PREVENTING EPILEPSY USING VIGABATRIN IN INFANTS WITH TUBEROUS SCLEROSIS COMPLEX (PREVeNT TRIAL)-NCT02849457

Protocol version 6.0 _ 7/2/2020

PREVENTING EPILEPSY USING VIGABATRIN IN INFANTS WITH TUBEROUS SCLEROSIS COMPLEX (PREVeNT TRIAL)

A randomized, double-blind, placebo-controlled seizure prevention clinical trial for infants with TSC

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KEYWORDS: Tuberous Sclerosis Complex, epilepsy, prevention, vigabatrin, electroencephalography

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Study Objectives:

Primary Study Objective:

The primary endpoint is the developmental impact of early versus delayed treatment with vigabatrin. To this endpoint we propose to demonstrate the effect of the intervening variable (early versus delayed treatment) on the developmental outcome at 24 months. The primary outcome measure for this objective will be the cognitive assessment score of the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) at 24 months of age.

Secondary Study Objectives:

1) Determine the effectiveness of early versus delayed treatment with vigabatrin in clinical seizure prevention. The outcome measure will be time to first clinical seizure following randomization. The prevalence of drug-resistant epilepsy will be assessed at 24 months for each group (vigabatrin early treatment vs. vigabatrin delayed treatment).
2) Determine the impact of early versus late treatment on the other components of the Bayley-III (receptive communication, expressive communication, fine and gross motor skills) and risk of autism spectrum disorders (ASD). The primary outcome measures for this objective will be subdomain scores of the Bayley-III, Vineland-II (Survey Interview Form), Beery Visual Motor Integration (VMI), and ADOS2 at 24 months. Additional exploratory analysis will be completed at 36 months to access changes observed at 24 months are consistent with those seen at 36 months and indicative of long-term outcome. At 36 months of age subjects will also complete the Peabody Picture Vocabulary Test (PPVT). During the COVID-19 Pandemic in order to minimize the risk of infection to observer and infant until the completion of the study the Brief Observation of Symptoms of Autism (BOSA) will be administered at 24- and 36-month visits. Additionally, the ADI-R will be administered at the 36-month visit.
3) Confirm vigabatrin safety as a preventative treatment for clinical seizures in infants with TSC. The outcome measure will be percentage of patients in each group (vigabatrin early treatment vs. vigabatrin delayed treatment) with treatment associated adverse events (AEs), serious adverse events (SAEs), and risk evaluation and mitigation strategy (REMS) measures.
4) Confirm of the feasibility of using EEG biomarkers to identify TSC infants at risk for developing epilepsy. The outcome measure will be the sensitivity and specificity of EEG epileptiform activity in predicting epilepsy outcomes at 24 months within treatment groups.

Study/Background/ Rationale:

TSC is a multisystem genetic disorder, in which 90-95% of the affected individuals have CNS involvement consisting of subependymal nodules, subependymal giant cell

astrocytomas (SEGA), multifocal areas of cortical dysplasia consisting of tubers and migration defects, and abnormalities of white matter organization. Clinically, epilepsy is manifest in 80-90%, of which half or more are drug-resistant[1]. In addition, intellectual disability, autism spectrum disorder, developmental delays, and psychiatric disorders are highly prevalent and demonstrate a strong association with early-onset epilepsy and severity in this population[2,3]. The molecular basis of TSC is known, arising from deficiency in proteins encoded by the TSC1 and TSC2 genes that form a protein complex that plays a critical role in the regulation of the serine-threonine kinase mammalian target of rapamycin (mTOR)[4,5]. Pharmacological inhibitors of mTOR have been demonstrated to reduce various tumor types and hamartomas characteristic of TSC[6-8]. Early studies have also suggested these drugs may have favorable impact on other TSC disease manifestations, including seizures and neurocognition[9-12], but definitive studies are still forthcoming.

As a rare disease, there is limited data regarding efficacy of current anticonvulsants for the treatment of epilepsy specifically in TSC. A clear exception is vigabatrin, in which multiple published studies have repeatedly demonstrated the efficacy of vigabatrin for the treatment of infantile spasms and partial-onset seizures in TSC.[1,13-18] Other FDA-approved treatments for infantile spasms in patients with TSC, such as adrenocorticotrophic hormone (ACTH), are less effective when compared to vigabatrin. Relatively few studies have compared both treatments directly to one another, and the large United Kingdom Infantile Spasms Study (UKISS) specifically excluded patients with TSC.[19] A smaller prospective treatment trial by Vigevano and Cilio for infants with new-onset infantile spasms found that vigabatrin was superior for patients with cerebral malformations and TSC, whereas ACTH was more effective for patients with hypoxic-ischemic injury.[20] This is consistent with the more recent retrospective study by Camposano *et al.*, in which spasms cessation occurred in 55% of those treated initially with vigabatrin but 13% of those initially treated with ACTH.[14] These and similar studies were the basis of the International League of Epilepsy Infantile Spasms US Working Group to recommend the use of vigabatrin as effective first-line therapy for infantile spasms, particularly in patients with infantile spasms and TSC.[21]

First developed in 1975, vigabatrin potentiates the action of an important, protective neurotransmitter in the brain called γ-aminobutyric acid (GABA). Human clinical trials began in 1979 and first regulatory approval occurred in the United Kingdom in 1989, followed by Australia (1993) and Canada (1994). Potential side effect concerns and industry decisions delayed FDA approval in the United States until 2009. The main side effect cited of concern with vigabatrin treatment is potential treatment-associated peripheral vision loss (vigabatrin-associated visual field loss, or VAVFL), estimated to occur in 10-15% of children by some measures, but clinically meaningful vision effects are estimated to be less than <1%.[18] Given the highly favorable benefit to risk ratio of vigabatrin in this population, vigabatrin is now the recommended first-line treatment for infantile spasms for patients with TSC by the International TSC Consensus Group and the European TSC Consensus for SEGA and Epilepsy Management.[22,23] However, current standard of care applies only to patients after clinical spasms are manifest.

Presently, there is no indication for pre-symptomatic treatment or treatment initiated on the basis of EEG findings alone.

The scientific basis for the use of vigabatrin as a potential treatment intervention that will slow the progression of epileptogenesis versus its efficacy for seizure control is based on the recent publication by Zhang *et al*. from the Michael Wong laboratory.[24] In the TSC1^{GFAP}CKO mouse model characterized by early seizures that are progressive and eventually lethal,[25] vigabatrin treatment increased GABA concentration, resulting in decreased seizures and increased survival.[24] These changes were associated with decreased activation of the mTOR pathway in both the cortex and hippocampal brain regions that were dose-dependent. Also, Russo et al reported that early treatment with vigabatrin had “antiepileptogenic” effects in a genetic rat model of absence epilepsy.[26] The exact mechanism of action in TSC is unclear, but together with its already established clinical efficacy in treating seizures, these data are the starting point for our proposal to investigate vigabatrin as an anti-epileptogenesis treatment for TSC.

Due to the high frequency of epilepsy in TSC that is often drug-resistant and associated with significant CNS-related comorbidities, interest in early, presymptomatic treatment has existed for decades. However, lack of supporting evidence and selection of a suitable agent has limited efforts that would justify initiating treatment with medication that has potential side effects in otherwise asymptomatic infants. Clinical characteristics of TSC and recent clinical developments now make such a treatment strategy rational and feasible:

(1) Many TSC patients are diagnosed before birth or at the time of birth due the presence of rhabdomyomas in the heart. A fetus or a newborn with multiple cardiac tumors has a 95% chance of having TSC.[27] Increased use of prenatal ultrasounds has significantly increased the detection of TSC in the fetus such that cardiac manifestations have become the most common presenting sign of TSC. [28] Therefore, there is essentially a newborn imaging screen available to detect individuals with TSC very early in life.

(2) More than 80-90% of individuals with TSC will experience seizures in their lifetime, with majority onset within the first 2 years of life.[1] Drug-resistant epilepsy is extremely common, estimated to occur in more than 50%. Epileptic encephalopathy and treatment-related side effects are also common, as are high likelihood of additional CNS comorbidities that have long-term implications on independence, quality of life, and overall health. Thus the majority of newborns and infants identified with TSC have an extremely high risk of significant negative outcomes as a result of their epilepsy for which current treatment strategies and standard of care are inadequate.

(3) Vigabatrin is already FDA-approved for use in TSC and therefore its use in infants is well-established. Medication-related safety risks and side effect profiles are known, and a risk evaluation and mitigation strategy (REMS) program with the FDA is in place to monitor and address potential vision-related concerns that might arise in treated individuals. Vigabatrin has a unique position in TSC because it is more efficacious for IS than other therapeutic options. Developmental outcomes are closely related to the presence of IS and suppression of IS, so based on the efficacy of vigabatrin in patients with TSC and IS, vigabatrin has the potential of being a disease modifying agent for neurodevelopmental outcomes in TSC. Vigabatrin mechanism of action is a GABA

potentiator, but is has superior efficacy to other GABA drugs such as phenobarbital and benzodiazepines suggesting another potential mechanism of action of vigabatrin in controlling IS in TSC. The TSC mouse model data has shown modest effect of vigabatrin as a mTOR inhibitor. However, the preclinical data of mTOR inhibitors as antiepileptogenic drugs in multiple mouse models for TSC are very convincing, suggesting that even a small effect of vigabatrin on mTOR is a significant candidate for antiepileptogenic therapy[24,25].

(4) Two recent publications: the first by Jozwiak et al. (2011), in which 14 TSC infants were treated with vigabatrin based on EEG abnormalities rather than waiting until onset of clinical seizures, demonstrated improved rates of seizure-freedom and intellectual ability compared to 31 historical controls treated only after onset of clinical seizures.[29] Although this study has significant limitations, it does support the potential for early treatment to positively change subsequent outcome trajectories of these at-risk infants. The second by Bombardieri et al. published a series of 10 children who received vigabatrin within one week of the onset of focal seizures or infantile spasms and had at least 30 months of neurodevelopmental follow up. They concluded that early control of seizures plays a pivotal role in preventing subsequent epileptic encephalopathy and reducing the impact of persistent seizures on developmental outcomes[30].

(5) Preliminary results of video EEG biomarker analysis from the NIH P20 grant-*Potential EEG Biomarkers and Antiepileptogenic Strategies for Epilepsy in TSC* (P20-NS080199) are compelling and support the use of the presence of epileptiform activity as an EEG biomarker for the PREVeNT Trial. The conventional EEG data analysis was focused on infants 6 to 24 months of age, when clinical seizures were more likely to emerge in the TSC population. The presence of inter-ictal EEG epileptiform activity and developing seizures had a sensitivity of 73.7% and specificity of 100%. The corresponding positive predictive value (PPV) for inter-ictal EEG epileptiform activity is 100% (that the subject with epileptiform discharges on EEG would develop seizures) and negative predictive value (NPV) is 64% (that the subject without epileptiform discharges on EEG would not develop seizures).

The central hypothesis of this Phase IIb trial is that early identification of electroencephalography (EEG) biomarkers and early treatment versus delayed treatment with vigabatrin in infants with TSC will have a positive impact on developmental outcomes at 24 months of age. It would also prevent or lower the risk of developing infantile spasms and refractory seizures. This preventative approach would be expected to result in more favorable long-term cognitive, behavioral, developmental and psychiatric outcomes and significantly improve overall quality of life. It is a randomized, double-blind, placebo-controlled clinical trial design. Successful completion of this trial will also advance the field by demonstrating the value of systematic surveillance with EEG in asymptomatic infants with TSC.

