Rare Diseases Clinical Research Network (RDCRN)

A Randomized Double-Blind Controlled Trial of Everolimus in Individuals with PTEN Mutations (EverolimusXUS257T)

Developmental Synaptopathies Consortium

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The RDCRN Data Management and Coordinating Center (DMCC) designated by the National Institutes of Health

Protocol Synopsis

Interventional Synopsis

Protocol Number:	7904
Protocol Title:	A Randomized Double-Blind Controlled Trial of Everolimus in Individuals with PTEN Mutations (EVEROLIMUSXUS257T)
Study Chair:	Antonio Hardan, M.D.
Statistician:	Booil Jo, Ph.D.
Consortium:	Developmental Synaptopathies Consortium

Participating Sites:	Boston Children's Hospital Cleveland Clinic Stanford University Medical Center
Activation Date:	June 9, 2017
Current Status:	Open to Enrollment
Sample Size:	40 randomized
Target Enrollment Period:	2 Years
Study Design:	Interventional, Phase I/II, Multi-Center
Primary Study Objective:	To evaluate the safety of everolimus compared with placebo in patients with PTEN mutations
Secondary Study Objective(s):	To evaluate the efficacy of everolimus on neurocognition and behavior in patients with PTEN mutations compared to placebo as measured by standardized, direct and indirect neurocognitive tools and behavioral measures (processing speed/working memory).
Study Population and Main Eligibility/ Exclusion Criteria:	Double-Blind Inclusion Criteria 1. Male and female outpatients between 5 and 45 years of age (inclusive); 2. Pathogenic PTEN mutation confirmed by clinical genetic testing; 3. Participant must be able to complete one of the following three standardized assessments: Conners' Continuous Performance Tasks (CPT-3-mean reaction time), Stanford Binet (SB-5; working memory), or the Purdue Pegboard Test; 4. Performance below the age-adjusted population mean on at least one of the above standardized measure: attention (CPT-3, mean reaction time), working memory (SB5), or fine motor skills (Purdue Pegboard Test; either dominant hand, non-dominant hand, or both hands); 5. Adequate bone marrow function as shown by: a. platelets ≥ 80,000/mm^3 b. absolute neutrophil count ≥ 1,000/mm^3 c. hemoglobin ≥ 9 g/dL 6. Adequate liver function as shown by: a. Total serum bilirubin < 1.5 x ULN b. AST and ALT levels < 2.5 x ULN c. INR ≤ 2 7. Adequate renal function: serum creatinine < 1.5 x ULN, 8. Signed informed consent obtained prior to any screening procedures; 9. Individuals on psychotropic and anti-epileptic medications should maintain a stable dose for at least 2 months prior to the screening visit; 10. Negative serum pregnancy test for females at screening and no plans to become pregnant or conceive a child while participating in the study. The effects of mTOR inhibitors on the developing fetus at the doses used in this study are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception prior to study entry and for the duration of the study. Estrogen-containing oral contraceptives are not recommended in women enrolled in this study. Abstinence or two effective non-estrogen or barrier methods of contraception (such as condoms + spermicidal foam) must be used; 11. No anticipated changes in the frequency and intensity of existing interventions such as behavioral and developmental treatments, in home services, and speech therapy;

	12. No planned changes in school placement; 13. For individuals under 18 or who are otherwise incapable, there must be an available caregiver who can reliably bring subject to clinic visits and provide trustworthy data 14. Able to communicate fluently in English Double-Blind Exclusion Criteria 15. Patients currently receiving anticancer therapies or who have received anticancer therapies within 4 weeks of the start of Everolimus (including chemotherapy, radiation therapy, antibody based therapy, etc.); 16. Known intolerance or hypersensitivity to Everolimus or other rapamycin analogs (e.g. sirolimus, temsirolimus); 17. Known impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of oral Everolimus; 18. Uncontrolled diabetes mellitus as defined by HbA1c >8% despite adequate therapy. Patients with a known history of impaired fasting glucose or diabetes mellitus (DM) may be included, however blood glucose and antidiabetic treatment must be monitored closely throughout the trial and adjusted as necessary; 19. Patient with uncontrolled hyperlipidemia: fasting serum cholesterol > 300 mg/dL OR >7.75 mmol/L AND fasting triglycerides > 2.5 x ULN. 20. Patients who have any severe and/or uncontrolled medical or psychiatric conditions (see section 4.6 for additional details) 21. Chronic treatment with corticosteroids or other immunosuppressive agents. Topical or inhaled corticosteroids are allowed; 22. Known history of or seropositivity for Hepatitis B, Hepatitis C, or HIV; 23. Patients who have received live attenuated vaccines within 1 week of start of Everolimus and during the study. Patient should also avoid close contact with others who have received live attenuated vaccines. Examples of live attenuated vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines; 24. Patients who have a history of another primary malignancy, with the exceptions of: a. non-melanoma skin cancer, b. and carcinoma in situ of the cervix, uteri, or breast from which the patient has been disease free for ≥3 years; 25. Planned changes to concomitant medications; 26. Prior or concomitant therapy with known or possible anti-mTOR activity, including rapamycin (sirolimus); 27. Concomitant therapy with strong inhibitor (e.g., cyclosporine and ketoconazole) or inducer of CYP3A; 28. Active infection at time of enrollment; 29. Patients with a history of non-compliance to medical regimens or who are considered potentially unreliable or will not be able to complete the entire study; 30. Patients who are currently part of or have participated in any clinical investigation with an investigational drug within 1 month prior to dosing;

	31. Pregnant or nursing (lactating) women; 32. Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant, must use highly effective methods of contraception during the study and 8 weeks after. Highly effective contraception methods include: a. A combination of any two of the following: 33. Use of oral, injected or implanted hormonal non-estrogen containing methods of contraception or; 34. Placement of an intrauterine device (IUD) or intrauterine system (IUS); 35. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository; 36. Total abstinence or; 37. Male/female sterilization. Women are considered post-menopausal and not of child-bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks prior to randomization. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child-bearing potential. 38. Male patients whose sexual partner(s) are WOCBP who are not willing to use adequate contraception, during the study and for 8 weeks after the end of treatment 39. Major surgery, radiation therapy, or stereotactic radio-surgery within previous 4 weeks at time of screening 40. Neurosurgery within prior 6 months at time of screening. Open-Label Inclusion Criteria 41. Patients who completed Double-Blind phase of the study and were assigned to the placebo treatment arm; 42. Verbal consent (and assent, as appropriate) obtained prior to any open-label phase study procedures.
Treatment	
Agent	Everolimus
Dosage, schedule, route of administration-	4.5mg/m^2/day, to the nearest 2.5mg, orally
Safety Issues-	The safety profile is characterized by manageable adverse events (AEs). These AEs are generally reversible and non-cumulative. Adverse events most frequently observed with everolimus are stomatitis, mouth ulcers, acne, infection, and menstrual irregularities. Adverse events that are also common include: rash, diarrhea, fatigue, asthenia, nausea, peripheral edema, decreased appetite, headache, dysgeusia, epistaxis, mucosal inflammation, pneumonitis, weight decreased, vomiting, pruritus, cough, dyspnea, dry skin, nail disorder, aggression, and pyrexia. Overall, the most frequently observed laboratory abnormalities include: decreased hematology parameters including hemoglobin,

	lymphocytes, platelets, and neutrophils; increased clinical chemistry parameters including cholesterol, triglycerides, glucose, aspartate transaminases, creatinine, alanine transaminases, and bilirubin; and decreased clinical chemistry parameters including phosphate and potassium. The majority of these AEs have been of mild to moderate severity (NCI CTC grade 1-2). Recommendations for dose adjustments, should any of these treatment related adverse events occur, are given in Table 3.
Primary Outcome Measures:	The primary endpoint will be study drop-out rate due to side effects, comparing everolimus vs. placebo. We will also determine the frequency of adverse events by type and severity. We predict that the rate of adverse events will be no more than 10% higher in the everolimus group compared to placebo but overall severity will be minimal.
Secondary Outcome Measures:	The main efficacy endpoint will be a neurocognitive composite. This composite will be computed in two ways: 1) an average for working memory (SB-5 working memory), processing speed (CPT mean reaction time) and fine motor (Purdue Pegboard-average of both hands) subtests, and 2) the same average as above excepted weighted 2/3 and an additional average based on all other available neurocognitive testing measures (receptive and expressive language, non-verbal ability, verbal learning, sustained attention, impulsivity, and visuomotor skills, etc.) weighted 1/3. Additional efficacy endpoints will examine the effect of everolimus on overall global clinical improvement, autism symptoms, other behavior problems, and adaptive abilities as measured by validated, standardized instruments including the Clinical Global Impressions-Improvement scale, Autism Diagnostic Observation Schedule – Second Edition (ADOS-2 calibrated severity score), Social Responsiveness Scale-Second Edition (SRS-2), Adult/Child Behavior Checklist (ABCL/CBCL), Wide Range Assessment of Memory and Learning-2 (WRAM-L2) and Vineland Adaptive Behavior Scales, 3rd Edition (VABS-III).
Statistical Considerations (sample size and analysis plan):	See data analysis section of protocol
Sponsors (federal, state, foundation and industry support):	National Institutes of Health; Novartis Pharmaceuticals; PTEN Research Foundation

1. Introduction

This is a Phase I/II 6-month, randomized, double-blind placebo-controlled trial of everolimus in patients, ages 5 to 45 years (inclusive) with a PTEN mutation, with safety and neurocognition as the primary endpoints.

Germline heterozygous phosphatase and tensin homolog (PTEN) gene mutations are associated with a spectrum of clinical disorders characterized by neurocognitive deficits, autism symptomatology, skin lesions, macrocephaly, hamartomatous overgrowth of tissues, and an increased risk of cancers. The neurocognitive and neurobehavioral deficits are associated with very high morbidity, health care cost, and impact on the quality of life of individuals with PTEN and their families. Currently, there are no approved agents for the treatment of these neurocognitive and neurodevelopmental deficits in PTEN.

This protocol addresses a key area of interest: Pharmacological treatments of a well-defined subgroup with autism spectrum disorder. It will leverage an existing investigation that aims at examining the natural history study of individuals with autism and germline heterozygous PTEN mutations (BCH IRB-P00013150: Natural History Study of Individuals with Autism and Germline Heterozygous PTEN Mutations). The purpose of this

phase II pilot study will be to establish short-term safety of everolimus treatment in individuals with germline PTEN mutations and evaluate associated cognitive and behavioral changes in this population to generate plausible hypotheses to be tested in a future Phase III confirmatory trial. This protocol also mirrors a previous clinical trial utilizing everolimus in the tuberous sclerosis population (BCH 10-06-0247 Randomized Double-Blind Phase 2 Trial of RAD001 for Neurocognition in Individuals with Tuberous Sclerosis Complex).

In this investigation, we propose a 6-month randomized, double-blind, multi-site study to evaluate the safety and efficacy of a rapamycin analogue, everolimus, to treat neurocognitive and social deficits in a total of 60 patients (age range 5-45 years) with a PTEN mutation with a maximum of 40 participants randomized to treatment. By maintaining a lower age limit of 5 years, we can ensure a more accurate assessment of cognitive and behavioral outcomes, streamline the neurobehavioral assessment battery, and with minimal loss to outcome measure scope and sensitivity, similar to the strategy we employed in a previous everolimus trial in tuberous-sclerosis complex (TSC) (NCT01289912), performed by PI Mustafa Sahin, M.D., Ph.D.

This investigation involves 3 participating sites (Stanford University, site PI: Antonio Hardan, M.D.; Cleveland Clinic, site PI: Rabi Hanna, M.D.; and Boston Children's Hospital, site PI: Mustafa Sahin, M.D., Ph.D.).

2. Objectives and Endpoints

2.1 Primary objective

To evaluate the safety of everolimus compared with placebo in patients with PTEN mutations focusing on NCI CTCAE Grade 3 and 4 adverse events, serious adverse events, and Grade 3 and 4 laboratory toxicities. The primary endpoint will be study drop-out rate due to side effects, comparing everolimus vs. placebo. Based on the published literature and our experience in prior TSC trials we hypothesize that the drop-out rate due to side effects in those receiving everolimus will be similar to those in the placebo group with minimal effect size (< 10% difference). We will also determine the frequency of adverse events by type and severity. We predict that the rate of adverse events will be no more than 10% higher in the everolimus group compared to placebo, but overall severity will be minimal.

2.2 Secondary objective

To evaluate the efficacy of everolimus on neurocognition and behavior in individuals with PTEN mutations compared to placebo as measured by standardized, direct and indirect neurocognitive tools and behavioral measures. The main efficacy endpoint will be a composite index score of neurocognitive function that will be computed in two ways. The first will be an average of measures evaluating working memory (Stanford-Binet Intelligence Scales, Fifth Edition; SB-5 working memory subscale), processing speed (Conners' Continuous Performance Test, Third Edition; CPT-3 mean reaction time), and fine motor skills (Purdue Pegboard Test average of both hands). The second will include the above average weighted by two-thirds and an average of the remaining standardized, norm-referenced neurocognitive measures (e.g., non-verbal ability, visuomotor skills, verbal learning, receptive and expressive language) weighted by one-third. We hypothesize that individuals receiving everolimus will show more improvement, relative to those taking placebo, in the composite index.

Additional efficacy endpoints will examine the effect of everolimus on overall global clinical improvement, autism symptoms, other behavior problems, and adaptive abilities as measured by validated, standardized instruments including the Clinical Global Impressions-Improvement scale, Autism Diagnostic Observation Schedule – Second Edition (ADOS-2 calibrated severity score), Social Responsiveness Scale - Second Edition (SRS-2), Adult/Child Behavior Checklist (CBCL ACF/CBCL), Wide Range Assessment of Memory and Learning, Second Edition (WRAML-2), and Vineland Adaptive Behavior Scales, Third Edition (VABS-III). We will also assess the effect of everolimus treatment on eye tracking tasks, resting state EEG, and task related EEG through auditory evoked potentials (AEP).

