Protocol Title

Stopping TSC Onset and Progression 2A: Epilepsy Prevention in TSC Infants

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I have read and approve this protocol. My signature, in conjunction with the signature of the investigator, confirms the agreement of both parties that the clinical study will be conducted in accordance with the protocol and all applicable laws and regulations including, but not limited to, the International Conference on Harmonisation Guideline for Good Clinical Practice (GCP), the Code of Federal Regulations (CFR), the ethical principles that have their origins in the Declaration of Helsinki, and applicable privacy laws.

_____ _____

Sponsor Signature Date

_____

Sponsor Name and Title (Print)

V. 7.0 INVESTIGATOR SIGNATURE

I have read and understand this protocol. My signature, in conjunction with the signature of the sponsor, confirms the agreement of both parties that the clinical study will be conducted in accordance with the protocol and all applicable laws and regulations including, but not limited to, the International Conference on Harmonisation Guideline for Good Clinical Practice (GCP), the Code of Federal Regulations (CFR), the ethical principles that have their origins in the Declaration of Helsinki, and applicable privacy laws.

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Investigator Signature Date

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Investigator Name and Title (Print)

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Institution (Print)

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1 ABSTRACT

Tuberous Sclerosis Complex (TSC) is caused by genetic mutation in *TSC1* or *TSC2,* resulting in dysregulation of the mechanistic target of rapamycin (mTOR) signaling pathway. mTOR inhibitors such as everolimus and sirolimus have been shown to be effective and well-tolerated for treatment of multiple clinical manifestations of TSC, including brain and renal tumors, lung disease, and most recently, medically-refractory partial complex epilepsy. Over the past decade, we have led successful efforts in the clinical development of mTOR inhibitors to treat TSC, with FDA and/or EMA-approval for subependymal giant cell astrocytoma (SEGA), angiomyolipoma, lymphangioleiomyomatosis (LAM), and partial seizures.

In all instances, treatment has been initiated *after* the development and progression of clinical disease manifestations. Given that the underlying genetic defect of TSC driving mTOR abnormal mTOR activation is present from birth, preventative treatment with everolimus or sirolimus *before* the onset of clinical symptoms could prove superior for optimizing outcomes and changing the course of the overall disease. This is particularly important for prevention of epilepsy, which affects more than 80% of individuals overall. Onset for the vast majority is in the first year of life, with more than 50% refractory to all currently available anticonvulsant medications. Those with early onset epilepsy are at greatest risk for lifelong developmental and cognitive impairment.

Our overall hypothesis is that early treatment with sirolimus is safe and effective for preventing epilepsy and associated developmental and cognitive delay in this high-risk population of TSC infants. This phase I clinical trial will evaluate sirolimus treatment in TSC infants 0-12 months of age, when onset of clinical seizures is highest and preventative treatment offers the greatest potential to protect long-term neurological outcome. Our primary aims are:

***Safety*:** To demonstrate safety and tolerability of sirolimus in infants with TSC that are 0-12 months of age.

The *Hypothesis* is that sirolimus will be safe and well tolerated in infants with TSC that are 0-12 months of age. Safety is defined as the frequency of severe/life-threating adverse events (grade ≥3, Common Terminology Criteria for Adverse Events, CTCAE v5.0) in infants treated with sirolimus compared. Tolerability is defined as the percentage of subjects that discontinue treatment or reduce dose because of an adverse event or serious adverse event (AE/SAE). We will also assess the number of days treatment is held but not discontinued because of an AE/SAE.

***Efficacy*:** To demonstrate that sirolimus prevents or delays epilepsy in infants with TSC that are 0-12 months of age.

The *Hypothesis* is that infants treated with sirolimus before the onset of clinical seizures and continued until 12 months of age. The primary endpoint for efficacy will be time to seizure onset. We will assess efficacy with regards to age at time of seizure onset, seizure type(s), and seizure frequency at the end of treatment (12 months of age) as additional secondary outcome measures, which also have previously been shown to directly correlate with long-term epilepsy severity and neurodevelopmental outcome in TSC.

Additional secondary objectives of the study are:

(1) To assess the effect of early sirolimus treatment in TSC infants 0-12 months of age on neurodevelopment and behavior, which closely associate with epilepsy in TSC.
(2) To assess the effect of early sirolimus treatment in TSC infants 0-12 months of age on TSC-associated electrophysiological (EEG) biomarkers of neuronal connectivity and explore whether normalization of biomarkers is associated with epilepsy prevention and structural biomarkers of neuronal connectivity measured by diffusion MRI (dMRI).
(3) To optimize treatment with sirolimus in TSC infants 0-12 months of age, identifying dosing that maintains sirolimus trough levels (C_{min}) where intolerable side effects are minimized while preserving treatment benefit.

2 BACKGROUND & RATIONALE

A key intracellular signaling pathway regulated by the mechanistic target of rapamycin (mTOR), essential for normal cell growth, differentiation, metabolism, function, and survival is responsible for many of the clinical manifestations of Tuberous Sclerosis Complex (TSC).[1] In the brain, subependymal giant cell astrocytomas (SEGA) arise from subependymal nodules in 10-15% of children with TSC and if untreated, can cause obstructive hydrocephalus.[2] Even more common in TSC is epilepsy, affecting 80-90% of affected individuals.[3] Seizures often present before 1 year of age as infantile spasms or focal seizures and too often is refractory to all available treatments and interventions.[3] Seizures in general and infantile spasms particularly have additional devastating impact on neurodevelopment and long-term cognitive and behavioral outcomes.[3-5] This is consistent with preclinical mouse models of TSC, in which seizures begin early and gradually worsen over time, to the point of intractability and early demise.[6] TSC mouse models also demonstrate cognitive and behavioral deficits similar to the human disease. In each case, deficits could be reversed and/or prevented with preemptive everolimus or sirolimus (rapamycin) treatment, small molecule pharmacological inhibitors of mTOR.[7-10]

Recent data from the NIH-funded TSC Autism Centers of Excellence Network (TACERN) not only confirms that 70% of diagnosed infants will develop seizures in the first year of life, but also that standardized, validated assessment tools at 12 month of age are able to identify measurable deficits in learning and behavior linked to early onset epilepsy in this population.[4, 11, 12] ***Furthermore, infants who do not develop seizures or whose seizures are completely or relatively controlled by 12 months of age are much more likely to develop normally through 24 months of age and beyond***. Unfortunately, such infants are the minority in TSC. The cumulative preclinical and clinical evidence strongly support targeted anti-epileptogenic therapies for TSC and such disease modifying, preventative treatment should be initiated before the onset of seizures in the first year of life before epilepsy becomes refractory to conventional treatment and before epilepsy-associated neurocognitive and behavioral deficits take hold.

As evidence emerged in the early 2000s that *TSC1* or *TSC2* mutations allow mTOR activity to go unchecked, we began to assess the effect of pharmacological inhibitors that rectify mTOR activity in patients with TSC. The first human TSC clinical experience with mTOR inhibitors was first reported in 2006, where sirolimus was found to be effective for reducing SEGA tumor volume in as little as 2 months.[13] Prospective clinical trials with sirolimus and everolimus, an ethyl-ester derivative of sirolimus, began in 2007. In 2010, the FDA and EMA approved everolimus for the treatment of SEGA in TSC.[14-19] Regulatory approval of everolimus for

angiomyolipoma and sirolimus for lymphangioleiomyomatosis (LAM) in TSC followed in 2012 and 2015, respectively.[20-23] Meanwhile, case reports emerged of sirolimus and/or everolimus reducing seizure frequency in TSC patients with refractory epilepsy.[24-26] After observing similar improvement in TSC patients treated for SEGA,[19] we initiated the first prospective multicenter clinical trial (Phase I/II) for everolimus specifically to treat refractory epilepsy in pediatric TSC patients 2-21 years of age. Following 3 months of treatment in which concurrent antiseizure medications remained unchanged, median seizure frequency was reduced 73%.[27] Furthermore, follow-up for an additional four years revealed the improved seizure frequency was sustained with continued treatment.[28] These pilot study results were confirmed in EXIST-3,[29, 30] an international multicenter phase III randomized, double-blind, placebo-controlled clinical trial leading to EMA (2017) and FDA (2018) approval of everolimus for the treatment of partial seizures in TSC.

This will be the first study to truly evaluate a targeted, disease-modifying drug therapy for preventing epilepsy in TSC using a rational, mechanism-based therapeutic approach. Clinical development of sirolimus and everolimus in TSC has been driven primarily by commercial and market exclusivity considerations by their respective manufacturers, limiting opportunities for direct comparison of the two mTOR inhibitors. To date, neither everolimus or sirolimus has demonstrated superiority over the other with regard to safety or efficacy in TSC and both are used interchangeably in clinical practice.[1] Safety in infants and toddlers is of particular concern, as all clinical trials in TSC have limited study to children > 2 years of age and adults; none have evaluated efficacy or safety of sirolimus in infants or toddlers and none have initiated treatment before clinical symptoms and disease progression are manifest. In older children and adults, the vast majority (>90%) of side effects are mild or moderate in severity (CTCAE grade 1 or 2), with stomatitis and lipidemia most prevalent. We identified 45 TSC infants 0-24 months of age (11.6 ± 7.6 months) from 19 TSC clinics treated with everolimus or sirolimus since 2010 for clinical indications.[31] Overall, treatment-related adverse events were common (78%) but as is the case in older populations the majority were mild or moderate in severity. However, the study was limited by its retrospective design and lack of control group for comparison. Well-designed, prospective studies are needed to adequately ensure there are no new adverse events or increased severity unique to the infant TSC population that would outweigh the potential benefit of epilepsy prevention in this population.

Incorporation of biomarkers of pharmacodynamic response to demonstrate drug-specific treatment effects would further advance development of sirolimus as a preventative, disease-modifying therapy in TSC. As our understanding of TSC increases, it is becoming increasingly clear that non-tuber abnormalities are strongly associated with neurological outcome[10, 32, 33]. We can measure potential treatment-related changes noninvasively with electroencephalography (EEG) and diffusion magnetic resonance imaging (dMRI). Both are routinely acquired as standard of care for TSC patients in this age range.[34] We have found conventional EEG is highly sensitive and specific identifying TSC infants at greatest risk for newly developing clinical focal seizures and/or infantile spasms.[12] In addition, quantitative EEG demonstrates abnormalities both regional and global connectivity and high frequency oscillations in TSC that may reflect changes in epileptogenicity and synaptic functionality.[35, 36] EEG assessment of functional connectivity is further reinforced by similar demonstration of structural connectivity in TSC, measured via dMRI. With dMRI, we have identified abnormalities in network organization and structural integrity associated with language deficits and autistic behaviors in TSC.[37, 38] Furthermore, dMRI can differentiate between typically developing children, children with TSC

alone, and those with both TSC and ASD.[39] Animal models of TSC demonstrate reduced myelination in white matter tracts, which could account for the increased diffusion observed in patients with the disorder.[9] Most relevant to this clinical trial, we have identified multiple dMRI measures of microstructural integrity of the corpus callosum, internal capsule, and geniculocalcarine tracts improve in SEGA patients treated with everolimus for 12-36 months.[40, 41] Thus, inclusion of EEG and dMRI as markers of target engagement represent a critical advancement over prior TSC clinical trials with mTOR inhibitors with the potential to provide important links between the molecular consequences of mTOR dysregulation and malleable brain networks amenable to treatment with mTOR inhibition. These same biomarkers potentially could be further developed as surrogates for target organ engagement by sirolimus and correlated with epilepsy prevention in TSC infants.

Preclinical studies suggest that the patient population most likely to benefit from treatment are young children in the critical period when epilepsy and neurobehavioral symptoms first emerge and synaptic plasticity is most robust.[42, 43] This is also supported by evidence from EXIST-3, where treatment was stratified by age and younger patients (< 6 years of age) were significantly more likely to reduce seizure frequency compared to older children and adults.[30] Younger patients also were more likely to achieve target dosing, and those with higher treatment exposure demonstrated the greatest age-associated improvement.[44] Yet as previously mentioned, safety and efficacy for mTOR inhibitors in younger children is limited, especially in those under the age of 2 years. Likewise, appropriate pharmacokinetic (PK) and pharmacodynamic (PD) characterization relating CNS kinetics to epilepsy outcomes in this age group is lacking. While high doses of mTOR inhibitors can prevent epilepsy and associated behaviors in TSC mouse models[10, 45, 46], few studies have attempted to define practical, clinically-relevant treatment protocols that could be translated into preventative therapy in TSC patients. To date, sirolimus and everolimus dosing in TSC clinical trials has been arbitrarily based on dosing to achieve target trough levels commonly used in solid organ transplantation (5-15 ng/ml)[19, 21, 27, 30, 47]. PK and PD studies in animal models and patients were primarily carried out during its clinical development for cancer and solid organ transplantation[48] and need to be validated in TSC, where the brain is the intended site of action. We have generated a preliminary population PK model for neonates and infants with vascular anomalies collected a patients using sirolimus data collected at Cincinnati Children's.[49] A secondary aim of the current study will be to update this PK model for TSC infants treated with a novel formulation of sirolimus (TAVT-18) and extend this to the primary and secondary outcome measures for PD analysis that determines optimal sirolimus dosing that maintains therapeutic benefit for epilepsy prevention without more exposure than necessary so as to minimize toxicity.

The proposed Phase I clinical trial is the first clinical trial to evaluate the potential of this mTOR inhibitor for preventing seizure onset and epilepsy progression in high-risk TSC infants. A previous version of this clinical trial protocol included a planned second stage, Phase IIb clinical trial comparing TAVT-18 to placebo in a randomized, double-blinded, placebo-controlled design. The latter has since been replaced by a stand-alone, Phase IIb clinical trial comparing a currently FDA-approved formulation of sirolimus with matching placebo known as the STOP2B: Sirolimus TSC Epilepsy Prevention Study (TSC-STEPS; clinicaltrials.gov NCT05104983; CCHMC IRB#2021-0438) made possible by separate funding from the FDA Office of Orphan Products Development (R01-FD007275).