Protocol description:

Study design is a Phase IIb prospective multi-center, randomized, placebo-controlled, double-blind clinical trial. The goal will be to enroll 80 infants with TSC who are less than

6 months of age prior to the onset of their first seizure (Appendix1: Figure 1-PREVeNT Trial Study Design).

INCLUSION CRITERIA:
1) ≤ 6months of age
2) No history of seizures or infantile spasms, or evidence of subclinical electrographic seizures on a previous video EEG
3) Meet genetic or clinical diagnostic criteria for TSC, the latter based on current recommendations for diagnostic evaluation, such as physical exam, neuroimaging, echocardiogram[28].

EXCLUSION CRITERIA:
1) Is greater than 6 months of age
2) Has not been diagnosed with TSC
3) History of seizures or infantile spasms, or evidence of subclinical electrographic seizures on a previous video EEG
4) Has received any anticonvulsant medication including vigabatrin, other anti-seizure therapeutic agent including cannabidiol
5) Has received an oral mTOR inhibitor such as everolimus or sirolimus
6) Has taken an investigational drug, including but not limited to cannabidiol, as part of a research study 30 days prior to enrollment, or plans on taking an investigational drug at any time during the duration of the study
7) Is currently enrolled, or plans on enrolling at any time during the duration of the study, in an experimental behavioral early intervention study
8) Has a history of being born prematurely (born less than <30 weeks gestation at the time of delivery)

Subjects who have consented and at the time of their baseline study visit and show evidence of electrographic seizures on their initial video EEG will exit from the PREVeNT study and proceed with initiation of management of their clinical condition. All other enrolled subjects will be followed until the age of 36 months with physical and neurologic exams, serial 1-hour awake and sleep video-EEGs, and ophthalmologic evaluation at baseline and defined intervals, every 6-12 weeks based on subjects chronological age.

Study visits and procedures are based on subjects' chronological age in months (±1 week if under 12 months of age and ±2 weeks if 12 months and older). Once enrolled, subjects enter a 'watchful waiting' protocol which includes baseline EEG at 1.5 month (~6 week) intervals until 12 months of age, then every 3 month (~12 week) intervals until 24 months of age. Subjects will continue in this protocol phase until treatment is justified.

Randomization will be stratified into two groups, according to age at time of emergence of EEG biomarkers: 1) <= 7 months; 2) >7 months of age. This is to account for natural history studies and data from our P20-NS080199 study that indicates mean age of clinical seizure onset less than 7 months of age, with a significant portion of TSC infants developing seizures between 6-12 months and still others after 12 months of age[1]. Accordingly, this randomization strategy will ensure we are able to evaluate age independently of timing of EEG change and treatment randomization.

For the accurate identification of clinical seizures, parents and caregivers will review a seizure recognition video at the time of study enrollment and retain it as a reference throughout the study. They will also be encouraged to video any suspicious events during the study period for review by clinicians of the investigation team. If at any point during the study the subject is suspected of having a clinical seizure, the parents/caregiver will be instructed to contact their site's study coordinator. The subject will be seen as soon as possible for a study visit and a video EEG will be completed for confirmation of clinical or electrographic seizure onset. Those subjects with either a documented clinical seizure on video or evidence of an electrographic seizure on video EEG will be transitioned to the Open-Label treatment phase.

At the emergence of abnormal epileptiform activity (*i.e.* sharp waves, spikes or polyspikes, spike and wave discharges, classic or modified hypsarrhythmia) prior to the onset of the first clinical or electrographic seizure, subjects will enter the protocol blinded-treatment phase (Arm A). Emergence of these specific, predetermined EEG biomarkers detected by video EEG will lead to a 1:1 randomization with vigabatrin or placebo. The vigabatrin and placebo will be dispensed as a sachet and those subjects randomized to vigabatrin will be titrated up to 100mg/kg/day by 50mg/kg/day every 3 days during the blinded phase of the study. The research pharmacist at each site will be unblinded during this phase of the study and the vigabatrin and placebo sachets doses will be identical in order to maintain the blind.

To further maintain the treatment blind, subjects who experience their first seizure after randomization to either vigabatrin or placebo will follow the same blinded 2 week transition to open label vigabatrin (dose increased to 150mg/kg/day in the open label phase), so that all patients regardless of randomization group will exit the blinded treatment phase in identical fashion. This way, neither the treating physician nor the parent/guardian would know which treatment group the child had been assigned. Only the PREVeNT trial independent medical monitor and research pharmacists at each site would have access to randomization assignments throughout the study. The blinded 2 week transition phase will also occur if the subjects in Arm A are seizure free at the time of their 24 month of age study visit. They will be tapered off the study drug (vigabatrin or placebo) without breaking the treatment blind and continue to be followed for the duration of the study (36 months) under best medical care (i.e. if seizures develop, vigabatrin may be initiated).

Both vigabatrin and placebo will be repackaged into identical aluminum foil sachets so as to maintain treatment blinding and distribute as appropriate to the investigational pharmacies at local participating sites for individual patient distribution and administration. For administration, the entire content of one sachet (500 mg active drug) is dissolved in 10 ml water for oral administration that is dosed according to body weight 50-150 mg/kg/day divided BID. Dosing will follow established recommended guidelines (50 mg/kg/day and increased as needed by 50 mg/kg/day every 3 days up to 100mg/kg/day during the blinded phase of the study, divided BID).

Only those subjects who have clinical seizures or electrographic seizures in the blinded treatment phase of the study will transition to open label vigabatrin to a dose of 150mg/kg/day.

Note** Subjects who have consented and at the time of their baseline study visit and show evidence of electrographic and/or clinical seizure(s) on their initial video EEG will exit from the PREVeNT study and proceed with initiation of management of their clinical condition.

If an enrolled subject has an electrographic or clinical seizure(s) prior to randomization and the previous EEG(s) have been normal they will move into the Open label phase of the study (Arm B). If the seizures are not controlled in the Open label phase at the vigabatrin maximum dose of 150mg/kg/day, the investigator may change the antiepileptic medication as clinically warranted to improve seizure control (including tapering off vigabatrin). These subjects will remain in the open arm of the study for the duration of the protocol (36 months).

Patients enrolled without prior clinical seizures but found on first baseline EEG to have electrographic or clinical seizures of which the parent and clinician were previously unaware will exit the PREVeNT trial and begin medical treatment by the site PI based on clinically accepted management guidelines for seizures in TSC infants. Based on the P20-NS080199 study, this group is estimated to be very small (<5%) and will not affect the total number of enrolled patients (N=80) eligible to be randomized to either placebo or vigabatrin treatment.

Enrolled subjects who never develop EEG abnormalities or clinical seizures (ARM C) will remain in the study and continue with regular clinical evaluations, EEG testing, and developmental assessments as scheduled (Table 2). Without EEG change or developing seizures, they will have neither been randomized nor treated with vigabatrin. These patients will serve as a negative control and be key for validating the sensitivity and specificity of EEG biomarkers as predictors of seizure risk and baseline disease characteristics attributable to TSC that are independent of epilepsy and AED treatment (TREATMENT ARM A).

All patients on placebo or vigabatrin will exit the blinded treatment phase (TREATMENT ARM A) at the onset of electrographic and/or clinical seizures or 24 months of age whichever comes first. Subjects experiencing clinical seizures will undergo a 2 week blinded transition to open label vigabatrin to a target dose of 150mg/kg/day, thus maintaining the blind. The treating clinician at that time may further optimize treatment with vigabatrin as tolerated, add adjunctive treatment, or transition to alternative AED according to best clinical judgment and established standard of care. All seizure treatments and dosing will be recorded and analyzed for synergistic effects, efficacy for specific seizure types, impact on long-term epilepsy and developmental outcome measures, and safety. Vigabatrin safety data will include the ophthalmologic examination data elements as outlined in the protocol and any potentially vigabatrin related changes

noted on routine clinical brain MRI that are obtained as part of clinical care during the subjects PREVeNT participation.

EEG Acquisition and Data Transfer:

Subjects will receive serial 1-hour video-EEG studies to monitor for the development and evolution of EEG abnormalities. All video EEG studies will be recorded for one hour, incorporating both sleep and wakefulness, at a sampling rate of 2000 Hz. A standard EEG acquisition protocol has been designed for all 15 sites to ensure uniform recording parameters as well as data collection with standard 23 electrodes placed according to the 10-20 international placement system. All subjects will receive the first EEG study within a 2 week window after study enrollment. Those subjects with evidence of epileptiform activity on their EEG will have repeated EEGs every 6 weeks until 12 months of age, then every three months until 24 months year of age, and a final EEG at 36 months. (Table 1).

Those subjects with no evidence of epileptiform activity on their EEG and remain seizure free will have repeated EEGs every 6 weeks until 12 months of age, then every three months until 24 months year of age, and a final EEG at 36 months. (Table 2).

In addition to the PREVeNT central EEG readers there should be a designated on-site EEG reader who will review the EEG and generate a clinical EEG report for the medical record at every study visit.

Prior to the subject being randomized into the blinded phase of the protocol the EEG report generated by the PREVeNT readers (Drs. Peters, Porter and Wu) will determine the subject's course in the study.

Once the subject enters the blinded phase of the study the study visit EEG should be read by the designated EEG reader at each site before the subject completes the designated requirements for the study visit. The EEG will continue to be read by the PREVeNT EEG readers but they will have up to 14 days to complete the study CRF. If the clinical EEG during the blinded phase of the study shows evidence of electrographic and/or clinical seizures the site clinical EEG reader should notify the site PI, site study coordinator. The site study coordinator will then notify the Prevent study coordinator (Jessica Krefting RN) and Dr. Bebin. Dr. Bebin will then notify the PREVeNT EEG reader on call to request an EEG review and confirmation of the EEG findings the subject completes the study visit. If there is a difference in interpretation between the site clinical EEG reader and the PREVeNT EEG reader the final determination will be made based on the PREVeNT EEG reader interpretation. This will determine if the subject will transition to the open-label phase of the protocol or not at this study visit.

If a subject develops clinical seizures during the blinded phase of the study their parent/LAR should contact the site study coordinator as soon as possible. It is strongly encouraged that the parent/LAR video any suspicious events that raise concern for seizures so they can be reviewed by the site PI. If it is determined by the site PI that the subject is having clinical seizures every effort should made to see the subject back in

clinic within 24-72 hours of receiving the call from the parent/LAR. The subject will return for an “Unscheduled Study Visit” which will include: physical examination, EEG, medication reconciliation, seizure classification, and determination if the subject is to transition into the Open Label phase of the protocol. If there are any questions regarding the subject’s transition to the Open Label Phase of the protocol Dr. Bebin should be contacted to discuss the subject course in the study.

The deidentified EEG data, without video, will be transferred to IEEG.org managed by the University of Pennsylvania for same day analysis by the blinded primary central EEG reviewer. There will be an assigned back up EEG reader to the primary central EEG reviewer in the rare instance that primary central EEG reviewer is not available. There will be 2 EEG readers (Dr. Brenda Porter and Dr. Peters) throughout the study. Prior to the subject randomization, the EEGs will be read the same day as the EEG is completed by either EEG reader, which will be pre-determined the week prior for any given week, and a CRF report will be generated. Once the subject has been randomized the EEGs will be read by both readers within 14 days of the EEG being completed. The primary central EEG reviewer’s interpretation of the EEG and identification of epileptiform activity will determine if the subject will be placed in Arm A for treatment randomization to vigabatrin or placebo.