2.3 Exploratory objective

To determine if PTEN-associated pathway molecules (PI3K/AKT, mTOR, MAPK), RNA levels, protein levels, and differences in the gut microbiome and mycobiome are affected by everolimus treatment and correlate with clinical improvements. For PTEN biochemistry, the primary endpoint will be relative change in total and

phosphorylated protein levels following treatment when compared to baseline in isolated peripheral blood mononuclear cells (PBMCs), which will then be correlated with everolimus levels. Other endpoints will be derived from each method and correlative studies will assess whether adverse events or neurocognitive changes are associated with a particular RNA, protein, microbiome or mycobiome profile or everolimus levels. The comprehensive analysis of these objectives will depend on the availability of additional funds, and at this time, samples will be collected and stored as per protocol.

3. Background

3.1 Overview of Germline Heterozygous PTEN Gene Mutations

Phosphatase and tensin homolog (PTEN) gene germline mutations are associated with a spectrum of clinical disorders characterized by neurocognitive deficits, intellectual disability, autism symptomatology, skin lesions, macrocephaly, hamartomatous overgrowth of tissues, and an increased risk of cancers.[1-11] In humans, PTEN-related research has historically focused on physical manifestations of the disease and the pathophysiology and treatment of the hamartomatous lesions that arise in affected patients and their predisposition for malignancy. In contrast, there is much less research to date focused on the behavioral and cognitive features, and their treatment.[2,4,8] Currently no effective therapies for individuals with germline PTEN mutations exist to target neurocognitive and social deficits, and effective interventions that target the core biologic alterations are crucial to optimize the short and long-term outcome of individuals affected with mutations in this gene.

PTEN is a dual specificity phosphatase that inhibits both the PI3K/AKT/mTOR and MAPK pathways, which, in turn, inhibit neural cell growth, proliferation, and synaptic function.[1,2] Historically, research efforts have focused on examining the physical manifestations resulting from these mutations, but there has been little interest in the behavioral and cognitive features and their treatment. In fact, the cancer risk is managed by high risk surveillance and early detection or prophylactic surgery and there is currently no effective treatment for the severe neurocognitive and social/communication deficits observed in PTEN mutation.[3,4]

Pervasive deficits in children with PTEN mutation include impairments in social development, cognitive functioning, and communication.[5] Active research is ongoing to identify disease-specific pharmacological treatment and medications, such as everolimus, are promising to target the core deficits observed in this disorder. Everolimus is an mTOR inhibitor that has been examined in different medical conditions including tuberous sclerosis. Because it may address underlying physiological abnormalities, everolimus may also improve core features of individuals with PTEN mutations and inform future research on pathophysiology of this disorder.

In patients with PTEN mutation, the mTOR pathway is abnormally upregulated and interferes with normal cell growth, proliferation, and function. Much is known regarding upstream and downstream regulation of mTOR. There are two structurally and functionally distinct multi-protein signaling complexes, mTORC1 (mTOR complex 1, rapamycin sensitive)31 and mTORC2 (mTOR complex 2, rapamycin insensitive). mTORC1 is mainly activated via the PI3 kinase pathway through AKT (also known as PKB, protein kinase B) and the tuberous sclerosis complex (TSC1/TSC2). Activated AKT phosphorylates TSC2, which leads to the dissociation of TSC1/TSC2 complex, thus inhibiting the ability of TSC2 to act as a GTPase activating protein. This allows Rheb, a small G-protein, to remain in a GTP bound state and to activate mTORC1. AKT can also activate mTORC1 by PRAS40 phosphorylation, thereby relieving the PRAS40-mediated inhibition of mTORC1.32,33 mTORC2 (mTOR complex 2) is activated through a currently unknown mechanism, possibly by receptor tyrosine kinase (RTK) signaling. It has been suggested that mTORC2 phosphorylates and activates a different pool of AKT that is not upstream of mTORC1. PHLPP phosphatase plays the role of a negative regulator. mTORC2 is rapamycin insensitive and is required for the organization of the actin cytoskeleton.31 Like TSC, PTEN deficiency is an attractive target for treatment with mTOR inhibitors due the PTEN's role through AKT to regulate specifically mTORC1, which is sensitive to rapamycin and analogs such as everolimus.

PTEN regulates a varied set of intracellular processes.[2,16-19] It is hypothesized that when dysfunctional, PTEN protein interacts with the protein of a second gene known as Tp53 to dampen energy production in neurons. This severe stress leads to a spike in harmful mitochondrial DNA changes and abnormal levels of energy production in the cerebellum and hippocampus, brain regions critical for social behavior and cognition. In addition, PTEN interaction with p53 triggers deficiencies in additional proteins linked to learning disabilities, including autism. Multiple lines of evidence support its role in cognitive development and in social behavior.[5,8,9,13]

While PTEN-associated pathways and their impact of cell growth and proliferation have been well described in the context of cancer,[6-8] much less attention has been given to understanding the role of PTEN in neurocognition,[5,9,10] It is not yet known which pathway components are most disrupted in human PTEN to cause neuronal synaptic dysfunction[8,11-13] and the associated autism spectrum disorder (ASD) and intellectual disability (ID) common with PTEN loss-of-function.[14,15]

3.2 Preclinical Studies of Neurocognition in PTEN Gene Mutations

Multiple murine CNS-conditional knock-out models have established the role of PTEN in learning and control of social behavior.[12,13] Using a germline model, female PTEN heterozygous animals exhibited decreased social behavior.[20] More recently, a germline model which results in inappropriate PTEN subcellular localization showed inappropriate social behavior and clumsiness, reminiscent of a subset of PTEN mutation positive ASD patients.[21-24] Additionally, PTEN loss in mature neurons have led to diminished social behavior, an effect replicated in a model of PTEN loss in neuronal precursors.[25,26] ENREF_5 The *Nse-cre x Pten*$^{loxP/loxP}$ model also shows decreased recognition learning and increased anxiety.[27] Interestingly, inhibition of mTOR complex 1, a downstream target of AKT signaling, improved social behavior in this model.[26]

3.3 Clinical Studies of Neurocognition in PTEN Gene Mutations

In humans, PTEN-related research has historically focused on physical manifestations of the disease and the pathophysiology and treatment of the hamartomatous lesions that arise in affected patients and their predisposition for malignancy. In contrast, there is much less research to date focused on the behavioral and cognitive features, and their treatment.[5,9,28] Currently no effective therapies for individuals with germline PTEN mutations exist to target neurocognitive and behavioral deficits, and effective interventions that target the core biologic alterations are crucial to optimize the short and long-term outcome of individuals affected with mutations in this gene.

3.4 Overview of Everolimus

3.4.1 Everolimus

Everolimus is a novel derivative of rapamycin. Everolimus has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation. In 2003, everolimus was approved in Europe (trade name: Certican®) via the Mutual Recognition Procedure (MRP) for the prevention of organ rejection in patients with renal and cardiac transplantation. Certican® is also approved in Australia, South Africa, the Middle East, Central and South America, the Caribbean and some Asian countries.

Everolimus 2.5mg, 5mg, 7.5mg, and 10mg tablets were approved under the trade name Afinitor® for patients with advanced renal cell carcinoma (RCC) after failure of treatment with Sutent® (sunitinib) or Nexavar® (sorafenib) in the US, EU, and several other countries and is undergoing registration in other regions worldwide.

In 2010, Afinitor® received United States (US) approval for patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC). Everolimus is also available as Votubia® in the European Union (EU) for patients with SEGA associated with TSC who require therapeutic intervention but are not candidates for curative surgical resection. Afinitor® was approved for “progressive pancreatic neuroendocrine tumor (PNET) in patients with unresectable, locally advanced, or metastatic disease” in 2011 in various countries, including the US and Europe. In 2012 Afinitor® received approval for the treatment of postmenopausal women with advanced hormone receptor-positive, HER2- negative breast cancer (advanced HR+ BC) in combination with exemestane, after failure of treatment with letrozole or anastrozole. Furthermore, in 2012, Afinitor® received approval for the treatment of adult patients with TSC who have renal angiomyolipoma not requiring immediate surgery. AFINITOR® DISEPERZ was approved in 2018 (April 10th) for adjunctive treatment of adult and pediatric patients aged 2 year and older with TSC-associated partial-onset seizures.

Everolimus is also approved to treat hamartomatous lesions in tuberous sclerosis complex (TSC), a genetic disorder with synaptic disruption and cognitive and behavioral features similar to those of PTEN. At the cellular and molecular level, everolimus acts as a signal transduction inhibitor. Everolimus binds to FKB12 to selectively

inhibit mTOR, a key and a highly conservative serine-threonine kinase. Everolimus selectively inhibits mTOR (mammalian target of rapamycin), specifically targeting the mTOR-raptor signal transduction complex. mTOR is a key serine-threonine kinase in the PI3K/AKT signaling cascade, which is known to be dysregulated in a wide spectrum of human cancers.[10]mTOR is present in all cells and is a central regulator of protein synthesis and ultimately cell growth, cell proliferation, angiogenesis and cell survival. mTOR is currently the only known target of everolimus.[29]

3.4.2 The role of mTOR pathway in tumorigenesis

The target of everolimus is mTOR (mammalian target of rapamycin), a serine-threonine kinase implicated in the PI3K/AKT pathway known to be active in numerous neoplastic conditions. Driven largely by growth factors acting upstream, the PI3K/AKT/mTOR pathway modifies downstream signaling events involved in the regulation of cell-cycling, cell growth and cell survival mechanisms.

An important aspect of the antitumor effect of everolimus is its potential to act on both tumor cells directly (to inhibit growth) and indirectly (by inhibiting angiogenesis). The observation of *in vivo* sensitivity of xenografts comprised of cells demonstrating resistance to everolimus *in vitro* is attributed to the drug's potential to act on the vascular component of the supporting peritumoral stroma. The anti-angiogenic property of everolimus has been confirmed through experiments demonstrating the effect of everolimus in countering VEGF-induced proliferation of human umbilical vein endothelial cells (HUVECs) *in vitro*, VEGF-driven angiogenesis in a chamber implant murine model and neovascularization in a murine orthotopic melanoma model.

At the cellular and molecular level, everolimus acts as a signal transduction inhibitor. The target of everolimus is mTOR (mammalian target of rapamycin), a serine-threonine kinase which is a member of the larger PI3K (phosphatidylinositol 3-kinase) family and present in all cells. Everolimus selectively inhibits mTOR, which regulates cell growth, proliferation and survival. The mTOR kinase is mainly activated via the phosphatidylinositol 3-kinase (PI3K) pathway through AKT/PKB and the tuberous sclerosis complex (TSC1/2). Mutations in these components or in PTEN, a negative regulator of PI3 kinase, may result in their dysregulation. Abnormal functioning of various components of the signaling pathways contributes to the pathophysiology of numerous human cancers. Various preclinical models have confirmed the role of this pathway in tumor development.

The main known functions of mTOR include:[30]

- mTOR functions as a sensor of mitogens, growth factors, energy and nutrient levels, facilitating cell-cycle progression from G1 - S phase in appropriate growth conditions.
- The PI3K (mTOR) pathway itself is frequently deregulated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors.
- The mTOR pathway is involved in the production of pro-angiogenic factors (e.g. VEGF) and in endothelial cell growth and proliferation.
- Through inactivating eukaryotic initiation factor 4E binding proteins and activating the 40S ribosomal S6 kinases (e.g. p70S6K1), mTOR regulates protein translation.

The regulation of mTOR signaling is complex and involves positive regulators, such as AKT, that phosphorylate and inactivate negative regulators such as the Tuberous Sclerosis Complex (TSC1/TSC2).

The PI3K/AKT/mTOR pathway is known to be dysregulated in numerous proliferative disorders including cancer. Molecular epidemiological studies have also shown that activation of the PI3K/AKT/mTOR pathway is frequently associated with worsening prognosis through resistance to treatment, disease extension and disease progression. A variety of preclinical models have confirmed the role of this pathway in tumor development. It has also been demonstrated that constitutional activation of kinases such as AKT can lead to inexorable development of cancers resembling those which in patients are characterized by frequent activation of the same kinases. This is complemented by the demonstration of the antitumor activity of kinase inhibitors acting on the pathway in *vitro* and *in vivo* preclinical models.

Table 1 Drug substance

Chemical name:	(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-

	methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.0^{4,9}]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone
International non-proprietary name	Everolimus

3.4.3 Preclinical Studies of Everolimus

Everolimus inhibits the proliferation of a range of human tumor cell lines *in vitro* including lines originating from lung, breast, prostate, colon, melanoma and glioblastoma. Everolimus also inhibits the proliferation of human umbilical vein endothelial cells (HUVECs) *in vitro*, with particular potency against VEGF-induced proliferation suggesting that everolimus may also act as an anti-angiogenic agent. The anti-angiogenic activity of everolimus was confirmed *in vivo*. Everolimus selectively inhibited VEGF-dependent angiogenic response at well-tolerated doses. Mice with primary and metastatic tumors treated with everolimus showed a significant reduction in blood vessel density when compared to controls.

Everolimus administered daily by mouth was a potent inhibitor of tumor growth, at well tolerated doses, in 11 different mouse xenograft models (including pancreatic, colon, epidermoid, lung and melanoma) and two syngeneic models (rat pancreatic, mouse orthotopic melanoma). These models included tumor lines considered sensitive and "relatively resistant" *in vitro*. In general, everolimus was better tolerated in mouse xenograft models than standard cytotoxic agents (i.e., doxorubicin and 5-fluorouracil), while possessing similar anti-tumor activity. Additionally, activity in a VEGF-impregnated subcutaneous implant model of angiogenesis and reduced vascularity (vessel density) of everolimus-treated tumors (murine melanoma) provided evidence of *in vivo* effects of angiogenesis.

All significant adverse events observed in toxicology studies with everolimus in mice, rats, monkeys and mini-pigs were consistent with its anticipated pharmacological action as an anti-proliferative and immunosuppressant, and were at least in part reversible after a 2- or 4-week recovery period with the exception of the changes in male reproductive organs, most notably testes.

In the published article, *Pharmacological Inhibition of mTORC1 Suppresses Anatomical, Cellular, and Behavioral Abnormalities in Neural-Specific Pten Knock-Out Mice*, Zhou et al. made a PTEN KO mouse that has neurological deficits. They treated the mice either at a presymptomatic stage (5–6 weeks old) or a symptomatic stage (adult 10–12 weeks old). They showed that rapamycin can rescue macrocephaly even in the adult group. Furthermore, rapamycin was able to significantly reverse neuronal hypertrophy in older mice, resulting in restoration of much of the hippocampal structure.