3 PRELIMINARY STUDIES

Everolimus for treatment of refractory epilepsy in TSC: Preclinical studies and early human case reports have demonstrated beneficial effects of mTOR inhibitors on seizures.[6, 9, 25, 50] We reported in 2013 the first prospective, multicenter, open-label, phase I/II human clinical trial designed to directly assess the benefit of everolimus on seizure control in patients with TSC (NCT01070316, N=20).[27] Overall, treatment was well-tolerated and seizures were reduced in 17 of 20 (85%) by a median of 73% ($p<0.001$). Improvement in seizure control was accompanied with improvement in multiple parent-reported measures of behavior and cognition assessed using the Nisonger Child Behavior Rating Form (NCBRF) and Quality of Life for Children with Epilepsy (QOLCE). Initial responders continued treatment up to an additional 48 months (n=18) and improved seizure control compared to baseline was maintained in the majority (14 of 18, 78%), with longer treatment resulting in greater efficacy over time.[27, 28] A follow-up Phase III randomized, placebo-controlled, double-blind clinical trial was initiated in 2012 comparing low-dose exposure (3-7 ng/mL) and high-dose exposure (9-15 ng/mL) with placebo (EXIST-3, n=366).[30] Upon completion of the main study phase (3 months), 28.2% and 40.0% of the low- and high-dose everolimus-treated groups reported ≥50% reduction in seizure frequency, compared to 15.1% in the placebo group ($p=0.0077$ and $p<0.0001$, respectively). The median response rate for the two everolimus dosing groups combined was 31% after 3 months but improved to 47% after 12 months.[51] In both epilepsy studies, the tolerability and safety profile of everolimus compared with placebo were similar to previous clinical trials for SEGA or renal angiomyolipoma in TSC.[14, 21]

Sirolimus for treatment of refractory epilepsy in TSC: Sirolimus is FDA approved for the treatment of LAM,[22, 23, 52] a progressive lung disease largely seen in adult women with TSC. Sirolimus has also been shown to be effective for treatment of TSC-associated SEGA,[24, 52] angiomyolipoma,[22, 52, 53] cutaneous hamartomas,[54, 55] retinal hamartomas,[56] and cardiac rhabdomomas.[57-60] Yet despite robust preclinical studies demonstrating reduced seizures in TSC animal models treated with sirolimus,[6, 50, 61] clinical development of mTOR inhibitors for the treatment of epilepsy in patients has almost exclusively focused on everolimus. Case reports and retrospective studies have reported reduced seizure frequency following the initiation of treatment with sirolimus,[24, 25] but to date there has been only one prospective clinical trial.[62] Twenty-three children with TSC ages 1.8-10.9 years were treated with open-label sirolimus for 6 months. Half were randomized to treatment immediately, the remainder to treatment 6 months later. Sirolimus treatment reduced seizure frequency 41% compared to standard care, greatest in those achieving a target trough level of 5-10 ng/mL, and 3 patients became seizure-free. The trial was limited, however, by failing to reach its inclusion goal of 30 children.

Safety of mTOR inhibitors in TSC Infants: Multiple prospective clinical trials have demonstrated safety and tolerability of mTOR inhibitors generally in TSC, including sirolimus[16, 19, 21-23, 27, 30, 52]. Because treatment in each of the trials was initiated after the onset of clinically significant symptoms, nearly all study participants were over the age of 2 years; safety in younger infants that would be ideally targeted prior to emergence of first seizures remains inadequately studied. There are increasing number of published case reports of successful treatment of TSC infants treated for other indications,[63-72] but no prospective studies to date. As part of an international collaboration of TSC specialty clinics, we collected retrospective safety data from TSC infants treated with everolimus (n=39) or sirolimus (n=11) for clinical indications since 2010,[31] when everolimus was first approved for treatment of SEGA. In this cohort, infants

treated with everolimus started as early as the first month of life; 18 started treatment between 0-12 months and 27 between 13-24 months. The average duration of treatment 27.3 ± 24.7 months. At least one treatment-related adverse event (AE) was reported in 78% of subjects (84% in those treated with everolimus compared to 63% for sirolimus). The most common AE reported was infections (URI, sinusitis, otitis, gastroenteritis, other infection types, ranging in frequency from 20-69%. All but 9% of infections were mild or moderate in severity. The next most common AE type were aphthous ulcers/stomatitis (31%), similarly mild or moderate in all but one case. The most common laboratory abnormality was hypercholesterolemia with or without hyperlipidemia, reported in 14%, and again mild or moderate in severity in all but one case. This profile of AE, both in type and severity, is consistent with TSC clinical trials with everolimus and sirolimus in older children and adults treated for SEGA, angiomyolipoma, LAM, and refractory epilepsy. In most instances, AE were managed by temporarily suspending treatment until AE resolved. However, 23% stopped everolimus because of adverse events, which is higher than that reported for older children and adults in published studies to date (typically between 4-6%).[27] Although the study was not designed to evaluate efficacy, treating clinicians reported that 29 of the 45 (64%) subjects demonstrated at least partial benefit. Of the remaining 16 subjects, 8 (18%) were unsure or undetermined benefit, and 8 did not improve or respond to treatment at time of data cut-off. Given that the type and severity of AE in this infant cohort was no different and response is similar to that of older children with TSC, it more likely parent and/or clinician discomfort with continuing treatment in the context of an AE rather than the AE type or severity itself. In other words, discontinuation seems to be driven more by the lack of evidence-based treatment guidance for this specific age group than unique tolerability or benefit experience in TSC infants. Thus, establishing a definitive safety profile through a well-designed, prospective clinical trial with randomized, placebo-controlled, double-blind design remains a critical need for clinical decision making and future clinical trials in this population.

Sirolimus Dosing for Infants in TSC: Lack of focused PK/PD studies is a major reason many promising preclinical treatments fail during later clinical development in human studies[73] and there is renewed emphasis for inclusion of the same throughout therapeutic drug development for prevention treatments in CNS disorders.[61, 74, 75] While high doses of mTOR inhibitors can prevent epilepsy and autism-associated behaviors in TSC mouse models,[6, 10, 50] few studies have attempted to define practical, clinically-relevant treatment protocols of mTOR inhibitors that could be translated into preventative 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, 52, 53] The assumption is that PK/PD characteristics for sirolimus in TSC are the same, although this has never been verified. Relevant to the current study, there is particular deficiency for very young children, where drug clearance can vary significantly due to developing organ function.[76] Sirolimus has been used successfully to treat congenital vascular anomalies at our center,[77-79] which can occur in patients with TSC.[80] Collaboration in these trials by the NIH-funded Pediatric Pharmacology Research Unit (PPRU) and the Laboratory of Applied Pharmacokinetics and Therapeutic Drug Monitoring at Cincinnati Children's has allowed us to develop age-appropriate precision sirolimus dosing for pediatric patients with congenital vascular anomalies.[81] More recently, we were able to extend our findings to neonates and infants.[82] For the current study, these same models will be validated for TSC and used to describe sirolimus concentrations relative to primary and secondary efficacy and safety outcome measures and identify the optimal dosing range that preserves treatment effect while minimizing the risk of adverse events.

TAVT-18, a novel formulation of sirolimus with improved bioavailability and reduced inter-patient variability: Dissolution properties of sirolimus are generally accepted to be a rate limiting factor of oral absorption. Currently, two formulations, oral suspension and oral tablets, are FDA-approved, both marketed under the trade name of Rapamune™ (Pfizer, Inc.) and used off-label for the clinical treatment of infants with TSC.[31] However, the systemic bioavailability is low, estimated to be 14% and 18%, respectively, for the Rapamune™ oral suspension and tablets.[83] TAVT-18 is a novel powder formulation of sirolimus being clinically developed by Tavanta Therapeutics, Inc. that improves sirolimus absorption from the GI tract. Only the active ingredient (sirolimus) is absorbed for TAVT-18, so there are no identified toxicities from TAVT-18 to date that differ from existing formulations of sirolimus.[83] In preclinical studies, TAVT-18 demonstrated significantly improved maximal plasma concentration (C_{max}, increased 3.7-fold) and exposure (AUC_{inf}, increased 2.0-fold) compared to Rapamune™, while reducing t_{max} nearly 10-fold (Table 1). As a result, less TAVT-18 is needed compared to Rapamune™ to achieve comparable therapeutic target drug levels.

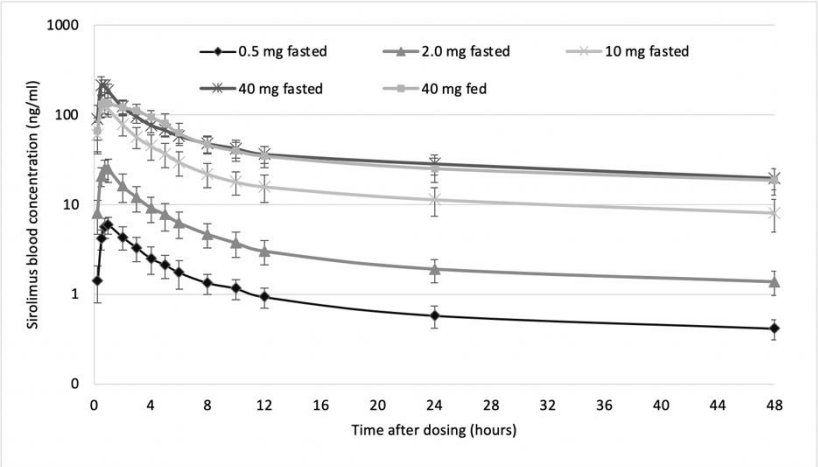
Table 1: PK parameters following oral administration of Rapamune™ or TAVT-18 in fasted rats (N=4) at 1.0 mg/kg/dose

Test compound	C_{max} (ng/ml)	t_{max} (h)	AUC_{inf} (ng/ml*h)	F_{rel} (%)
Rapamune®	6.9±0.3	4.8±0.4	144±14	
TAVT-18	25.9±9.0*	0.5±0.0**	307±59*	209

*: $p < 0.05$ **; **: $p < 0.005$

A Phase 1 clinical trial with TAVT-18 has also been conducted to evaluate the safety, PK and food effect of single doses oral doses of 0.5, 2 and 10 mg (fasted) and 40 mg (fasted and fed). TAVT-18 was well tolerated in this study, with no severe adverse events (AEs) reported, and no subject withdrawn as a result of an AE. The majority of AEs reported were mild in severity and considered unrelated to TAVT-18. Inter-individual variability also is reduced with TAVT-18 and therapeutically relevant plasma concentrations (C_{max}) and exposures ($AUC_{0\text{-}48h}$) are dose-proportional up to 10 mg in healthy adults (Figure 1, n=32).[84]

Figure 1. Mean (± standard deviation) blood sirolimus concentrations in the first 48 hours following the administration of the novel formulation at the indicated dose and prandial state.



The lower overall dosing that is needed to achieve therapeutic sirolimus levels, combined with less inter-patient variability, supports TAVT-18 is an attractive alternative to conventional sirolimus formulations such as Rapamune™ for preventative epilepsy treatment in TSC infants.

Alternative Strategies for Epilepsy Prevention in TSC: In 2010, Bombardieri *et al.* published a case series of 10 TSC children demonstrating the benefits of early recognition of seizures and treatment with the anticonvulsant vigabatrin, which inhibits GABA transaminase to increase GABAnergic signaling at the neuronal synapse,[85] was associated with more favorable long-term neurological outcome.[86] Shortly thereafter, Jóźwiak *et al.* demonstrated treatment with vigabatrin before the onset of clinical seizures in TSC infants was associated with improved neurological outcome when compared to historical controls treated after the onset of clinical seizures.[87] Prospective open-label (EPISTOP, NCT02098759) and randomized, double-blind, placebo-controlled (PREVeNT, NCT02849457) are underway in Europe and the United States, respectively, but results are not yet known. Our investigator team leads the PREVeNT study and have purposely designed this clinical trial with matching enrollment criteria, visit schedules, assessment procedures, and outcome measures to allow direct comparison between these two studies of preventative treatment for TSC infants that utilize distinct mechanisms of action.

4 STUDY OBJECTIVES AND OUTCOME MEASURES

4.1 Primary Endpoints

1. ***Safety*:** The primary endpoint will be the percentage of subjects reporting severe (CTCAE v5.0 grade ≥3) adverse event (AE) or serious adverse event (SAE), when treated with sirolimus (TAVT-18). Additional safety-related endpoints include:
    a. Percentage of subjects that reduce or discontinue treatment due to an AE or SAE (any grade).
    b. Number of days treatment is withheld due to an AE or SAE (any grade).

2. ***Efficacy*:** The primary endpoint for efficacy will be time to seizure onset, when treated with sirolimus (TAVT-18). Additional efficacy-related endpoints include:
    a. Age at time of seizure onset
    b. Percentage of subjects with infantile spasms (past or current) at the end of the treatment period (12 months of age).
    c. Seizure frequency at the end of the treatment period (12 months of age).

4.2 Secondary Endpoints

1. To evaluate the effect of early sirolimus (TAVT-18) treatment in TSC infants on epilepsy-associated neurodevelopment outcomes using the TSC-associated Neuropsychiatric Disorders (TAND) Checklist, Vineland Adaptive Behavioral Scales (VABS), Bayley Scales of Infant and Toddler Development (Bayley-4), Preschool Language Scale, Fifth Edition (PLS-5), and Autism Diagnostic Observation Schedule, Second Edition (ADOS-2).