The Central EEG reviewer’s interpretation will be communicated back to each site. The primary central EEG reviewer will be available to each site as needed for clarification or any discrepancy of the individual site readers EEG interpretation. Should either the primary central EEG reviewer or the backup EEG reader be uncertain of epileptiform discharges in rare cases, these two readers will consult each other and reach a consensus.

Video EEG will be viewed digitally in the standard time scale of 30 mm/sec and standard filter settings of 1 Hz low frequency filter (high pass) and 70 Hz high frequency filter (low pass), along with a 60 Hz notched filter. Each video-EEG study will be scored, based on age-appropriate norms, as either: normal or abnormal. Abnormalities will be further characterized in terms of specific background abnormalities (e.g. generalized or focal slowing), epileptiform abnormalities (e.g. focal, multifocal, or generalized spike discharges, electrographic seizures), and presence of hypsarrhythmia. EEG data will be collected using a modified NINDS Common Data Element Epilepsy Tools.

Vigabatrin and Placebo:

The investigational drug product to be used in this study is vigabatrin, marketed in the US under the trade name Sabril by Lundbeck Pharmaceuticals. Lundbeck will supply vigabatrin for this study directly to the Investigational Pharmacy at Cincinnati Children’s Hospital (CCHMC). It is supplied as a white granular powder enclosed in 50x60 mm child-resistant aluminum foil packets (sachet). It is non-hygroscopic, freely soluble in water, and thermodynamically stable with an established shelf life of more than 36 months at room temperature. Povidone is the primary inactive excipient. The investigational pharmacy at CCHMC will produce identical white granular powder with indistinguishable

physical appearance, color, solubility, and taste to the vigabatrin powder supplied by Lundbeck. Both vigabatrin and placebo will be repackaged into identical aluminum foil sachets so as to maintain treatment blinding and distribute as appropriate to the investigational pharmacies at local participating sites for individual patient distribution and administration. For administration, the entire content of one sachet (500 mg active drug) is dissolved in 10 ml water for oral administration that is dosed according to body weight 50-150 mg/kg/day divided BID. Dosing will follow established recommended guidelines (50 mg/kg/day and increased as needed by 50 mg/kg/day every 3 days up to a maximum dose of 100 mg/kg/day, divided BID in the blinded phase and 150mg/kg/day in the Open Label Phase).

Subjects randomized to vigabatrin in Arm A (Figure 1) will be treated with vigabatrin 100mg/kg/day or placebo until 24 months of age or until they show evidence of clinical seizures or electrographic seizures on video EEG. Based on the published EEG biomarker data for infants with TSC, the subjects randomized to our primary randomized trial population, Arm A (who have evidence of epileptiform activity in their EEG) will be at high risk for developing epilepsy (approaching 100%).

If the subjects in Arm A are seizure free at 24 months of age, they will be tapered off the study drug (vigabatrin or placebo) without breaking the treatment blind and continue to be followed for the duration of the study (36 months).

If electrographic or clinical seizures occur while on study drug, they will transition into the Open label phase of the study (Arm B) and continue to be followed until 36 months of age as per the PREVeNT protocol schedule. If the seizures are not controlled in the Open label phase at the vigabatrin maximum dose of 150mg/kg/day, the investigator may change the antiepileptic medication as clinically warranted to improve seizure control (including tapering off vigabatrin). These subjects will remain in the open arm of the study for the duration of the protocol (36 months).

Based on the TSC-EBS data, it is estimated that 30% of subjects (24 subjects) in Arm C will not be treated but continue in the study until the age of 36 months (Table 2).

See Pharmacy Manual for details of packaging and labeling, product tracking and accountability, and product disposal and destruction.

Developmental Assessments:

The developmental leadership team will oversee the implementation of the developmental assessments and monitor the quality and consistency across all sites.

All enrolled subjects, regardless of which arm of the PREVeNT protocol, will complete the Bayley-III assessment scale and Vineland -II (Survey Interview Form), at 6 months, 12 months, 24 months and 36 months of age. The Bayley Social Emotional Adaptive Behavior Assessment will be completed at the 6 month study visit. The Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) which will be completed at 12,

24 and 36 months of age. The blind will be maintained for the clinical psychologist performing the developmental assessments throughout the study. They will be blind to treatment arm, subject's seizure control and concomitant medications. In addition to the primary outcome analysis to determine the impact of early vs. delayed treatment with vigabatrin on neurodevelopmental outcome at 24 months, the inclusion of the 12-month time-point allows additional exploratory analysis to determine if problematic areas of cognitive function and/or developmental domains are evident before 24 months of age. Additional developmental assessments will be completed which include the subdomain scores of the Bayley-III, Vineland (Survey Interview Form), Beery Visual Motor Integration (VMI), Child Behavior Check List (CBCL) and ADOS2, and/or Brief Observation of Symptoms of Autism (BOSA)* and DSM-5 checklist at 24 months. At 36 months of age subjects will complete the same assessments as 24 months with the addition of the BOSA* and ADI-R if the ADOS is not administered, as an exploratory analysis to access whether changes observed at 24 months are consistent with those seen at 36 months and indicative of long-term outcome and those subjects at risk for autism or ASD. At 36 months of age subjects will also complete the Peabody Picture Vocabulary Test (PPVT). When appropriate instruments are available, measures can be administered to Spanish speaking individuals.

*During the COVID-19 Pandemic and for the remainder of the study the BOSA and DSM5-checklist was added to the 24 and 36 month study visit. This would enable the study to complete the Autism assessments at the 24 and 36 month visit using a valid assessment tool while complying with the COVID-19 safety measures. Because of the requirement to wear a facemask during the subject's study visit would invalidate the ADOS-2 result. In its place the BOSA will be administered over a 15-20 min session by trained ADOS-2 research reliable psychologists, since it requires the same clinical skill and judgement required for the ADOS-2 administration and scoring. The ADI-R will be completed at the 36 month study visit if the ADOS is not administered to aid the Autism diagnostic accuracy with the BOSA. (See Appendix 3 page 36)

A summary of the neurodevelopmental assessment will be provided to the site investigators after each assessment. If the subject shows evidence of developmental delays they will be referred for early intervention services, including physical, occupational and speech therapy. The investigators are aware that this could potentially bias the results but it is ethically imperative that referrals to early intervention services be initiated when warranted. The proportion referred and the early intervention services implemented will be tracked over the duration of the study

Ophthalmologic Assessments:

Since approval of vigabatrin for the treatment of infantile spasms in 2009, the FDA has required a risk evaluation and mitigation strategy (REMS) that includes recommended ophthalmologic evaluations every 3 months. For the current study, this recommended safety guidelines of the REMS program will be followed, but we recognize that this creates some unique challenges within a double-blinded, placebo-controlled randomized study. All subjects enrolled in the PREVeNT Trial will follow a standard ophthalmologic protocol.

At each site, the ophthalmologic examination will be completed by a pediatric ophthalmologist or neuro-ophthalmologist who has experience in TSC infant ophthalmologic examinations.

The protocol will include a baseline eye exam within 4 weeks of initial enrollment in study. This will include: visual acuity, pupillary reaction and dilated funduscopic examination with fundus photography. Electroretinography (ERGs) will **not** be obtained as part of the ophthalmologic examination at any of the participating sites. It should be noted that the FDA risk evaluation and mitigation strategy (REMS) program does not require ERGs.

Subjects randomized to the Blinded Treatment phase (Arm A) will be treated with vigabatrin 100mg/kg/day or placebo until 24 months of age, or have a clinical seizure or electrographic seizure on EEG as determined by the Central EEG Reader. During this phase of the study, the established FDA REMS guidelines will be followed. Eye examinations will be completed within 4 weeks after randomization (Arm A) and then every three months while the subject is in the double blind phase of the study. The eye exams will include: visual acuity, pupillary reaction and dilated funduscopic examination. A final eye exam will be completed after the subject has been off study drug for 3-6 months. The final exam will include: visual acuity, pupillary reaction and dilated funduscopic examination.

If the subject continues into the Open-label phase (Arm B) of the study and remains on vigabatrin, the REMS guidelines will be followed as long as they are treated with vigabatrin. A final eye exam will be completed once they have been off vigabatrin 3-6 months in the Open label phase of the study. The final exam will include: visual acuity, pupillary reaction and dilated funduscopic examination with fundus photography.

If the subject remains in Arm C (seizure free and Normal EEG) they will have a baseline eye exam within 4 weeks of the initial enrollment in the study. A repeat eye exam will be done at 12 months of age, 24 months of age and 36 months of age.

If at any time during the study the ophthalmologist detects visual changes in the subject, they will contact the site PI directly. At that point, the site PI and ophthalmologist will discuss and recommend further ophthalmologic assessments such as Pediatric optical coherence tomography (OCT) under anesthesia and fundus photography. The parents/LAR, examining ophthalmologist, site PI, and NIH DSMB will review the ophthalmologic examination results and consider all options including the decision to withdraw from the study at that point.

PREVeNT Trial Background for Vision Testing and Creation of eCRFs

Background

The PREVeNT trial will require evaluation of the visual function of patients enrolled in the study because of previous reports of adverse effects of vigabatrin upon the anterior afferent visual system. However, more recent work from the FDA/Lundbeck Risk

Evaluation Mitigation Strategy [REMS] program and the prospective "Lundbeck Vision Study" has disclosed a much lower incidence of visual defects than previously reported in the literature.

The discrepancy between the past studies and the more recent work is explained by obtaining baseline evaluations of the patients eligible for vigabatrin compared to the prior case reports that were cross-sectional in nature and failed to evaluate the patients' baseline visual status and also failed to account for the now well-recognized high variability of automated visual field testing. Studies by Gonzalez et al; Sergott et al, the REMS reports over the past 6 years since FDA approval and the Lundbeck vision study have consistently demonstrated that 20-40% of patients considered appropriate for vigabatrin therapy have abnormalities of the afferent visual system at baseline as measured by visual acuity, visual fields, funduscopic examination and optical coherence tomography [OCT].

Based upon these new data, the REMs program and the product label are now being considered for revisions by the FDA. Although it is premature to assume that vigabatrin is without any risk to the visual system, the current data suggest that it may be safer than originally thought at least over a 24 month cumulative exposure.

The following protocol is being proposed to protect the vision of the subjects participating in the PREVeNT trial. The protocol will then be converted to a standard operating procedures [SOP] format and electronic clinical research forms [eCRFs] to insure that the subjects are evaluated in a standardized manner at all the clinical sites and that the data can be analyzed throughout and at the conclusion of the trial.

Age Appropriate Methods for Evaluation of Visual Function [Based upon the recommendations of the American Academy of Ophthalmology]

Method	Abnormalities	Recommended Age	
		Newborn to 6 months	6 months until Able to cooperate With testing
Red Reflex & Bruckner test	Absent, white, dull opacified, asymmetric	X	X
External Exam	Structural abnormality such as ptosis, proptosis	X	X

Pupils	Irregular shape, anisocoria, afferent defect	X	X
Visual acuity Fix and follow	Failure to fix and follow, failure to maintain central fixation	X Cooperative infant 3 months or older	X
Corneal reflex	Asymmetric or displaced	X	X
Retinoscopy & Strabismus	Significant Myopia, hyperopia Anisometropia	Glasses & amblyopia therapy at discretion of the pediatric ophthalmologists	X
Dilated funduscopic examination	Retinal hamartomas Optic atrophy Photos if possible	X	X

Optical Coherence Tomography

To be performed if high index of suspicion for decreased visual function. Will require general anesthesia.