Another article published by Nguyen and Anderson, *mTOR-dependent alterations of Kv1.1 subunit expression in the neuronal subset-specific Pten knockout mouse model of cortical dysplasia with epilepsy*, showed that PTEN loss alters Potassium channel expression in the in the hippocampus. They demonstrate that mTOR inhibition with rapamycin treatment at early (4-5 weeks) and late (9-10 weeks) stages of the pathology normalized Kv1.1 protein levels in NS-Pten KO mice to WT levels

3.4.4 Brain Penetration

Everolimus penetration into brain tissue has been evaluated in mice and rats. Non-tumor bearing BALB/c mice were administered everolimus once at 5 mg/kg orally or 1 mg/kg intravenously, and blood and tissues were obtained at times after drug administration. The extent of penetration (AUC_{brain}/AUC_{blood}) was 1.8% and 5.2% after oral and intravenous administration. Despite the relatively low penetration, everolimus brain tissue concentrations (69 ng/g after oral; 8 ng/g after intravenous) are within range of the IC_{50} values for PTEN-/- glioblastomas for at least 9 hours and above the IC_{50} values for PTEN+/+ mice for at least 2 hours.[34,35]

In Wistar rats, the brain everolimus distribution was related to drug dose and time after intravenous drug administration. At everolimus dosages up to 1 mg/kg, the blood and brain tissue concentrations increased linearly; thereafter, a nonlinear increase was noted in both. The higher everolimus brain tissue concentrations at higher intravenous dosages are consistent with a saturation of an efflux pump present in brain capillary endothelial cells. The kinetics of everolimus brain uptake were established by measuring blood and brain tissue

concentrations of ^{3}H everolimus at various times after an intravenous bolus dosage. Everolimus was rapidly distributed in the brain with slow efflux. At 168 hours after the dose, significant brain levels of 6 ng/g were detected, without any significant blood levels. Although up to 24 hours no metabolites are noted in the brain, at 168 hours they represent 60% of total drug in the brain.[34]

Although everolimus penetration into the brain is low, after a 1.0 mg intravenous dose of everolimus in Wistar rats, brain tissue concentrations exceeded the in vitro antiproliferative IC_{50} for HUVEC cells and a panel of PTEN-/- glioblastoma cell lines for 168 hours, and selected PTEN+/+ cell lines for 24 hours.[35,36]

3.4.5 Everolimus Pharmacokinetics

The pharmacokinetic characteristics of everolimus have been extensively investigated in the context of the drug's development as an immunosuppressant in solid organ transplantation where everolimus was administered twice daily as a part of an immunosuppressant multi-drug regimen consistently including cyclosporin A and glucocorticoids. Recent Phase I studies provide steady-state pharmacokinetics for both the weekly and daily schedules at varying dose levels in patients with advanced cancers.

Everolimus is rapidly absorbed after oral administration, with a median time to peak blood levels (t_{max}) of 1-2 hours post dose. The extent of absorption is estimated to be above 11%. The area under the blood concentration-time curve (AUC) is dose-proportional over the dose range tested while maximum blood concentration C_{2h} appears to plateau at dose levels higher than 20 mg. The terminal half-life in cancer patients averaged 30 hours, which is similar to that in healthy subjects. Inter-patient variability is moderate with the coefficient of variation (CV) of approximately 50%. A high-fat meal altered the absorption of everolimus with 1.3-hour delay in $t_{max,}$ a 60% reduction in C_{2h} and a 16% reduction in AUC. In whole blood, approximately 80% of everolimus is contained in red blood cells. Of the fraction of drug contained in plasma, 74% is protein-bound. The apparent distribution volume (Vz/F) after a single dose was 4.7 L/kg. Everolimus is eliminated by metabolism, mainly by hydroxylation, then excreted into the feces >80%.

Everolimus is mainly metabolized by CYP3A4 in the liver and to some extent in the intestinal wall. Everolimus is also a substrate of P-glycoprotein (P-gp). Therefore, absorption and subsequent elimination of systematically absorbed everolimus may be influenced by medicinal products that interact with CYP3A4 and/or P-glycoprotein. *In vitro* studies showed that everolimus is a competitive inhibitor of CYP3A4 and of CYP2D6 substrates, potentially increasing the concentrations of medicinal products eliminated by these enzymes. In two phase III clinical trials in patients following kidney transplantation, strong inhibitors of CYP3A4 (azoles, antifungals, cyclosporine, erythromycin) have been shown to reduce the clearance of everolimus therapy thereby increasing everolimus blood levels. Similarly, Rifampin, a strong inducer of CYP3A4, increases the clearance of everolimus thereby reducing everolimus blood levels. Caution should be exercised when co-administering everolimus with CYP3A4 inhibitors or inducers.

Patients with PTEN may be treated with P450 enzyme-inducing antiepileptic drugs (EIAEDs) which lead to an increase of everolimus apparent clearance. Commonly used EIAEDs which are used in this population include:

1. Phenytoin (Dylantin®, Dilantin Kapseals®, Dilantin® Infatabs®, Eptoin®, Epanutin®, Diphenin®, Dipheninum®, Phenytek®),
2. Mephenytoin (Mesantoin®),
3. Carbamazepine (Tegretol®, Biston®, Calepsin®, Carbatrol®, Epitol®, Equetro®, Finlepsin®, Sirtal®, Stazepine®, Telesmin®, Teril®, Timonil®, Trimonil®, Epimaz®, and Degranol®),
4. Phenobarbital (Luminal®),
5. Pentobarbital (Nembutal®),
6. Primidone (Mysoline®),
7. Oxycarbazepine (Trileptal®).

Patients receiving EIAEDs show decreased plasma levels of several medications when administered at conventional doses. This may, in turn, lead to ineffective dosing.

Everolimus pharmacokinetics in transplant patients were investigated in special populations such as subjects with hepatic or renal impairment, various ethnic groups and pediatric renal transplant patients. In subjects with

mild or moderate hepatic impairment, mean AUC to everolimus is increased 2-fold whilst renal impairment does not affect the pharmacokinetics of everolimus. Age, weight (both over the adult range) and gender do not affect the pharmacokinetics of everolimus to a clinically relevant extent. Also, pharmacokinetics does not alter in Asian patients whereas black patients have 21% higher clearance compared to non-blacks. A single, escalating-dose study in Japanese subjects did not show a significant difference in dose normalized systemic exposure.

The pharmacokinetic parameters derived for everolimus given daily are summarized in Table 2.

Table 2 Steady-state everolimus pharmacokinetics (daily dosing)

Parameter	5 mg	10 mg
N	4	6
t_{max} (h)	1 (1)	1 (1-6)
C_{min}^{ss} (ng/mL)	5.4 ± 1.8	13.2 ± 7.9
C_{max}^{ss} (ng/mL)	32 ± 9	61 ± 17
C_{max}^{ss}/Dose (ng/mL/mg)	6.4 ± 1.8	6.1 ± 1.7
$AUC\tau^{ss}$ (ng·h/mL)	238 ± 77	514 ± 231
$AUC\tau^{ss}$/Dose (ng·h/mL/mg)	48 ± 15	51 ± 23
C_{avg}^{ss} (ng/mL)	9.9 ± 3.2	21.4 ± 9.6

Values are median (range) for t_{max} and mean ± standard deviation for all others.
Dose-normalized parameters are per mg. τ is 24 h

3.4.6 Pharmacodynamic Studies

Pharmacokinetic/pharmacodynamic modeling based on inhibition in a peripheral biomarker (S6 kinase inhibition in peripheral blood mononuclear cells) suggests that 10 mg daily should be an adequate dose to produce a high-degree of sustained target inhibition. Furthermore, molecular pharmacodynamic (MPD) studies using immunohistochemistry (IHC) in biopsied tumor tissue assessed the degree of inhibition and its duration (for p-S6, p-4E-BP1 and p-AKT expression) with the daily and weekly dosing. The pathologist was blinded for the biopsy sequence. There was almost complete inhibition of p-S6 at all doses and schedules studied (p=0.001). Preliminary results suggest a dose-related decrease in p-4E-BP1 and increase in p-AKT expression with maximal effect at 10 mg daily and ≥ 50 mg weekly.

3.4.7 Phase I investigation of Safety, Tolerability, and Molecular Pharmacodynamic effects in patients with advanced solid tumors

In this study, protocol CRAD001C2107, molecular changes were subsequently investigated through serial biopsying of tumors before and while on treatment.[37] Biopsying of tumors on treatment took place at week 4 of treatment (pharmacokinetic steady-state). All patients underwent a 24-hr post-dose biopsy. Patients following the weekly regimen had a further biopsy on Day 4-5 during the same week. Molecular activity was measured by immunohistochemistry. In the absence of a reliable technique for measuring mTOR phosphorylation itself, the phosphorylation status of downstream markers S6 and eIF4G, for which reliable antibodies exist, was selected as reflecting the immediate pharmacodynamic effect of everolimus. Also measured were changes in the phosphorylation status of upstream AKT and the proliferation index Ki67. The daily regimen was associated with a high inhibition of p-S6 and p-eIF4G at 5mg/d and a complete inhibition at 10mg/d. In patients on the weekly schedule, p-S6 inhibition was complete and sustained at all dose levels while that of p-eIF4G was complete and sustained at 50mg/d but not at 20mg/wk. On both regimens numerous patients demonstrated apparent up-regulation of AKT which tended, however, not to persevere in the patients at 50mg/wk. The proliferation index was reduced in most patients, recovering in some of those on the 50mg/wk regimen.

3.4.8 A randomized, double-blind, placebo-controlled study of RAD001 in the treatment of patients with subependymal giant cell astrocytes (SEGA) associated with Tuberous Sclerosis Complex (TSC)

In the phase I Study CRAD001C2413, (IND #70,714) everolimus was administered orally at a daily dose of 2.1, 3, 5 and 6.5 mg/m2 in cohorts of three to six patients per dosage level (Fouladi et al., 2007). The MTD for this population was 5 mg/m^2. At the starting dose of 3 mg/m^2 readily reversible grade 3 and 4 dose-limiting toxicities (DLTs) at 3 mg/m^2 included reversible hypokalemia (n = 1, grade 4) and hypophosphatemia (n = 2, grade 3) were observed in platinum pretreated patients, resulting in dose de-escalation to 2.1 mg/m^2. The definition of DLT was amended to exclude grade 3 or 4 electrolyte abnormalities that resolved to grade 2 or below within 7

days of interrupting treatment, allowing further dosage escalation. Dose de-escalation to 2.1 mg/m^2 led to no further DLTs in three assessable patients. Three more assessable patients were then enrolled at 3 mg/m^2 and 5 mg/m^2, each with no DLTs. At 6.5 mg/m^2, the DLTs included grade 3 events of elevation of ALT (n = 1), mucositis (n = 1), and diarrhea (n = 1). Thus, three more patients were enrolled at 5 mg/m^2, with no observed DLTs, establishing 5 mg/m^2 as the recommended MTD. No additional grade 4 side effects were reported. Additional grade 3 effects at any time during trial therapy were ALT elevation (1 patient, 5mg/m^2), reduced hemoglobin (2 patients at 3 mg/m^2), infection (3 patients at 3 mg/m^2), leucopenia (2 patients at 3mg/m^2), mucositis (one patient), hypokalemia (1 patient at 3mg/m^2, 1 patient at 5mg/m^2), hyponatremia (1 patient at 3mg/m^2), anorexia (1 patient at 3 mg/m^2), dizziness (1 patient at 3mg/m^2), hyperglycemia (1 patient at 2.1 mg/m^2, 1 patient at 5 mg/m^2), nausea (1 patient at 3 mg/m^2) and pain in the oral cavity (1 patient at 5 mg/m^2). The adverse events seen were consistent with the known safety profile of everolimus in adults.[38]

Everolimus pharmacokinetics in children were also found to be comparable to adults. Everolimus was absorbed rapidly, with maximum concentrations achieved as early as 30 minutes after administration. The maximum everolimus whole-blood concentrations and AUC at each dose level were variable, but increased with dose.

3.4.9 Phase I Study of Everolimus in Pediatric Patients with Refractory Solid Tumors

Everolimus has been investigated in one phase I/II study (28 patients) in patients with TSC who have subependymal giant cell astrocytoma (SEGA) [CRAD001C2485]. Additionally, two phase III studies have been conducted in patients with TSC: study CRAD001M2301 in patients with TSC who have SEGA (117 patients) and study CRAD001M2302 in patients with renal angiomyolipoma (118 patients). CRAD001MIC02 is an ongoing, expanded access phase IIIb study for patients with TSC associated SEGA. Currently, there is a fourth phase III study CRAD001M2304 ongoing in which everolimus will be tested to evaluate whether its use as adjunctive treatment results in reducing seizure frequency in patients with TSC who have refractory seizures.

Data on a small series of TSC patients who had systematic SEGAs which were treated with daily rapamycin therapy showed tumor size reduction by 46-63% within 2.5 to 5 months (Franz et al. 2006). All lesions exhibited regression and, in one case, necrosis. Interruption of therapy for one patient resulted in regrowth of the SEGA, but further regression of another 62-75% was seen upon the resumption of therapy. These results represented a new therapeutic paradigm for the treatment of SEGAs in the TSC patients with rapamycin and related agents.

Therefore, in 2007, everolimus entered clinical development for TSC. Study C2485, conducted by Cincinnati Children's Hospital Medical Center (CCHMC), is a prospective, non-randomized, open-label, investigator-initiated, single-center 28-patient trial designed to evaluate the safety and efficacy of everolimus in patients ≥ 3 years of age with SEGA associated with TSC. The primary efficacy endpoint was the change from baseline in volume of primary SEGA lesion at 6 months, as determined by central radiology review. At 6 months, 9 out of 28 patients (32%, 95% CI: 16% to 52%) had a ≥ 50% reduction in the tumor volume of their largest SEGA lesion. The median duration of response for these 9 patients was 11.8 months (range 3.2 to 39.1 months). Response rate continues to improve as 58.3% of patients (14 of 23) who took everolimus for at least four years have experienced a reduction of ≥ 50% in the size of their largest SEGA relative to baseline. One patient has experienced a SEGA progression (defined as an increase of at least 25% to a value greater than baseline). The median time from first response to progression/censoring was 37 months (range: 6-63 months). No patient developed a new lesion and none required surgical resection or other therapy for SEGA.

