a prospective, open-label, phase 1 clinical trial of sirolimus to test the feasibility of a precision dosing strategy to achieve targeted sirolimus levels in TSC infants and to assess its safety, tolerability, and potential efficacy as a preventive treatment for epilepsy in this population.

Methods

Subject recruitment

The Stopping TSC Onset and Progression 2: Epilepsy Prevention in TSC Infants (STOP2A) trial is an open-label phase 1 clinical trial design to verify dosing and safety for TAVT-18 (sirolimus) powder for oral solution along with measuring efficacy in reducing seizure onset. The trial is registered with clinicaltrials.gov (NCT04595513). The initial trial design for STOP2A allowed for enrollment of six infants under 6 months of age and was to be followed by a second-stage randomized, double-blind, placebo-controlled trial enrolling 60 infants. During STOP2A, new funding and a change in sirolimus supply required separating the second-stage trial from STOP2A. The second stage opened for enrollment in the fall of 2021 under Stopping TSC Onset and Progression 2B: Sirolimus TSC Epilepsy Prevention Study (TSC-STEPS) (clinicaltrials.gov NCT05104983). With this change, the number of infants enrolled in STOP2A was reduced to five total (all infants enrolled at the time of the switch).

Participants were recruited through the TSC Centers of Excellence at Cincinnati Children's Hospital Medical Center (CCHMC) and the McGovern Medical School, University of Texas Health Science Center at Houston (UTHealth). However, all infants were enrolled at CCHMC. Infants with confirmed clinical and/or genetic diagnosis of TSC[33] were enrolled if they were younger than 6 months and had no prior history of clinical or electrographic seizures, no prior or current treatment with antiseizure medications, no clinical indication for mTOR inhibitor therapy (such as congenital SEGA or clinically significant cardiac rhabdomyomas), and no significant perinatal complications or problems. The study was reviewed and approved by the institutional review boards at CCHMC and UT Health. Parents of enrolled infants provided informed consent prior to being screened and before undergoing any study procedures.

Study design

Infants were treated with TAVT-18 for a period of 14 days. TAVT-18 is a proprietary formulation of sirolimus in clinical development by Tavanta Therapeutics, Inc.[34] TAVT-18 was supplied from the manufacturer in powder form in premeasured vials. The infant's family added 20 mL of water to the powder, mixed, and then orally administered the prescribed dose. The infant's family kept a diary of dosing information, recording exact dosing times, time of last feeding, and dose administered. Documentation was reviewed at each visit for adherence, and remaining drug supply was measured to verify dosing compliance.

Infants with no severe adverse events (SAEs) during the initial 14-day treatment period were allowed to continue open-label treatment so that additional extended exposure safety data could be collected until 12 months of age. At 12 months of age, clinicians could offer the option to start commercially available sirolimus. Outcomes were followed through 24 months of age.

Precision dosing

We sought to develop a predictive dosing model for early sirolimus (TAVT-18) treatment in infants by measuring steady-state blood trough concentration (ng/mL) corresponding to dose-normalized sirolimus dose (mg/m^2/day), based on prior studies treating infants with congenital vascular malformations.[35,36] The starting dose was predetermined based on age and body surface area to target a goal concentration of 10 ng/mL (Supporting Information: Table S1). Postdose levels were drawn at 1, 3, 6, and 24 h after the initial dose. Infants were brought back on day 7 (±1 day) for blood trough level, 1 h postdose level, and 3 h postdose level measures and again on day 14 (±1 day) for a single blood trough level. Individualized dosage adjustments were made based on day 7 and day 14 blood concentration results with the model-informed precision dosing approach. Briefly, each infant's demographics, sirolimus dosing history, and blood concentration data were entered into clinical PK/PD (pharmacokinetic/pharmacodynamic) modeling software MwPharm++ (Mediware, Czech Republic). Individual PK parameters were estimated with the Bayesian estimation using a previously published pediatric PK model including maturation function to account for the development of sirolimus clearance in infants.[35,36] The individualized dosing recommendation was estimated to target a 10 ng/mL trough concentration based on the PK prediction. This process was repeated, with dosing adjustments based on measurement of blood trough levels, patient age, and body size at each subsequent study visit one month posttreatment initiation and thereafter based on chronological age: 3, 6, 9, and 12 months. Levels were considered within the target range if they were between 5 and 15 ng/mL. Infants for which treatment was held temporarily due to an adverse event (AE) did not require a repeat sirolimus whole blood trough level to be checked when resuming treatment after the AE

resolved if sirolimus dose was unchanged. For infants who were unable to tolerate the protocol-defined dosing schedule, dose adjustments were permitted in order to keep the infant on the study drug.

Study procedures

Infants had follow-up visits with safety labs, physical exam, diary review, and electroencephalography (EEG) at one-month posttreatment initiation and 3, 6, 9, and 12 months of age. Depending on when the infant was enrolled in the study, the visits at 3 and 6 months of age may not have been completed or were combined with the one-month posttreatment initiation visit. A comprehensive developmental and behavioral assessment battery providing an abbreviated global assessment was conducted at the end of treatment (12 months of age). This assessment was repeated at 24 months of age to examine the durability of effect. The assessments battery and scheduling was designed to match other TSC-related studies in this age group[2,11,37–39] to allow future comparison of cohorts; it consisted of the TSC Associated Neuropsychiatric Disorders-Lifetime Checklist version (TAND-L),[40] Vineland Adaptive Behavioral Scales, 3rd Edition (VABS-III),[41] and Bayley Scales of Infant and Toddler Development, 4th Edition (Bayley-4)[42] at 12 months. These were repeated at 24 months along with the Preschool Language Scale, 5th Edition (PLS-5)[43] and Autism Diagnostic Observation Schedule, 2nd Edition (ADOS-2).[44,45] MRI brain was also performed at both 12 and 24 months.