Examination

1. Documentation of the child's level of cooperation with the examination required to interpret the results

2. Testing of sensory function should be performed before any dissociating techniques such as covering an eye to check monocular visual acuity or cover testing to test ocular alignment. Testing for alignment should be done before dilating drops are instilled.

3. Binocular Red Reflex [Bruckner] Test

    a. In a darkened room, the direct ophthalmoscope light is directed towards both eyes from approximately 18-30 inches.
    b. A symmetric red reflex in each eye is normal

c. Opacities of the reflex are always abnormal
d. The reflex will vary upon retinal pigmentation so variation will be present by race/ethnicity
e. Significant hyperopia demonstrates an inferiorly placed brighter crescent in the reflex
f. Significant myopia presents as superiorly displaced reflex
g. Also used to assess for ocular misalignment—esotropia, exotropia, and hypertropia.

4. Fixation

a. Visual acuity testing in this age group requires a qualitative assessment of fixation and tracking movements of the eyes. The child's attention is drawn to the examiner's or caregiver's face [infants < 3 months] to a hand-held light, toy, or other fixation target.
b. Fixation behavior is recorded for each eye as "fixes and follows" or "central, steady, and maintained".

Fixation preference graded by the energy the child objects to occlusion of one eye relative to the other eye.

Biomarker Blood Samples, Genetic testing and Bio-specimen Sharing:

Biomarker blood samples:

Serial Blood sample collection will be optional and collected at each subject's enrollment in the study, at the time of the subject's randomization to vigabatrin or placebo (Arm A), at clinical seizures onset, and a final sample after 3 months of seizure control or 3 months of uncontrolled seizures in the open label phase of the study (Arm B). For this group of subjects it will be a total of 4 blood samples over the course of the study. For subjects randomized but remain seizure free on study drug will have three blood samples (at enrollment, randomization and at 24 months of age).

Those subjects who remain seizure free and have a normal EEG will have a second biomarker sample collected at 12 months of age and a final sample at 24 months of age (Arm C). Samples will be stored in the TS Alliance Biosample Repository (a research resource supported by the National Institutes of Health/National Institute of Neurological Disorders and Stroke), for a future research initiative that will test whether we can detect increased mTOR activity and protein synthesis in peripheral lymphocytes from individuals with TSC.

Genetic testing:

Blood samples from the patient and parents if available will be collected after the subjects' enrollment, and stored at the TS Alliance Central Biobank Repository. DNA will be extracted from these samples and will be used in genetic studies to identify tuberous sclerosis complex disease phenotype modifiers in the future Network collaborative grant proposals. Proposals for sample use will be reviewed by a committee including site PIs and PREVeNT Biorepository Committee which includes a representative from NIH and the TS Alliance. This is optional for subjects and their parents participating in the study and will not impact their enrollment eligibility.

TSC genetic testing will be obtained as part of the clinical diagnostic evaluation of each subject, if it has not already been completed. If the patient's insurance will cover genetic testing of the *TSC1* and *TSC2* genes, this will be done as part of the routine clinical care and will be billed through insurance. If this is not possible DNA samples for those subjects needing testing of *TSC1*/*TSC2* will be sent to UAB Medical Genomics Laboratory which is a CLIA certified laboratory to undergo genetic testing of *TSC1*/*TSC2* using next generation sequencing. The DNA will have been extracted and stored at the TS Alliance Central Biobank Repository. The results will be given to the site PI, subject's parent(s)/LAR and entered into the DCC's database.

We will collect the necessary demographic and clinical data information from the human participants for genotype-phenotype analyses and this information will be maintained in the Data Coordinating Center (DCC). We plan to deposit de-identified genotyping data in NIH designated databases (e.g. dbGaP). We will share research data in accordance with NIH and NDAR data sharing policy. Identifiers will be removed from the final data set to fully protect the human participants. We will evaluate each data request to ensure that special circumstances do not exist that would permit anyone to deduce the identification of individuals from the remaining data. If such case exists, we will make the data available under a data-sharing agreement that provides for (1) a commitment to using the data only for research purposes and not to identify any individual participant; (2) a commitment to securing the data using appropriate technology; and (3) a commitment to destroying or returning the data after analyses are completed.

After central extraction/processing, biorepository specimens will be securely labeled, cataloged, and stored according to standard operating procedures appropriate for the specific biosample type and intended future investigations thereof. The TSC PREVeNT Biorepository will utilize liquid nitrogen and/or -80ºF freezer cryostorage equipment with automatic emergency electrical backup and a remote notification temperature alarm for long-term storage. Depending on local procedures and capability, permanent preservation of fixed tissues and their storage may be performed locally or shipped for central catalog and storage, as detailed in the study manual of operations. Processing may include receipt and storage of frozen biosamples, centrifugation, separation, aliquoting, labeling and storage, histology/molecular processing, microtomy, and tissue staining for analysis and quality control analysis. Procedures may also include the isolation and quality management of RNA, DNA or proteins, digital imaging of stained

tissue sections, also for quality assessment and/or measurable analysis of small molecules or labeled analytes. In some cases immunohistochemistry or protein profiles may performed to assess the quality of the fluids or tissues. During all procedures, the biosample identification is managed using barcode labels to reduce transcription error. Biosample identification numbers are unique for each aliquot and are de-identified from any subject PHI.

For access and analysis to repository tissues and associated de-identified clinical data, there will be a repository use committee, comprised of the Lead PI (Bebin), PREVeNT PIs (Sahin, Krueger, Wu, Porter, Koenig, Frost), and Dr. Bruce Korf, Dr. Hope Northrup and TS Alliance Representative (Roberds), and NIH Representative (Laura Mamounas). The Repository Use Committee will be responsible for approving release of PREVeNT study blood samples or, genetic material, and clinical data to both PREVeNT and external investigators. The TSC Project Leader (Bebin) will serve as chair of the committee. A specimen request form will be required that includes among other data, the name of the principal investigator making the request, funding source for the proposed analysis, a research synopsis, IRB approval of the research project, and justification for the required samples. At the time a de-identified sample is requested, the requesting investigator may also request de-identified clinical data, if needed. Completion of the specimen request form and majority approval by the Repository Use Committee is required before samples will be distributed. The request form specifies that the investigator must not try to re-identify the subjects from whom the samples are derived. Under no circumstance may an investigator provide these samples for use to additional investigators unless specified otherwise in an approved sample request. Repository committee members will excuse themselves during discussions in any case that they themselves have requested samples. The chair of the committee, with agreement from at least one other member of the committee, will have the authority to approve minor changes to requests (i.e. addition of a small number of samples to an already approved study). Once the Repository Use Committee approves the scientific/technical merit of a specimen request, the samples will be released to the requesting investigator and/or institution.

Acquired specimens of the TS Alliance Central Biobank Repository may continue to be used in accordance with the study protocol and the signed informed consent active at the time the specimens were obtained, even if the subject withdraws consent or fails to complete the entire study. Subjects withdrawing consent from the study and wishing that previously collected specimens be removed from the biorepository must provide written request for specimen removal to the study principal investigator (Dr. Bebin). The written request for specimen removal should include the subject's name and date of birth, the name of the person submitting the request and relationship to the subject, and the specimen(s) requested to be removed from the TSC Alliance Central Biobank Repository. Upon receipt and verification, we will do our best to remove the requested specimens and associated clinical data from the biorepository and destroy them.

Study Organization and Data Management: (Appendix 1:Figure 2- PREVeNT Trial Organization)

The PREVeNT Principal Investigator and Clinical Coordinating Center (CCC) Director, Martina Bebin, MD, will maintain responsibility for the overall conduct of the study and communications with the NINDS. The CCC will be responsible for coordinating the activities of the Steering Committee (SC), Data Coordinating Center (DCC), the EEG Analysis Core, the Pharmacy Core and the Genetic Research Laboratory Core. The Research Pharmacy core will be led by Dr. Darcy Krueger, EEG Analysis core led by Dr. Joyce Wu and Dr. Brenda Porter and Dr. Jurriaan Peters will assist in EEG analysis, Developmental Assessment Leadership Team will coordinate all the developmental assessments throughout the study and ensure consistency in the assessments across all 15 sites. The Data Coordinating Center led by Dr. Gary Cutter, the TSC Genetic Research Laboratory by Drs. Korf and Messiaen. Dr. Robert Sergott will serve as a consultant regarding the ophthalmologic assessments throughout the PREVeNT Trial.

The CCC will provide site management oversight, including the following duties: site evaluation; subcontract development and execution; providing the protocol and study documents to the site investigators for submission to the IRB; obtaining documentation of IRB approval prior to shipping study materials; ensuring patient informed consent is properly obtained; and ensuring proper clinical site monitoring.

The EEG data will be stored and analyzed using the University of Pennsylvania IEEG.org Portal. They will provide technical support for each of the 15 sites for data processing, conversion and upload to the IEEG.org platform via Amazon's Elastic Computing Cloud (EC2). The software engineer will ensure that IEEG.org will support upload of the data from each study site within 1-2 hours of data collection. Data will be stored and accessible to the study investigators on IEEG.org for the length of the study. The University of Pennsylvania IEEG.org group, which includes a software engineer, will develop tools and features on IEEG.org portal that is specific for the needs and goals of this project.

The Data Coordinating Center (DCC) will provide full service electronic data capture (EDC), data management, and reporting services as it currently does for the current TSC-EBS study (P20-NS080199). The extent of these data management services include: database specification, development and testing; validation rules programming; data management plan development and maintenance; ongoing manual data review; and data cleaning and locking. The DCC will implement a quality assurance plan to provide feedback to the clinical sites in order to maintain and improve the quality of the study database. In addition, the DCC will provide statistical analysis and DSMB reports. The PREVeNT Trial Steering Committee will consist of Dr. Bebin as Study PI, Dr. Robert Flamini-Medical Safety Monitor (MSM), Dr. Wu, Dr. Krueger and Dr. Cutter. The Data Quality Committee will routinely review the monthly data summaries from the trial and monitor the quality and identify any related study issues that warrant a protocol amendment or further analysis or review. NIH will select the PREVeNT Trial DSMB which will review the quarterly DCC reports and communicate with Study PI and MSM.

Estimate of Key timelines:
Year 1, Q1/2: Study initiation, implementation of Data Coordinating Center, IEEG.org for EEG data transfer/ storage, and enrollment of subjects 1-4
Year 1, Q3/4: Subjects 5-28 will be enrolled=Total enrollment 28 subjects at the end of Year 1
Year 2, Q1/2: Subjects 29-52 enrolled=total 56 enrolled, Subjects 1-4 complete the 12 month visit
Year 2, Q3/4: Subjects 53-76 enrolled=total 80 subjects, Subjects 5-28- complete 12 month time point
Year 3, Q1/2: Subjects 1-4 reach the 24 month time point and Subjects 29-52 reach the 12 month time point.
Year 3, Q3/4: Subjects 5-28 complete the 24 month time point, Subjects 53-76 reach the 12 month time point
Year 4, Q1/2: Subjects 1-4 reach 36 month time point; Subjects 29-52 reach 24 month time point
Year 4, Q3/4: Subjects 5-28 reach 36 month time point, Subjects 53-76 reach 24 month time point;

*Analysis begins for the PREVeNT trial using the baseline, 12 and 24 month data set.
Year 5, Q1/2: Subjects 29-52 reach 36 month time point,
Year 5, Q3/4: Subjects 53-76 reach 36 month time point,
*Analysis will begin for the 36 month developmental assessments and primary and secondary outcome measures.