The safety and efficacy of everolimus in patients with TSC who have SEGA was studied further in [CRAD001M2301], a randomized (2:1), double-blind, placebo-controlled trial of everolimus conducted in 117 pediatric and adult patients with SEGA and TSC. The main efficacy outcome measure was SEGA response rate based on independent central radiology review. After 6-months of study treatment, 35% of the patients treated with everolimus had at least a 50% reduction in SEGA volume compared to none in the placebo group (Franz et al 2013). Based on recent data, the response rate after at least 12-months of everolimus treatment has increased to 48%. SEGA progression was seen in nine of the all patients treated with everolimus (8.1%). In five patients, progression occurred after cessation of treatment or was associated with C_{min} values which were markedly reduced or at zero. In one case of progression, the patient developed hydrocephalus that was successfully treated, in the absence of SEGA growth. In one case, progression reversed with further treatment.

3.5 Study Rationale

Currently, no effective therapies for individuals with germline PTEN mutations exist to target neurocognitive and behavioral deficits, and effective interventions that target the core biologic alterations are crucial to optimize the short and long-term outcome of individuals affected with mutations in this gene.

Like TSC, PTEN deficiency is an attractive target for treatment with mTOR inhibitors due the PTEN's role through AKT to regulate specifically mTORC1, which is sensitive to rapamycin and analogs such as everolimus.

Use of a neurocognitive composite score to determine efficacy has several advantages. Assuming that everolimus treatment does not impair some functions while improving others, a composite score is likely to be sensitive, highly reliable, can be computed in all patients even if 1 or 2 measurements are missing or could not be completed, and has good domain coverage to capture possible variability across patients in the specific neurocognitive domains showing improvement. An example of the latter advantage, is if some individuals show improvements in processing speed while others show improvements in working memory. The neurocognitive composite would identify improvements in both of these sets of patients. As such, the neurocognitive composite may be more sensitive to change than any individual measure. Furthermore, a composite primary efficacy outcome will not require additional multiple comparison correction.

Exploratory measures were chosen based on their potential to serve as objective outcome measures in future trials. While none of these measures have specific evidence with PTEN mutation cases, they have some data regarding abnormalities within autism or developmental disability populations. In the cases of remote eye gaze tracking, resting EEG, and auditory evoked potentials, these measures are also reasonably inexpensive, can be acquired rapidly, and can be acquired in the majority of PTEN patients.

4 Design and Methods

4.1 Study Rationale

This is a phase II, double-blind, randomized, parallel group, placebo-controlled, three center study evaluating treatment with everolimus versus placebo in 40 patients with PTEN gene mutations, ages 5-45 years (inclusive). It is expected that 60 patients will need to be screened to have 40 who will meet inclusion and exclusion criteria.

There are four different phases in this study: pre-treatment (screening), a 6-month blinded treatment phase, a 6-month open label phase for patients initially randomized to placebo, and a follow-up phase. Each of these phases is described in detail below.

4.2 Pre-Treatment Phase (Screening)

At screening, the investigator or his/her designee will assign a unique number to patients being considered for the study, as outlined in the 7904 Manual of Procedures. The patient/parent must provide a signed Informed Consent Form prior to any study screening evaluations being performed. Once the patient/parent provides a ***signed informed consent form*** and ***eligibility is confirmed (all inclusion/exclusion criteria have been verified),*** the investigator or his/her designee will register the patient for randomization.

Screening evaluations will include demography, relevant medical history/current medical conditions, a physical examination (including a neurological examination), suicidal ideation/behavior assessment, vital signs, laboratory assessments, and other additional study entry evaluations. Primary endpoint neurocognitive data will be collected for screening purposes (SB-5, CPT-3, and Purdue Pegboard Test). Once the patient is confirmed as being eligible to be randomized, the baseline visit will be scheduled to occur within 6 weeks of the screening visit.

Participants will be screened for signs and/or symptoms of cancer. If a patient presents at the screening visit with a mass that may be malignant or benign, the study physician will refer them to the appropriate health care providers. If the mass is benign, and the patient meets all other inclusion/exclusion criteria, the patient may be enrolled in the study. Additional information about cancer screening can be found in the Manual of Procedures.

Participants will be screened for suicidal ideation and/or behaviors using the Columbia Suicide Severity Rating Scale (C-SSRS). Participants that are determined to have significant suicidal ideation and/or behaviors, as

defined in Section 4.6.5, will be ineligible to participate in the trial, and will be referred to the appropriate healthcare provider per the principal investigators discretion.

A complete list of screening evaluations is provided in the schedule of evaluations. All of the above assessments/procedures must be conducted and evaluated prior to randomization.

4.3 Blinded Treatment Phase

All baseline procedures and evaluations should be completed within 6 weeks of the screening visit. If a patient's safety lab results are out of range, and the investigator has reason to believe there were situational factors affecting the values, the screening labs may be repeated. The safety evaluations may be completed at a lab that is geographically close to the participant and either provide the results of that analysis directly to the study team, or a sign a release so the study team can obtain the results from the lab. If a patient's baseline visit is more than 6 weeks after the screening visit the participant must have the safety labs re-drawn and processed, have the physical and neurological exam re-done, and the study team will confirm that there are no changes in the medical history or changes to the patient's current medications. If the screening safety evaluation and/or lab results are out-of-range, the participant will not be eligible to participate in the study. Patients who meet the study eligibility criteria following screening will be randomized to receive everolimus or placebo. The randomization ratio is 1:1, with one patient being randomly assigned to everolimus for every one patient randomly assigned to placebo. Treatment assignment via the RDCRN Members' Website should occur prior to the Baseline visit to allow adequate time for pharmacy preparation. ***Patients must start treatment within 7 days of the baseline visit***, given that the participant still meets all inclusionary criteria and has not developed any exclusionary criteria. The medication should be taken at the same each day with a light, low-fat breakfast (i.e. 8:00 am with light breakfast).

The duration of blinded phase this study is 6 months. Patients will have their first daily dose of everolimus or placebo within 7 days of Visit 2 (baseline) and will continue on treatment for 6 months unless an intolerable toxicity occurs, withdrawal of consent, or investigator decision to discontinue the patient from study treatment.

Starting dose and adjustments are defined in Section. 6.2.

Safety evaluations are routinely performed (visit 3/month 1, visit 5/month 3, and visit 8/month 6). Patients must be in a fasting state at the time of blood sampling for all laboratory evaluations including the lipid profile. Hematology and biochemistry assessments are required at the screening visit, the Month 1 visit, the Month 3 visit, and the Month 6 visit. All blood samples obtained at each visit will be sent to LabConnect, LLC and associated testing facilities for analysis. If safety labs are out of range while the participant is randomized, a clinical assessment by the physician will take place that might include a repeat of blood work. Withdrawal from the study will be considered by the principal investigator if clinically indicated and after discussion with the study team. Complete details regarding all study required safety assessments are provided in Section 7.15.

A PK sample will be collected at the Month 1 visit (one month ± 14 days from the baseline visit), when possible at steady-state, prior to dose administration during this visit, within 24 ± 4 hours after the last dose. Participants who receive a dose adjustment after this visit will work with study staff to get another PK sample taken at the study site or locally, by a designated and trained remote health care provider, 2 weeks (± 1 week) after the new dose has been taken. A PK sample may also be collected from a patient experiencing an adverse event or side effect. The decision to obtain this sample will be made by the site physician.

Visits 4, 6, and 7 (months 2, 4, and 5) can be in-person, or by phone if that is preferred by the family. If performed by phone, a phone interview will be performed to collect safety and other data, review AEs, and assess changes in mood or behavior. All applicable CRFs will be completed. Modified visits 3, 5, 8 (months 1, 3, and 6) can also be conducted by phone/video conference if the family cannot make it to the study site within the designated window due to extenuating unforeseen circumstances.

Tests that will be conducted during the blinded treatment phase include laboratory tests for safety, physical exams (including a neurological assessment), vital signs, and neuropsychological assessments. If unforeseen circumstances (i.e., unexpected personal reasons) prevent the patient from complying with the established visit schedule, the site can re-schedule the visit (within ± 14 days of the expected visit date). The reason(s) for any visit or treatment delays will be documented in the CRF for the appropriate visit. All on-site visits (screening,

baseline, month 1, month 3, and month 6) must be completed by the participant, and any missed visits would warrant termination from the study. A summary of the neuropsychological results may be made available at study completion.

At the end of the double-blind phase (or early termination), the treatment code will be broken and the participant will be informed of their assigned treatment arm, using the process outlined in the 7904 Manual of Procedures. Participants who received the active compound will enter the follow-up phase of the study (section 4.5) and will be referred back to their treating physicians. Individuals who were in the placebo group will be invited to enter Phase B, a 6-month open-label extension trial (section 4.4). The inclusion of the open-label phase may increase the acceptability of the project by families and increase their participation in the primary, controlled trial. All participants that received everolimus during the blinded treatment phase will enter the follow-up phase of the trial and will be referred back to their treating physicians. A summary of patient's participation in the study will be provided, if needed.

4.4 Open Label Phase

Individuals in the placebo group will be invited to enter the 6-month open-label extension trial that will follow the same schedule as the blinded treatment phase. The final visit, or the Month 6 visit, in the blinded treatment phase will serve as the baseline visit of the open label phase if the participant chooses to enter the open label phase. Safety labs obtained at the end of the blinded phase will be used as baseline safety labs for the open-label phase. Similarly, the scores from the patient's developmental assessments will be used as both the 6-month blinded treatment phase data, and the baseline open-label phase data. Study staff should be prepared to dispense open label medication at this visit. If the patient is unable to enter the open-label phase at the final visit of the blinded treatment phase, the patient can come back within 2 weeks (+/- 2 weeks) of this visit date. Treatment, assessments, and outcomes of the open-label phase will also be identical to the double-blind phase.

The next visit will be the Month 1 visit. A PK sample, as well as specified safety labs, will be drawn on site and sent to LabConnect LLC. If the participant receives a dose adjustment at the time of this visit, study staff will work with the participant/family to get another sample taken at the study site or locally by a trained phlebotomist. The Month 3 visit and the Month 6 visit will include safety labs, developmental testing, optional EEG/AEP and eye tracking procedures, physical and neurological exams, vital signs, and other study questionnaires. A PK sample may also be collected from a patient experiencing an adverse event or side effect. The decision to obtain this sample will be made by the site physician.

The Month 2, Month 4, and Month 5 visits can be in person or by-phone if that is preferred by the family. If performed by phone, a phone interview will be performed to collect safety and other data, review AEs, and assess changes in mood or behavior. All applicable CRFs will be completed.

If unforeseen circumstances (i.e., unexpected personal reasons) prevent the patient from complying with the established visit schedule, the site can re-schedule the visit (within ± 14 days of the expected visit date). The reason(s) for any visit or treatment delays will be documented in the CRF for the appropriate visit. All on-site visits (month 1, month 3, and month 6) must be completed by the participant, and any missed visits would warrant termination from the study. Under extenuating unforeseen circumstances, visits 3, 5, 8 (months 1, 3, and 6) can be conducted by phone/video conference as a modified visit as to still capture obtainable data and maintain patient safety. A summary of the neuropsychological results may be made available at study completion.

At the final visit of the open label phase, or the 6 month visit, participants will exit the trial. As done in the blinded treatment phase, they will receive a letter

4.5 Follow-Up Phase

All patients will have a follow-up phone call scheduled 28 days (± 14 days) after the last dose of the study treatment to follow for AEs and SAEs that may have occurred after discontinuation from the study treatment. See section 6.3.15 for additional details.

4.6 Patient Selection and Inclusion/ Exclusion Criteria

Patients must have screening evaluations performed within the 6 weeks of the baseline visit and must still meet all inclusion and exclusion criteria at the time of the baseline visit. Results of all screening evaluations, which ensure that all inclusion and exclusion criteria have been satisfied, must be reviewed by the site's Principal Investigator or his/her designee prior to enrollment of that patient. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to any screening activities. The following criteria apply to all patients enrolled onto the study, unless otherwise specified**.**

4.6.1 Double-Blind Inclusion Criteria

1. Male and female outpatients between 5 and 45 years of age (inclusive);
2. Pathogenic PTEN mutation confirmed by clinical genetic testing;
3. Participant must be able to complete one of the following three standardized assessments: Conners' Continuous Performance Tasks (CPT-3), Stanford Binet (SB-5; working memory), or the Purdue Pegboard Test;
4. Performance below the age-adjusted population mean on at least one of the above standardized measures: attention (CPT-3, mean reaction time), working memory (SB5, working memory subscale), or fine motor skills (Purdue Pegboard Test; either dominant, non-dominant, or both hands);
5. Adequate bone marrow function as shown by:
    a. Platelets ≥ 80,000/mm^3
    b. Absolute neutrophil count ≥ 1,000/mm^3
    c. Hemoglobin ≥ 9 g/dL;
6. Adequate liver function as shown by:
    a. Total serum bilirubin <1.5 x ULN
    b. AST and ALT levels < 2.5 x ULN
    c. INR ≤2;
7. Adequate renal function: serum creatinine < 1.5 x ULN;
8. Signed informed consent obtained prior to any screening procedures;
9. Individuals on psychotropic and anti-epileptic medications should maintain a stable dose for at least 2 months prior to the screening visit;
10. Negative serum pregnancy test for females at screening and no plans to become pregnant or conceive a child while participating in the study. The effects of mTOR inhibitors on the developing fetus at the doses used in this study are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception prior to study entry and for the duration of the study. Estrogen-containing oral contraceptives are not recommended in women enrolled in this study. Abstinence or two effective non-estrogen or barrier methods of contraception (such as condom + spermicidal foam) must be used;
11. Medically stable with no active medical problem such as unstable seizure or cardiovascular disease as evidenced by a history;
12. No anticipated changes in the frequency and intensity of existing interventions such as behavioral and developmental treatments, in home services, and speech therapy;
13. No planned changes in school placement;
14. For individuals under 18 or who are otherwise incapable, there must be an available caregiver who can reliably bring subject to clinic visits and provide trustworthy data;
15. Able to communication fluently in English.