Adverse events and safety reporting

Safety was monitored throughout the study through laboratory and clinical parameters. SAEs were reviewed in real time by an independent medical monitor. Additionally, all AEs were reviewed every six months by a data safety and monitoring board established to protect and safeguard interest of all participants. All AEs were noted and graded according to the Common Toxicity Criteria for Adverse Events (CTCAE v5.0). For the purposes of the study, AEs were defined as the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after beginning the study drug, whether or not considered treatment related. SAEs were defined as any AE that was fatal or life-threatening, resulted in persistent or significant disability/incapacity, constituted a congenital anomaly/birth defect, or required inpatient hospitalization or prolonged existing hospitalization. Medical conditions/diseases present before beginning the study drug were considered an AE only if they worsened after starting the study drug. Epilepsy and seizures as a primary outcome of interest were not reported as an AE unless accompanied by additional symptoms or events not ordinary in the course of epilepsy management in patients with TSC. Out-of-range lab results that were considered clinically significant, induced clinical signs or symptoms, or required intervention and/or changes in study treatment also were reported as an AE.

Study endpoints

The primary endpoint was safety of TAVT-18 at 12 months. Safety was assessed by the percentage of infants reporting SAEs (grade > 3). Secondary safety endpoints included percentage of infants that reduced or discontinued treatment and the number of days treatment was withheld due to an AE or SAE. We also explored efficacy as a secondary endpoint. Efficacy was measured as the time to seizure onset after initiating treatment with sirolimus, as well as determination of infant age at time of seizure onset, percentage of infants with infantile spasms, and seizure frequency at the end of treatment (12 months) and final assessment (24 months). Composite and contributing subdomain scores for each cognitive and neurodevelopmental outcome measure were also determined at 12 and 24 months.

Statistical analysis

This study was not powered for comparative analysis, as its purpose was to inform potential safety concerns and optimize sirolimus dosing for the larger, follow-up randomized, placebo-controlled clinical trial that is now underway (TSC-STEPS). In this study, descriptive statistics were used unless otherwise described.

Results

Patient characteristics

Five infants were enrolled in STOP2A (Table 1). The median age at treatment initiation was 1.5 months (range 1–4 months). All completed treatment with TAVT-18 through 12 months of age. After 12 months of age, four of five infants continued treatment with clinically sourced sirolimus through 24 months of age. All infants completed final assessments at 24 months of age.

A diagnosis of TSC was suspected in all infants prenatally upon detection of cardiac rhabdomyomas during routine ultrasound of the mother. The diagnosis was then confirmed postnatally via genetic testing, in which four had pathogenic or likely pathogenic variants in the *TSC2* gene and one had a clinical diagnosis and a variant of unknown significance in the *TSC2* gene that was predicted to be

pathogenic. Postenrollment, one infant was found to have large deletion of chromosome 16 spanning both *TSC2* and *PKD1* genes (contiguous gene deletion syndrome); the infant was doing well on treatment and thus completed the study.

Three of the five had epileptiform discharges detected on EEG prior to starting treatment with TAVT-18. None had electrographic or electroclinical seizures at baseline. None had electrographic evidence of hypsarrhythmia. Clinically, four of the five also had readily identifiable evidence of structural lesions of the brain characteristic of TSC consisting of cortical and subcortical tubers and subependymal nodules (Table 1). Additional nonneurological manifestations at the baseline exam attributable to TSC included hypopigmented macules or poliosis ($n = 3$), renal cysts ($n = 2$), and retinal hamartomas ($n = 1$, but three infants had not had formal eye exams at baseline, and another infant was later found to have retinal hamartomas).

Precision dosing of TAVT-18 (sirolimus)

TAVT-18 was dosed for a targeted sirolimus blood trough level of 10 ng/mL, with dosage adjustments at defined intervals between 0 and 12 months of age to maintain dosing within a range of 5–15 ng/mL (Figures 1 and 2). With initial median dose of 0.25–0.45 mg/m^2/dose given twice daily, measured trough levels 24 h after treatment initiation in all infants were below target, ranging from 1.2 to 1.9 ng/mL (Figure 1). Trough levels were in range for three infants, albeit all less than 10 ng/mL, day 7 (median 5.1 ng/mL, range 3.7–8.0 ng/mL, Figure 1). Each infant had dosage adjustment based on these results and returned for repeat trough level assessment on day 14. By day 14, all infants were within the target range (median 8.8 ng/mL, range 7.9–13.5 ng/mL) on dosing between 0.25 and 0.89 mg/m^2/dose given twice daily (Figures 1 and 2). This repeated pattern of trough level assessment, dosage adjustment, and reassessment every 30–90 days until

Table 1. Participant characteristics. There were no eye exams at baseline for three of the participants.

					N
Total subjects enrolled in study					5
Sex					
Male					1
Female					4
Ethnicity					
Hispanic or Latino					1
Non-Hispanic or Latino					4
Not reported/unknown					0
Race					
American Indian/Alaska Native					0
Asian					0
Black or African American					0
Native Hawaiian or Other Pacific Islander					0
White					5
Not reported/unknown					0
Subject ID	001	002	003	004	005
Individual subject baseline data					
Age at enrollment (months)	1	4	2	1	2
Genetic mutation	*TSC2* del exons 4-42	*TSC2* c.1372 C > T	*TSC2* c.4074dup	*TSC2* c.1385 G > A[a]	*TSC2* c.482-3 C > G
Epileptiform activity	No	Yes	Yes	No	Yes
Tubers	Yes	Yes	Yes	No	Yes
SEN	Yes	Yes	Yes	No	Yes
SEGA	No	No	No	No	No
Hypopigmented skin lesion or poliosis	Yes	Yes	Yes	No	No
Renal cyst	Yes	No	No	Yes	No
Retinal hamartoma	Yes	No			
Cardiac rhabdomyoma	Yes	Yes	Yes	Yes	Yes

Abbreviations: SEGA, subependymal giant cell astrocytoma; SEN, subependymal nodule.

[a]Subject 004 was identified as having a newly described variant of *TSC2* and as such was initially classified as having a variant of unknown significance. Protein prediction software, however, indicated the novel variant to be pathogenic, and review of genetic testing results with a geneticist, who is also the lead investigator for the DNA Core of the TSC Clinical Research Consortium, agreed as to its likely pathogenicity and thus meeting study eligibility for inclusion.