Statistical Methods, Sample Size and Analysis Plan:

Monitoring Recruitment
The table below shows the number screened and the lower limit of a 95% confidence interval for the number randomized. For example, if we screen 10 patients, we expect 6.3 to be biomarker positive (62.5% of the cohort based on the pilot). If the expected is 6.25 (6.3), then if we observe 0 or 1 of the 10 biomarker positive screened who are eligible, we can conclude that the rate of biomarker positivity is statistically likely to be less than 62.5% and we may not reach our target numbers for randomization. The Study and DSMB will monitor recruitment in this manner to enlist early monitoring concerns and discussions of potential solutions, including adding clinical sites or extending recruitment. An analysis of the EEG biomarker data will be done after the first 28 subjects have reached 12 months of age (toward the end of year 2) to estimate whether additional subjects would be needed for Arm A (randomization arm).

Number Screened	Epileptiform Activity	Using Poisson and Normal Approx
	Expected (62.5% screened)	Lower Limit of 95% CI
10	6.3	1.4

15	**9.4**	**3.4**
20	**12.5**	**5.6**
25	**15.6**	**7.9**
30	**18.8**	**10.3**
35	**21.9**	**12.7**
40	**25.0**	**15.2**
45	**28.1**	**17.7**
50	**31.3**	**20.3**
55	**34.4**	**22.9**
60	**37.5**	**25.5**
65	**40.6**	**28.1**
70	**43.8**	**30.8**
75	**46.9**	**33.5**
80	**50.0**	**36.1**

Primary Study Objective:

The primary endpoint of this Phase IIb feasibility trial will be the demonstration of the prevention of seizures and the subsequent cognitive assessment component of the Bayley-III. The other components of the Bayley (receptive communication, expressive communication, fine and gross motor subscores) will be used as secondary and supportive endpoints. The primary goal of this Phase IIb trial will be to provide the evidence needed for a Phase III pivotal trial. Thus, the study will need to demonstrate the purported mechanism by which an improved clinical outcome is achieved, prevention of seizures, leading to a difference in cognitive outcomes. The rationale for not using a composite outcome at this stage of evaluation is that it will be simpler to focus on a single most promising outcome measure of early treatment and one that is clinically meaningful. The other components of the Bayley-III will be evaluated in a multivariate composite as part of the secondary outcomes and exploratory outcome.

The sample size for this feasibility trial is derived from the resources available to support the trial as per this NINDS mechanism, the primary goal of demonstrating a reduction in seizures and the power to assess the differences in cognitive scores on the Bayley-III. To assess the plausibility of recruitment, we looked at the pilot data from our ongoing TSC

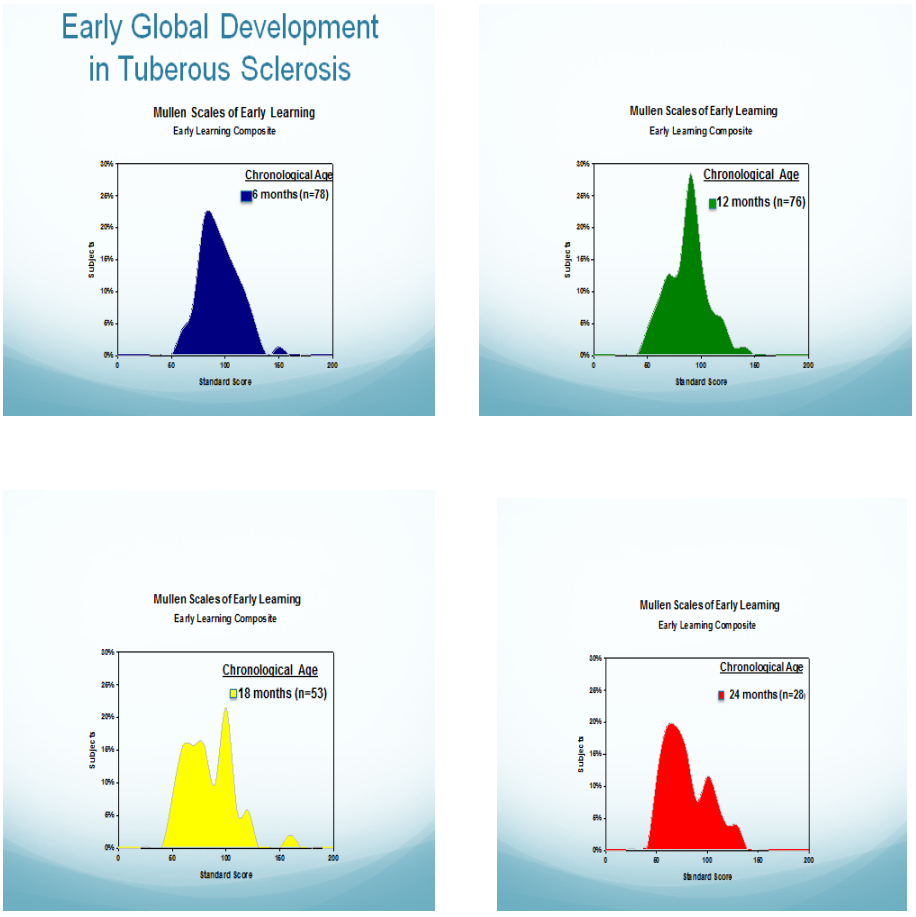
Project. To date, we have enrolled 40 patients, 1 patient dropped out; 15 continue without seizures to date; 19 developed well controlled seizures, and 5 have developed refractory seizures. Thus, 25 of the 40 or almost two thirds of the enrolled patients have developed seizures to date. Reducing this number with earlier intervention would seem consistent with achieving a positive effect on the cognitive outcomes as assessed by the Bayley-III.

We expect to be able to recruit between 75 and 80 participants over the course of the recruitment period. Based on these numbers we expect 37.5% (based on our P20 data where15/40 remained seizure free) to be biomarker free and never randomized. Thus, we expect to lose to randomization between 28 to 30 participants yielding approximately 48 patients to be randomized (75-28 = 47 and 80-30=50) or 24 to 25 participants per group.

To estimate sample size needed, we first assess the ability to demonstrate the feasibility that the intervening variable, seizures, can be prevented with early treatment. In the placebo arm, we expect that all patients will develop seizures, but to be conservative we assume that 2/24 in the placebo arm do not develop a seizure within the time frame, making the proportion of seizure occurrence 0.917. In the vigabatrin arm, we assume it mirrors the proportion well controlled in our ongoing TSC project, thus our seizure rate will be estimated at 5/24 (19 remain seizure free to date) or 0.208. A Fisher's exact test with a 0.05 two-sided significance level will have 99% power to detect the difference between a Group 1 proportion, p_1, of 0.917 and a Group 2 proportion, p_2, of 0.208 when the sample size in each group is 24.

The percent difference for seizures used in the power calculation is based on data collected during our current TSC project. We note that this is a large difference. However, it is conservatively estimated through discounting our expected 100% seizure rate in the control arm to 91.7% and using our observed data for the treatment arm, which under a strict protocol could be higher. Even if the vigabatrin treatment is less effective than expected and 10/24 (0.417) in the treatment arm experience seizures, we still have greater than 90% power to detect this treatment effect.

We proposed the percent difference in cognitive scores also based on our ongoing work. We have found that the distribution of Mullen Scales of early development show that by two years there is a substantial downward shift in scores (bottom right figure (red graph) below for the 24 month results). The mean at 6 months was 89.9 (sd=18.1) and mean at 24 months 75.7 (sd=22.1). The estimated standard deviation of the change is 4 points (assuming a correlation of 0.60 between the two time points as is consistent with the correlations of Bayley's over time). With the mean decline of over 14 points from 6 months to 24 months on average, with a standard deviation of 4 points, there is a great deal of potential for an intervention to achieve an effect size of 0.889 (our proposed effect size based on differences amongst disease groups on the Bayley's). This effect size would be approximately 3.5 points on the Mullen Scale. This would amount to a percent reduction in the decline of 3.5/14.2 or about 25% of the natural decline, which seems reasonable for a drug intervention. Thus, our pilot data once again provide reasonable evidence that our intervention effects are achievable.



In addition, for the cognitive outcomes, using a t-test on the final Bayley-III Cognitive Scores shows that when the sample size is 24 participants per arm there is 85% power to detect an effect size on the cognitive component of the Bayley III of 0.885 when the Type I error is 0.05, two tailed. The plausibility of achieving this effect size was assessed using published data from the Bayley III manual. In children with Pervasive Developmental Disorders, the average cognitive score is 5.7 (sd 2.9) compared to matched controls with a mean of 11.0 (sd 3.0)[31]. The effect size for the difference between this disorder and the matched controls is 1.77 [(11.0-5.7)/3.0], a difference double the effect size detectable with the sample size available for this Phase IIb trial. While it is unlikely that early treatment would return these patients to normal levels, we are hypothesizing as the alternative that we will achieve an effect size of approximately 50% of the difference compared to normal scores. That is, we will use a sample size necessary to detect an effect size of 0.885.

To further examine the ability of the sample size to achieve the goals of the study, we first examined the sensitivity of the design to our assumption of the standard deviation by examining the standard deviation against other groups of special interest.

The Bayley-III cognitive test battery shows that the standard deviations vary from a low of 1.9 at an assessment in Downs Syndrome Children to 3.5 amongst SGA infants. The table below shows the standard deviations for various special groups.

PDD	CP	DD	Language impaired	Perf At Risk	Asphyx	Fetal Alcohol	SGA	Premie
2.9	3.1	1.9	2.4	3.2	3.1	2.7	3.5	3.1

The average standard deviation is 2.9, the median is 3.1, so again, using 3.0 in our calculations seemed reasonable.

We next estimated the sensitivity of the trial in terms of the power for testing the time-averaged difference (TAD) between two means in a *repeated measures* design. In the TAD model, we assumed the mean difference starts to become evident at 3 months and is continuing or enlarging at 12, 24 and 36 months. The correlation amongst Bayley-III Cognitive assessments within person was varied from 0.10 to 0.80.

The power for this study is actually slightly higher than 85% because the analysis approach planned will make use of repeated measures analyses enabling more information to be gleaned from the data. Information and thus power is gained because partial data are used from the annual cognitive assessment measurement on those completing the trial. Thus, the sample size provides further protection against minor deviations from the assumptions used to calculate the feasibility based on the sample size.

BOSA PRELIMINARY DATA Based on the ADOS (Reference BOSA Manual version 6-25-20)

As of 7/1/2020 no BOSA data has yet been collected or analyzed. Preliminary data analyses were conducted by MAKING BETTER MEASURES Group at UCLA led by Dr. Cathy Lord.

They used binary ADOS algorithm codes scored from full standardized ADOS administrations. Based on the findings with the ADOS, it was determined that a cut-off estimate of 5 for Modules T,1 and 2 yielded the best pairing of sensitivity and specificity. It is important to note that these cut-offs are only estimates and were determined using ADOS data (not BOSA data). They may also not reflect scores from much briefer and less nuanced BOSA administration. Thus the information should only be used to inform clinical decisions when using the BOSA in combination with other sources of information. These estimates are intended to give the physicians a sense of what may come and may change as data is collected on BOSA administration.

Given the age of the participants, the PREVeNT study will be using two BOSA versions:

1) BOSA-MV which is appropriate for individuals of any age who are minimally verbal (i.e., nonverbal or use only single words/rote phrases) and includes sets of free play toys and bubbles.
2) BOSA-PSYF which is appropriate for individuals of any age who use flexible phrase speech or verbally fluent children under the age of 6-8 and includes sets of free play toys, a dollhouse with figurines and bubbles.