4.6.2 Double-Blind Exclusion Criteria

1. Patients currently receiving anticancer therapies or who have received anticancer therapies within 4 weeks of the start of Everolimus (including chemotherapy, radiation therapy, antibody based therapy, etc.);
2. Known intolerance or hypersensitivity to Everolimus or other rapamycin analogs (e.g. sirolimus, temsirolimus);
3. Known impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of oral Everolimus;

4. Uncontrolled diabetes mellitus as defined by HbA1c > 8% despite adequate therapy. Patients with a known history of impaired fasting glucose or diabetes mellitus (DM) may be included, however blood glucose and antidiabetic treatment must be monitored closely throughout the trial and adjusted as necessary;
5. Patient with uncontrolled hyperlipidemia: fasting serum cholesterol > 300 mg/dL OR > 7.75 mmol/L AND fasting triglycerides > 2.5 x ULN.
6. Patients who have any severe and/or uncontrolled medical or psychiatric conditions such as:
    a. Unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction ≤6 months prior to start of Everolimus, serious uncontrolled cardiac arrhythmia, or any other clinically significant cardiac disease
    b. Symptomatic congestive heart failure of New York heart Association Class III or IV
    c. Active (acute or chronic) or uncontrolled severe infection, liver disease such as cirrhosis, decompensated liver disease, and active and chronic hepatitis (i.e. quantifiable HBV-DNA and/or positive HbsAg, quantifiable HCV-RNA),
    d. Known severely impaired lung function (spirometry and DLCO 50% or less of normal and O_2 saturation 88% or less at rest on room air)
    e. Active, bleeding diathesis;
    f. unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction ≤6 months prior to start of Everolimus, serious uncontrolled cardiac arrhythmia, or any other clinically significant cardiac disease
    g. Symptomatic congestive heart failure of New York Heart Association Class III or Iactive (acute or chronic) or uncontrolled severe infection, liver disease such as cirrhosis, decompensated liver disease, and active and chronic hepatitis (i.e. quantifiable HBV-DNA and/or positive HbsAg, quantifiable HCV-RNA),
    h. known severely impaired lung function (spirometry and DLCO 50% or less of normal and O_2 saturation 88% or less at rest on room air),
    i. severe psychiatric disorders, such as bipolar disorders or schizophrenia,
    j. active substance-abuse,
    k. active, bleeding diathesis;
7. Chronic treatment with corticosteroids or other immunosuppressive agents. Topical or inhaled corticosteroids are allowed;
8. Known history of or seropositivity for Hepatitis B, Hepatitis C, or HIV
9. Patients who have received live attenuated vaccines within 1 week of start of Everolimus and during the study. Patient should also avoid close contact with others who have received live attenuated vaccines. Examples of live attenuated vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines;
10. Patients who have a history of another primary malignancy, with the exceptions of:
    a. non-melanoma skin cancer
    b. carcinoma in situ of the cervix, uteri, or breast from which the patient has been disease free for ≥3 years;
11. Planned changes to concomitant medications;
12. Prior or concomitant therapy with known or possible anti-mTOR activity, including rapamycin (sirolimus);
13. Concomitant therapy with strong inhibitor (e.g., cyclosporine and ketoconazole) or inducer of CYP3A;
14. Active infection at time of enrollment;
15. Patients with a history of non-compliance to medical regimens or who are considered potentially unreliable or will not be able to complete the entire study;
16. Patients who are currently part of or have participated in any clinical investigation with an investigational drug within 1 month prior to dosing;
17. Pregnant or nursing (lactating) women;
18. Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant, must use highly effective methods of contraception during the study and 8 weeks after. Highly effective contraception methods include:
    a. A combination of any two of the following:
        i. Use of oral, injected or implanted hormonal non-estrogen containing methods of contraception or;
        ii. Placement of an intrauterine device (IUD) or intrauterine system (IUS);
        iii. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository;
    b. Total abstinence or;

c. Male/female sterilization.
Women are considered post-menopausal and not of child-bearing potential if they have had 12 months or natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks prior to randomization. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child-bearing potential.

19. Male patients whose sexual partner(s) are WOCBP who are not willing to use adequate contraception, during the study and for 8 weeks after the end of treatment
20. Major surgery, radiation therapy or stereotactic radio-surgery within previous 4 weeks at time of screening
21. Neurosurgery within prior 6 months at time of screening.

4.6.3 Open-Label Phase Inclusion Criteria

1. Patients who completed Double-Blind phase of the study and were assigned to the placebo treatment arm;
2. Verbal consent (and assent, as appropriate) obtained prior to any open-label phase study procedures.

4.6.4 Screening for Hepatitis B, Hepatitis C, and HIV

Study teams will review a list of risk factors for Hepatitis B, Hepatitis C, and HIV with participants/families. If the participant meets any of these risk factors, a serology blood sample will be drawn at the screening visit. If the participant is positive for any of the viruses, they will be ineligible to participate in the study.

Patients that meet any of the risk factors below will have a blood sample drawn for a serology panel that will test for Hepatitis B (HBV-DNA, HBsAg, HBs Ab, and HBc Ab), Hepatitis C (quantitative RNA-PCR and HCV Ab), and HIV (HIV I/II).

- Currently live or have lived in Asia, Africa, Central and South America, Eastern Europe, Spain, Portugal, and Greece
- Had an operation in a developing country at any time
- Received a blood transfusion prior to 1990
- Are current or past IV drug user
- Have body piercing or tattoos
- Have a biological mother diagnosed with HIV
- Have a biological mother diagnosed with hepatitis B or hepatitis C
- Have a history of hepatitis B infection (e.g., dark urine, jaundice, right upper quadrant pain) or hepatitis C
- Have had household contact with hepatitis B or hepatitis C infected patient(s)
- Have current or prior high-risk sexual activity
- Have current or prior dialysis

Hepatitis B, Hepatitis C, and HIV screening will be done at the discretion of the principal investigator at each site, using these factors. If a patient tests positive for HIV, the Principal Investigator at each enrolling site is responsible for reporting the result to the appropriate public health agency based on IRB and state regulations. The Principal Investigator will inform the patient or parent of the positive result and reporting plan. Patients that screen positive for HIV will be provided counseling.

4.6.5 Columbia Suicide Severity Rating Scale (C-SSRS)

In light of the recent evidence linking psychotropic medications to suicidal behaviors, it is important to closely monitor the mental well-being of patients taking medications that target the central nervous system[61]. Therefore, assessment of suicidal ideation/behaviors will be completed during the screening phase and will be monitored during the trial. The C-SSRS will be administered to assess the presence of suicidality throughout the entirety of the trial.

The Columbia-Suicide Severity Rating Scale (C-SSRS) is an interview that prospectively assesses suicidal ideation and behavior using a semi-structured interview to probe patient responses. The C-SSRS is intended to

be used by individuals who have received training in its administration. Ultimately, the determination of the presence of suicidal ideation and/or behavior depends on the judgment of the individual administering the scale.

A trained investigator will administer the C-SSRS. Patients 13 years of age and older will complete the scale for themselves. Parents/caregivers will complete the interview for patients that are under the age of 13, or for patients unable to understand and complete the assessment due to cognitive impairment. Additionally, the study physician will proactively assess patients for suicidality and mood disturbances through discussions with the patient and/or parent. Any positive responses will be further investigated, and appropriate medical treatment will be initiated.

The C-SSRS will be completed at screening, Month 1, Month 3 and Month 6 of the blinded phase, and Month1, Month 3 and Month 6 of the open label phase. For the screening visit, the C-SSRS will be evaluate the last 12 months. For Month 1, Month 3, and Month 6 of the double-blind and open-label phases, the C-SSRS will assess any changes from the previous visit. If, at any assessment, the Suicidal Ideation (questions #1-5) score is a 4 or above, or the patient (or parents) answers “yes” to any Suicidal Behavior item (actual attempt, interrupted attempt, aborted attempt, preparatory acts or behavior, or suicidal behavior), the patient must be referred to a health care professional for further assessment and/or treatment. If the participant meets any of these criteria at the screening visit, they will be excluded from the study. Once the participant has started study medication, the decision about whether the participant should be withdrawn from study treatment will be determined by the site PI, in collaboration with one of the site’s mental health professionals and Dr. Hardan. To note, any study investigator or research staff can refer a participant to the designated site mental health professional for any concerns about suicidality, even if the patient does not meet the criteria detailed above.

The patient should not be allowed to leave the study center until the results of the C-SSRS are reviewed and the patient is not considered to be at risk. If there is doubt about whether a patient is at risk, the Investigator should obtain appropriate psychiatric consultation prior to releasing the patient.

In the event of a suicide attempt or successful suicide, the investigator will complete a serious adverse events form.

4.7 Early Termination

If the participant, legal guardian, or the study physician make the decision to have the participant exit the study early, a follow-up visit must be scheduled 28 days (+/- 14 days) after termination. At this visit, the study physician should review the patient’s medical history, concomitant medications, and the DOTES. If the patient exited the trial due to an adverse event, this event should be reviewed and if it is still present the study team must follow-up with the patient/legal guardian for 56 days or until the event has subsided. At the completion of this follow-up visit, the participant may be unblinded and the results may be disclosed to the participant.

5. Recruitment Methods

Recruitment will leverage the observational study, The Natural History of Individuals with Autism and Germline Heterozygous PTEN mutations, and will occur at 3 sites: Cleveland Clinic, Stanford University, and Boston Children’s Hospital, with Cleveland Clinic expected to recruit at least 50% of the sample based on extensive experience with PTEN patients at this site. Over the last 5 years, we have recruited and comprehensively evaluated 35+ patients with PTEN ASD only from referrals to Cleveland Clinic and word of mouth. We anticipate that the majority of these individuals would participate in the proposed study.

We will supplement our current recruitment efforts with several complementary strategies. First, Dr. Charis Eng (Cleveland Clinic) has developed a registry of > 400 individuals with PTEN mutations that includes > 50 PTEN cases with ASD or evidence of developmental delay. To date, this registry has not been tapped, but it will be used to recruit individuals for the proposed study. Dr. Eng’s lab also regularly screens individuals at risk of PTEN mutations using an online risk calculator and extensive referral network – growing her registry. Second, the two additional sites, Stanford University and Boston Children’s Hospital, also have experience with PTEN ASD patients and have an identified population that is interested in participating in this trial. Both of these sites will also leverage their relevant clinics to advertise and recruit for this study. Third, we have obtained support

from a community for caregivers of PTEN ASD patients (www.ptenlife.com) and the largest online group devoted to supporting families and patients affected with and by PHTS (www.ptenworld.com). Both groups have agreed to assist in recruitment. Finally, we will employ multiple advertising and recruiting tools both locally and nationally to raise awareness of the study.

Potential subjects will be recruited through the same methods as listed above at the other participating sites. Recruitment will begin as soon as IRB approval is obtained.

The study coordinator, principal investigator, co-investigators and sub-investigators at each site will be responsible for recruitment efforts. An introductory letter to families with known diagnosis of PTEN and who otherwise meet eligibility criteria with an opt-out postcard as well as advertisements on advocacy group websites will be utilized for recruitment at each site. We will also utilize the Rare Diseases Clinical Research Network Developmental Synaptopathies Consortium (RDCRN DSC) contact registry, developed as a part of the natural history study, to inform individuals interested in research opportunities about the trial. This registry already has more than 80 distinct patients entered.

5.1 Retention Strategies

To retain participants throughout the 6-month trial, sites will use procedures that have proven successful in previous studies, such as phone calls to debrief after the most recent visit and phone calls or emails to prepare for the upcoming visit. Individual sites will seek IRB approval for each of the methods that they use prior to distribution. Whenever possible, study staff at each site should also try to schedule study visits on a day that is convenient for the family. Additionally, caregivers, or adult participants, will receive feedback on their child's, or their, neurocognitive profile and changes over time at the end of the trial. Since there is a 50/50 chance of participants receiving everolimus or placebo, it is important for study staff to make sure the family is aware of the open-label phase of the study. Families should also be reminded that all travel expenses, throughout the entirety of the trial, will be covered. Finally, study staff should always express gratitude and thanks to both the patient and his/her family for participation in the study.

6. Description of Study Treatments or Exposures/Predictors

6.1 Everolimus Administration

Patients will be randomized to receive either everolimus or placebo. Patients will be treated with blinded study treatment for 6 months, or until unacceptable toxicity or discontinuation for any other reason.

For the duration of the trial, everolimus and placebo will be supplied to the research pharmacies at each site directly from Novartis. Everolimus and placebo will be formulated as identical tablets of 2.5mg or 5mg strength, blister-packed under aluminum foil in units of 10 tablets. Medication labels will comply with US legal requirements for investigational drug products and will be printed in English. The storage conditions for study drug will be described on the medication label.

The study drug everolimus and/or placebo will be self-administered (by the patient or patient's parent/guardian). The investigator should promote compliance by instructing the patient to take the study drug exactly as prescribed and by stating that compliance is necessary for the patient's safety and the validity of the study. The patient should be instructed to contact the investigator if he/she is unable, for any reason, to take the study drug as prescribed. Everolimus should be administered orally once daily at the same time every day, consistently with a light, low-fat meal. Everolimus or placebo tablets should be opened only at the time of administration as drug is both hygroscopic and light-sensitive. The extent of absorption of everolimus through topical exposure is not known. Therefore, patients/caregivers are advised to avoid contact with the everolimus or placebo tablets and should wash their hands thoroughly before and after administration.

For the Open-label phase: Everolimus is formulated as tablets for oral administration of 2.5mg, 5mg, and 10mg strength. Tablets are blister-packed under aluminum foil, which should be opened only at the time of administration as drug is both hygroscopic and light-sensitive. Refer to label for expiration date and storage conditions.

6.2 Treatment Course Information

The average starting dose will be 4.5 mg/m^2/day of trial therapy (either everolimus or placebo), rounded to the nearest 2.5mg dose. The BSA should be calculated (section 7.15) based on an accurate height and weight measurement performed according to institutional guidelines. Leftover study medication, as well as all used blister packs, should be collected at each study visit, and drug should be accounted for at this time. If 2.5mg tablets become unavailable during the trial, patients prescribed a dose including 2.5mg tablets will be instructed to alternate between a higher and lower dose. The site physician will instruct the patient to take the higher dose on Monday, Wednesday, and Friday. The patient will take the lower dose on Tuesday, Thursday, Saturday, and Sunday. For example, a patient prescribed a dose of 7.5mg will take 10mg on Monday, Wednesday, and Friday, and 5mg on Tuesday, Thursday, Saturday, and Sunday.