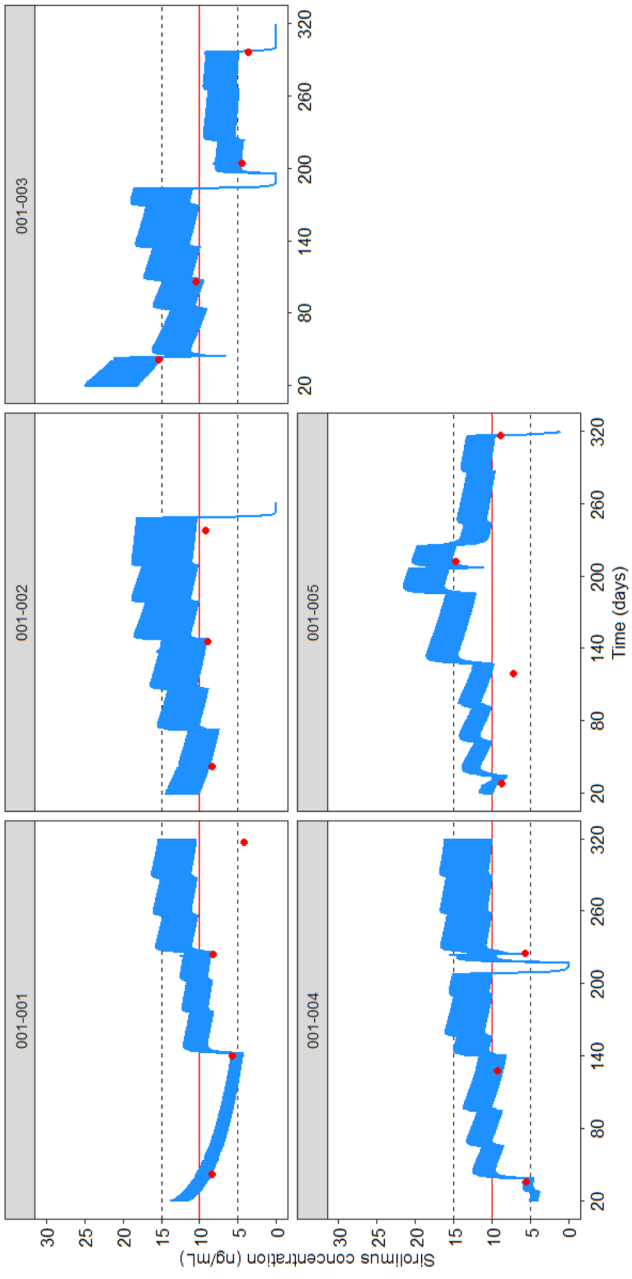

Figure 1. Predicted versus measured sirolimus blood levels using precision dosing strategy. For each participant, predicted concentration profile for given dose is shown in blue, as the dose was adjusted over time throughout the study (0–12 months). Measured (actual) sirolimus levels in whole blood drawn at study visits are shown as red dots. The precision dosing strategy, which targeted a blood trough level of 10 ng/mL, is shown with the horizontal red line. Accepted range (within target) blood trough levels (between 5 and 15 ng/mL) is shown with horizontal dotted lines.

Figure 2. Model-informed precision dosing recommendations. For each participant, the dosing recommendation of TAVT-18 (sirolimus) in mg/m^2 at study start is shown. Circles indicate the dosing recommendation at poststudy start. Blue arrows show when trough blood levels were measured and new dosing recommendations were calculated. In participant 001-003, a dosing recommendation was given based on the model at six months poststudy start (red arrow), but the clinical team decided to reduce the recommended dosing due to AEs (gray shaded area, correlating with reduced levels in Figure 1). D, day; M, month.

12 months of age continued, in which success in maintaining targeted trough levels was greater than 87% (Figure 1). The model-informed precision dosing needed per infant varied threefold to maintain levels at 9 months of age (last dose adjustment from a trough level, occurring at approximately six months poststudy start) ranging from 0.47 to 1.69 mg/m^2. This variability of dosing across infants continued through the end of the treatment phase (12 months of age, approximately nine months after starting the study) and ranged from 0.73 to 2.08 mg/m^2 (Figure 2). Overall, all but one out-of-range trough level could be explained clinically. Clinical reasons for out-of-range trough levels included premature transition to commercial sirolimus at the 12-month study visit ($n = 1$) and medication being held or reduced due to an AE ($n = 2$). Only one participant had the model-informed precision dosing recommendation rejected and a lower dose given due to AEs (Figure 2).

Safety profile and tolerability of TAVT-18 (sirolimus)

Overall, treatment with TAVT-18 between 0 and 12 months was well tolerated. A total of 2710 of 2941 doses were administered (92%), and only 218 doses (7.4%) over that time span were held specifically due to an AE (Supporting Information: Table S2). All five infants experienced at least one AE. In total there were 92 AEs, with 34 being possibly, probably, or definitely related to treatment (Table 2). Only two were considered grade 3 (severe), all of which related to elevated lipids on safety screening labs (one infant at 14 days of treatment and another at 30 days of treatment). In each case, blood triglyceride levels were within acceptable range prior to the elevated result; both infants continued therapy without the need for dosage adjustment, and their levels normalized at subsequent scheduled blood draws. There were no life-threatening AEs (grade 4) or deaths (grade 5). The most common mild/moderate AEs (grade 1 or 2) were fever and infection, followed by GI disturbances and irritability, irrespective of whether the AE was suspected to be related to treatment. Mouth or lip sores (n = 9) were among the most common treatment-associated AEs; one infant had multiple episodes that improved after the sirolimus dose was reduced improved. All AEs were resolved by study completion at 24 months of age.