2. To evaluate the effect of early sirolimus (TAVT-18) treatment in TSC infants on electrophysiological biomarkers of neuronal connectivity with EEG, we will measure frequency of interictal and ictal epileptiform activity, mean coherence, average path length, clustering coefficient, and global efficiency. We also will explore if these EEG biomarkers of functional connectivity correlate with white matter tracts associated with neurodevelopmental and/or neurocognitive impairment in TSC[39] and treatment with mTOR inhibitors in TSC.[40, 41]

3. To develop a predictive dosing model for early sirolimus (TAVT-18) treatment in TSC infants using TAVT-18, we will measure steady-state blood trough concentration (ng/mL) corresponding to actual and dose-normalized sirolimus dose (mg/m^2/day).

5 STUDY DESIGN AND ORGANIZATION

This trial originally planned to use a two stage, phase I clinical trial design, with the first stage to employ an open-label clinical trial design to verify dosing for TAVT-18 (sirolimus) powder for oral solution in TSC infants (N=5) that would then be carried forward to inform appropriate initial dosing, dosing frequency, and dosing adjustments for the second stage—a randomized, double-blind, placebo-controlled multi-site trial design—to evaluate the safety and efficacy of early sirolimus treatment to prevent epilepsy in TSC infants until 12 months of age (N=60). However, with the elimination of the planned second stage which has now been replaced by STOP2B: TSC-STEPS, the study design now consists of a single stage, phase I clinical trial design only. Subjects will be between 0-6 months of age at time of treatment initiation and meet clinical and/or genetic diagnostic criteria for TSC and have no prior history of clinical seizures at time of enrollment. Treatment will be continued until subjects reach 12 months of age. The TSC Clinic at Cincinnati Children's Hospital Medical Center (CCHMC) will serve as the single clinical site.

Additional key investigators of the study:

- Dr. Hans Greiner (CCHMC) will direct EEG acquisition and data transfer quality control, processing, and blinded central analysis
- Dr. Jamie Capal (UNC, Chapel Hill) will direct neuropsychological assessments and data transfer quality control, processing, and blinded central analysis
- Dr. Alexander (Sander) Vinks (CCHMC) will direct PK/PD sample acquisition and quality control, processing, and blinded central analysis
- Dr. Simon Warfield (Boston Children's Hospital) will direct dMRI acquisition and data transfer quality control, processing, and blinded central analysis
- Dr. Paul Horn (CCHMC) will serve as study biostatistician

Operations will be managed through the Clinical Coordinating Center (CCC) and Data Management Center (DMC) at CCHMC to ensure the optimal scientific rigor, efficient execution and management of the study, and analyses.

An independent medical monitor (MM) and Data Safety Monitoring Board (DSMB) will be established to ensure patient safety is appropriately monitored and prioritized throughout the study.

6 BENEFITS AND RISKS

6.1 Risk and Benefit Analysis

This study is greater than minimal risk, but with the potential for direct benefit.

6.2 Potential Benefits

Potential Benefits to Subjects and Others

There may be some benefit to research subjects from the therapeutic intervention and evaluations as part of this research. When available and as long as disclosure does not have the potential to impact the accuracy of future assessments of the subject during the course of the study, results of research-specific procedures and assessments performed during the course of this study may be provided to parents and clinicians.

Importance of the Knowledge to be Gained

The most important information to be gained from this research will be the possibility of developing a targeted, disease-modifying drug therapy to prevent seizure onset and epilepsy progression in infants with TSC.

6.3 Potential Risks

EEGs are routine clinical tests that are considered safe and carry negligible risk. EEGs have a minor risk of skin irritation due to adhesive used for sensor attachment but are routinely performed as part of clinical care for subjects with TSC in this age group.

MRIs are routine clinical tests that are considered safe and carry negligible risk. For subjects with TSC in this age group, the MRI performed yearly as part of clinical care routinely includes sedation. This sedation will be extended during the research portion of the MRI for no more than 15 minutes.

For blood sample collection to measure sirolimus levels and laboratory studies, there is a minor risk of pain, bleeding, bruising, or infection. When possible, blood draws will be combined with venipuncture already being performed for clinical purposes.

As part of this study there is a minor risk for a loss of confidentiality. Good clinical practice standards and institutional rules will be observed throughout all aspects of the study to maintain subject confidentiality. No individually identifiable subject data will be distributed to non-research personnel.

7 STUDY DURATION

The trial is for 3 years. Subject recruitment will occur during year 1 and completion of scheduled clinical visits and data analysis will occur in years 2-3.

8 SELECTION AND WITHDRAWAL OF SUBJECTS

Evaluable subjects are those who have initiated treatment with sirolimus, complete the initial

follow-up visit 1 month after initiating treatment, and complete the final assessment at 12 months of age.

Male and female TSC infants 0-6 months of age will be enrolled. There will be no exclusion based on race or sex.

Subjects will be recruited through the TSC clinics which are geographically distributed to allow nationwide recruitment, supplemented by and in partnership with the advocacy organization Tuberous Sclerosis Alliance (TS Alliance) website, *Perspective* magazine, and social media efforts (Facebook and Inspire, online community support page). We will continue existing strategies to improve recruitment and retention that have been successful in our TACERN and PREVeNT studies of TSC infants, which have been able to recruit a similar number of TSC infants under 12 months of age over a similar time period proposed for the current study. Successful recruitment strategies utilized in the past and continued here include leveraging existing outreach and clinical support programs at each center, financial support when available for qualified travel expenses, scheduling visit times and places that accommodate the needs of the family, providing regular feedback to families about the ongoing progress of their children, and optimization of overall TSC clinical medical care.

We may modify recruitment strategies as needed if the current pandemic or other events affect the ability for subjects to travel or willingness to be seen at a research site on schedule or if protocol-required assessments/procedures cannot be performed in a validated manner. Telehealth for clinical assessment of subjects, local collection of sirolimus levels and safety laboratory studies, and increased use of parent-reported measures or personal protective equipment/physical distancing-friendly tools are such strategies in current use by other TSC studies during the COVID-19 pandemic.

8.1 Inclusion Criteria

1. 0-6 months of age at the time of enrollment (subject must be <7 months of chronological age at time of randomization and treatment initiation). Corrected age must be at least 39 weeks (calculated by subtracting the number of weeks born before 40 weeks gestation from the chronological age).
2. Has a confirmed diagnosis of TSC based on established clinical or genetic criteria[88]

8.2 Exclusion Criteria

1. Prior history of seizures (clinical or electrographic) at the time of enrollment or identified on baseline EEG
2. Has been treated in the past or is currently being treated at the time of enrollment with conventional anticonvulsant medications (AEDs), systemic (oral) mTOR inhibitors (such as rapamycin, sirolimus, or everolimus), ketogenic-related special diet, or another anti-seizure therapeutic agent, device, or procedure
3. Has taken any other investigational drug as part of another research study, within 30 days prior to the baseline screening visit
4. Has a significant illness or active infection at the time of the baseline screening visit
5. Has a history of significant prematurity, defined as gestational age <30 weeks at the time of delivery, or other significant medical complications at birth or during the neonatal period that other than TSC would convey additional risk of seizures or

neurodevelopmental delay (i.e. HIE, severe neonatal infection, major surgery, prolonged ventilatory or other life-saving supportive care or procedures)

6. Abnormal laboratory values at baseline (i.e., renal function, liver function, or bone marrow production) that are in the opinion of the investigator clinically significant and may jeopardize the safety of the study subject
7. Prior, planned or anticipated neurosurgery within 3 months of the baseline visit
8. Has a TSC-associated condition for which mTOR treatment is clinically indicated (i.e. SEGA or AML)
9. Subjects who are, in the opinion of the investigator, unable to comply with the requirements of the study

8.3 Subject Withdrawal

Subjects may be withdrawn from the study for any of the following reasons:

- Excessive toxicity, prolonged interruption in treatment with study drug, or emergency unblinding due to an adverse event (see section 12)
- Non-compliance with study procedures or reporting
- Non-compliance with study drug dosing
- Withdrawal of consent
- Subjects lost to follow up, with documentation of due diligence
- Any unforeseen event that in the opinion of the treating physician and/or the PI, will prevent the research subject from taking part in this study

9 STUDY TREATMENT AND DOSING

9.1 STUDY MEDICATION (TAVT-18 SIROLIMUS)

The half-life of sirolimus (TAVT-18) is estimated to be >30 hours,[84] allowing for convenient twice daily oral dosing. Medication should be taken in the fed state, defined as within 30 minutes of feeding. An individualized PK model-informed age-adjusted dosing scheme with a starting dose based on age at the time of enrollment (see Table 2) will be utilized, which is based on previous experience and simulation analysis of data obtained from our previous studies treating infants with congenital vascular anomalies with Rapamune™ [49, 77, 82] and preclinical and human studies with TAVT-18.[83, 84] These targeted doses are estimated based on the assumption that the oral bioavailability of TAVT-18 is 2-times higher compared to Rapamune™. The target pre-dose whole blood trough sirolimus concentration (C_{min}) will be 10 ng/mL with an upper tolerance range of 10-15 ng/mL. This target is consistent with prior published experience from infants treated with sirolimus for clinical indications[31] and previous clinical trials in TSC patients treated for SEGA or epilepsy.[24, 31, 62]

Table 2: Estimated sirolimus (TAVT-18) starting doses for the indicated target concentration range[82]

Age group (months)	Dosing for target concentration range = 10-15 ng/ml
0-1	0.2 mg/m^2/dose, administered twice daily (total daily dose = 0.4 mg/m^2)
1-2	0.25 mg/m^2/dose, administered twice daily (total daily dose = 0.5 mg/m^2)
2-3	0.3 mg/m^2/dose, administered twice daily (total daily dose = 0.6 mg/m^2)
3-4	0.35 mg/m^2/dose, administered twice daily (total daily dose = 0.7 mg/m^2)

4-6	0.45 mg/m^2/dose, administered twice daily (total daily dose = 0.9 mg/m^2)

Steady state whole blood trough levels should be drawn in the morning, approximately 24-hours since last dose of study medication, after the subject has been on continued dosing for 7-14 days. Sirolimus trough level may be drawn at the participating study center or drawn locally and sent to the reference laboratory, whichever is easier for the subject. Subjects for which treatment is held temporarily due to an AE but resumed at same dosing as prior to the AE (see section 12) do not require a repeat sirolimus whole blood trough level to be checked when resuming treatment after the AE has resolved.

Whole blood sirolimus trough levels will be assessed at defined intervals after the initial dose (+1, +3, +6, +24 hours). Infants will continue daily dosing at the same dose, orally twice/daily within 30 minutes of feeding, for an additional 6 days (±1 day), when the steady state sirolimus whole blood trough levels will be assessed at defined intervals of +1, and +3 hours post-dose. Based on resultant steady-state measured trough level at day 7, sirolimus dosing will be adjusted per protocol (see next paragraph) to achieve the desired precision dosing target of 10 ng/ml. Treatment at the adjusted dosing will continue an additional 6 days (±1 day), after which the steady state sirolimus trough level will be measured again (day 14). Subject recruitment and participation is an essential component to the success of the overall study, but it is recognized enrollment is unlikely unless given opportunity to continue treatment beyond 14 days given the potential for direct benefit (see section 6.2 above) provided no individual- or group-level safety concerns are encountered. Accordingly, after the day 14 sirolimus trough level, participants can elect to continue open-label treatment with TAVT-18 (sirolimus) up to 12-months of age, providing additional extended exposure dosing and safety data for TAVT-18. For additional details, see section 11.2 below.

During open-label treatment with TAVT-18 (sirolimus), participants will have already had their first steady sirolimus whole blood trough concentration measured at 7 and 14 days after initiating treatment and subsequent dosing will be individually adjusted using real-time drug concentration measurements in combination with a Bayesian population model-based target optimization approach.[124] Sirolimus trough levels also will be obtained in conjunction with safety labs (CBC with differential, comprehensive metabolic panel, lipid profile, amylase, and lipase) drawn at each clinical follow-up visit (1, 3, 6, 9, and 12 months). Patients will keep a diary of dosing information recording exact dosing times, time of last feeding, and dosing amount administered. Any missed doses will be reviewed at each visit to document adherence. Sirolimus concentrations will be determined at the CLIA-certified laboratory located at Cincinnati Children's using a validated tandem mass spectrometry assay performed using electrospray on a Waters Quattro Micro API triple quadrupole mass spectrometer interfaced with an Acquity Ultra Performance Liquid Chromatography instrument (Water Corporation, Milford, MA). The dynamic range of the assay is 0.5–100.0 ng/mL. As we did previously,[89] a central physician not involved in patient care or study assessments will monitor sirolimus results and provide protocol-specified dosing adjustments in real time Patients may continue treatment clinically (non-research) with sirolimus after the 12-month follow-up visit if clinically indicated. However, research study medication will not be provided beyond the final follow-up visit, so sirolimus must be sourced through normal clinical supply channels in such instances.

For subjects who are unable to tolerate the protocol-defined dosing schedule, dose adjustments are permitted following guidelines in Table 4 (section 12) in order to keep the subject on study drug. If study drug is interrupted due to toxicity (AE), study drug should not be resumed until recovery to grade ≤ 1 is achieved. Depending on AE type and grade, study drug may be re-introduced at the initial dose or a lower dose level (see Table 4). Any changes must be recorded on the appropriate CRF. Additionally, if any surgery is planned, study drug should be interrupted for one week prior to surgery and be re-started as soon as possible following wound healing (default is 2 weeks post-procedure, but may be shortened to 1 week for minor procedures and extended as far as 4 weeks under special circumstances).

Treatment compliance will be monitored via patient diaries and drug accountability procedures. Treatment compliance will be reviewed at each clinical follow-up visit and calculated as the ratio of actual drug taken/expected drug taken. For calculation of treatment compliance, days expected will be corrected to account for days when subject is instructed by study staff to hold medication due to an AE. Subjects unable to provide verification of study dosing compliance via medication diaries and/or drug accountability procedures may be removed from the study due to noncompliance. Likewise, subjects repeatedly with a corrected treatment compliance ratio <75% or >125% despite retraining may also be removed from the study due to noncompliance.