A maximum volume of 3 ml of blood will be drawn for trough everolimus PK levels when necessary. The blood draws will be timed to occur at 24 ± 4 hours after ingestion of the drug. Blood collection for PK samples may be conducted during a scheduled visit on-site or via a designated and trained remote healthcare service. Everolimus levels will be measured at ARUP laboratories in Salt Lake City, UT, in conjunction with LabConnect, LLC. When necessary, a kit for remote collection of blood samples can be sent to the participant and returned per the shipping instruction by express mail.

Dose adjustments in the blinded phase of the trial can occur in both placebo (randomized modification) and active (based on PK findings) arms and will be at the discretion of a central unblinded investigator, Sarah Spence, M.D. or back-up unblinded investigator, Kiran Maski, M.D. In the event both Drs. Spence and Maski are unavailable, Dr. David Franz, Medical Review Officer, will serve as the unblinded investigator for the purposes of PK-driven dose adjustments. An unblinded research nurse will assist the unblinded physicians and the Medical Review Officer in their roles and will make sure that communications between them and the different sites is occurring in a timely manner.

Dose adjustments will be permitted, based on safety findings and PK levels. The site PI will initiate any safety level related dose adjustments according to the protocol, in collaboration with the Medical Review Officer as appropriate. PK based dose adjustments will be initiated by the unblinded physician. All dose adjustments will be made using the 2.5mg tablets (i.e. increase by 2.5mg, decrease by 2.5mg, or maintain dose). If a dose adjustment is made for safety purposes, the unblinded physicians and Medical Review Officer should be made aware of any dose modifications as soon as possible.

Sites may substitute 5mg tablets (or 10mg tablets in open label) for 2.5mg tablets where necessary, as long as the single dose level increase or decrease is maintained. For example, an adjustment from 7.5mg to 10mg can be made by prescribing two 5mg tablets (or one 10mg tablet in open label) rather than one 5mg and two 2.5mg tablets. Following a dose adjustment, sites can mail the extra 5mg tablets to the participant in between visits to make up for the remaining days until the next site visit. One level dose increase would be increasing the dose by 2.5mg and a one level dose decrease would be decreasing the dose by 2.5mg as stated above.

Placebo Arm: Placebo dose adjustment procedures are defined in the unblinded dose adjustment protocol – accessible only to the unblinded physician(s) and the DMCC. The site team will be blinded with respect to treatment assignment at all times. Dr. Maski, Dr. Spence, and Dr. Franz will receive instructions regarding dose modifications for participants on placebo from the DMCC. When PK values become available for individuals in placebo arm, Dr. Maski or Dr. Spence will refer to the provided instructions and notify site PI/coordinator of dose status (increase, decrease, or maintain) for individuals assigned to a dose adjustment.

Active Treatment Arm: Based on the everolimus PK level, Dr. Maski, Dr. Spence, or Dr. Franz will determine whether a dose adjustment is warranted, i.e. the value falls outside the range of 5-15 ng/ml. The unblinded physician(s) will notify the local research team and PI how to proceed with next dose (increase by 2.5mg, decrease by 2.5mg, or maintain dose). Following any dose adjustments, a blood sample will be redrawn two weeks (+/- one week) after the modification. The unblinded physician will assess the everolimus PK level and notify the local study team to maintain or adjust the dose accordingly to achieve the target range of 5-15ng/ml. This process will be repeated until the everolimus level falls within the target range.

Dose adjustments in the open label phase of the study will be made by the site principal investigator. The PK value taken at the Month 1 visit will be released to the site physician by the unblinded coordinator and dose adjustments will be made based on the value.

A detailed explanation of other permitted dose adjustments can be found in Section 7.7.3.

6.3 Known Undesirable Effects of Study Drug/Treatment

Overall, safety data available from completed, controlled and uncontrolled studies indicate that everolimus is generally well tolerated at weekly or daily doses. The safety profile is characterized by manageable adverse events (AEs). These AEs are generally reversible and non-cumulative.

Adverse events most frequently observed with everolimus are stomatitis, mouth ulcers, acne, infection, and menstrual irregularities. Adverse events that are also common include: rash, diarrhea, fatigue, asthenia, nausea, peripheral edema, decreased appetite, headache, dysgeusia, epistaxis, mucosal inflammation, pneumonitis, weight decreased, vomiting, pruritus, cough, dyspnea, dry skin, nail disorder, aggression, and pyrexia.

Overall, the most frequently observed laboratory abnormalities include: decreased hematology parameters including hemoglobin, lymphocytes, platelets, and neutrophils; increased clinical chemistry parameters including cholesterol, triglycerides, glucose, aspartate transaminases, creatinine, alanine transaminases, and bilirubin; and decreased clinical chemistry parameters including phosphate and potassium.

The majority of these AEs have been of mild to moderate severity (NCI CTC grade 1-2). Recommendations for dose adjustments, should any of these treatment related adverse events occur, are given in Table 3.

6.3.1 Adverse Drug Reactions (ADRs) Reported for Previous Tuberous Sclerosis Complex Studies

The most frequent ADRs (incidence ≥ 1/10 and suspected to be related to treatment by the investigator) from the pooled safety database are (in decreasing order): stomatitis, amenorrhea, upper respiratory tract infections, hypercholesterolemia, nasopharyngitis, acne, menstruation irregular, sinusitis, and pneumonia
The most frequent grade 3/4 adverse reactions (incidence ≥ 1/100 to < 1/10 and suspected to be related to treatment by the investigator) were stomatitis, amenorrhea, pneumonia, neutropenia, pyrexia, and gastroenteritis viral.

The below table shows the incidence of ADRs based on pooled data in patients receiving everolimus in the TSC studies (including both the double-blind and open-label study and extension periods). ADRs are listed according to MedDRA system organ class. Frequency categories are defined using the following convention: very common (≥ 1/10); common (≥ 1/100 to < 1/10); uncommon (≥ 1/1,000 to < 1/100); rare (≥ 1/10,000 to < 1/1,000); very rare (< 1/10,000); not known (cannot be estimated from the available data). Within each frequency grouping, ADRs are presented in order of decreasing frequency.

Infections and infestations
- Very common - Upper respiratory tract infection, nasopharngitis, sinusitis, pneumonia
- Common - Otitis media, urinary tract infection, pharyngitis, cellulitis, pharyngitis streptococcal, gastroenteritis viral, gingivitis
- Uncommon - Herpes zoster, bronchitis viral

Blood and lymphatic system disorders
- Common - Neutropenia, anemia, leukopenia, lymphopenia, thrombocytopenia

Immune system disorders
- Uncommon - Hypersensitivity

Metabolism and nutrition disorders
- Very common - Hypercholesterolemia
- Common - Hyperlipidemia, decreased appetite, hypophosphatemia, hypertriglyceridemia

Psychiatric disorders
- Common - Insomnia
- Uncommon - Aggression

Nervous system disorders
Common - Headache, dysgeusia

Vascular disorders
Common - Hypertension, lymphedema

Respiratory, thoracic and mediastinal disorders
Common - Cough, epistaxis
Uncommon - Pneumonitis

Gastrointestinal disorders
Very common - Stomatitis
Common - Diarrhea, nausea, vomiting, abdominal pain, oral pain, flatulence, constipation, gastritis

Skin and subcutaneous tissue disorders
Very Common - Acne
Common - Rash, dermatitis acneiform, dry skin
Uncommon - Angioedema

Renal and urinary disorders
Common - Proteinuria

Reproductive system and breast disorders
Very Common - Amenorrhea C, menstruation irregular C,
Common - Menorrhagia, vaginal hemorrhage, ovarian cyst, menstruation delayed C

General disorders and administration site conditions
Common - Fatigue, pyrexia, irritability

Investigations
Common - Blood lactate dehydrogenase increased, blood luteinizing hormone increased
Uncommon - Blood follicle stimulating hormone increased

Includes very common: stomatitis, mouth ulcerationaphthous stomatitis; uncommon: gingival pain, glossitis, lip ulceration.

Includes common: rash, rash erythematous.

Includes uncommon: erythema, rash macular, rash maculo-papular, rash generalized.

Frequency is based upon number of women age 10 to 55 years of age in the safety pool.

6.3.2 Management of Infections

Everolimus has immunosuppressant properties and may predispose patients to bacterial, fungal, viral or protozoal infections, including infections with opportunistic pathogens. Patients taking everolimus are therefore at an increased risk of infection. In oncology patients, some infections have been severe, and rarely have had a fatal outcome. Physicians should be aware of the increased risk of infection, and should warn patients and their caregivers to be vigilant for signs and symptoms of infection, and to seek medical attention immediately should such signs or symptoms occur. Treat pre-existing infections prior to starting treatment with everolimus. Should an infection occur, anti-infective should be prescribed as clinically appropriate, and in the case of clinically significant infection, consideration should be given to withholding study medication until resolution of the infection.

As everolimus is an immunosuppressant, live vaccinations should be avoided (as recommended by the Center for Disease Control) (FluMist®, MMR, Varicella, human papillomavirus or HPV, rotavirus or rotarix vaccine, smallpox or vaccinia, rabies vaccine, yellow fever, typhoid Ty21a and BCG). It is okay and recommended for

participants to receive a flu shot every year (unless contraindicated) as that is an inactivated vaccine. This precaution pertains to the risk for inadequate antibody response more than risk for disease from the immunization.

6.3.3 Management of Skin Toxicity

For patients with grade 1 toxicity, no specific supportive care is usually needed or indicated. Rash must be reported as an AE. Patients with grade 2 or higher toxicity may be treated with the following suggested supportive measures at the discretion of the investigator: oral minocycline, topical tetracycline, topical clindamycin, topical silver sulfadiazine, diphenhydramine, oral prednisolone (short course), topical corticosteroids, or pimecrolimus.

6.3.4 Management of Hypersensitivity Reactions

Hypersensitivity reactions manifested by symptoms including, but not limited to, anaphylaxis, dyspnea, flushing, chest pain or angioedema (e.g. swelling of the airways or tongue, with or without respiratory impairment) have been observed with everolimus.

6.3.5 Angioedema with Concomitant Use of Angiotensin-Converting Enzyme (ACE) Inhibitors

Patients taking concomitant ACE inhibitor therapy may be at increased risk for angioedema.

6.3.6 Renal Failure Events

Cases of renal failure (including acute renal failure), some with fatal outcome, occurred in patients treated with everolimus. Renal function of patients should be monitored particularly where patients have additional risk factors that may further impair renal function. Elevations of serum creatinine, usually mild, and proteinuria have been reported in patients taking everolimus. Monitoring of renal function, including measurement of blood urea nitrogen (BUN), urinary protein, or serum creatinine, is recommended prior to the start of everolimus therapy and periodically after.

6.3.7 Management of Mouth Ulcers/Stomatitis/Oral Mucositis

Stomatitis/oral mucositis/mouth ulcers due to everolimus should be treated using appropriate locally available supportive care. Please note that investigators in earlier trials have described the oral toxicities associated with everolimus as mouth ulcers, rather than mucositis or stomatitis. If your examination reveals mouth ulcers rather than a more general inflammation of the mouth, please classify the adverse event as such. Please follow the paradigm below for treatment of stomatitis/oral mucositis/mouth ulcers:

1. For mild toxicity (grade 1), use conservative measures such as **non-alcoholic mouth wash or salt water (0.9%) mouth wash** several times a day until resolution.
2. For more severe toxicity (grade 2 in which case patients have pain but are able to maintain adequate oral alimentation, or grade 3 in which case patients cannot maintain adequate oral alimentation), the suggested treatments are **topical analgesic mouth treatments (i.e., local anesthetics such as benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or phenol)** with or without **topical corticosteroids,** such as triamcinolone oral paste 0.1% (Kenalog in Orabase®).
3. Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.
4. Antifungal agents must be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (ketoconazole, fluconazole, itraconazole, etc.) should be avoided in all patients due to their strong inhibition of everolimus metabolism, therefore leading to higher everolimus exposures. Therefore, topical antifungal agents are preferred if an infection is diagnosed. Similarly, antiviral agents such as acyclovir should be avoided unless a viral infection is diagnosed.

For additional details about the treatment of stomatitis as an everolimus adverse drug reaction (ADR), see the 7904 Study Manual of Procedures.

6.3.8 Management of Hyperlipidemia and Hyperglycemia

Management of hyperlipidemia should take into account the pre-treatment status and dietary habits of the patient. Blood tests to monitor hyperlipidemia must be taken in the fasting state. Hyperlipidemia and hypertriglyceridemia should be treated according to local best clinical practice. Grade 3 hypercholesterolemia (> 400 mg/dL or 10.34 mmol/L) or grade 3 hypertriglyceridemia (>5 × ULN) should be treated as clinically indicated with a 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase inhibitor (e.g. atorvastatin, pravastatin) or appropriate lipid-lowering medication, in addition to diet. Patients should be monitored clinically and through serum biochemistry for the development of rhabdomyolysis and other adverse events as required in the product label/data sheets for HMG-CoA reductase inhibitors.

Note: Concomitant therapy with fibrates and an HMG-CoA reductase inhibitor is associated with an increased risk of a rare but serious skeletal muscle toxicity manifested by rhabdomyolysis, markedly elevated creatine phosphokinase (CPK) levels and myoglobinuria, acute renal failure and sometimes death. The risk versus benefit of using this therapy should be determined for individual patients based on their risk of cardiovascular complications of hyperlipidemia.

Grade 3 hyperglycemia has been observed in patients receiving everolimus therapy. In almost all cases the affected patients had an abnormal fasting glucose at baseline. Based on this finding, it is suggested that optimal glucose control should be achieved before starting a patient on study drug, and that glucose control should be monitored during the trial. More frequent monitoring is recommended when everolimus is co-administered with other drugs that may induce hyperglycemia.

6.3.9 Management of Diarrhea

Diarrhea attributed to everolimus toxicity may be treated with supportive care, such as loperamide, initiated at earliest onset (for example, 4 mg orally followed by 2 mg orally every 2 hours until resolution of diarrhea). Other medications for diarrhea may be used as needed.