Seizure and developmental outcomes with TAVT-18 (sirolimus)

Efficacy of preventive treatment with TAVT-18 was determined by monitoring the time to develop seizures, seizure type, and seizure frequency. Through 12 months while on treatment with TAVT-18, two infants developed seizures (Table 3). One of these infants developed infantile spasms at 2 months of age and responded successfully to vigabatrin treatment but required an increased dose to resolve focal motor seizures with impaired awareness that subsequently developed. The other infant developed infantile spasms at 10 months of age that resolved with vigabatrin treatment without any recurrence or new seizures thereafter. Between 12 and 24 months of age, a third infant who had transitioned to commercial sirolimus treatment developed focal seizures consisting of behavior

Table 2. Adverse events (AEs) during treatment with sirolimus by category, grade, and relatedness to treatment (definitely, probably, or possibly).

	Grade 1		Grade 2		Grade 3		Grade 4		Total	
	Total *N*	Related *N* (%)	Total *N*	Related *N* (%)	Total *N*	Related *N* (N%)	Total *N*	Related *N* (N%)	Total *N*	Related *N* (N%)
Gastrointestinal (incl. mouth sores)	12	2 (17)	8	5 (62)	0	0 (0)	0	0 (0)	20	7 (35)
General disorders (incl. fevers and irritability)	2	0 (0)	23	0 (0)	0	0 (0)	0	0 (0)	25	0 (0)
Immune system disorders	0	0 (0)	1	0 (0)	0	0 (0)	0	0 (0)	1	0 (0)
Infections	4	2 (50)	8	7 (88)	0	0 (0)	0	0 (0)	12	9 (75)
Metabolism and nutrition disorders (lipidemia)	6	6 (100)	0	0 (0)	2	2 (100)	0	0 (0)	8	8 (100)
Psychiatric disorders (insomnia)	1	0 (0)	1	0 (0)	0	0 (0)	0	0 (0)	2	0 (0)
Respiratory disorders	6	3 (50)	5	2 (40)	0	0 (0)	0	0 (0)	11	5 (45)
Skin disorders (incl. lip sores)	7	1 (14)	5	4 (80)	0	0 (0)	0	0 (0)	12	5 (42)
Vascular disorders (hypertension)	0	0 (0)	1	0 (0)	0	0 (0)	0	0 (0)	1	0 (0)
Total	38	14 (38)	52	18 (35)	2	2 (100)	0	0 (0)	92	34 (37)

Table 3. Seizure characteristics in participants treated with early sirolimus during infancy.

Subject ID	001	002	003	004	005
Seizure efficacy data					
Age at 1st seizure (days)	--	319	592	--	88
Duration of sirolimus treatment prior to 1st seizure (days)	--	178	511	--	26
Lifetime total # of seizures prior to 12 months of age	0	11	0	0	18
Lifetime total # of seizures prior to 24 months of age	0	11	215	0	19
Monthly total # of seizures at 24-month visit (# of seizures in the previous 4 weeks)	0	0	0	0	0
Seizure types developed by 24 months of age	--	Infantile spasms only	Focal seizures only	--	Infantile spasms and focal seizures

arrest. Full seizure control was achieved in this infant with the combined treatment of vigabatrin and lacosamide. The remaining two infants never developed any seizures through 24 months of age; interestingly, these same infants were the only ones with normal EEG prior to initiating treatment with TAVT-18. Collectively, all infants' seizures were either resolved or fully controlled on seizure medications at both the 12- and 24-month timepoints.

Preventive treatment with TAVT-18 was also measured through developmental assessments at 12 and 24 months of age (Supporting Information: Table S3). Overall disease burden by parent rating using the TAND-L Checklist severity rating was low at both 12 months (median 2, range 0–5) and 24 months (median 1, range 0–2). Global assessment of development using the Bayley-4 revealed median cognitive scores for all subdomains between 87 and 90 (ranges 70–112) at 12 months, with four of five infants in the normal range (>80). Results remained similar at 24 months of age (medians 74–95, ranges 77–100). Adaptive behaviors measured using the VABS-III showed similar patterns mostly in normal ranges at 12 months (median 91, range 86–102) and 24 months (median 88, range 66–128). At 24 months, total language was normal for four of five infants as measured using the PLS-5 (median 97, range 74–123). Four of five infants also scored below the range for autism or autism spectrum on the ADOS-2 at 24 months (Supporting Information: Table S3). One infant obtained an ADOS-2 score in the autism spectrum range, but the clinical team recommended repeat assessment when the child was older to resolve uncertainty with the categorization.

Discussion

This phase 1 clinical trial is the first to prospectively evaluate the potential of an mTOR inhibitor, sirolimus, for preventing seizure onset and epilepsy progression in high-risk TSC infants. There is an increasing number of reports describing TSC infants who were successfully treated for various indications,[16,32,46–53] but none of these has been prospective. Previously we collaborated with TSC clinics around the world to collect retrospective safety data from TSC infants and toddlers treated with everolimus ($n = 39$) or sirolimus ($n = 11$) for clinical indications in which infants were treated as early as the first month of life, but most were treated later in the first year or after 12 months.[32] In comparison, this study initiated treatment exclusively in infants, with all starting treatment between 1 and 4 months of age. Duration of exposure, however, was similar to the previous retrospective analysis, approximately 2 years. Both the prior study and this one also reported at least one treatment-related AE in most participants, but nearly all classified as mild or moderate severity. The types of AEs in both studies were consistent, with infections, mouth sores (aphthous ulcers/stomatitis), and hypercholesterolemia/hyperlipidemia most common. This profile of AEs, in frequency, type, and severity, is consistent with previous clinical trials conducted in older children and adults with TSC using open-label oral everolimus or sirolimus to treat SEGA, renal angiomyolipoma, LAM, or medically refractory epilepsy.[22,49,54–56] As in those studies, most AEs in this study were managed successfully by suspending treatment until the AE resolved and resuming treatment without having to reduce dosing. In the infant who experienced recurrent side effects, reducing the dose to target blood concentration around 5 ng/mL allowed the patient to tolerate sirolimus without return of the AE.