9.2 CONCURRENT MANAGEMENT OF EPILEPSY

The purpose of the study is to determine if sirolimus is safe and effective for prevention of epilepsy in at-risk infants diagnosed with TSC. The primary endpoint for efficacy epilepsy prevention is time to first seizure (seizure onset). However, subjects treated with sirolimus may still develop seizures if (1) our primary hypothesis is incorrect and sirolimus is not preventative or (2) our hypothesis is correct and seizure onset is delayed but sirolimus does not fully eliminate seizures from ever occurring. It is this very reason that our study will evaluate additional secondary efficacy disease-modifying outcome measures relating to epilepsy (seizure type and seizure frequency) and neurodevelopment at 12 months of age.

Accordingly, new onset of seizures (clinical or electrographic) does not require discontinuation of study medication. New onset of seizures should be evaluated and treated with conventional anticonvulsant medications (note that mTOR inhibitors are excluded) according to recommended clinical practice guidelines and the managing clinician's best judgement. In most instances, this will be initiation of vigabatrin, recommended first line therapy for infantile spasms in TSC and an effective treatment for focal epilepsy which is also common at this age.[12, 34] Rescue medications are also allowed. In all instances, prescribed treatment for seizures (medication, diet, and/or procedures) will be recorded in the appropriate CRF, including medication/treatment/procedure name, start/end dates used, total daily dose prescribed (including dosage adjustments, when applicable). Medications temporarily utilized or prescribed for use on as needed basis (PRN) will be recorded and include the date/time/dose for each use.

Another clinical scenario with relevance to the current study is the potential for off-label, prophylactic treatment with vigabatrin in TSC infants who have not yet experienced clinical or electrographic seizures. Off-label prophylactic treatment with vigabatrin has the potential to directly interfere with primary and indirectly affect secondary outcome measures of this study evaluating the preventative benefit of sirolimus. Jóźwiak et al (2011) published a case series of 14 TSC infants prophylactically treated with vigabatrin following abnormal EEG, compared to

historical controls treated conventionally with vigabatrin after the onset of clinical seizures.[87] In that study, the pre-emptive vigabatrin treatment did not always prevent seizures from occurring but still resulted in favorable seizure control and developmental outcomes at 24 months of age. Some patients since 2011 as a result have been treated with prophylactic vigabatrin in the absence of clinical or electrographic seizures. However, the Jóźwiak study results have yet to be replicated and validation efforts using prospective, randomized, double-blind clinical trials currently are underway in Europe (EPISTOP) and the United States (PREVeNT). Until then, off-label prophylactic treatment with vigabatrin is not recommended in current international guidelines nor considered current standard of care.[34] Accordingly, such prophylactic treatment with vigabatrin (or any other anticonvulsant medication or anti-seizure therapy prescribed for the same purpose of epilepsy prevention) before the onset of first seizure is specifically prohibited. To be clear, this restriction does not apply to vigabatrin or any other anticonvulsant or anti-seizure treatment(s) after the onset of first clinical seizure (see above), as withholding such treatment would be unethical and contrary to current treatment guidelines.[34]

10 STUDY DRUG SUPPLY

10.1 Description of Study Drug

The investigational drug product to be used in this study is TAVT-18, a proprietary formulation of sirolimus in clinical development, by Tavanta Therapeutics, Inc. It is provided in powder formulation consisting of 16.7% sirolimus as active ingredient along with 50.0% Povidon K90 and 33.3% sodium lauryl sulfate as excipients. The excipients ensure that sirolimus disperses instantaneously in water, which is then administered as an oral solution. Both excipients used are pharmaceutical grade and are listed on the FDA Inactive Ingredients Used in Approved Drug Products list.[90] TAVT-18 will be supplied from the manufacturer in powder form in pre-measured vials. The central investigational pharmacy at Cincinnati Children's will then oversee the labeling of the TAVT-18 in pre-measured vials containing 500 ug of TAVT-18 (sirolimus) for single use. TAVT-18 is stable in powder form for at least 12 months when stored at 2-8 °C.

Printed instructions and supplies will be provided to the parent for TAVT-18 preparation and administration. When not in use, TAVT-18 (sirolimus) will be kept in the refrigerator. At time of administration, the parent/caregiver will add 20 ml water and invert the vial 2-3 times to reconstitute the oral solution with a concentration of 0.025 mg/ml. The reconstituted solution is ready for administration right after the reconstitution and is stable at room temperature for at least 2 hours. Sirolimus stability is known to be reduced at lower pH (e.g. pH <3) that is ameliorated by raising pH by as little as 1-2 points.[91] Stomach pH in infants typically ranges between 2-5[92] and although there was little difference between fasted and fed states with regard to mean t_{max} (< 1 hr) in adults treated with TAVT-18 (sirolimus),[84] we want to reduce this potential added source of absorption variability to the present study. Accordingly, TAVT-18 (sirolimus) will be administered to infants in the fed state (within 30 minutes of feeding), where breastmilk or formula (pH 7.0-7.4)[93] is sufficient to sufficiently raise stomach pH above the minimum desired threshold (pH > 4).[91]

10.2 Study Drug Labelling and Packaging

TAVT-18 will be packaged and labeled per institutional guidelines. Study medication labels will be labeled as "Sirolimus (TAVT-18) for Oral Solution" and include all additional required labeling as an investigational product, including source (Cincinnati Children's Hospital),

quantity, administration instructions, storage conditions, Lot#, Expiration date, Name of Clinical Trial, PI Name, and FDA IND#.

10.3 Study Drug Administration, Compliance and Accountability

Medication will be dispensed to subjects at each visit, with enough supply to cover the visit window time period. Subjects will be instructed on how to prepare and administer study drug in accordance with the protocol. Dispensing of study drug will only be done by designated and qualified individuals at that site location. The study drug is to be taken as a suspension only. For PK/PD analyses, time and dose amount will be captured via CRF when corresponding blood trough levels are obtained (see section 9 above regarding dosing and blood trough level monitoring). An IRB-approved Dosing and Administration instructions for use will be provided to subject families that outlines specific instructions for preparation and use.

If the subject misses a dose (scheduled dose not administered within 12 hours of expected timing), the dose will be skipped. The missed dose will be recorded as not taken in the subjects' medication diary. If the dose is missed/held due an AE, that will be recorded in the subject's medication diary also. Study drug compliance will be assessed by the investigator and/or study team designee at each visit. Parents/LARs will be expected to return both unused and used study drug to the site at each scheduled visit. All doses taken and any adjustments in doses are to be captured on the drug diary CRF.

Subjects who take 75-125% of their drug, excluding days in which they are instructed to temporarily hold study medication by study investigators due to an AE (see section 12), will be considered compliant. Subjects who take <75% or > 125% of their drug, excluding days in which they are instructed to temporarily hold study medication by study investigators due to an AE (see section 12), or subjects for which drug compliance cannot be verified, may be withdrawn from the study (see section 8.3 above).

Upon withdrawal from or completion of the study, any unused study medication is to be returned by the subject.

10.4 Study Drug Distribution, Return, and Destruction

The CCHMC Investigational Pharmacy will serve as central pharmacy and be responsible for providing the site with a pharmacy binder and applicable training documents for site staff to review before the initial shipment of drug dispensed. The pharmacy binder will include (1) dispensing guidelines, (2) enrollment logs, (3) pharmacy personnel and training logs, (4) study medication shipment and receipt forms, (5) site master inventory logs, (6) physician ordering templates, (7) study medication resupply forms, and (8) study medication return forms.

All medication provided to the site from the central pharmacy will be used only for this trial and accountability logs will be maintained at the site. CRFs will be completed each time study medication is received or dispensed. At the conclusion of the trial and at appropriate times throughout duration of the trial, accountability will be reconciled (monitored). Accountability logs may need to be faxed or emailed to the central pharmacy or the sponsor when requested for overall study medication reconciliation.

The central pharmacy will keep an expiration log for all study medication.

11 STUDY PROCEDURES

11.1 Subject Numbering

Each subject will be assigned a unique study ID when the subject is first enrolled and remains the identifier for the study duration. Study IDs will not include protected health information (PHI) or PHI-derived identifiers nor elements that could be used to determine randomization assignment.

11.2 Initial Open-label PK Study (Stage 1)

Five infants will be enrolled for detailed TAVT-18 (sirolimus) dosing and pharmacokinetic (PK) analysis and preliminary safety profile. Open-label, non-randomized treatment initially will be for 14 days, during which sirolimus trough levels and safety labs will be collected at baseline and at defined intervals along with continuous AE/SAE reporting.

Following verification of baseline safety laboratory studies (CBC with differential, complete metabolic panel, lipid profile, amylase, and lipase), initial dosing will be based on age at the time of enrollment (see Table 2), dosed orally within 30 minutes of feeding, twice/daily (see section 9.1). Whole blood sirolimus trough levels will be assessed post-dosing at defined intervals (+1, +3, +6, +24 hours) on day 1. Infants will continue daily dosing at the same dose, orally twice/daily within 30 minutes of feeding, for an additional 6 days (±1 day), when the steady-state sirolimus whole blood trough level will again be assessed at +1 and +3 hours post-dose. Based on resultant steady-state measured trough level at day 7, sirolimus dosing will be adjusted per protocol to achieve the desired precision dosing target of 10 ng/ml (see section 9.1). Dosing will continue for an additional 7 days and the final PK assessment for Stage 1 analysis will be assessed on day 14 (±1 day). Safety labs will be repeated at the +7 days and +14 days assessment visits. In addition, AE and SAE safety data will be collected throughout.

Subjects with no significant AE/SAE during the initial 14-day treatment period will be allowed to continue open-label treatment so as to allow collection of additional extended exposure safety data until 12 months of age, provided sirolimus trough levels within the target range are able to be achieved and maintained (see section 9.1 above).

11.3 Interim Analysis and DSMB Determination of Study Continuation

After the last (5^{th}) subject completes the 14-day open-label treatment study visit, a detailed PK-dosing and preliminary safety analysis report will be prepared by the study team for review by the study sponsor, drug supplier, and DSMB. Specific criteria for continuation of the study (Go/No-Go criteria) are not specified for this Phase I study, but the report must address the following key questions to the DSMB's satisfaction:

(1) Is 0.2-0.45 mg/m^2 TAVT-18 (sirolimus) dosed orally 2x/day appropriate for initial dosing in TSC infants 0-6 months of age to achieve a target sirolimus trough levels between 5-15 ng/ml?

(2) Does the proposed real-time, individualized PK model-based precision-dosing strategy for TAVT-18 (sirolimus) in TSC infants perform adequately in order achieve the target steady-state sirolimus trough level of 10 ng/ml?

(3) Are there any unique safety events or related concerns that have emerged in TSC infants that were not anticipated which significantly alter the potential benefit-to-risk profile for TAVT-18 (sirolimus) in this population?
(4) Have any practical issues with the planned study design been identified which were not anticipated that could impact the feasibility or successful completion of the primary objectives of the study?

The DSMB may request additional information or analysis from the investigators, modification to the study design, or study discontinuation.

11.4 Treatment Assignment and Randomization (Stage 2)

This section of the protocol has been deleted, as the Phase IIb (Stage 2) portion of the study has been replaced by STOP2B: TSC-STEPS operating under a separate protocol (CCHMC IRB# 2021-0438).

11.5 Study Visit Schedule

A summary of study visit schedule and study procedures is summarized in Table 3.

Baseline Visit (Screening/Treatment Initiation)
To be eligible, infants 0-6 months of age must have a confirmed diagnosis of TSC based on established clinical and/or genetic criteria.[88] During the baseline visit, eligibility criteria will be verified, baseline medical history/demographics, vital signs, and any existing laboratory or diagnostic testing results will be collected. A clinical examination along with a routine video-EEG and safety laboratory tests (with option for additional blood samples to be collected at the same time and stored at the TSC Biorepository located at the Van Andel Research Institute for future genetic and/or molecular studies) will be performed. Dispensation of study drug will occur following confirmation of eligibility. Genetic mutation information will be collected, but genetic testing will not be required to be positive if clinical criteria are fulfilled since up to 15% of subjects will clinically-definite TSC have no mutation identified through conventional genetic testing.[88, 94, 95]

Follow-up Visits
In-person study visits will occur at screening/baseline, Day 7, +1 month following treatment initiation, and then at defined intervals thereafter according to subject's chronological age: 3 months, 6 months, 9 months, and 12 months. Visit schedule windows are +/- 2 weeks from the scheduled visit date, and if the +1 month follow-up visit and the next defined age interval follow-up visits are within 4 weeks of each other, they may be combined into a single in-person study visit. At each visit, a clinical examination with interval medical history that includes concomitant medications and any additional interventional therapies received (including type of intervention and average hours/week of each), safety laboratory studies including sirolimus level, optional biorepository samples, EEG, and study drug accountability will be completed. Seizure diaries and adverse event monitoring and reporting will also be maintained continuously throughout the study and reviewed at each visit. At the in-person follow-up visit at 12 months of age, dMRI, EEG, TAND Checklist, VABS, and Bayley-4 will be performed. To verify durability of treatment effect, at the in-person follow-up visit at 24-months of age, repeat developmental assessment with the addition of the PLS-5 and ADOS-2, accompanied by clinical

examination and an interval medical history that will include any additional treatment interventions, epilepsy history, and therapies, will also be performed.