6.3.10 Dosing Modifications In Case of Treatment Related Toxicities

Table 3 Criteria for dose modification/re-initiation in case of toxicity suspected to be related to study treatment

Toxicity	Actions
Pneumonitis	See Table 5
Hyperlipidemia and/or hypertriglyceridemia	Any grade: Treat according to best clinical practice. No specific dose reductions are needed.
Hyperglycemia	Any grade: Treat according to best clinical practice. No specific dose reductions are needed.
Stomatitis	Grade 2: Interrupt study drug until resolution to ≤ grade 1. Restart at same dose. Grade 3: Interrupt study drug until resolution to ≤ grade 1. Reintroduce study drug at the next lower dose level**. Discontinue study drug if stomatitis does not recover to ≤grade 1 within 4 weeks. Grade 4: Discontinue study drug

Other toxicities	Grade 2 and 3: Interrupt administration until resolution to ≤grade 1. Restart at same dose. Grade 4: Hold study drug until recovery to ≤ grade 1. Reintroduce study drug at the lower dose level**, if available.
Toxicity requiring interruption for > 6 weeks	Permanently discontinue treatment.
No specific dose adjustments are recommended for Grade 1 toxicity. However, physicians should always manage patients according to their medical judgment based on the particular clinical circumstances. **To determine the next lowest dose level, please refer to Table. 5.	

Table 4 Dose modification guidelines for hematologic toxicities

Toxicity	Actions
Thrombocytopenia Platelet Count	• ≥ 75000/mm^3: No change • 50000/mm^3 to 75000/mm^3: Hold study drug until recovery to **≥ 75000/mm^3.** Reintroduce study drug at the same dose level • < 50000/ mm^3: Hold study drug until recovery to **≥ 75000/mm^3.** Reintroduce everolimus at the next lower dose level, if available
Absolute Neutrophil Count (ANC)	• ≥ 1000/ mm^3: No change • 500/mm^3 to 1000/ mm^3: Hold study drug until recovery to ≥ 1000/mm^3. Reintroduce study drug at the same dose level • < 500/ mm^3: Hold until recovery to ≥ 1000/ mm^3. Reintroduce everolimus at the next lower dose level, if available.
Febrile Neutropenia	Hold further dosing until ANC ≥ 1250/mm^3 and no fever. Then resume dosing at the next lower dose level** if available.
Toxicity requiring interruption for >6 weeks	Permanently discontinue treatment.
Physicians should always manage patients according to their medical judgment based on the particular clinical circumstances.	

6.3.11 Management of Non-Infectious Pneumonitis

Pneumonitis is a recognized adverse effect of rapamycins (sirolimus, temsirolimus, everolimus). Numerous case reports in the literature suggest that rapamycin-associated pneumonitis is relatively non-aggressive, limited in extent and reversible upon drug discontinuation. In the largest completed phase III trial using everolimus in oncology (in patients with metastatic renal cell carcinoma), the frequency of clinically apparent pneumonitis was 14%. 4% of cases were CTC grade 3, and 0 were CTC grade 4. To date, three cases of pneumonitis have had a fatal outcome in more than 4,000 adult oncology patients treated with everolimus. All three cases were associated with overwhelming systemic infection or disease progression.

Individuals participating in this trial will be questioned at each study visit as to the presence of new or changed pulmonary symptoms consistent with lung toxicity. If an investigator suspects a patient may be developing pneumonitis, the patient should be managed according to Table 5. Investigations such as pulmonary function tests, CT chest and referral to a pulmonologist should be considered.

Table 5 Management of non-infectious pneumonitis

Worst Grade Pneumonitis	Required Investigations	Management of Pneumonitis	Study Treatment Dose Adjustment
Grade 1	CT scans with lung windows and pulmonary function testing including: spirometry, DL_{CO}, and room air O_2 saturation at rest. Repeat CT scan at least every 12 weeks until return to within normal limits.	No specific therapy is required	Administer 100% of study treatment dose.
Grade 2	CT scan with lung windows. Consider pulmonary function testing including: spirometry, DL_{CO}, and room air O_2 saturation at rest. Repeat CT scan at least every 4-12 weeks until return to within normal limits. Consider bronchoscopy with biopsy and/or BAL	Symptomatic only. Consider corticosteroids if symptoms are troublesome.	Reduce study treatment dose by 1 dose level until recovery to ≤ Grade 1. Study treatment may also be interrupted if symptoms are troublesome. Patients will discontinue study treatment if they fail to recover to ≤ Grade 1 within 3 weeks.
Grade 3	CT scan with lung windows and pulmonary function testing including spirometry, DL_{CO}, and room air O_2 saturation at rest. Repeat at least every 4-8 weeks until return to within normal limits. Bronchoscopy with biopsy and/or BAL is recommended.	Consider corticosteroids if infective origin is ruled out. Taper as medically indicated.	Hold treatment until recovery to ≤ Grade 1. May restart study treatment within 3 weeks at a reduced dose (by one level**) if evidence of clinical benefit.
Grade 4	CT scan with lung windows and required pulmonary function testing including spirometry, DL_{CO}, and room air O_2 saturation at rest. Repeat at least every 4-8 weeks until return to within normal limits. Bronchoscopy with biopsy and/or BAL is recommended if possible.	Consider corticosteroids if infective origin is ruled out. Taper as medically indicated.	Discontinue treatment.

6.3.12 Follow-Up for Toxicities

Patients whose treatment is interrupted or permanently discontinued due to an adverse event or abnormal laboratory value suspected to be related to study treatment must be followed at least weekly until the adverse event or abnormal laboratory resolves or returns to grade 1. If a patient requires a dose delay of ≥ 6 weeks from the intended day of the next scheduled dose, then the patient must be discontinued from the study.

All patients will be followed for adverse events and serious adverse events for 28 days following the last dose of study drug. Beyond these 28 days, any serious adverse events that are suspected to be related to the study drug that are ongoing at the follow-up call will continue to be monitored for an additional next 8 weeks (56 days) or until the event resolves. Any changes in medication/therapy given during the follow-up period of 28 days will be recorded on the CRF.

7. Description of Study Procedures

7.1 Written Informed Consent

Written informed consent will be obtained from each participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment.

7.2 Process of Consent

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

Parents/guardians/subjects will be provided with a copy of the informed consent, and assent form (if applicable), that explains the purpose of the study, the study procedures, and assessments. Parents/guardians/subjects will also be provided with the contact information of the investigator and qualified personnel who can assist with their questions and concerns.

7.3 Remote Consent Process

Remote consenting procedures will be used for re-consenting participants that are no longer coming to the research site for study related visits. Consenting by remote process should not be used for initial consent, nor for participants actively attending study visits in-clinic. Study staff will use secure methods to communicate and transmit information, such as scanning, faxing, or mailing the documents, each participant or their parent(s)/guardian(s) will be provided with two blank copies of the informed consent/assent documents: one to keep and one to return. Once the participant/parent/guardian has received the consent documents, an authorized research staff member will explain the study and review the consent document, highlighting the changes that have been made to this consent form version, with each participant by phone or videoconference. If assent is required, the authorized staff must have a discussion with both parent/legal guardian and child participant.

Once all questions have been answered and the authorized staff member feels confident that each participant/parent/guardian understands the study and the changes, the participant (adult) and/or a parent/guardian (child) will sign and date the consent form. The signed consent form will be returned to the site via email, fax, or mail. If there is a separate assent form, the participant will need to sign, date, and return the assent with the consent form. When the signed consent form is returned to the study team, the staff member who provided the explanation of the study will sign the appropriate signature line, using the current date. Please note, the site signature date should not be back-dated to coincide with the participant's signature.

It is recommended that site staff document that consent was obtained remotely, via phone or videoconference, with the current date and date the form(s) were mailed/emailed/faxed back. This can be accomplished with a note under the PI signature line on the consent form. Example: "Discussed with [patient name and parent/guardian name] via telephone/videoconference on [insert date], and received sign consent form on [insert date]."

Authorized staff should document the entire informed consent/assent process for each participant in a memo-to-file.

7.4 Patient Numbering

Each patient in the study will be uniquely identified. A Local ID number should be assigned as described in the 7904 Manual of Procedures.

When the patient has signed the informed consent form, the investigator or his/her staff will log onto the electronic data capture system and provide the requested identifying information including the Local ID number for the patient. Participants will maintain the same Local ID for both the Double Blind and Open Label phases, as applicable.

7.5 Randomization Procedure

Subjects will be randomized 1:1 to everolimus or placebo after meeting all eligibility criteria. A designated member of the research team will communicate to the participant that randomization has occurred and sufficient study drug will be dispensed to cover the interval until the next study visit. This is a double-blind study. The study design allows patient unblinding during the double-blind phase only in very precise circumstances (section 7.6.1).

Randomization data are to be kept strictly confidential until the time of unblinding at discontinuation or at the time of final analysis. These data will not be accessible to anyone involved in the conduct of the study with the exception of the unblinded physicians, the Medical Monitor, the Data Safety and Monitoring Committee (DSMC), and the Data Management Coordinating Center (DMCC). The DSMC will review safety data as outlined in section 15. The identity of treatment arms will be concealed by the use of study drugs (everolimus and matching placebo) that are identical in packaging, labeling, schedule of administration and in appearance.

Prior to Baseline (Visit 2), the investigator or his/her designee will log onto the data system to verify that the patient fulfills all eligibility criteria and randomize the patient to a treatment arm. The system will assign a randomization arm, as described in the 7904 Manual of Procedures. The pharmacy will be responsible for dispensing the active drug or placebo based on the procedures outlined in the 7904 Pharmacy Manual of Procedures. The randomization will be stratified by age. Age will be stratified by two levels. Level 1 will be participants aged 5-21 years old, and level 2 will be 22-45 year olds. Permuted block sizes of two and four will be used. At the conclusion of the 6-month double-blind phase, the blind will be broken immediately. Individuals on the active drug will exit the study, and those who were on placebo will be invited to join the open label phase.

7.6 Double-Blind Treatment Assignment Unblinding

Prior to the Month 6 visit or early termination, the site will obtain the unblinded treatment assignment via the procedures described in the 7904 Manual of Procedures. At the conclusion of the double-blind phase or early termination, the blind will be broken immediately. Individuals on the active drug will exit the study, and those who were on placebo will be invited to join the open label phase (section 4.4).

7.6.1 Emergency Unblinding Treatment Assignment

In general, circumstances that might lead to emergency unblinding are rare. Most often, study drug discontinuation and knowledge of the possible treatment assignments are sufficient to treat a study patient who presents with an emergency condition. One unusual circumstance in which unblinding might be necessary is when a patient requires emergency surgery and the anesthesiologist needs to know all medications that the patient has been exposed to in order to make proper decisions about treatment and support during the surgery.

Emergency unblinding should only be done when necessary in order to treat the patient. Emergency code breaks are performed using the database, as described in the 7904 Manual of Procedures.

It is the investigator's responsibility to ensure that there is a procedure in place for emergency unblinding. The investigator will inform the patient how to contact his/her backup in cases of emergency when he/she is unavailable.

Study drug must be discontinued after emergency unblinding. The investigator is not allowed to place emergency unblinded patients into open-label everolimus therapy.

7.7 Treating the Patient

7.7.1 Study Drug Administration

Everolimus or placebo will be dispensed by the study center personnel on an outpatient basis. Patients will be provided with study drug at Visit 2 (Baseline), and each subsequent on-site visit for self-administration at home.

The first dose of everolimus or placebo must be taken within 7 days of Visit 2 (Baseline). Patients will be instructed to take everolimus or placebo by mouth regularly, with the dose being taken at the same time in the morning every day. Everolimus or placebo tablets should be swallowed whole with a glass of water. The tablets should not be chewed or crushed. Any dietary habits around the time of everolimus or matching placebo intake should be as consistent as possible throughout the study. The extent of absorption of everolimus through topical exposure is not known. Therefore, patients/caregivers are advised to avoid contact with the everolimus or placebo tablets and should wear gloves and should wash their hands thoroughly before and after administration. If vomiting occurs, no attempt should be made to replace the vomited dose. Patients should be instructed that if they miss a dose one day, they must not take any extra dose the next day.

Patients should be requested to bring their unused study drug, including the empty blister packs, to the clinic at each visit.

Patients will receive treatment with study drug for 6 months or until the occurrence of unacceptable toxicity or the investigator or patient decides that continuation is not in the best interest of the patient. Interruption for toxicity should follow the instructions in Table 3.

7.7.2 Treatment Compliance

Compliance should be verified by the investigator's staff through counting the number of tablets consumed between visits. Compliance will be assessed by the investigator or his/her designee at each visit using pill counts and review of the drug diary. The site must maintain an overall drug accountability log for the study, as well as individual accountability records for each patient. The dose, amount dispensed, amount taken, and amount remaining unused must be recorded in the source document. The patient will return all unused study drug at each dispensing visit, except Visit 2/Baseline, and at the End of Study visit.

- Patients will be requested to bring their unused medication including empty packaging to the clinic at each visit.
- All doses taken by the patient and all dose changes during the study must be recorded on the Dosage Administration Record CRF and the Dose Adjustment Log CRF, respectively.
- The investigator or his/her designee must keep documentation (overall drug accountability log for the study as well as individual study drug accountability records for each patient) of tablets administered, tablets used, dose changes, dates dispensed and intervals between visits.

7.7.3 Other Permitted Study Drug Adjustments

Standard dosing modifications will be made as described in Section 6.2.

For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments are permitted, as described in Tables 3, 4, and 5, in order to keep the patient on study drug. In addition, if any surgery is planned, trial therapy should be interrupted one week prior to surgery and should be re-started as soon as possible after wound healing.

Dose modifications initiated by the site investigator (such as in response to a toxicity or safety lab level) will not require an everolimus PK level, unless the duration of the change lasts more than two weeks. If this was to occur, and a PK sample needed to be collected, the sample should be true trough. A true trough level is ~24 hours (+/- 4 hours) after the last dose was taken, and after 4 days of consistent dosing.

If treatment is interrupted due to toxicity, study drug should not be resumed until recovery to grade $\leq$ 1 is achieved. If recovery takes 6 weeks or more, the patient should be discontinued from the study. If recovery takes less than 6 weeks, study drug can be reintroduced at the initial dose or a lower dose level depending on the toxicity type and grade (see Tables 3 and 4). These changes must be recorded on the Dosage Administration Record and Dose Adjustment Log CRFs, as appropriate.

Dose adjustments (reduction, interruption, or possible dose re-escalation to starting dose) may also occur based on safety findings. All doses taken by the patient and all dose changes during the study must be recorded on the CRFs. Dose adjustments will also be made in the placebo group according to the placebo adjustment protocol.