Multiple preclinical and clinical studies have demonstrated beneficial effects of mTOR inhibitors on seizures.[25–28] In TSC mouse models exhibiting seizures and subsequent cognitive and behavioral deficits, symptoms were either reversed or prevented altogether with mTOR inhibitors.[25,26,57–59] Our study utilized sirolimus, which is now approved by the Food and Drug Administration (FDA) for the treatment of LAM,[22,23,54] a progressive lung disease occuring primarily in adult women with TSC, and as a topical treatment for TSC-associated facial angiofibromas.[60] Sirolimus has also been

effective for treatment of TSC-associated SEGA,[54,61] angiomyolipoma,[22,49,54] retinal hamartomas,[62] and cardiac rhabdomyomas.[63–66] Yet despite robust preclinical studies demonstrating reduced seizures in TSC animal models treated with sirolimus,[25,28,67] clinical development of mTOR inhibitors for the treatment of epilepsy in patients has almost exclusively focused on everolimus.[31,56] However, a few reports have documented similar results with sirolimus.[27,61,68] In the only prospective clinical trial involving 23 children between ages 1 and 11 years, sirolimus treatment reduced seizure frequency by 41% compared with standard care, and three patients became seizure-free.[68] More is needed to assess the effects of sirolimus on epilepsy in early childhood or infancy; however, as sirolimus and everolimus have only minor structural differences, they have the same pharmacologic effect and thus, despite individual differences in tolerability, can likely be viewed as equivalent in clinical practice. Two active clinical trials at the Medical University of Warsaw and Children's Memorial Health Institute in Poland are evaluating the safety and efficacy of rapamycin (sirolimus) in TSC. One of the trials is a placebo-controlled study to assess the efficacy and safety of rapamycin in drug-resistant epilepsy associated with TSC in individuals ages 3 months to 50 years (RaRE-TS) (NCT05534672). The other is a two-arm, randomized, double-blind and double-dummy, placebo-controlled study evaluating the efficacy, tolerability, and safety of vigabatrin versus rapamycin as a preventive treatment in infants with TSC (ViRap) (NCT04987463). In this clinical trial participants are randomized to receive vigabatrin or rapamycin based on the presence of epileptiform activity on baseline video EEG.

Our prior retrospective study in infants and toddlers included more patients treated with everolimus than sirolimus and did not separate epilepsy-specific outcomes, but treating clinicians reported uncontrolled epilepsy as the primary reason for initiating treatment.[69] Overall, 29 of the 45 (64%) patients demonstrated at least partial benefit. While not adequately powered to provide a clear evaluation of efficacy, our results in this study are consistent. Only two of the five infants (40%) developed seizures in the first year compared with a historical prevalence of seizures by age 12 months of 55% in TSC.[7] In addition, seizures in the infants who developed them were well-controlled with medication when the study ended at 24 months of age, compared with a rate of medically refractory epilepsy of 33% of those with prior seizures in a similarly aged TSC cohort without early mTOR treatment (TACERN, $N = 117$).[12] Cognitive and neurodevelopment outcomes were similarly promising, with none of the five infants having composite cognitive and language scores <70 on the Bayley-4 or PLS-5 at 24 months, compared with rates of 45% and 38%, respectively, in the TACERN cohort.[37] The likelihood for TACERN participants to demonstrate significant signs and symptoms of autism on the ADOS-2 was 35%, whereas our study identified concern for autism in only one of five participants (20%).

Lack of focused PK/PD studies is a major reason many promising preclinical treatments fail during later clinical development in human studies,[70] and there is renewed emphasis for inclusion of these studies throughout therapeutic drug development for prevention treatments in CNS disorders.[67,71,72] While high doses of mTOR inhibitors can prevent epilepsy and autism-associated behaviors in TSC mouse models,[25,28,59] few studies have attempted to define practical, clinically relevant treatment protocols of mTOR inhibitors that could be translated into preventive therapy in TSC patients. Sirolimus dosing in TSC clinical trials to date has been based on dosing to achieve target trough levels commonly used for solid organ transplantation (C_{min} 5–15 ng/mL).[22,23,49,54] The assumption is that PK/PD characteristics for sirolimus in TSC are the same, although this has never been verified. Relevant to our study, there is particular deficiency for very young children, where drug clearance can vary significantly due to developing organ function and maturation of drug metabolism.[50] Building on our previous work using sirolimus to treat infants with congenital vascular malformations at our center,[52,53,73] which also can occur in patients with TSC,[74] we successfully implemented a protocol that targeted a blood trough level of 10 ± 5 ng/mL (measured goal 5–15 ng/mL). With PK model–informed precision dosing strategies, we were able to achieve a target blood trough level within 7–14 days after treatment initiation and maintained this level 94% of the time through 12 months of age. By comparison, in the EXIST-3 clinical trial evaluating everolimus to treat medically refractory epilepsy,[75] only 49% of participants with a blood trough level goal between 3 and 7 ng/mL was achieved after seven days of treatment. After three months, successful achievement of the targeted goal level improved to only 67%. In the EXIST-3 cohort with a goal trough level between 9 and 15 ng/mL, successful levels were achieved in 59% after seven days, but this rate decreased to only 29% at three months. In TSC-STEPS, we are further refining the precision dosing protocol to improve clinical feasibility and scalability by reducing the number of draws to seven and 30 days after treatment initiation and then every three months thereafter and extending the precision dosing protocol through 24 months of age.

Conclusion

This work provides preliminary evidence that preventive sirolimus is both safe and tolerated in infants with TSC and consistent with previous retrospective studies with mTOR inhibitors in this population, while also being consistent with prospective clinical trials in older children and adults with TSC. Model-informed precision dosing can be used to

rapidly achieve and maintain target sirolimus blood trough levels through 12 months of age. Effects of sirolimus on epilepsy prevention and cognitive/neurodevelopmental outcomes when initiated as early in life as possible, before the onset of EEG abnormalities and clinical seizures, is promising and supports the need for larger clinical trials to confirm safety and efficacy of sirolimus in infants with TSC. TSC-STEPS (clinicaltrials.gov NCT05104983), the follow-up phase 1/2b multicenter, placebo-controlled clinical trial using a commercially supplied, FDA-approved formulation of sirolimus, is already underway to confirm and extend these exciting results.