The sensitivities at a cut-off of 4 or 5 ranged from 0.80 to 0.93 (Module T: 0.84, Module 1: 0.93, Module 2: 0.86, Module 3: 0.84, Module 4: 0.80). Thus, clinicians should take note that similar to the ADOS-2, there will be individuals who have autism who will have scores lower than the cut-offs, particularly those who do not display repetitive behaviors during the BOSA. For this reason, it is very important to supplement the BOSA with the ADI-R caregiver interview.

The specificity at a cut-off of 4 or 5 also ranged widely (Module T: 0.76, Module1: 0.84, Module 2: 0.80, Module 3: 0.76, Module 4: 0.65), though the AUC's consistently exceeded 0.80. These low specificities mean that false positives will be common when comparing the BOSA scores to these estimated cut-offs and should be cross checked with other sources of information.

Analyses

The primary analyses for this Phase IIb trial consists of two testing hypotheses: time to onset of a first clinical seizure, documenting that the intervening variable (i.e. vigabatrin treatment) does indeed reduce or delay clinical seizures and comparison of the developmental outcomes at 24 months. While vigabatrin is used to control seizures and thus suppress them once seizure onset has been identified, it is presumed that if given prior to occurrence of clinical seizures occurring such a delay will be observed, but this is untested. Thus, the study endpoint of time to first clinical seizure is an essential step in determining the impact on developmental ability. The first step in the analyses will include demonstrating a difference between the proportion seizure free in the treatment arm versus placebo. This will be assessed at two years of follow-up using a chi square test and an intention to treat analysis. Supportive analyses will use a time to first seizure analysis using Cox regression methods to evaluate the treatment effects. Kaplan Meier analyses will provide graphical and overall test of the incidence of seizures. Cox models with age, gender, baseline number of epileptiform discharges included with treatment group will be attempted keeping in mind the relatively small sample size. Tests will use 2 sided Type I errors of 0.05.

The next key analysis to move to a go decision for Phase III will focus on the Bayley-III. While ideally the primary assessment would be made at age 5 or 6, there will be insufficient follow-up to make such an assessment in this grant period. We will perform assessment of the Bayley-III using the standardized cognitive scores over time using the constrained longitudinal data analysis (cLDA) method proposed by Liang and Zeger[32]

which assumes a common mean across treatment groups at Baseline and a different mean for each treatment at each of the post-Baseline time points (12, 24, and 36 months for those who have them). In this model, we use a response vector that includes the time 0 or the baseline measure and assessments at each post-baseline observation time point. Each time point is considered a categorical variable so that no overall linear restrictions are imposed on the means over time.

The unanticipated pandemic which has interrupted almost all studies has caused us to develop an alternative assessment during the pandemic for safety of the participants as well as providing some information on our primary endpoint if a key time point visit occurs during this time period. Conversion to approximate ADOS-2 scores will be attempted using internal and data external to PREVeNT. If such linkage is not adequate for mapping between the two methods of assessment, imputation will be done using this information as a covariate to aid in the imputation. Since it is unknown at this time, how many visits will be impacted by this study interruptions, a formal plan has not been put into place. If an exam is not one of the endpoint exams, the impact will be far less than if it is a final visit for the participant. This will be assessed and the final plan decided before breaking the blind. Before the final analysis begins, we will contact Making Better Measures and Dr. Cathy Lord regarding additional data on the BOSA measure for accessing autism.

Additional analyses will focus assessing whether the treatment effect is symptomatic or potentially a disease modifying effect assessed by a comparison of the early differences (12 months minus baseline) compared to late differences (24 month minus 12 month or 36 minus 12). Corresponding 95% confidence intervals (CIs), and nominal (unadjusted) P-values will be estimated using the cLDA model. If the contrast between the early and late differences is significant, this is consistent with a disease modifying effect.

A second consideration is the observation time during the trial. Since the patients will be put on active therapy following an event, a second consideration is the duration of seizure suppression, that is, the time after an event has occurred up until two years. This is effectively, the medication possession ratio (MPR =total time on vigabatrin treatment/total follow-up time). We expect the delayed treatment group on average to have Vigabatrin treatment for slightly less than 1-2 years and effectively 2-3 years for early treatment group. This is based on assuming a uniform distribution of occurrence of the clinical seizures. Clearly, if there were no significant differences between the early and late treatment in this parameter, we would expect a negative outcome. However, this parameter may be useful in understanding the extent of the differences observed. The MPR, if there is a relationship to the primary outcome, may be useful for imputation of missing data (which we expect to be quite low). This variable can be entered into the cLDA model and assessed using the procedures of MacKinnon for testing mediation.[33-37]The mediation model would say that the effect of the treatment on the outcome of the cognitive scores is mediated through the MPR and not the earlier intervention. The sample size may not allow a clear answer to this question, but if earlier intervention before the onset of a clinical seizure is key in preservation of cognitive function, then the mediation will not be demonstrated.

Drug resistant epilepsy: For the current study, drug resistant epilepsy is defined as the "failure of adequate trials of two tolerated appropriately chosen and used antiepileptic drug schedules (whether as monotherapy or in combination) to achieve sustained seizure freedom as put forth by the International League Against Epilepsy[31.] This will be compared between the early and delayed treatment groups using Fisher's exact test.
Additional developmental measures and ASD Risk: The sub-scores on the Bayley-III and the composite score estimated using Hotelling's T^2. The power for this endpoint will vary, but is expected to be similar to the primary. In the children with Pervasive Developmental Disorders, the effect sizes versus normal matched controls were 1.77, 1.67, 1.17 and 1.33 for the Receptive communication, Expressive Communication, Fine and gross motor sub-scores respectively. The ADOS2 will be completed at 24 and 36 months of age as a neurodevelopmental assessment for symptoms of ASD. The results from Study Arm A (seizure free, no treatment), Arm B (VGB versus. Placebo), and subjects with electrographic seizures at baseline EEG who move directly into Open Label treatment will be compared increasing our power to assess differences (Figure1).

Determination of vigabatrin safety as a preventative treatment for clinical seizures in infants with TSC will be assessed using comparison between the treatment groups and as a summary of exposure time on the drug. The outcome measure will be percentage of patients in each group (vigabatrin early treatment vs. vigabatrin delayed treatment) with treatment associated adverse events (AEs), serious adverse events (SAEs), and risk evaluation and mitigation strategy (REMS) measures. Treatment related AEs and SAEs will be monitored and tabulated. Given the expectation that the delayed group will cross-over to active treatment sooner than the early treatment group, adverse events will be characterized using life table approaches to ensure adjustment for exposure time. Thus, counts of individuals with AEs, total number of specific AEs and rate per months of follow-up will all be provided and assessed for any differences and for the safety population (early plus late on person years of exposure basis). Events of specific interest and risk evaluation and mitigation strategy (REMS) measures will be compared, including any and all ophthalmologic problems that may arise. Data from the negative control group (estimated to be approximately 15-20 patients) will also be used to compare the occurrence of AEs and SAEs.

EEG biomarker feasibility: The outcome measure will be the sensitivity and specificity of EEG epileptiform activity in predicting outcomes at 24 months for each treatment group. To confirm the feasibility of using EEG biomarkers to identify TSC infants at risk for developing epilepsy, sensitivity and specificity of EEG epileptiform activity at each time-point for predicting subsequent development of epilepsy by 24 months of age will be conducted within each treatment arm. Successful treatment in the vigabatrin arm should produce significantly poorer specificity and lower positive predictive value (PPV) by mitigating the predictive power of the EEG epileptiform activity. Exploratory analysis will further evaluate the timing of EEG change with time to seizure onset and type of EEG change with epilepsy type and treatment response as a covariate in the Cox proportional hazards assessment of time to clinical seizure. Additional analysis will evaluate the separate impact of TSC clinical features and concurrent AEDs. Our estimate is that 75% will develop the EEG biomarker prior to clinical seizures, some of the enrolled infants will

develop neither EEG biomarker nor seizures and some will develop seizures prior to being identified with the EEG biomarker or never develop the EEG biomarker despite the onset of clinical seizures. If the incidence of developing the EEG biomarker relative to the incidence of seizures without the EEG biomarker is very low, then the efficacy of treatment would have to be great to warrant the cost of monitoring. Thus, evaluation of this aim will be a tradeoff between biomarker incidences versus the treatment benefit. Descriptive statistics and numbers needed to treat (NNT) will be used to evaluate this endpoint.

Additional measures and analyses considerations: We will use descriptive statistics to quantify the percentage of patients requiring unblinded treatment after randomization and the duration of blinded treatment within the study period. We will also determine the percentage of randomized patients at 24 and 36 months of age with drug-resistant epilepsy as a clinically meaningful and significant correlate to the time to first seizure measure. As noted above, Cox regression models will be attempted with the pre-treatment covariates from the baseline and index EEG (that is, the biomarker findings that initiated the randomization) to examine if there are gradients in the predictors and levels of risk/time to events that can be predicted. These analyses will be done both within the treatment group and with the interaction of treatment and biomarkers included in the model. Since subject randomization is based on EEG findings, not seizure onset and the most common age of seizure onset is 3-7 months based on published natural history studies and the data from our P20-NS080199 study.[1] The subjects will be stratified into two groups for the analysis: 1) subjects randomized based on EEG biomarkers <12months, 2) subjects randomized based on EEG biomarker >12 months of age. There will be 2 additional groups in the analysis: subjects with normal EEGs, seizure free and never randomized and subjects with electrographic seizures at baseline, never randomized but treated with vigabatrin (the 28-30 children not randomized). Additional analysis will be done to measure the impact of concomitant AEDs used during the study.

Adverse Event Reporting

Baseline conditions at study entry will be noted and documented during screening/baseline medical history. Changes in health status and disease progression will be collected as part of interval history and will not be evaluated as adverse events. Clinical data such as medication use, seizure history, and EEG activity will be collected as part of interval medical history and recorded for the determination of disease development.

Adverse events will be defined as those relating to study procedures including neurodevelopment and EEG will be tracked on an Adverse Event log. Adverse Events will be categorized using the Common Terminology Criteria for Adverse Events (CTCAE) CTCAE version 4.0.

Each procedure related adverse event (AE) will also be graded for severity as follows:

Mild-Discomfort noticed, but no disruption of normal daily activity.
Moderate-Discomfort sufficient to reduce or affect normal daily activity.

Severe-Incapacitating, with inability to work or to perform normal activity.

Adverse Events (AEs) will also be determined to be *expected* or *unexpected* events. An *unexpected adverse event* is any adverse event occurring in subjects participating in the study, for which the nature, severity, or frequency of the event is not consistent with the foreseeable risks associated with the procedures involved in the research, as described in the study-related documents or literature regarding the procedures, or the expected natural progression of any underlying disease, disorder, or condition of the subject experiencing the adverse event.

Adverse events that require capture are to be reported per local IRB requirements and reported on a regular basis as outlined in the Manual of Procedures.

For this study, a *Serious Adverse Event* (SAE) is defined as any untoward medical occurrence that results in death; is life threatening; requires inpatient hospitalization or prolongation of an existing hospitalization; results in persistent or significant disability/incapacity; is any other untoward significant medical event, which may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require interventions to prevent one of the outcomes listed above. Also included are untoward behavioral, psychosocial or legal events which may jeopardize the subject, as determined by the investigator. SAEs at least possibly related to study participation, should be reported within 1-2 business days of the site's knowledge of the event. Each site should report related SAEs to their institution as per local and institutional requirements.