Between in-person study visits, interim phone visits will be conducted as often as needed, but not less frequently than 1x/month throughout the entire length of study. Phone visits will occur more frequently for patients enrolling and initiating treatment as follows: 1) for those enrolling between 0-3 months of age, phone calls will occur weekly the first month of treatment and every other week thereafter until the patient is at least 3 months of age, then monthly thereafter; 2) for those enrolling between 4-6 months of age, phone calls will occur weekly the first month of treatment and then monthly thereafter. Phone visits will consist of update of medical history, discovery and documentation of any interval AE not previously reported, review of seizure and drug diaries, and preparations for next scheduled study visit. If a new or worsening AE with severity grade ≥3 is revealed during an interim phone visit (or other communication between study team and subject parent/caregiver), study medication must be held until subject is able to undergo appropriate clinical evaluation by qualified medical professional and cleared by study investigators to resume treatment. All AE and stopping/resuming study medication will be reported using the appropriate CRF and reporting requirements outlined in Section 12.

After treatment initiation, follow-up PK assessment and dose-adjustment visits will take place at defined intervals. During Stage 1, scheduled follow-up PK assessment and dose-adjustment visits will occur on day 7 and day 14. Thereafter, PK assessment and dose-adjustment visits will in conjunction with in-person clinic visits (+1 month after treatment initiation and then according to chronological age (3, 6, 9, and 12 months)). During Stage 2, scheduled follow-up PK assessment and dose-adjustment visits will occur 7-14 days after treatment initiation then in conjunction with in-person clinic visits (+1 month after treatment initiation and then according to chronological age (3, 6, 9, and 12 months)). Scheduled PK assessment and dose-adjustment visit windows 7-14 days after treatment initiation are +/- 1 week. All other scheduled PK assessment and dose-adjustment visit windows are +/- 2 weeks from the scheduled visit date, and if the +1 month PK-a visit and the next defined age interval follow-up visits are within 4 weeks of each other, they may be combined into a single study visit. For both Stage 1 or Stage 2, unscheduled PK assessment and dose-adjustment visits will occur 7-14 days after TAVT-18 (sirolimus) dose is adjusted in accordance to protocol to achieve target blood trough level of 10 ng/ml (see section 9.1 above) or due to AE/SAE (see section 12 below).

TABLE 3: Schedule of Study Procedures/Events

Procedure	**Screening/Baseline (0-6 months of age)**	**+7-14 days**	**+1 month**	**Age: 3 months**	**Age: 6 months**	**Age: 9 months**	**Age: 12 months**	**Age: 24 months**
Eligibility/ Informed Consent	X							
Baseline History/Demographics	X							
Interval Medical History			X	X	X	X	X	X
Safety Laboratory tests[1]	X		X	X	X	X	X	
Sirolimus level[2]		X	X	X	X	X	X	
Vital Signs	X		X	X	X	X	X	X
Clinical Exam	X		X	X	X	X	X	X
Adverse Event Review	X		X	X	X	X	X	
Seizure Diary Review			X	X	X	X	X	
EEG[3]	X		X	X	X	X	X	X
dMRI[4]							X	X

Neuropsych assessment[5]							X	X
Dispense Study Drug	X		X	X	X	X		
Interim phone vists[6]								

Subjects may be enrolled into the study at any time between the ages of 0-6 months and will receive TAVT-18 (sirolimus) until 12 months of age. Visit window for follow-up visits is ± 2 weeks.

[1]Includes option for additional bloodwork for exploratory measures; subjects not taking study medication within the previous 48 hours of the study visit due to AE or other reasons will not have sirolimus level assessed.
[2]Sirolimus trough levels should be drawn in the morning, approx. 24hrs after last dose of study medication. The initial level will be drawn 7-14 days after subject has been on steady dosing and in conjunction with safety labs at each clinic follow-up visit (1,3,6,9,12 mths). Similarly, trough levels will be drawn at 7-14 days after any dose modification, until target concentration is reached. For convenience, levels can be drawn at the study center or drawn locally and sent to the reference laboratory.
[3]Routine EEG (60 minutes duration). EEG acquisition schedule for study is consistent with current TSC clinical guideline recommendations
[4]dMRI is 15-minute add-on sequence to conventional MRI that is recommended clinically every 1-3 years in TSC.
[5]Developmental assessment consists of TAND Checklist, VABS, and Bayley-4 at the 12 months of Age visit and TAND Checklist, VABS, Bayley-4, PLS-5, and ADOS-2 at the 24 months of Age visit
[6]Monthly calls will occur in between in-person visits. During the first month in the study, phone calls will occur weekly.

End of Treatment

At the end of the treatment period (when each subject reaches 12 months of age), participants will stop taking the study drug (TAVT-18). Due to the extended half-life of sirolimus, gradual weaning is not necessary. In the absence of significant complications or contraindications, parents who wish to pursue continued treatment with sirolimus after their final study visit as an off-label clinical treatment using commercially-available, FDA-approved medication may do so through consultation with the primary TSC clinician. However, such is not part of the current research study and no research study medication will be provided after completion of the final study visit at 12 months of age.

Post-treatment follow-up

A follow-up assessment will occur 30 days after the end of treatment visit by phone, which will include questions about any new or persistent side effects after discontinuing treatment. We are interested in correlating primary and secondary outcome measures at 12 months (this study) with potential longer-term epilepsy-related outcomes. Thus, enrolled subjects will repeat developmental assessment (TAND Checklist, VABS, Bayley-4, PLS-2 and ADOS-2) at 24 months, along with collection of interval medical history related to epilepsy (seizure types and frequency, epilepsy and cognitive/behavior/developmental treatment(s), etc. In addition, as long as the current study protocol remains active and provided proper subject consent, subjects may be contacted annually to obtain any new additional follow-up clinical history, lab results, EEG or brain imaging source data and reports, and other clinical data pertinent to epilepsy or neurodevelopmental outcomes beyond 24 months of age.

11.6 Visit Procedures

Clinical Evaluations

The following clinical data specifically will be collected: Basic demographics, medical/family history, vital signs, physical and neurological examination, current seizure history (type and frequency), prior and concomitant medications, adverse events, interventional therapies. Seizure diaries will be given to parents/guardians to record seizure type and frequency following randomization, and these will be returned at each visit. A study drug diary will also be given to parents/guardians to record treatment administration and these likewise be returned to the sites at

each visit. Outside test results or specialist evaluation notes obtained as part of the subjects' clinical care may be included in the research medical/family history, including but not limited to clinical imaging studies, laboratory tests, genetic testing results, and medical procedures.

Laboratory Evaluations

Laboratory evaluations will be done at baseline, 1 month, 3 months, 6 months, 9 months, and 12 month visits. These are standard of care laboratory assessments for this population, including: CBC with differential, comprehensive metabolic panel, lipid profile, amylase, lipase and concurrent anticonvulsant levels (if any). Sirolimus trough level measured in whole blood (C_{min}) will also be determined for any follow-up visit if patient has taken study medication within the previous 48 hours, utilizing a central laboratory in order to maintain treatment assignment blinding (CCHMC). At each blood draw, the time/amount of last medication(s) dose, medication preparation procedures, time since last feeding, and any other identifiable parameters with potential impact on testing results will be captured via CRF.

Opportunity to collect biological tissue for future research from well-characterized patients with rare diseases such as TSC is extremely limited. Accordingly, we will include the option for participants in this study to provide blood and tissue for future genetic and molecular studies, as we have done in all previous NIH-funded TSC clinical trials conducted by our clinical research consortium. During time of scheduled research or clinical blood draws, extra blood may be collected for this purpose. Likewise, when tissue specimens left over from clinical procedures that would otherwise be discarded may be collected. The TSC Biorepository is funded by the TS Alliance and physically located at the Van Andel Research Institute (VARI) under the direction of Dr. Scott Jewell and accredited by the Biorepository Accreditation Program of the College of American Pathologists (#8017856). Providing blood or biological specimens is optional, will not require any additional effort on the part of the patient, and will not interfere with any required clinical or study procedures. Collection and shipping kits are provided by the TSC Biorepository, and at all times accompanying demographic and accompanying phenotypic data is devoid of any protected health information so as to maintain patient confidentiality at all times. Following established protocols and procedures, specimens are prepared at shipped overnight to VARI. Blood is separated into white blood cells and plasma, from which DNA and RNA are isolated. Other tissue samples are stored as either fresh frozen or preservative-fixed specimens.

When blood is drawn, the sirolimus trough will be done first, followed by safety testing, followed by optional biorepository specimens. Drawing of the blood tests will be ordered in this way to ensure the most important samples for patient dosing and safety are collected first, while not exceeding institutional limits. Total blood draw volume over the 12 month study period is likely to range from 20-40mL.

Clinically significant abnormalities prior to initiating treatment with study medication that are not exclusionary will be recorded as part of the medical history and current medical conditions in the source documents and CRFs. New significant abnormalities identified after the start of study medication will be recorded as adverse events in the source documents and CRFs. All laboratory reports will be retained in the source documents in the subject charts.

Neurodevelopmental/Behavioral Evaluations

A comprehensive neuropsychological and behavioral assessment battery providing an abbreviated global assessment, using the TAND Checklist, VABS, and Bayley-4 will be

performed at the end of treatment (12 months of age). The same neuropsychological and behavioral assessment battery will be performed at 24 months to assess durability of treatment effect. Components of the TAND Checklist capture achievement of major developmental milestones and presence/absence of early problem behaviors. Components of the VABS assess adaptive behaviors. Components of the Bayley-4 include measurements of motor, cognitive, language, social-emotional, and adaptive behavior development. Components of the PLS-5 assess language skills for children. Components of the ADOS-2 assess children for autism spectrum disorder (ASD). Both the TAND and VABS are administered through parent interview or automatic capture (e.g., iPAD) and reviewed with the parent at time of collection. The Bayley-4 and PLS-5 are a combination of parent interview and child observation. The ADOS-2 is conducted through child interaction and observation.

TAND Checklist: The TAND Checklist consists of a set of questions that can be used to guide a 15-minute conversation between the healthcare provider and a parent, caregiver, or individual with TSC. The checklist captures basic developmental milestones, current behavior, psychiatric diagnosis, perceived intellectual disability, scholastic difficulties, neuropsychological skills, and psycho-social functioning. It also includes parent/caregiver/self-rating and healthcare provider ratings of the overall impact of TAND. As a screening tool, the TAND Checklist has been shown to have face validity, content validity, and transferability for capturing essential and relevant TAND features in different populations of TSC[96]. Domain scores correlated strongly with the Strength and Difficulties Questionnaire and BRIEF behavior rating index, two additional standardized parent-report measures. **(**Administration time: approximately 15 minutes)

Vineland Adaptive Behavioral Scales (VABS): VABS will be administered at the end of treatment visit when subjects are 12 months of age. The VABS is a caregiver-interview that assesses social, communication, motor, and daily living schools.[4] Test booklets with scores will be maintained with the source documents. Raw and age equivalency adaptive behavior composite (ABC) and 4 subscale scores will be entered in the CRFs. (Administration time: approximately 25 minutes)

Bayley Scales of Infant and Toddler Development, Fourth Edition (Bayley-4): The Bayley-4 includes a core battery of five scales: adaptive behavior, cognitive, language, motor, and social-emotional. The Bayley-4 is a combination of parent/caregiver interview/input along with observation of the child performing activities[114]. Test booklets with scores will be maintained with the source documents. Subtest level raw and scaled scores, domain composite scores, and percentile ranks will be entered into the CRFs. (Administration time: approximately 30-70 minutes)

Preschool Language Scale, Fifth Edition (PLS-5: Zimmerman, 2011): The Preschool Language Scale will be completed at the 24-months of Age visit. This interactive, play-based assessment provides information about language skills for children birth through age 7. (Administration time: approximately 10-30 minutes)

Austism Diagnostic Observation Schedule, Second Edition (ADOS-2; Lord, 2000): ADOS-2 will be completed at 24-months of Age visit. The ADOS-2 is a semi-structured, interactive observation schedule designed to assess individuals who may have an ASD and consists of 5 modules. The specific module to be used at each time point is determined by the ADOS

administrator at the time of these assessment. The Toddler Module is for infants between 12-30 months of age who do not consistently use phrase speech, whereas Module 1 is for children 31 months or older who do not consistently use phrase speech. Module 2 is for children of any age who use phrase speech but not verbally fluent. The revised diagnostic algorithms will be computed and the established cut-off scores used to differentiate ASD and non-ASD children and can be compared across modules. It is routine practice for ADOS-2 assessments to be digitally recorded and later reviewed by the research psychologist team members for scoring, standardization, and quality assurance purposes. (Administration time: approximately 60 minutes)

SPECIAL LANGUAGE CONSIDERATIONS FOR DEVELOPMENTAL ASSESSMENTS: TAND Checklist, VABS, Bayley-4, PLS-5, and ADOS-2 should be done in the subject's native language (defined as primary spoken language in the subject's home) by qualified administrator fluent in the subject's native language. In non-native English speakers where this is not available, these can be administered in English if parent is fluent in the same or can be administered through a qualified medical interpreter in the parent's native language, but noted as such on the corresponding CRF. Inability to obtain a specific developmental assessment due to language barrier is not a reason for exclusion from study participation, but any developmental assessments not performed should be recorded as a procedural study deviation on the appropriate CRF.

Encephalography (EEG)

Encephalography (EEG) with video is recommended at the time of TSC diagnosis and as clinically indicated thereafter for surveillance of seizure risk and response to treatment.[34] EEG (60 minutes, included extended epochs of both wake and sleep states) are scheduled to be acquired at every visit, but at minimum will be performed at baseline and at end of treatment (12 months). Sites in this network will utilize identical EEG acquisition protocols and procedures established by the TSC Clinical Research Consortium that are designed to yield comparable results across different equipment platforms.[12] Recordings will utilize 21 gold-plated electrodes placed according to the International 10-20 system with a digital sampling rate at a rate of 2000Hz with no filters, displayed at an effective time scale of 30 mm/sec with 1Hz low frequency and 70Hz high frequency display filters on the left screen, and on the right screen a maximum time scale of 100 mm/sec, 30Hz low frequency filter (high pass) and 100Hz high frequency filter (low pass). These parameters were designed to yield comparable data on Stellate, Nihon-Kohden, XLTEK/Natus EEG systems throughout the consortium. Any additional EEG obtained for clinical purposes between scheduled visits will also be collected for research analysis. The digitized EEG source data will be transferred to CCHMC for central processing and analysis.