Author Contributions

Jamie K. Capal: Conceptualization; investigation; methodology; visualization; writing—original draft; writing—review and editing. **David M. Ritter**: Conceptualization; data curation; formal analysis; investigation; writing—original draft; writing—review and editing. **David Neal Franz**: Conceptualization; writing—review and editing. **Molly Griffith**: Data curation; project administration. **Kristn Currans**: Data curation; investigation; methodology; writing—review and editing. **Bridget Kent**: Data curation; investigation; writing—review and editing. **E. Martina Bebin**: Conceptualization; funding acquisition; investigation; writing—review and editing. **Hope Northrup**: Conceptualization; investigation; writing—review and editing. **Mary Kay Koenig**: Conceptualization; investigation; writing—review and editing. **Tomoyuki Mizuno**: Data curation; formal analysis; methodology; writing—review and editing. **Alexander A. Vinks**: Data curation; investigation; methodology; writing—review and editing. **Stephanie L. Galandi**: Data curation; formal analysis; methodology; writing—review and editing. **Wujuan Zhang**: Data curation; formal analysis; investigation; writing—review and editing. **Kenneth D.R. Setchell**: Data curation; formal analysis; investigation; methodology; writing—review and editing. **Kelly M. Kremer**: Data curation; investigation; writing—review and editing. **Carlos M. Prada**: Data curation; investigation; methodology; writing—review and editing. **Hansel M. Greiner**: Investigation; writing—review and editing. **Katherine Holland-Bouley**: Data curation; investigation; methodology; writing—review and editing. **Paul S. Horn**: Formal analysis; writing—original draft; writing—review and editing. **Darcy A. Krueger**: Conceptualization; funding acquisition; investigation; methodology; project administration; supervision.

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Conflicts of Interest

J. K. C. serves on the strategic working group for Marinus Pharmaceuticals. D. A. K. has received consulting fees from Biocodex, Jazz Pharmaceuticals, and Longboard Pharmaceuticals. He also serves on the board of directors of the TSC Alliance and the medical and scientific advisory committee of the Smith-Kingsmore Syndrome Foundation. M. K. K. receives research funding from Jazz Pharmaceuticals, Noema Pharmaceuticals, and Marinus Pharmaceuticals and has both consulted and provided speaking engagements for Jazz Pharmaceuticals and Marinus Pharmaceuticals. A. A. V. is a partner and clinical pharmacology consultant with NDA Partners. D. N. F. is an *Annals of the Child Neurology Society* editorial board member. The remaining authors declare no conflicts of interest.

ORCID

Jamie K. Capal http://orcid.org/0000-0002-7579-375X
David M. Ritter http://orcid.org/0000-0001-7723-3636

References

1. Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia*. 2010;51(7):1236-1241.
2. Capal JK, Bernardino-Cuesta B, Horn PS, et al. Influence of seizures on early development in tuberous sclerosis complex. *Epilepsy Behav*. 2017;70(Pt A):245-252.
3. Jozwiak S, Goodman M, Lamm SH. Poor mental development in patients with tuberous sclerosis complex: clinical risk factors. *Arch Neurol*. 1998;55(3):379-384.
4. Hussain SA, Schmid E, Peters JM, et al. High vigabatrin dosage is associated with lower risk of infantile spasms relapse among children with tuberous sclerosis complex. *Epilepsy Res*. 2018;148:1-7.
5. Camposano SE, Major P, Halpern E, Thiele EA. Vigabatrin in the treatment of childhood epilepsy: a retrospective chart review of efficacy and safety profile. *Epilepsia*. 2008;49(7):1186-1191.