The SAEs will also be reviewed by the medical safety monitor (Dr. J. Robert Flamini) for relatedness to the study. All SAEs that are at least "possibly, probably, or definitely" related to the study will be reported to the site IRB (if applicable) and lead PIs as soon as possible. The lead PI, Dr. E. Martina Bebin, will be asked to review these *related* SAE reports within 72 hours of receipt of report.

Data and Monitoring Plan

Data management system features for project management and clinical site end users
A distributed data management system, where the clinical sites are responsible for the entry and management of data from their own center, is both cost-effective (as it removes a majority of the query process) and results in higher quality data, with continual re-education of staff. At the site level, participant data will likely be recorded first on paper forms and a web-based integrated system will be used to implement such a distributed data management system, where the system:

- *Establishes eligibility*: As the local coordinator enters critical components of the eligibility criteria, each is confirmed to ensure that participants with protocol violations are not permitted to be randomized without specific approvals that can be automated to allow for real time decision making so that participants are only minimally inconvenienced and the short time-window of eligibility is not missed. A participant ID, in the form ccc-pppp (center-participant), is automatically assigned with this initial

encounter and is used to track the participant throughout their interactions with the system. As these data (and all subsequent data) are entered, range and validity checks (including cross-form checks) to identify invalid data are performed and the coordinator is queried for correct values – thereby providing immediate communication to the clinical center to resolve inconsistencies within the data.

- *Randomizes the participant:* Participants who are candidates for this trial will be recruited using well established protocols to identify these participants. Inclusion and exclusion criteria forms will be completed prior to randomization to ensure eligibility. Informed consent and/or assent for participation will be carried out by the participant or legally authorized representative in circumstances where the potential participant is not competent to do so. Participants will be randomized using the web-based integrated data management system. Usually a permuted block randomization scheme will be employed, with stratification by clinical site and block sizes of 2, 4, and 6 (assuming 2 groups). Nevertheless, the coordinator will enter critical elements of eligibility to ensure the identification of the participant and the eligibility of the participant. After the entry of these data, the coordinator will be asked to confirm that the participant is to be irrevocably randomized, and, after confirmation, the treatment PID2 will be provided.
- *Assists in the management of participant flow in the center:* After randomization, the system updates the schedule for treatment, tests and assessments for the rest of the hospital stay or clinic visit, and future follow up contacts. The scheduling portion of the system provides real-time reports of expected treatments to be delivered and visits and participant encounters for the clinic or on the telephone. In addition, the system prints participant-specific forms for each encounter, ensuring that the correct forms (and only the correct forms) are completed.
- *Tracks drug supplies:* The system records the use of the drug in the inventory system that is jointly maintained by the DCC, trial Central Pharmacy, and the site pharmacies. The Central Pharmacy will also have access to portions of the web-based system to review and monitor the drug inventory at each site, and regular reports are produced, showing drug inventory levels and identifying sites requiring resupply.
- *Provides a platform for data entry:* Following a participant encounter, the case report forms (CRFs) are brought to any computer connecting to the internet, and are entered in a web-based system that transmits data to the database (that resides at the DCC at UAB) in real-time with immediate transfer to prevent lost data. The system tracks each CRF through its life-cycle where it is first due to be printed, partially completed, completed and “confirmed” (*i.e.*, all data fields are completed and all pass data range and validity checks), and finally locked. The system will be built upon an existing platform in place at UAB, where a matrix of rows for data items or forms and columns for participant contacts is created and is color coded, allowing the coordinator to instantly know of forms pending or overdue for specific participants. Once a form is locked, the site can review, but not change, data on the form. In order to change data, a request has to be made to the DCC to unlock the specific variables on the form. This is one place in the system where, in order to reinforce the importance of accurate form completion and locking, it has been made intentionally burdensome to the clinic.
- *Assists in ensuring data quality:* Not only does the system assist in ensuring data quality through enforcing the participant schedule with visits entered within windows,

but it also monitors and tracks other components of data quality, including missed visits and missed forms and data lag for entry (*i.e.*, overdue forms). In addition, the system generates reports on the status of data quality to the local site (at both a summary and a participant-level report), which can then be forwarded to other trial and Network committees as needed.

- *Supports the clinical center sites in the transfer of data to central reading centers (as required or needed):* Triggered when responses on the CRFs are entered into the system, the web-based system can identify, manage and track large digital files and also track these data through the reading process to ensure that the central facility provides results for all participants submitting information.
- *Supports transfers of participants from one site to another:* Based on our experience in trials, we have been able to plan our system to seamlessly allow for movement of participants from one location to another either due to participant relocation or a site closure (both have occurred in our trials). Our estimated rates of participant moves are about 5 percent, and when not planned for in advance, often cause ad hoc solutions and confusion; however, in the PREVeNT Trial the procedures will already be in place.
- *Assists in ensuring participant safety:* With each AE/SAE in the trial, a tracking system of the event is generated. When SAE CRFs are entered, the safety monitor is notified of the event through an automatically generated email as are pre-specified key individuals (the Chair or members of the DSMB, NINDS representative, appropriate DCC, etc.). Reminders are sent until the email is opened and the alert acknowledged online in the DCC web-based system. The safety monitor has access to the details of the SAE through a separate interface to the system, and as he/she codes the SAE (determining likelihood of association with trial drug, severity of the SAE, the expected/unexpected nature of the event, and the resolution of the event), the event can be closed and locked in the system. If appropriate, tracking of the event over time is also monitored to determine its resolution or continued severity, and also the duration of the process.

  Each site will use trained personnel from their institution who are independent of this trial to conduct monitoring visits twice a year. The UAB Center for Clinical and Translational Science (CCTS) group will serve as UAB site monitor. This includes both regulatory and clinical. Each site will be monitored at the site level and summary monitoring report will be emailed to UAB addressing any action items. All monitoring reports will be sent to UAB IRB.

Human Subject Protection

Compliance with Good Clinical Practice (GCP) guidelines for the conduct and monitoring of this observational study will occur through the performance of the ethical and regulatory requirements presented in ICH E6, Good Clinical Practice: Consolidated Guideline. The study (protocol, informed consent, advertisements, and subject information sheets) should be reviewed and approved by the Institutional Review Board (IRB). Any 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 review, and regulatory inspection(s) by providing direct access to source documents.

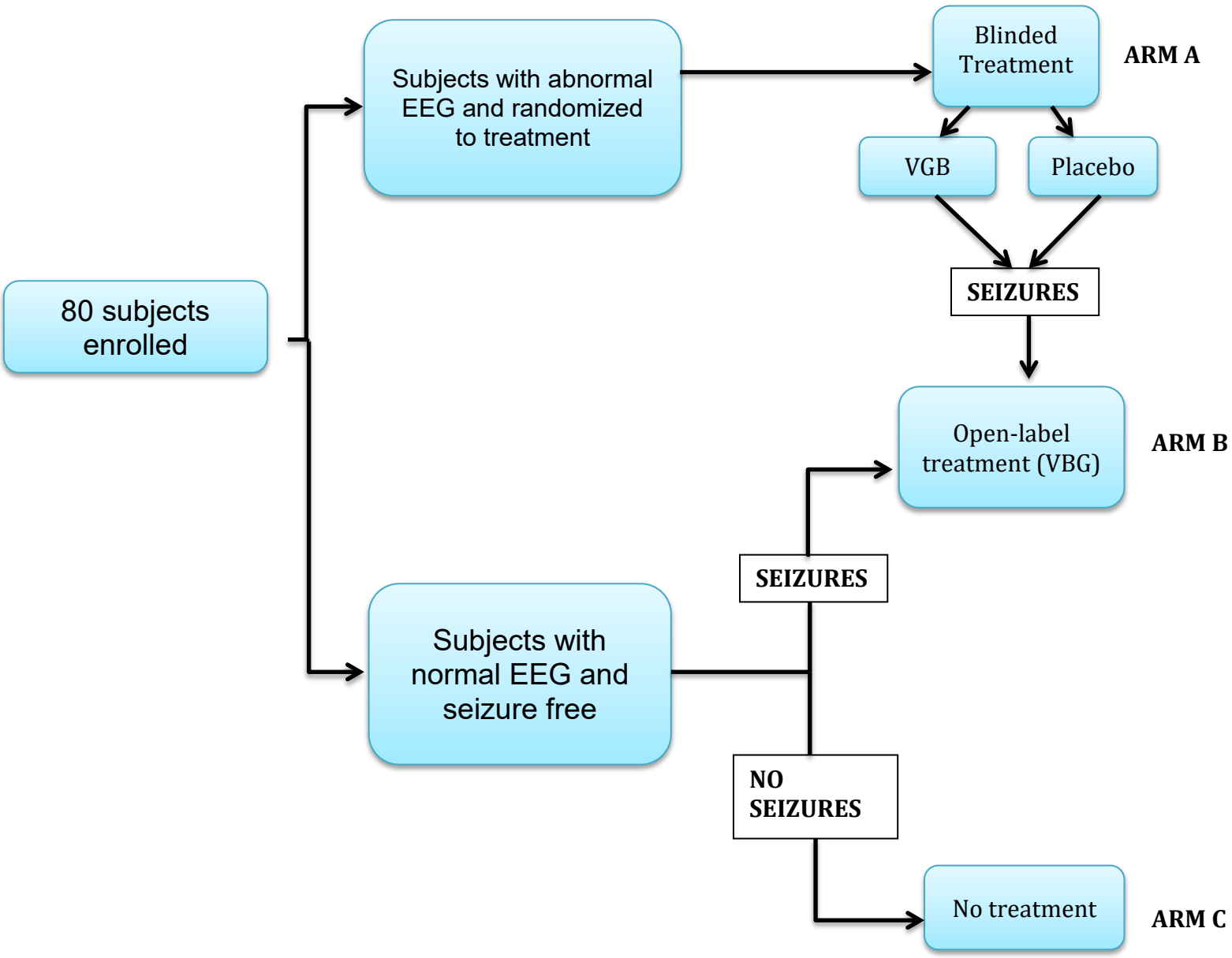
Privacy and Confidentiality

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."