Diffusion MRI (dMRI)

Brain MRI is recommended in TSC infants annually as standard clinical care to monitor for developing or progressing SEGA.[97] We have developed a diffusion MRI (dMRI) protocol,[98] that is an optimally distributed set of gradient directions and strengths, and an algorithm that identifies diffusion tensor (DTI) and diffusion compartment (DCI) parameters at every voxel from the same acquisition.[99, 100] Each year during clinical MRI, we will include this research dMRI sequence (scan acquisition time ≤15 minutes). The TSC Clinical Research Consortium uses human phantoms as well as ACR phantoms to align the parameters for collection of diffusion MRI data at participating sites. Such an approach has allowed us to obtain high

reproducibility across sites in previous multicenter TSC studies, including TACERN.[101] Additionally, if a clinical MRI scan with DTI is performed prior to enrollment in the study, these scans may also be included in the planned analysis.

12 ASSESSMENT OF SAFETY

Safety will be monitored using laboratory and clinical parameters at each visit and periodic phone calls as indicated in the Study Schedule (Table 3). Data will be reviewed regularly by an independent medical monitor who has no other involvement in study execution or analysis or TSC patient clinical care. In addition, a dedicated Data Safety and Monitoring Board (DSMB) will be established to protect and safeguard the interests of all study patients and to oversee trial progress. The DSMB board composition at minimum will include separate clinical specialists with experience in pediatric epilepsy, TSC clinical care, and treatment with mTOR inhibitors. The independent medical monitor also will review any serious adverse events and determine if unblinding and reporting to the FDA and DSMB is required. The DSMB will receive regular reports from the trial on any injuries or adverse events and any developments that jeopardize the continued success of the trial and integrity of the data. The DSMB will meet twice yearly to examine study data and additionally may meet on an ad hoc basis as needed. The DSMB will be provided with a summary report of all AEs, deviations and SAEs as well as safety laboratory evaluations for review during the meetings.

12.1 Adverse Events, Safety Evaluations, and Reporting

Baseline conditions (e.g. an elevated laboratory value) at study entry will be noted and graded per the CTCAE criteria. For the purposes of this study, an adverse event (AE) is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after beginning the study drug, whether or not considered drug related. Medical conditions/diseases present before beginning study drug are only considered adverse events if they worsen after starting study drug. Epilepsy and seizures as primary outcome of interest will not be 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 are considered clinically significant, induce clinical signs or symptoms, or require concomitant intervention or changes in study treatment also will be reported as adverse events.

Following informed consent, all AEs will be captured, graded per the specified parameters, and assigned causality. Adverse events will be assessed in accordance with The Common Toxicity Criteria for Adverse Events (CTCAE v5.0). If the event is not found in the CTCAE criteria, the event will be captured in the "other" category of CTCAE. The occurrence of adverse events should be sought by non-directive questioning of the subject at each visit throughout the study. Adverse events may also be detected when they are volunteered by the subject during or between visits, though physical examination, laboratory tests, or other assessments. Each event should be evaluated to determine:

1. Severity grade: CTCAE 1-5
2. Relationship to study drug: suspected/not suspected
3. Duration: start and end dates (or ongoing at the time of study completion)
4. Action taken: no action taken, study drug dosage adjusted/interrupted, study drug permanently discontinued, concomitant medication taken, non-drug therapy given, hospitalization/prolonged hospitalization

5. Whether it is a serious adverse event (SAE) as defined by the FDA and described below in section 12.2.

All adverse events should be treated appropriately. If any concomitant medication or non-drug therapy is given as a result, these should be recorded on the appropriate CRF.

Once an adverse event is detected, it should be followed until resolution, with an assessment made at each study visit to make note of any changes in severity, suspected relationship to study drug, interventions require, and the outcome. In addition, all adverse events will be recorded and subjects will be followed for 30 days after the last dose of study drug. At the end of the 30-day period, the Principal Investigator will determine if the patient is stable and the tracking of ongoing AE's can be discontinued. New AEs will not be captured beyond the 30-day period. At the end of the 30-day period, AEs that have not resolved (e.g. returned to at least baseline) will be documented as ongoing.

Periodic summary reports of AEs will be provided to the independent medical monitor, DSMB, and Tavanta Therapeutics, Inc. —the supplier for TAVT-18 for this study. Patient confidentiality will be maintained at all times. No PHI will be included in AE summary reports.

All adverse events are to be reported as follows, unless they meet the definition of a Serious Adverse Event described in section 12.2:

- FDA Reporting: Annually
- DSMB: Per DSMB Charter
- IRB: Per IRB requirements
- Study sponsor, independent medical monitor, and drug supplier: Monthly

12.2 Serious Adverse Events, Safety Evaluations, and Reporting

For the purpose of this study, a serious adverse event (SAE) is defined by the FDA as one which:

- Is fatal or life-threatening
- Results in a persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
- Requires inpatient hospitalization or a prolongation of an existing hospitalization (greater than 24 hours) unless the hospitalization is for:
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
    - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of an SAE given above and not resulting in a hospital admission
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition

Every SAE, regardless of suspected causality, occurring after the subject has provided informed consent until 30 days after treatment has stopped must be reported to the sponsor within 24 hours of learning of its occurrence. SAEs will also be reported within 24 hours of learning of its occurrence to the drug supplier and medical safety monitor. The medical monitor will determine

if steps to identify and treat the SAE by study investigators are appropriate, if additional investigations are required, and if treatment unblinding or treatment discontinuation is necessary.

Any SAEs occurring after the 30-day period following end of treatment should only be reported to the sponsor and medical safety monitor if the investigator suspects a causal relationship to the study drug.

SAEs that are considered to be unexpected and at least possibly related to study drug are required to be reported to the FDA within 7 calendar days of the sponsor's initial receipt of the information if characterized as fatal or life-threatening. Non-fatal or non-life-threatening events must be reported no later than 15 calendar days after the sponsor determines that the serious suspected adverse reaction (SSAR) or other information qualifies for reporting.

All SAEs are to be reported to the IRB and the DSMB as per policy and Tavanta Therapeutics, Inc. Patient confidentiality will be maintained at all times when reporting SAE.

12.3 General Guidelines for Management of Adverse Events

Adverse events most frequently observed with sirolimus are rash, stomatitis/oral mucositis/aphthous ulcers, fatigue, headache, anorexia, nausea, vomiting, diarrhea, and infections. Non-infectious pneumonitis, although rare, has also been reported with this class of medications. The most frequently observed laboratory abnormalities include neutropenia, thrombocytopenia, hypercholesterolemia, and/or hypertriglyceridemia. The majority (>95%) of these AEs have been of mild to moderate severity (CTCAE grade 1-2). General recommendations for management of adverse events are provided in Table 4, separated according to CTCAE grade.

Table 4: Criteria for dose medication management in case of toxicity suspected to be related to study treatment

AE CTCAE Grade	Actions
CTCAE Grade 1 (mild)	No specific action required. However, physicians should always manage patients according to their medical judgement based on the particular circumstances. May consider temporary interruption of therapy, in which case restart study drug at same dose after symptoms improve/resolve.
CTCAE Grade 2 (moderate)	If toxicity is tolerable, no dose adjustment required. Initiate appropriate medical therapy and monitor. If toxicity becomes intolerable, temporary dose interruption until recovery to grade ≤1. Re-start study drug at same dose unless AE is recurrent, in which case consider re-starting study drug at lower dose.
CTCAE Grade 3 (severe)	Interrupt study drug until symptoms resolve to grade ≤1. Initiate appropriate medical therapy and monitor. Consider re-starting study drug at a lower dose, unless AE is recurrent, in which case re-start study drug at a lower dose or consider discontinuation.
CTCAE Grade 4 (life-threatening)	Temporary dose interruption until recovery to grade ≤1. Initiate appropriate medical therapy and monitor. Consider discontinuation or re-start study drug at a lower dose. If toxicity recurs at Grade 4, discontinue study drug.

Subjects with frequent and/or severe AE may require reduction in treatment medication at the discretion of the study investigator. Recommendations by the independent physician monitoring whole blood trough results to increase dosing may also be deferred at the discretion of the study investigator if history of current or higher dosing previously attempted during the course of the study was associated with significant and/or recurrent AE. Reasons for dosing reductions due to an AE and/or deferment of dosing recommendations to increase dosing under such circumstances should be clearly documented in the research chart using the provided CRF.

12.4 Specific Guidelines for SELECTED Treatment-related AE

Infections

Sirolimus is a mild immunosuppressant, and therefore subjects who take study drug may at an increased risk of infection, including bacterial and viral pneumonias. Opportunistic infections have not been reported in TSC clinical trials with sirolimus (or everolimus). Otitis media, upper respiratory infections, and gastroenteritis are most common. Investigators should be aware of the increased risk of infection and should warn subjects and their caregivers to be vigilant for signs/symptoms of infection and to seek medical attention should signs/symptoms occur. Should an infection occur, subjects should be evaluated clinically by their pediatrician or similarly qualified medical professional. Upon confirmation of infection, anti-infectives should be prescribed as appropriate and consideration be given to withholding study medication until infection is resolved.

Stomatitis/Oral mucositis/Mouth ulcers

Stomatitis/oral mucositis/mouth ulcers due to sirolimus is the most commonly reported treatment-related side effect in prior TSC clinical trials and should be treated using appropriate locally available supportive care. Please note that investigators in earlier trials have described the oral toxicities associated with mTOR inhibitors as mouth ulcers, rather than mucositis or stomatitis. If examination reveals mouth ulcers rather than a more general inflammation of the mouth, the adverse event will be classified as such.

1. For mild toxicity (grade 1 or asymptomatic occurrences): No symptomatic treatment may be needed, but study medication may be held temporarily until resolution. Dosage reductions upon re-start may be required if recurrence of any grade, including grade 1, is frequent.
2. For more severe toxicity (grade 2 in which case patients have pain but are able to maintain adequate oral alimentation, or grade 3 in which case patients cannot maintain adequate oral alimentation), treatment medication should be held until recovery to grade ≤ 1. In addition, symptomatic treatment to alleviate symptoms may be considered and include any or all of the following:
    a. Oral sulcrafate (i.e. Carafate®), 5-10 ml applied to discrete aphthous ulcers (or oral swish & spit, but OK if swallowed) up to 5x/day.
    b. Topical corticosteroids, such as 0.1% triamcinolone oral paste (i.e., (Kenalog in Orabase®), applied directly to discrete aphthous ulcers 1-4x/day. Mucosal surface to which the paste is to be applied must be dried of saliva for medication to adhere and be absorbed. Alternative topical oral steroids (i.e. 0.5 mg/5ml oral dexamethasone solution) can also be used in older populations as swish and spit (in this population-direct aerosolized spray to mucosal surface may be more appropriate) treatment up to 4x/day. However, the infant population of the current study makes such swish and spit strategies impractical.

   c. Local topical analgesic mouth treatments (i.e., local anesthetics such as benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or phenol). Should be applied directly to discrete lesions rather than swallowed.
3. Subjects experiencing frequent mouth sores during treatment may benefit from prophylactic daily intake of supplemental amino acid L-lysine. Standard dosing for L-lysine has not been established, but typical dosing in infants would be 125 mg 1-2x/day.
4. Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents. Some foods which are spicy or acidic may also exacerbate symptoms.

Hyperlipidemia/hyperglycemia

Grade 3 hyperglycemia has been observed in patients receiving sirolimus therapy and is the most common laboratory-related AE reported in previous TSC clinical trials. In almost all cases the affected patients had an abnormal fasting glucose at baseline. Based on this finding, it is suggested that optimal glucose control should be achieved before starting a patient on study drug, and that glucose control should be monitored during the trial. Management of hyperlipidemia should take into account the pre-treatment status and dietary habits of the patient. For example, breast milk is high in cholesterol and is critical to brain development; long-term breastfed babies fare better than others when it comes to healthy hearts. Infant formulas, on the other hand, are low in cholesterol but many include supplemental fatty acids to partially compensate for the difference. To minimize immediate feeding effects on circulating blood levels, blood tests to monitor hyperlipidemia must be taken in the fasting state when possible (minimum 4 hours since last breastfeeding or 6 hours since last formula feeding, infant cereals, or baby food) for infants under 1 year of age. Cholesterol and triglyceride results drawn in non-fasting states is non-standardized and cannot be reliably used for clinical decision making or AE reporting. For the purposes of the current study, upper limit of normal (ULN) in fasting infant for total cholesterol is 200 mg/dL (5.18 mmol/L) and total triglyceride is 150 mg/dL (1.69 mmol/L). Non-fasting cholesterol or triglyceride levels > 4X ULN should be rechecked in the fasting state. As for all AE involving laboratory values, Grade 1 AE is ≥1X ULN. Grade 2 AE is ≥ 2.5X ULN. Grade 3 AE is ≥ 5X ULN. Grade 4 AE is ≥ 10X ULN. Grade 1 and 2 AE for cholesterol or triglycerides do not require any specific intervention, but dietary modification may be considered. In addition to diet modification if possible, Grade 3 hypercholesterolemia or hypertriglyceridemia should be treated by lowering daily dose of treatment medication. Grade 4 hypercholesterolemia or hypertriglyceridemia should prompt holding study medication temporarily until levels normalize and then can be resumed at a lower daily dose. Although sirolimus-associated hyperlipidemia/hypercholesterolemia can be treated with HMG-CoA reductase inhibitors in older children and adult patients, such treatment is not recommended for the 0-12 month-old infant population of the current study.