6. Wu JY, Peters JM, Goyal M, et al. Clinical electroencephalographic biomarker for impending epilepsy in asymptomatic tuberous sclerosis complex infants. *Pediatr Neurol*. 2016;54:29-34.
7. Wu JY, Goyal M, Peters JM, et al. Scalp EEG spikes predict impending epilepsy in TSC infants: a longitudinal observational study. *Epilepsia*. 2019;60(12):2428-2436.
8. Jóźwiak S, Kotulska K, Domańska-Pakieła D, et al. Antiepileptic treatment before the onset of seizures reduces epilepsy severity and risk of mental retardation in infants with tuberous sclerosis complex. *Eur J Paediatr Neurol*. 2011;15(5):424-431.
9. Moavero R, Kotulska K, Lagae L, et al. Is autism driven by epilepsy in infants with tuberous sclerosis complex? *Ann Clin Transl Neurol*. 2020;7(8):1371-1381.
10. Jozwiak S, Słowińska M, Borkowska J, et al. Preventive antiepileptic treatment in tuberous sclerosis complex: a long-term, prospective trial. *Pediatr Neurol*. 2019;101:18-25.
11. Bebin EM, Peters JM, Porter BE, et al. Early treatment with vigabatrin does not decrease focal seizures or improve cognition in tuberous sclerosis complex: the PREVeNT Trial. *Ann Neurol*. 2023:1-12.
12. Ihnen SKZ, Capal JK, Horn PS, et al. Epilepsy is heterogeneous in early-life tuberous sclerosis complex. *Pediatr Neurol*. 2021;123:1-9.
13. MacKeigan JP, Krueger DA. Differentiating the mTOR inhibitors everolimus and sirolimus in the treatment of tuberous sclerosis complex. *Neuro-Oncol*. 2015;17(12):1550-1559.
14. Franz DN, Leonard J, Tudor C, et al. Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. *Ann Neurol*. 2006;59(3):490-498.
15. Franz DN, Belousova E, Sparagana S, et al. Efficacy and safety of everolimus for subependymal giant cell astrocytomas associated with tuberous sclerosis complex (EXIST-1): a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet*. 2013;381(9861):125-132.
16. Franz DN, Agricola K, Mays M, et al. Everolimus for subependymal giant cell astrocytoma: 5-year final analysis. *Ann Neurol*. 2015;78(6):929-938.
17. Franz DN, Belousova E, Sparagana S, et al. Long-term use of everolimus in patients with tuberous sclerosis complex: final results from the EXIST-1 study. *PLoS One*. 2016;11(6):e0158476.
18. Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med*. 2010;363(19):1801-1811.
19. Krueger DA, Wilfong AA, Holland-Bouley K, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Ann Neurol*. 2013;74(5):679-687.
20. Bissler JJ, Kingswood JC, Radzikowska E, et al. Everolimus long-term use in patients with tuberous sclerosis complex: four-year update of the EXIST-2 study. *PLoS One*. 2017;12(8):e0180939.
21. Bissler JJ, Kingswood JC, Radzikowska E, et al. Everolimus for angiomyolipoma associated with tuberous sclerosis complex or sporadic lymphangioleiomyomatosis (EXIST-2): a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet*. 2013;381(9869):817-824.
22. Bissler JJ, McCormack FX, Young LR, et al. Sirolimus for angiomyolipoma in tuberous sclerosis complex or lymphangioleiomyomatosis. *N Engl J Med*. 2008;358(2):140-151.
23. McCormack FX, Inoue Y, Moss J, et al. Efficacy and safety of sirolimus in lymphangioleiomyomatosis. *N Engl J Med*. 2011;364(17):1595-1606.
24. Koenig MK, Hebert AA, Roberson J, et al. Topical rapamycin therapy to alleviate the cutaneous manifestations of tuberous sclerosis complex: a double-blind, randomized, controlled trial to evaluate the safety and efficacy of topically applied rapamycin. *Drugs R D*. 2012;12(3):121-126.
25. Zeng LH, Xu L, Gutmann DH, Wong M. Rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex. *Ann Neurol*. 2008;63(4):444-453.
26. Meikle L, Talos DM, Onda H, et al. A mouse model of tuberous sclerosis: neuronal loss of Tsc1 causes dysplastic and ectopic neurons, reduced myelination, seizure activity, and limited survival. *J Neurosci*. 2007;27(21):5546-5558.
27. Muncy J, Butler IJ, Koenig MK. Rapamycin reduces seizure frequency in tuberous sclerosis complex. *J Child Neurol*. 2009;24(4):477.
28. Meikle L, Pollizzi K, Egnor A, et al. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci*. 2008;28(21):5422-5432.
29. Krueger DA, Wilfong AA, Mays M, et al. Long-term treatment of epilepsy with everolimus in tuberous sclerosis. *Neurology*. 2016;87(23):2408-2415.
30. Franz DN, Lawson JA, Yapici Z, et al. Everolimus for treatment-refractory seizures in TSC: extension of a randomized controlled trial. *Neurol Clin Pract*. 2018;8(5):412-420.
31. French JA, Lawson JA, Yapici Z, et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study. *Lancet*. 2016;388(10056):2153-2163.
32. Krueger DA, Capal JK, Curatolo P, et al. Short-term safety of mTOR inhibitors in infants and very young children with tuberous sclerosis complex (TSC): multicentre clinical experience. *Eur J Paediatr Neurol*. 2018;22(6):1066-1073.
33. Northrup H, Aronow ME, Bebin EM, et al. Updated international tuberous sclerosis complex diagnostic criteria and surveillance and management recommendations. *Pediatr Neurol*. 2021;123:50-66.
34. Basa-Dénes O, Angi R, Kárpáti B, et al. Dose escalation study to assess the pharmacokinetic parameters of a nano-amorphous oral sirolimus formulation in healthy volunteers. *Eur J Drug Metab Pharmacokinet*. 2019;44(6):777-785.