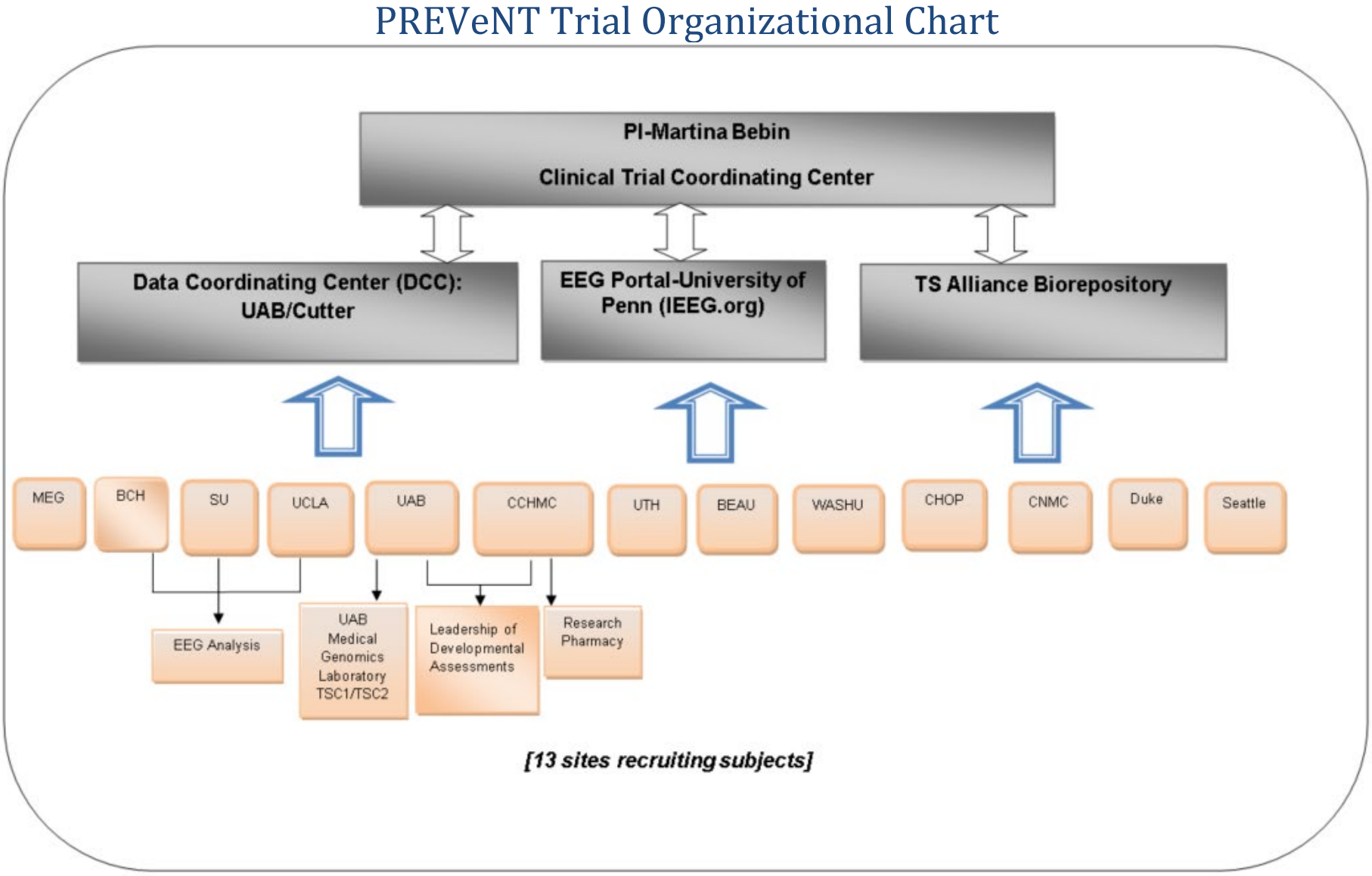
The investigator, his or her staff and associates, and the appropriate regulatory agencies may use the information included in this protocol as necessary for the conduct of the trial and the safety of subjects. The parent/legal guardian may obtain the results of clinical and procedures obtained for this research study if (1) results are available and (2) disclosure does not have the potential to impact the accuracy of future assessments of the subject during the course of the study.

Data file records that are to be shared between sites will be encrypted before sharing so that they will be undecipherable if intercepted in transit. All subject data entered into the study database will be identified only by a unique identifier for research records. Any publications will reflect only unique identifiers. 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.

Appendix 1: Figure 1: PREVeNT STUDY DESIGN



Appendix1: Figure 2- Organizational Chart



Appendix 2: Table 1-Study Visit Schedule Enrollment and Randomization to vigabatrin or placebo

Study Visits	**Screening/Baseline (1st visit)**				**Study Visits (Study Follow-up Period)**											
	~6wks (1.5m)	~12wks (3m)	~18wks (4.5m)	~24wks (6m)	~12wks (3m)	~18wks (4.5m)	~24wks (6m)	~30wks (7.5m)	~36wks (9m)	~45wks (10.5m)	~52wks (12m)	~65wks (15m)	~78wks (18m)	~91wks (21m)	~104wks (24m)	~156wks (36m)
					If 6wks is BSL, then 12wks is the second visit (FU). If 12wks is BSL, then 18wks is the second visit (FU). If 18wks is BSL, then 24wks is the second visit (FU). If 24wks is BSL, then 30wks is the second visit (FU).											
Informed consent	✓	✓	✓	✓												
Eligibility Assessment	✓	✓	✓	✓												
TSC Genotype Information	✓	✓	✓	✓												
Parent's Genetic Blood Sample	✓	✓	✓	✓												
Biomarker blood collection[2]	✓	✓	✓	✓												
Family History & Demographics	✓	✓	✓	✓												
Medical History	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Physical exam	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VEEG	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Concomitant Medications	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Eye Exam[1]	✓	✓	✓	✓												
Bayley Scales of Infant and Toddler Development (3rd edition)[3]				✓			✓				✓				✓	✓
Beery-Buktenica Developmental Test of Visual-Motor Integration (6th edition)															✓	✓
Autism Diagnostic Observation Schedule (2nd edition)															✓	✓

Study Visits	**Screening/Baseline (1st visit)**				**Study Visits (Study Follow-up Period)**											
	~6wks (1.5m)	~12wks (3m)	~18wks (4.5m)	~24wks (6m)	~12wks (3m)	~18wks (4.5m)	~24wks (6m)	~30wks (7.5m)	~36wks (9m)	~45wks (10.5m)	~52wks (12m)	~65wks (15m)	~78wks (18m)	~91wks (21m)	~104wks (24m)	~156wks (36m)
					If 6wks is BSL, then 12wks is the second visit (FU). If 12wks is BSL, then 18wks is the second visit (FU). If 18wks is BSL, then 24wks is the second visit (FU). If 24wks is BSL, then 30wks is the second visit (FU).											
Peabody Picture Vocabulary Test 94th edition)																✓
Child Behavioral Checklist															✓	✓
Vineland Adaptive Behavior Scale (2nd edition)				✓			✓				✓				✓	✓

1. Follow up eye exams will be completed within 4 weeks after randomized to vigabatrin or placebo and then every 3 months. A final eye exam will be completed after subject has been off study drug for 3-6 month as per the REMS recommendations. For those patients who come off Vigabatrin at 24 months they will have to have final eye exam between 27-30 months of age.

2. Biomarker samples will be collected at each subject's enrollment, subject randomization to vigabatrin or placebo, clinical seizures onset, and a final sample after 3 months of seizure control after randomization in Arm B or 3 months of uncontrolled seizures in the open label phase of the study.

3. Bayley III (Social-Emotional and Adaptive Behavior Questionnaire) will be completed at 6 month visit and the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) will be completed at 12, 24 and 36 months of age.

Appendix 2: Table 2- Study Visit Schedule for Subjects with Normal EEG and Seizure Free

Study Visits	**Screening/Baseline (1st visit)**				**Study Visits (Study Follow-up Period)**											
	~6wks (1.5m)	~12wks (3m)	~18wks (4.5m)	~24wks (6m)	~12wks (3m)	~18wks (4.5m)	~24wks (6m)	~30wks (7.5m)	~36wks (9m)	~45wks (10.5m)	~52wks (12m)	~65wks (15m)	~78wks (18m)	~91wks (21m)	~104wks (24m)	~156wks (36m)
					If 6wks is BSL, then 12wks is the second visit (FU). If 12wks is BSL, then 18wks is the second visit (FU). If 18wks is BSL, then 24wks is the second visit (FU). If 24wks is BSL, then 30wks is the second visit (FU).											
Informed consent	✓	✓	✓	✓												
Eligibility Assessment	✓	✓	✓	✓												
TSC Genotype Information	✓	✓	✓	✓												
Parent's Genetic Blood Sample	✓	✓	✓	✓												
Biomarker blood collection[2]	✓	✓	✓	✓												
Family History & Demographics	✓	✓	✓	✓												
Medical History	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Physical exam	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
VEEG	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Concomitant Medications	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Eye Exam[1]	✓	✓	✓	✓							✓				✓	✓
Bayley Scales of Infant and Toddler Development (3rd edition)[4]				✓			✓				✓				✓	✓
Beery-Buktenica Developmental Test of Visual-Motor Integration (6th edition)															✓	✓
Autism Diagnostic Observation Schedule (2nd edition)															✓	✓

Study Visits	**Screening/Baseline (1st visit)**				**Study Visits (Study Follow-up Period)**											
	~6wks (1.5m)	~12wks (3m)	~18wks (4.5m)	~24wks (6m)	~12wks (3m)	~18wks (4.5m)	~24wks (6m)	~30wks (7.5m)	~36wks (9m)	~45wks (10.5m)	~52wks (12m)	~65wks (15m)	~78wks (18m)	~91wks (21m)	~104wks (24m)	~156wks (36m)
					If 6wks is BSL, then 12wks is the second visit (FU). If 12wks is BSL, then 18wks is the second visit (FU). If 18wks is BSL, then 24wks is the second visit (FU). If 24wks is BSL, then 30wks is the second visit (FU).											
Peabody Picture Vocabulary Test 94th edition)																✓
Child Behavioral Checklist															✓	✓
Vineland Adaptive Behavior Scale (2nd edition)				✓			✓				✓				✓	✓

1. Follow up eye exams will be completed within 4 weeks of the initial enrollment in the study. A repeat eye exam will be done at 12 months of age, 24 months of age and 36 months of age.
2. Biomarker samples will be collected at each subject's enrollment. Those subjects who remain seizure free and have a normal EEG will have a second biomarker sample collected at 12 months of age and a final sample at 24 months of age.
3. Telephone Contact – will be done every 3 months after the 1 month of age visit until the final visit at 36 months.
4. Bayley III (Social-Emotional and Adaptive Behavior Questionnaire) will be completed at 6 month visit and the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) will be completed at 12, 24 and 36 months of age.

APPENDIX 2: Table 3- <u>COVID-related modifications for PREVeNT ASD-focused assessments beginning 7/1/2020 and going forward:</u>

Visit	BOSA & DSM5 Checklist	ADOS	ADI-R	Clinical Certainty Rating
24 months (ADOS permitted)	X	X		X
24 months (ADOS not permitted)	X			X
36 months (ADOS permitted)	X	X		X
36 months (ADOS not permitted)	X	X	X	X

- The BOSA and DSM5 checklist should be administered at every 24- and 36-month visit moving forward, even once the ADOS-2 is re-instated.
- The ADI-R is being added at the 36-month visit to aid diagnostic accuracy with the BOSA when the ADOS is not being administered.
- If no ADI research-reliable evaluator available at your site, this can be done via zoom or phone by Sarah O'Kelley (UAB) or another ADI evaluator within the PREVeNT network.
- When administering both the BOSA and the ADOS-2, the BOSA should be administered/coded by someone who is BOSA-trained but not the same person who does the ADOS-2 for that child
    - Video of the BOSA could be uploaded for an independent rater to score
    - All BOSA assessments will be video recorded to evaluate test reliability as part of the PREVeNT data analysis.
- After 7/1/2020 the BOSA and ADOS can be done at the 24 month study visit only if permitted by each individual PREVeNT site

Protocol Version 6.0 Summary of Changes

Before Amendment	After Amendment
Sites *Children's Hospital of Orange County* and *Icahn School of Medicine at Mount Sinai* were participating sites.	Removed from page 1 and Organizational Chart on page **37** *Children's Hospital of Orange County* and *Icahn School of Medicine at Mount Sinai* as participating sites – no subjects were enrolled at these sites.
	Page 4 - Modified Secondary Objectives under #2 to add During the COVID-19 Pandemic in order to minimize the risk of infection to observer and infant until the completion of the study the Brief Observation of Symptoms of Autism (BOSA) will be administered at 24- and 36-month visits. Additionally, the ADI-R will be administered at the 36-month visit. See also page 14 under Development Assessments, pages 26-27, BOSA Preliminary Data Based on the ADOS, page 28 Analyses, and Appendix 2: Table 3.
	Page 45, Added Reference #38 relating to Brief Observation of Symptoms of Autism (BOSA) Version: 6-25-20.

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