Non-infectious pneumonitis

Pneumonitis is a recognized adverse effect of rapamycin derivatives (i.e. everolimus, sirolimus) in adults. In human clinical trials for TSC to date, rates of non-infectious pneumonitis are low (<1%), but in oncology studies frequency has been reported as high as 14%. Individuals participating in this trial will be questioned at each study visit as to the presence of new or changed pulmonary symptoms consistent with lung toxicity. If an investigator suspects a subject may be developing pneumonitis, other potential mimics should first be excluded (viral URI, reactive airway disease, pneumonia). In unresolved cases when other causes are determined not

to be responsible, study medication should be temporarily withheld while investigations such as CT chest and/or referral to a pulmonologist are considered.

13 CONCOMITANT ADMINSTRATION OF MEDICATIONS

With the exception of experimental therapies, use of most concomitant medication/therapy deemed necessary for the care of the subject is allowed (see special considerations regarding concurrent anticonvulsant treatments in section 9.2 above). The investigator should instruct the subject family to consult with the study investigator prior any new medications he/she takes after the start of the study drug, including natural supplements (nutraceuticals) and over-the-counter medications. All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) administered within 30 days of starting study drug, throughout the entire study period, and up to 30 days after study drug discontinuation must be listed on the appropriate CRF.

Investigational or commercial anti-proliferative and immunosuppressive agents other than study drug (including other mTOR inhibitors, e.g., everolimus, temsirolimus) are prohibited.

Sirolimus is metabolized by isozyme CYP3A4 and P-glycoprotein (PgP). Co-administration with moderate or strong inducers/inhibitors/substrates of CYP3A4 or PgP should be avoided (refer to Table 5). Exception is use of anti-epileptic medications that are clinically-indicated. If a subject is treated long-term with one of these medications (anticipated or actual duration ≥2 weeks) a sirolimus whole blood trough level should be repeated.

Seville orange, star fruit, grapefruit and their juices affect CYP3A4 and PgP activity should be avoided.

Sirolimus may affect the response to vaccinations, making the response to the vaccination less effective. Recombinant and inactive vaccinations may be given at any time during the course of the study, including annual non-live influenza vaccination that is recommended by the CDC for at-risk populations that include infants diagnosed with TSC. However, live vaccines should be avoided while patient is treated with sirolimus. As current CDC recommendations do not recommend any live vaccines (MMR, Varicella) before the age of 12 months, this should not be impacted by participation in this study. However, if there is reason for live vaccine to be administered, it should be administered 1-2 weeks prior to initiation of sirolimus or delayed until 1-2 weeks after sirolimus is discontinued upon study completion at 12 months of age. If live vaccination must be administered while taking sirolimus, then study medication should be discontinued 1-2 weeks prior to vaccination with the live vaccine and restarted 1-2 weeks afterward.

Table 5: Clinically-relevant Drug Interactions with Sirolimus: Substrates, Induces and Inhibitors of CYP3A and/or PgP

CYP3A4 INDUCERS

Strong inducers: avasimibe, carbamazepine,enzalutamide, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's wort (*Hypericum perforatum*)

Moderate inducers: bosentan, efavirenz, etravirine, genistein, lersivirine, lopinavir, modafinil, nafcillin, ritonavir,semagacestat, talviraline, thioridazine, tipranavir

Weak inducers: amprenavir, aprepitant, armodafinil (R-modafinil), bexarotene, boceprevir,brivacetam,clobazam, danshen, dexamethasone, Echinacea, eslicarbazepine, garlic (*Allium sativum*), gingko (*Ginkgo biloba*), ginseng, glycyrrhizin, methylprednisolone, nevirapine, oxcarbazepine, pioglitazone, prednisone, pleconaril, primidone, quercetin, raltegravir, ritonavir,rufinamide, sorafenib, Stribild (combo of elvitegravir, cobicistat, emtricitabine, and tenofovir), sulfinpyrazone,telaprevir, terbinafine,ticagleror,ticlopidine,topiramate, troglitazone, vemurafenib, vicriviroc/ritonavir, vinblastine

CYP3A4 INHIBITORS

Strong inhibitors: boceprevir, clarithromycin, cobicistat, conivaptan,danoprevir/ritonavir,eltegravir/ritonavir,elvitegravir,grapefruit juice, indinavir,indinavir/ritonavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, saquinavir/ritonavir, telaprevir, telithromycin, tipranavir/ritonavir, troleandamycin, voriconazole

Moderate inhibitors: Amprenavir, aprepitant, atazanavir, atazanavir/ritonavir,casopitant, cimetidine, ciprofloxacin, crizotinib, cyclosporine, darunavir, darunavir/ritonavir, diltiazem, dronedarone, erythromycin, fluconazole, fosamprenavir, grapefruit juice (Citrus parasidi fruit juice), imatinib,lomitapide, netupitant,nilotinib,*Schisandra sphenanthera*, tofisopam, verapamil

Weak inhibitors: almorexant, alprazolam, alprazolam, amiodarone, amlodipine, amlodipine, atorvastatin, azithromycin, berberine, bicalutamide, bicalutamide, blueberry juice, cilostazol, cilostazol, cimetidine, clotrimazole, clozoxazone, cranberry juice, cyclosporine, delavirdine, everolimus, fluoxetine, fluvoxamine, fosaprepritant, ginkgo, goldenseal, isoniazid, isoniazid, ivacaftor, lacipidine, linagliptin, nilotinib, oral contraceptives, pazopanib, peppermint oil, propiverine, ranitidine, ranitidine, ranolaxine, ranolazine, resveratrol, roxithromycin, Seville orange, simeprevir, sitaxentan, tabimorelin, tacrolimus, teriflunomide, ticagrelor, tipranavir/ritonavir, tolvaptan, zileuton

Dual PgP/CYP3A4 INHBITORS

amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, elacridar, erythromycin, felodipine, fluvoxamine, ginkgo (*Ginkgo biloba*), indinavir, indinavir/ritonavir, itraconazole, mibefradil, milk thistle (*Silybum marianum*), nelfinavir, nifedipine, nitrendipine, paroxetine, quercetin, quinidine, ranolazine, rifampin, ritonavir, saquinavir/ritonavir, *Schisandra chinensis*, St John's wort (*Hypericum perforatum*), talinolol, telaprevir, telmisartan, ticagrelor, tipranavir/ritonavir, tolvaptan, verapamil

PgP INDUCERS

avasimibe, carbamazepine, efavirenz, genistein, phenytoin, quercetin, rifampin, St John's wort

PgP INHIBITORS

alogliptin,canaglifozin, cremophor RH40, curcumin, ketoconazole, lapatinib, lopinavir/ritonavir, mirabegron, propafenone, simepravir, valspodar, vandetanib, voclosporin

PgP SUBSTRATES

afatinib, alfuzosin, aliskiren, alogliptin, ambrisentan, apixaban, apremilast, aprepitant, atorvastatin acid, atorvastatin, azithromycin, boceprevir, bosentan, carvedilol, caspofungin, ceritinib, cerivastatin, citalopram, colchicine, CP-481,715, cyclosporine, dabigatran, digoxin, docetaxel, domperidone, doxepin, doxorubicin, eribulin, everolimus, fentanyl,fexofenadine, fidaxomicin, fluvastatin, fosamprenavir, gatifloxacin, idelalisib, iloperidone, indacaterol,indinavir, irbesartan, lacosamide, lapatinib, levetiracetam, levofloxacin, linagliptin, linezolid, loperamide, losartan, maraviroc, mirabegron, moxifloxacin, naloxegol, nateglinide, nevirapine, nintedanib, lodaterol, paclitaxel, pantoprazole, paroxetine, pazopanib, phenytoin, posaconazole, pravastatin, proguanil, quinidine, quinine, ranolazine, riociguat, risperidone, ritonavir, rivaroxaban, saquinavir, silodosin, simeprevir, simvastatin, sirolimus, sitagliptin, sofosbuvir, sorafenib, tacrolimus, telaprevir, tenofovir, ticagrelor, tipranavir, tolvaptan, topotecan, umeclidinium, valsartan, vardenafil, vincristine, voclosporin, voriconazole
Reference: Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated April 2015, which summarizes DDI data from three sources including the FDA's "Guidance for Industry, Drug Interaction Studies", the University of Washington's Drug Interaction Database, and Indiana University School of Medicine's Drug Interaction Table."

14 DATA AND SAFETY MONITORING BOARD

A Data and Safety Monitoring Board will be established to protect all study subjects, monitor the overall conduct of the trial, advise the investigators in order to protect the integrity of the trial, and supervise the conduct and analysis of all interim analyses. DSMB membership may include members from the same institution but none may be otherwise involved or affiliated with the study nor have direct clinical or scientific collaboration with a member of the study team. Composition of the DSMB will be determined by the DSMB chair in accordance with the DSMB charter, but must include at least one or more each of the following: biostatistician, child neurology and/or epilepsy specialist, TSC clinician with experience prescribing/managing mTOR inhibitors, pharmacologist, and general pediatrician.

Initially, the DSMB will review the protocol and will have the opportunity to request changes or clarifications in the study design and conduct prior to study start. The DSMB will meet prior to study initiation to review the protocol and confirm the adequacy of safety monitoring. During the study, the DSMB will review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity that might jeopardize the continued success of the trial and integrity of the data. This includes mandatory review of Stage 1 PK analysis and preliminary safety report (see section 11.3 above) before enrollment is allowed to begin for Stage 2 of the study.

The DSMB charter outlines the membership, responsibilities, frequency of meetings, and the process for review of study data at selected intervals. Additionally, the charter includes the format of the formal report that will be provided by the DSMB chair to the study sponsor outlining the conclusions and recommendations of the DSMB following each meeting.

15 DATA AND MONITORING PLAN

15.1 Data Management

The Data Management Center (DMC) at Cincinnati Children's will provide full data management support to the project. The DMC has dedicated space within the Division of

Biostatistics and Epidemiology (DBE) and was formed in 2002. The DMC currently consists of three Managers of Data Management operations and 25+ staff members (Clinical Research Data Specialists, Clinical Research Database Programmers and Data Coordinators), six of which are Certified Clinical Data Managers. DMC staff are active members of the Society of Clinical Data Management, the Drug Information Association, and the Society of Clinical Trials and subscribe to Good Clinical Data Management Practices. Data management support services include grant review, budgeting and resourcing for data management operations, protocol review, case report form design and development, database development using Medidata Rave ®, documentation, data cleaning and preparation for analysis.

The project will use Medidata Rave ® as its Electronic Data Capture (EDC) software. Medidata Rave® is a robust EDC platform for capturing, managing and reporting clinical research data. As a single platform, Medidata Rave® combines easy-to-use EDC and advanced clinical data management capabilities. Rave's® extensive capabilities — including wide support of industry data standards, flexibility to implement any data management workflow and a rich set of on-demand data extraction and ad hoc reporting tools — provide a robust platform to manage site-, patient- and lab-reported data from EDC and other third party systems via smooth integrations, and rapidly make it available for analysis and submission. The early visibility to reliable trial data enables study teams to safely and quickly make sound decisions and bring life-enhancing treatments to patients earlier.

The Rave® system includes a robust query management system based on the data quality checks identified in the Data Quality Plan and programmed during database design. Values that are considered potentially inaccurate are flagged by the system and a query is created and sent to the site, all within the Rave® interface. All queries and data changes are documented in the audit trail and available for reporting purposes, such as identifying common data queries and query rates per site. In addition, the data management team will provide frequent monitoring, described as process control, of the database throughout the study to assure completeness and accuracy of the data. This will allow corrective action to be taken quickly during the course of the study if problems with the data collection process are identified.

Rave® allows for seamless uploads of external data sources into the system to eliminate the need to enter data that is already available in electronic formats and integrates this additional clinical data for data cleaning and quality control. Documented procedures will be developed for the collection, transfer, loading, and validation of any external data. Written specifications will identify mandatory fields including the appropriate variables needed to merge data.

Rave® provides 42 standard reports that will be available to investigators at any time. This allows investigators to track their progress on such key statistics such as enrollment, data entry, query status and audit trails.

15.2 Data Quality Control

All the developmental assessments and secondary outcome measured described above have been previously performed by the collaborative research team. Quality control is the highest priority in data collection; therefore, as in previous TSC Clinical Research Consortium studies including TACERN, RDRCN-DSC, and PREVeNT, we will take multiple steps to ensure data collected is of the highest quality and fidelity. This includes all personnel performing assessments be

professionals trained and certified in the appropriate use and administration of each neuropsychological instrument.

15.3 Study Monitoring and Auditing

Monitoring will be performed to ensure the study is conducted, documented, and reported in accordance with the IRB approved protocol, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines, and applicable FDA regulatory requirements.

Targeted on-site monitoring will occur at least annually, with the schedule for monitoring at each site to be determined by the overall risk level of the study, identified site-level risks, and issues that may arise during the course of the study.

On-site monitoring will include source data verification and source data review as specified in the Monitoring Plan. The study monitor will perform verification and/or review of critical site processes as indicated, including:

- Eligibility of enrolled research subjects
- Protocol adherence and appropriate reporting of protocol deviations
- Study drug accountability
- Safety information and reporting
- Consent process and documentation
- Record keeping of essential documents
- Completeness and accuracy of data entry and data query resolution
- Investigator oversight

16 STATISTICAL ANALYSIS PLAN

16.1 Data Analysis

Primary Endpoint Analysis and Statistical Considerations (Safety):

The hypothesis is that sirolimus will be safe in infants with TSC up to 12 months of age. We predict that most AE/SAE will be mild/moderate (CTCAE grade 1 or 2) and as was the case for the treatment of SEGA and epilepsy in TSC,[30, 47]. Descriptive statistics (percentages and 90% CI based on Newcombe's modification of Wilson scores[102]) will be used to describe the frequency of individual AEs by type and severity (CTCAE grade). Treatment tolerability will also be measured as a safety-related endpoint. Tolerability is distinct in that it represents the patient's ability to continue treatment despite the presence of one or more significant or recurrent AEs rather than a measure of the AE frequency or severity itself. Tolerability will be measured as the percentage of subjects that permanently reduce or discontinue treatment because of an AE or SAE. We will also determine the number of days treatment is held because of an AE or SAE. We will also assess the number of days treatment is held but not discontinued because of an AE or SAE. Since this measure of tolerability has no precedent, to our knowledge, there is no non-zero value that we think should or should not be contained within the CI.