35. Mizuno T, Emoto C, Fukuda T, Hammill AM, Adams DM, Vinks AA. Model-based precision dosing of sirolimus in pediatric patients with vascular anomalies. *Eur J Pharm Sci*. 2017;109s:S124-S131.
36. Mizuno T, Fukuda T, Emoto C, et al. Developmental pharmacokinetics of sirolimus: Implications for precision dosing in neonates and infants with complicated vascular anomalies. *Pediatr Blood Cancer*. 2017;64(8). doi:10.1002/pbc.26470
37. Capal JK, Williams ME, Pearson DA, et al. Profile of autism spectrum disorder in tuberous sclerosis complex: results from a longitudinal, prospective, multisite study. *Ann Neurol*. 2021;90(6):874-886.
38. Schoenberger A, Capal JK, Ondracek A, et al. Language predictors of autism spectrum disorder in young children with tuberous sclerosis complex. *Epilepsy Behav*. 2020;103(Pt A):106844.
39. Capal JK, Horn PS, Murray DS, et al. Utility of the autism observation scale for infants in early identification of autism in tuberous sclerosis complex. *Pediatr Neurol*. 2017;75:80-86.
40. de Vries PJ, Whittemore VH, Leclezio L, et al. Tuberous sclerosis associated neuropsychiatric disorders (TAND) and the TAND Checklist. *Pediatr Neurol*. 2015;52(1):25-35.
41. Sparrow SSCD, Saulnier C. *Vineland Adaptive Behavior Scales*. 3rd ed. Pearson; 2016.
42. Nancy B. *Bayley Scales of Infant and Toddler Development*. 4th ed. Pearson; 2019.
43. Zimmerman I, Steiner V, Pond R. *Preschool Language Scale Fifth Edition (PLS-5)*. Pearson; 2011.
44. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S. *Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) Manual (Part I): Modules 1–4*. Western Psychological Services; 2012.
45. Lord CLR, Gotham K, Guthrie W. *Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) Manual (Part II): Toddler Module*. Western Psychological Services; 2012.
46. Colaneri M, Quarti A, Pozzi M. Everolimus-induced near-resolution of giant cardiac rhabdomyomas and large renal angiomyolipoma in a newborn with tuberous sclerosis complex. *Cardiol Young*. 2016;26(5):1025-1028.
47. Aw F, Goyer I, Raboisson MJ, Boutin C, Major P, Dahdah N. Accelerated cardiac rhabdomyoma regression with everolimus in infants with tuberous sclerosis complex. *Pediatr Cardiol*. 2017;38(2):394-400.
48. Dahdah N. Everolimus for the treatment of tuberous sclerosis complex-related cardiac rhabdomyomas in pediatric patients. *J Pediatr*. 2017;190:21-26.
49. Davies DM, De Vries PJ, Johnson SR, et al. Sirolimus therapy for angiomyolipoma in tuberous sclerosis and sporadic lymphangioleiomyomatosis: a phase 2 trial. *Clin Cancer Res*. 2011;17(12):4071-4081.
50. Emoto C, Fukuda T, Mizuno T, et al. Characterizing the developmental trajectory of sirolimus clearance in neonates and infants. *CPT: Pharmacometrics Syst Pharmacol*. 2016;5(8):411-417.
51. Rensing N, Han L, Wong M. Intermittent dosing of rapamycin maintains antiepileptogenic effects in a mouse model of tuberous sclerosis complex. *Epilepsia*. 2015;56(7):1088-1097.
52. Adams DM, Trenor 3rd CC, Hammill AM, et al. Efficacy and safety of sirolimus in the treatment of complicated vascular anomalies. *Pediatrics*. 2016;137(2):e20153257.
53. Hammill AM, Wentzel M, Gupta A, et al. Sirolimus for the treatment of complicated vascular anomalies in children. *Pediatr Blood Cancer*. 2011;57(6):1018-1024.
54. Dabora SL, Franz DN, Ashwal S, et al. Multicenter phase 2 trial of sirolimus for tuberous sclerosis: kidney angiomyolipomas and other tumors regress and VEGF-D levels decrease. *PLoS One*. 2011;6(9):e23379.
55. Krueger DA, Care MM, Holland K, et al. Everolimus for subependymal giant-cell astrocytomas in tuberous sclerosis. *N Engl J Med*. 2010;363(19):1801-1811.
56. Krueger DA, Wilfong AA, Holland-Bouley K, et al. Everolimus treatment of refractory epilepsy in tuberous sclerosis complex. *Ann Neurol*. 2013;74(5):679-687.
57. Di Nardo A, Kramvis I, Cho N, et al. Tuberous sclerosis complex activity is required to control neuronal stress responses in an mTOR-dependent manner. *J Neurosci*. 2009;29(18):5926-5937.
58. Ehninger D, Han S, Shilyansky C, et al. Reversal of learning deficits in a Tsc2+/- mouse model of tuberous sclerosis. *Nat Med*. 2008;14(8):843-848.
59. Tsai PT, Hull C, Chu Y, et al. Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature*. 2012;488(7413):647-651.
60. Wataya-Kaneda M, Ohno Y, Fujita Y, et al. Sirolimus gel treatment vs placebo for facial angiofibromas in patients with tuberous sclerosis complex: a randomized clinical trial. *JAMA Dermatol*. 2018;154(7):781-788.
61. Cardamone M, Flanagan D, Mowat D, Kennedy SE, Chopra M, Lawson JA. Mammalian target of rapamycin inhibitors for intractable epilepsy and subependymal giant cell astrocytomas in tuberous sclerosis complex. *J Pediatr*. 2014;164(5):1195-1200.
62. Zhang ZQ, Shen C, Long Q, et al. Sirolimus for retinal astrocytic hamartoma associated with tuberous sclerosis complex. *Ophthalmology*. 2015;122(9):1947-1949.
63. Breathnach C, Pears J, Franklin O, Webb D, McMahon CJ. Rapid regression of left ventricular outflow tract rhabdomyoma after sirolimus therapy. *Pediatrics*. 2014;134(4):e1199-e1202.
64. Ninic S, Kalaba M, Jovicic B, et al. Successful use of sirolimus for refractory atrial ectopic tachycardia in a child with cardiac rhabdomyoma. *Annals Noninvasive Electrocardiol*. 2017;22(4):e12435.
65. Patel C, Abraham S, Ferdman D. Rapid regression of prenatally identified intrapericardial giant rhabdomyomas with sirolimus. *CASE*. 2018;2(6):258-261.

66. Weiland MD, Bonello K, Hill KD. Rapid regression of large cardiac rhabdomyomas in neonates after sirolimus therapy. *Cardiol Young*. 2018;28(3):485-489.
67. Galanopoulou AS, Buckmaster PS, Staley KJ, et al. Identification of new epilepsy treatments: issues in preclinical methodology. *Epilepsia*. 2012;53(3):571-582.
68. Overwater IE, Rietman AB, Bindels-de Heus K, et al. Sirolimus for epilepsy in children with tuberous sclerosis complex: a randomized controlled trial. *Neurology*. 2016;87(10):1011-1018.
69. Krueger DA, Capal JK, Curatolo P, et al. Short-term safety of mTOR inhibitors in infants and very young children with tuberous sclerosis complex (TSC): multicentre clinical experience. *Eur J Paediatr Neurol*. 2018;22:1066-1073.
70. Kleiman RJ, Ehlers MD. Data gaps limit the translational potential of preclinical research. *Sci Transl Med*. 2016;8(320):320ps1.
71. Simonato M, Brooks-Kayal AR, Engel Jr. J, et al. The challenge and promise of anti-epileptic therapy development in animal models. *Lancet Neurol*. 2014;13(9):949-960.
72. O'Brien TJ, Ben-Menachem E, Bertram 3rd EH, et al. Proposal for a "phase II" multicenter trial model for preclinical new antiepilepsy therapy development. *Epilepsia*. 2013;54(Suppl 4):70-74.
73. Ricci KW, Hammill AM, Mobberley-Schuman P, et al. Efficacy of systemic sirolimus in the treatment of generalized lymphatic anomaly and Gorham-Stout disease. *Pediatr Blood Cancer*. 2019;66(5):27614.
74. Jenkins D, McCuaig C, Drolet BA, et al. Tuberous sclerosis complex associated with vascular anomalies or overgrowth. *Pediatr Dermatol*. 2016;33(5):536-542.
75. Franz DN, Lawson JA, Yapici Z, et al. Everolimus dosing recommendations for tuberous sclerosis complex-associated refractory seizures. *Epilepsia*. 2018;59(6):1188-1197.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