There are methodological challenges in safety assessment from a statistical perspective in any clinical trial, especially those that are limited in size or duration.[103] It is no different in TSC,

where every clinical trial evaluating mTOR inhibitors that has led to an FDA-approved indication has reported AE/SAE frequency in descriptive terms without accompanying analysis of statistical significance between groups.[21, 30, 47] Individually and collectively, these studies also have shown that most treatment-related AE and SAE in TSC are mild or moderate in severity. For the current study, we likewise will report all treatment-related AE, by category and severity.

Primary Endpoint Analysis and Statistical Considerations (Efficacy):
The hypothesis is that sirolimus will prevent or delay seizure onset in infants with TSC up to 12 months in age. To evaluate efficacy, we will determine time to seizure onset after treatment initiation in infants treated with sirolimus.

It was originally planned for this analysis to consist entirely of subjects enrolled into Stage 2 (n=60), comparing time to seizure onset between the two groups (treatment vs. placebo). With Stage 2 now eliminated from this protocol, we will instead use a contemporary, prospectively evaluated historical control group (n=32) from our TACERN/TSC-EBS natural history study for comparison (Wu *et al*. *Epilepsia* 2019. 60:2428-36), in which infants were similarly enrolled 0-6 months of age and followed until 24 months of age. Instead of time to seizure onset for the comparative analysis, we will use age of seizure onset as the outcome of interest as there was no treatment in the TACERN/TSC-EBS studies to use as a start point. The average age of seizure onset in the TACERN/TSC-EBS control group was 7.4 ±4.4 months. For statistical significance, we will use t-test or Wilcoxon rank sum test for the differences, depending on distribution (parametric vs. nonparametric). In addition, survival analysis will be conducted with statistical comparison between treatment groups utilizing the long-rank test. Seizure frequency, and history of infantile spasms at 12 months of age have been shown to be predictive of long-term neurodevelopmental outcome and seizure control at 24-36 months of age.[4] Therefore, we will similarly compare seizure frequency at 12 months of age using the t-test/Wilcoxon rank sum test and the proportion of subjects who develop infantile spasms (past or current) using Fisher's exact test.

Secondary Endpoint Analysis and Statistical Considerations:
As described above for primary outcome measures, descriptive statistics will be determined for each composite and individual component measures of the TAND Checklist, VABS, Bayley-4, PLS-5 and ADOS-2 for neurodevelopment and behavior; EEG for electrophysiological connectivity; and dMRI for structural connectivity.

EEG data for conventional analysis (interictal and ictal epileptiform activity) will be sampled at 500 samples/channel/second, 0.5 Hz low pass and 100 Hz high pass filters, and a 60 Hz notch filter, with impedance less than 5 Kohm for all electrodes. Persyst software (San Diego, CA) will be used to digitally view each tracing by a single central reader in the standard timescale of 30 mm/sec and standard filter settings of 1 Hz low-frequency filter (high pass) and 70 Hz high frequency (low pass), along with a 60 Hz notched filter. EEG will be classified according to age-appropriate norms, as either normal or abnormal. Conventional EEG abnormalities will be further evaluated in terms of the presence or absence of background abnormalities, such as generalized or focal slowing, epileptiform discharges (focal, regional, bilateral, or generalized spike or spike and wave discharges), hypsarrhythmia (classic or modified), voltage attenuation, as well as clinical and/or electrographic seizures, in accordance with the NINDS common data element tools for epilepsy. The association between epileptiform discharges and primary efficacy outcome measures will be analyzed using multiple methods, including time-to-event

survival analysis, multivariable Cox proportional hazard analysis to assess the contribution of concurrent clinical or genetic variables on the response to treatment, and logistical regression analysis to determine the strength of associations.

EEG data for quantitative analysis (power, coherence, path length, clustering, and global efficiency) will be imported into Brain Vision Analyzer (BVA) software (Brain Products GmbH) to remove offsets, optimize scaling, and re-reference the data into the desired montage. The power spectral density of the artifact-free bipolar pair EEG data will be calculated using the fast Fourier transform (FFT) function. The 512-point FFT is calculated for artifact-free two-second epochs with a rectangular window, DC de-trending applied to each segment of data, 0.5 Hz overlap at the limits of the band, and yielding a frequency resolution of 0.5Hz. Power will be calculated in six frequency bands, corresponding to delta (0.5-4Hz), theta (4-8Hz), alpha (8-12Hz), beta (12-20Hz), gamma (30-50Hz), ripple frequencies (100-250Hz), and fast ripple frequencies (250-500Hz).

Supplementation of traditional DTI from dMRI data with DCI allows us to characterize changes in free water content, multiple oriented fascicles per voxel, and diffusivity parameters per fascicle,[104-107] using the concepts of statistical modelling of infinitesimal microenvironments,[108] and the well-established minimum requirements for compartment models.[109] DIAMOND[99, 100] is a finite mixture model of compartments, where the signal arising from each of the compartments is represented by a statistical distribution of 3-dimensional infinitesimal microenvironments. Our model order selection algorithm determines from the dMRI data, exactly the number of compartments that are present at each voxel.[110] DCI enables better delineation of white matter pathways relevant to CNS disease in TSC;[111] allows measurement of the free water volume and improves compensation for partial volume averaging; provides increased sensitivity to detect loss of microstructural integrity; provides intra-voxel orientational heterogeneity and allows tractography of all fascicle orientations; and provides measures of diffusivities from the imaging data.

Sirolimus (TAVT-18) dosing and corresponding trough levels for the sirolimus (TAVT-18) treatment group will be analyzed for dose-dependent relationships to primary efficacy and safety outcome measures. We will define the developmental trajectory of sirolimus (TAVT-18) in very young infants ('projected model' line) using actual and dose-normalized sirolimus (TAVT-18) dose and corresponding trough concentrations at each sampling time, as done previously in patients with congenital vascular anomalies[49]. We will then use validated population modeling approaches supported by the U.S. Food and Drug Administration to simulate drug-treated patient populations of interest.[112, 113] Mixed (random and fixed) effects modeling approach where the data are pooled with individual subject characteristics and maintained during the analysis will be employed to capture both inter-patient and intra-patient (also known as residual) variability. Exploratory covariate analyses using PK/PD modeling approach will be performed with variables considered to include (but are not limited to) age, gender, ethnicity, total bodyweight (TBW), body mass index (BMI), body surface area (BSA), hematocrit, disease status, time since last feeding, and concomitant medications. The fit of the population PK and PK/PD models will be evaluated both graphically and statistically. The final model will then be developed by applying a direct-link model to describe sirolimus concentrations and changes in primary efficacy and safety outcome measures to identify optimal dosing range for preservation of observed treatment effect compared to treatment-associated risk of AE.

Our consortium is actively involved in the NIH-funded epilepsy prevention clinical trial evaluating the pre-emptive treatment with vigabatrin in TSC infants (PREVeNT). The current study design is purposefully similar to that trial (age at time of enrollment, clinical epilepsy and neurodevelopment assessments between 0-12 months of age, timing of serial blood and EEG acquisitions). The underlying premise of the two studies, however, is distinct. PREVeNT specifically targets early epileptogenesis with a conventional anticonvulsant, vigabatrin, that is initiated *after* EEG abnormalities are identified. In contrast, the present study initiates treatment *after* TSC diagnosis, as the proposed treatment (sirolimus) targets the underlying molecular defect caused by genetic mutation of *TSC1* or *TSC2* that is present from birth. Thus, we will be able to directly compare outcome measures and biomarkers to explore the significance of timing (*before* vs. *after* EEG change) and mechanism (targeting the mTOR pathway vs. GABAnergic effect of vigabatrin) to prevent epilepsy onset and progression in TSC infants.

16.2 Study Size and Power Calculations

The original study was powered for the planned enrollment of Stage 2, in which 60 subjects were to be randomized 2:1 for treatment with TAVT-18 (sirolimus) vs. placebo. These power calculations remain valid for STOP2B: TSC-STEPS (CCHMC IRB#2021-0438) that has replaced Stage 2 of the current study. However, with enrollment under the current protocol now limited to Stage 1 only (n=5), this study is no longer powered for definitive analysis and becomes a true pilot study aimed at obtaining preliminary data with regard to potential treatment safety concerns and an estimation of patient variability in treatment response. Analyses for statistical significance will still be carried out with the modifications as outlined above, but it is expected that unless the treatment effect size is very large compared to a similarly matched historical cohort from the TACERN/TSC-EBS study, statistical significance will not be achieved with such a low number of treated individuals in this study. Nonetheless, descriptive analysis as a pilot study using sirolimus (TAVT-18) in a patient population (TSC infants 0-12 months of age) that has hereto never previously been studied in a prospective fashion still has immense scientific and clinical value. Furthermore, should the TAVT-18 sirolimus formulation be further developed as a potential clinical treatment for this indication, the descriptive statistics will provide essential data for estimating potential treatment effect size and interpatient variability values that will prove essential for determining study size and study power.

17 HUMAN SUBJECT PROTECTION

Compliance with Good Clinical Practice (GCP) guidelines for the conduct and monitoring of this clinical trial will occur through observation of the ethical and regulatory requirements presented in ICH E6, Good Clinical Practice: Consolidated Guideline. The study (protocol, informed consent, advertisements, subject information sheets and Investigator CV and credentials) should be reviewed and approved by the Institutional Review Board (IRB) or ethics committee. Changes to the protocol will be approved by the IRB. Subjects must sign written informed consent prior to being screened, before undergoing any study procedures.

The investigators and institutions affiliated with this study will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s) by providing direct access to source documents.

17.1 Privacy and Confidentiality

Subject confidentiality will be maintained by the investigator, the investigator's associates and co-workers, and by all administrators who are part of the project. Confidentiality will be maintained according to ICH E6; 4.8.10, part O: "Records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the subject's identity will remain confidential."

The investigator, his or her staff and associates, and the appropriate regulatory agencies may use the information included in this protocol as necessary for the conduct of the trial and the safety of subjects. The subject/legal guardian may obtain the results of clinical labs, tests and procedures obtained for this research study.

Subjects will be identified only by a unique identifier for research specimens collected and shipped to analysts outside the consortium. Any publications will reflect only unique identifiers. All information related to this study will be kept in secure and locked offices.

Clinical data on paper forms will be stored separate from the patient chart. The data on paper will be kept locked. Any data on computer will be accessible only by password access. Only members of the research team will have access to these files.

17.2 Informed Consent Process

Informed consent is an ongoing process that includes the signing of an informed consent document. Parents/LARs are required to sign an informed consent prior to being screened, and before undergoing any study procedures or assessments, in accordance with International Conference on Harmonisation (ICH) E6; 4.8, "Informed Consent of Trial Subjects." When substantial modifications are made to the informed consent, the DSMB or IRB may require that all subjects currently enrolled in the study will be re-consented; ICH E6; 4.8 guidelines would still apply.

Parents/LARs will be provided with a copy of the informed consent that explains the purpose of the study, the study procedures, and assessments. Parents/LARs will also be provided with the telephone numbers of the investigator and qualified personnel who can assist with their questions and concerns.

The following is the consent process that will be used for this project:

- Subjects are recruited from the patient populations at each participating center.
- A copy of the informed consent is given to the parents/LARs before signing the consent. The content of these documents, and the nature of the study, is discussed with the parents/LARs before obtaining their signature. If there are no questions regarding the studies or the content of the consent forms, the parent and/or guardian is requested to sign the consent in the presence of a member of the investigation team.
- Parents/LARs are given copies of the consent with signatures to keep.

- For advertisements used for recruitment purposes for this study, prior approval will be obtained from the institutional review board (IRB).

18 FUNDING SOURCES

The primary source for funding this clinical trial is the Clack Foundation and private donations in smaller amounts from individual patients and families donating to general TSC research at Cincinnati Children's Hospital. This funding supports the necessary infrastructure and resources necessary for successful completion of the study, including all study visits and procedures for the primary safety, efficacy, developmental/behavioral assessment, and later PK/PD endpoint analyses. Tavanta Therapeutics, Inc. is providing TAVT-18 (sirolimus) drug supply for the entire study and supplemental funding to support study visits and procedures for the initial open-label, detailed PK analysis portion of the study (Stage 1). The TS Alliance, a national patient advocacy not-for-profit organization, provides funding for tissue and blood biosample acquisition, shipping, processing, and storage at the TSC Biorepository in Grand Rapids, Michigan.

19 SUBJECT COMPENSATION

Subjects will not be compensated for participating in this trial. Limited travel reimbursement for qualifying expenses may be available. Clinical procedures and tests described above that are clinically indicated (meaning they would be done even if the subject was not participating in this study) may be charged to the subject and/or their medical insurance. Examples include but are not limited to EEGs, MRI, laboratory studies, clinical evaluations, and/or additional developmental assessments. However, procedures and tests that would only be done because of participation in this research study, such as measurement of sirolimus levels, will not be charged to the subject and/or their insurance.

20 PUBLICATION PLANS

Manuscript(s) and abstract(s) prepared from the data collected during this trial will be prepared by the study investigators using standard publication guidelines.

The investigators on the clinical trial will be given the first and exclusive opportunity to analyze, present and publish data collected from their investment of time and effort. It is the obligation of the study investigators to disseminate results and it is expected that the investigators will write manuscripts including study design and primary results as well as other methodology and preplanned analyses of secondary outcomes.

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