7.7.4 Concomitant Medications

Patients must be instructed not to take any additional medications (over-the-counter, herbal, or other products) during the study without prior consultation with the investigator. All medications taken within 30 days of starting study treatment should be reported on the Concomitant Medications/Significant Non-Drug Therapies CRF. The following guidelines must be adhered to during the study:

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

• Co-administration with moderate or strong inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole, ritonavir, erythromycin, fluconazole) or inhibitors of P-glycoprotein (PgP) must be avoided (refer to Table 9 and Table 10)

• Co-administration with strong inducers of CYP3A4, other than antiepileptics, must be avoided. Table 9 lists clinically relevant CYP3A inhibitors, inducers and the definition of strong and moderate inhibitors/inducers.

• Seville orange, star fruit, grapefruit and their juices affect P450 and PgP activity. Concomitant use should be avoided

Everolimus may affect the response to vaccinations making the response to the vaccination less effective. As everolimus is an immunosuppressant, live vaccines should be avoided while a patient is treated with everolimus.

Otherwise, the use of other concomitant medication/therapy deemed necessary for the care of the patient is allowed. The investigator should instruct the patient to notify the study site about any new medications he/she takes after the start of the study drug. All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) administered after the patient starts everolimus and for up to 84 days (12 weeks) after study drug discontinuation must be listed on the CRF.

A letter will be provided to each randomized patient's primary care physician outlining medications to avoid.

Table 9 Clinical relevant drug interaction: inducers and inhibitors of isoenzyme CYP3A

I N D U CE R S
Strong inducers: avasimibe, carbamazepine, enzalutamide, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's wort (*Hypericum perforatum*)
Moderate inducers: bosentan, efavirenz, etravirine, genistein, lersivirine, lopinavir, modafinil, nafcillin, ritonavir, semagacestat, talviraline, thioridazine, tipranavir
Weak inducers: amprenavir, aprepitant, armodafinil (R-modafinil), bexarotene, boceprevir, brivacetam, clobazam, danshen, dexamethasone, Echinacea, eslicarbazepine, garlic (*Allium sativum*), gingko (*Ginkgo biloba*), ginseng, glycyrrhizin, methylprednisolone, nevirapine, oxcarbazepine, pioglitazone, prednisone, pleconaril, primidone, quercetin, raltegravir, ritonavir, rufinamide, sorafenib, Stribild (combo of elvitegravir, cobicistat, emtricitabine, a tenofovir), sulfinpyrazone,telaprevir, terbinafine, ticagleror, ticlopidine, topiramate, troglitazone, vemurafenib, vicriviroc/ritonavir, vinblastine

I N H I B I T O R S

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

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

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

Table 10 Clinical relevant drug interactions medicated by PgP

PgP Substrates	PgP Inhibitors	PgP Inducers
afatinib, alfuzosin, aliskiren, alogliptin, ambrisentan, apixaban, apremilast, aprepitant, atorvastatin acid, atorvastatin, azithromycin, boceprevir, bosentan, carvedilol, caspofungin, ceritinib, cerivastatin, citalopram, colchicine, CP-481,715, cyclosporine, dabigatran, digoxin, docetaxel, domperidone, doxepin, doxorubicin, eribulin, everolimus, fentanyl, fexofenadine, fidaxomicin, fluvastatin, fosamprenavir, atifloxacin, idelalisib, iloperidone, indacaterol, indinavir, irbesartan, lacosamide, lapatinib, levetiracetam, levofloxacin, linagliptin, linezolid, loperamide, losartan, maraviroc, mirabegron, moxifloxacin, naloxegol, nateglinide, nevirapine, nintedanib, lodaterol, paclitaxel, pantoprazole, paroxetine, pazopanib, phenytoin, posaconazole, pravastatin, proguanil, quinidine, quinine, ranolazine, riociguat, risperidone, ritonavir, rivaroxaban, saquinavir, silodosin, simeprevir, simvastatin, sirolimus, sitagliptin, sofosbuvir, sorafenib, tacrolimus, telaprevir, tenofovir, ticagrelor, tipranavir, tolvaptan, topotecan, umeclidinium, valsartan, vardenafil, vincristine, voclosporin, voriconazole	alogliptin, canaglifozin, cremophor RH40, curcumin, ketoconazole, lapatinib, lopinavir/ritonavir, mirabegron, propafenone, simepravir, valspodar, vandetanib, voclosporin	avasimibe, carbamazepine, efavirenz, genistein, phenytoin, quercetin, rifampin, St John's wort

Reference: Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated April-2015 which summarizes DDI data from three sources including the FDA's "Guidance for Industry, Drug Interaction Studies," the University of Washington's Drug Interaction Database, and Indiana University School of Medicine's Drug Interaction Table.

7.7.5 Concomitant Medication Dose Adjustments

Samples for trough everolimus PK levels (drawn 0 to 2 hours before the next scheduled dose) will be taken two weeks (+/- one week) after the following events:

1. everolimus dose increase to a higher level than previously taken
2. reduction in the dose of a CYP3A4 or pGP inducer (e.g. reduction in anticonvulsant dose)
3. starting, or increasing the dose of, a CYP3A4 or pGP inhibitor

Trough is not required for dose re-escalation to a previously-used dose; or for dose decreases.

7.7.6 Study Drug Interruption or Discontinuation

An intent to treat approach will be used. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

The term “interruption” refers to a patient stopping the study medication during the course of the study, but then re-starting it at a later time in the study (as determined by their study doctor).

The term “discontinuation” refers to a patient’s premature and permanent withdrawal from the study treatment. The reason for discontinuation from treatment will be recorded. The patient may discontinue participation in the study for any of the following reasons:

1. adverse event(s)
2. abnormal laboratory value(s)
3. abnormal test procedure result(s)
4. protocol deviation
5. subject withdrew consent
6. Withdrawal by the investigator
7. lost to follow-up
8. administrative problems
9. intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g.-mental status change, large pleural effusion).
10. death

If a patient has discontinued the study drug due to an unacceptable adverse event or an abnormal laboratory value, he/she should not have withdrawal of consent recorded as the reason for discontinuation. Instead, the reason for discontinuation must be recorded as due to adverse event or abnormal laboratory value.

All patients must have evaluations for 28 days after the last dose of study treatment. Participants that have ongoing adverse events at the 28 day follow-up visit will continue to be followed for an additional 8 weeks (56 days) to record any SAEs that are suspected to be related to study drug. Patients lost to follow up should be recorded as such on the CRF. Patients who discontinue study drug before completing the study should be scheduled for a visit as soon as possible, at which time all of the assessments listed for End of Treatment visit will be performed. At a minimum, all patients who discontinue study treatment, including those who refuse to return for a final visit, will be contacted for safety evaluations during the 28 days following the last dose of study drug. Patients will also be asked a series of neurocognition follow-up questions by phone and will complete the CBCL and DCDQ at the final follow-up call.

7.7.7 Withdrawal from the study and study evaluation completion

Patients may voluntarily withdraw from the study or be dropped from it at the discretion of the investigator at any time.

As a general rule, if a patient discontinues study drug and later is withdrawn from the study, the reasons for study evaluation completion may include the following:

- Protocol deviation
- Subject withdrew consent
- Lost to follow-up
- Death
- Use of non-study systemic anti-PTEN therapy
- Administrative problems

For patients who are lost to follow-up, the investigator should show due diligence by documenting in the source documents steps taken to contact the patient, e.g., dates of telephone calls, registered letters, etc.

7.7.8 Study Discontinuation

The National Institutes of Health (NIH), Data and Safety Monitoring Committee (DSMC) and local IRBs (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- Early stopping rules have been met
- Accrual has been met
- The study objectives have been met
- The Study Chair / Study Investigators believe it is not safe for the study to continue
- The DSMC suspends or closes the trial
- The NIH suspends or closes the trial
- The FDA suspends or closes the trial

7.8 Good Clinical Practice and Human Subjects Training

All personnel conducting human research as part of the Consortium will have completed an approved training course on human subjects' protection as required by their local institution; training is updated, and will be current at the time they are engaged in Consortium clinical activities. All personnel are required to read and agree to adhere to the FDA Good Clinical Practice Guidelines. Documentation of trainings will be maintained by each site in their regulatory binder.

7.9 Neuropsychological Evaluations

The measures included were selected to cover the full age range included in the trial. This combination of age and developmental level does mean that floor or ceiling effects may be seen in a small proportion of study participants on some of the measures. The approach in the study will be to use very experienced testers to administer measures. The testers will be asked to attempt all direct measures on all participants, with the acknowledgement that, in some instances it may not be possible to complete all tasks. All measures will be re-administered as per study protocols, regardless of previous floor or ceiling effects.

7.9.1 Training and Reliability of Independent Evaluations (IEs)

At each site, an experienced team will conduct study procedures guided by an operations manual. IEs, who are blind to treatment condition, will rate 3 measures in this study: CGI-S, CGI-I, and ADOS-2. Similar to previous studies, IEs will be trained to reliability criterion on the CGI-S, CGI-I, and ADOS-2 on 3 to 4 vignettes at the start and yearly after that [42,43]_ENREF_16. If IEs fail to meet reliability criterion, they will be re-trained and given a second set of 3 vignettes for scoring. This procedure will be repeated as necessary to ensure that each rater has demonstrated reliability.

7.9.2 Research Report

Upon request, participants/parents will be provided with a research report with a summary of assessment scores. This report will be provided at the end of treatment.

7.9.3 Main Efficacy Outcomes

Neurocognitive Composite

A composite neurocognitive score will be generated based on performance on objective measures. The composite index score will be computed in two ways. The first will be an average of measures evaluating working memory (SB-5 working memory subscale), processing speed (CPT-3 mean reaction time), and fine motor skills (Purdue Pegboard Test-average of both hands). The second will include the above average weighted by two-thirds and an average of the remaining standardized, norm-referenced neurocognitive measures (e.g., non-verbal ability, visuomotor skills, verbal learning, receptive and expressive language) weighted by one-third. The first method will be preferred if nearly complete data is observed on the 3 primary constructs (working memory, processing speed, and fine motor skills) in randomized patients. The second will be preferred if missing data is present on >20% of randomized patients. In order for any patient to be included in the study they must complete measures from at least one of the three primary efficacy constructs. If patients cannot complete the full Stanford Binet, the Mullen Scales of Early Learning may be substituted. In a similar

fashion, if the CPT-3 cannot be completed, the processing speed index of the Wechsler scales may be substituted as an alternative measure of processing speed.

Measures were chosen based on previous empirical findings in a cross-sectional cohort study of PTEN patients with ASD. In this study, measures of working memory and processing speed were differentially impaired relative to other cognitive measures, including IQ. Furthermore, the cognitive deficits seen in these patients were related to reduced PTEN protein levels and brain white matter abnormalities. Specifically, in a cross-sectional mediational model, reduced PTEN protein levels led to greater brain white matter abnormalities which, in turn, led to greater cognitive difficulties. Although not evaluated using standardized testing, in our prior cohort of PTEN patients with ASD, every patient had a history of fine motor difficulties, occupational therapy, and/or observations during testing of significant fine motor weaknesses. Motor difficulties are also consistent with brain white matter abnormalities. Thus, the neurocognitive index will include measures that have both empirical and biological bases for inclusion as outcome measures.

In the second version of the neurocognitive composite, the primary justifications for including the remaining neurocognitive measures are: 1) if significant missing data is seen on measure of processing speed, working memory, and fine motor skills, including a minor component of additional neurocognitive measures will ensure more reliably obtained data is contributing to the measurement of change, and 2) PTEN expresses in all of the cells of the body, the brains of PTEN patients showed widespread changes that included white matter abnormalities but also regional gray matter changes, and mouse models of PTEN loss have suggested that dendrite and synaptic dysfunction is also a consequence. Thus, it is reasonable to expect a range of neurocognitive functions might improve. Inclusion of other neurocognitive measures as the minority part of the index will ensure that improvements in other domains, such as expressive language, also have an opportunity to contribute to individual patient outcome measurements. Given that we do not yet know with certainty which neurocognitive functions are most likely to improve in this whole brain condition, allowing for possible improvement in other domains seems prudent.

Use of a neurocognitive composite score has several advantages. Assuming that everolimus treatment does not impair some functions while improving others (an unlikely scenario), a composite score is likely to be highly reliable, can be computed in all patients even if 1 or 2 measurements are missing or could not be completed, and has good domain coverage to capture possible variability across patients in the specific neurocognitive domains showing improvement. An example of the latter advantage, is if some individuals show improvements in processing speed while others show improvements in working memory. The neurocognitive composite would identify improvements in both of these sets of patients. As such, the neurocognitive composite may be more sensitive to change than any individual measure. Furthermore, a composite primary efficacy outcome will not require additional multiple comparison correction.

7.9.4 Additional Secondary Efficacy Outcomes

Attention Improvement
Conners' Continuous Performance Test, Third Edition (CPT-3/K-CPT-2): Discriminability index (d') and Omissions

Processing Speed Improvement
Conners' Continuous Performance Test, Third Edition (CPT-3/K-CPT-2): Mean Reaction Time
Weschler Processing Speed Index Subtests: Processing speed if CPT cannot be administered

Impulsivity Improvement
Conners' Continuous Performance Test, Third Edition (CPT-3/K-CPT-2): Commissions and Bias (B')

Working Memory Improvement
Stanford-Binet Intelligence Scales, Fifth Edition (SB-5) Working Memory: Standard Score

Global Clinical Improvement
Clinical Global Improvement Scale; Severity and Improvement subscales

Improving Global Cognitive Ability

Stanford-Binet Intelligence Scales, Fifth Edition (SB-5) or Mullen Scales of Early Learning; Full scale, verbal and nonverbal ability (IQ)

Improving Long-Term Memory

Wide Range Assessment of Memory and Learning, Second Edition (WRAML-2) – Verbal Learning: Scaled Score

Improving Language

Peabody Picture Vocabulary Test (PPVT-4): Standard Score
Expressive Vocabulary Test Second Edition (EVT-2): Standard Score

Improving Motor Deficits

Purdue Pegboard (Pegs): Dominant and non-dominant hand combined standard scores
Developmental Coordination Disorder Questionnaire (DCDQ): Total score

Targeting Autism Symptoms

Autism Diagnostic Observation Schedule (ADOS): Calibrated severity scores

An interviewer-based, semi-structured observational schedule for the assessment of individuals with possible autism spectrum disorders. The ADOS is widely regarded as an excellent phenotyping and diagnostic aid, and is used in many large-scale autism studies throughout the world, including the AGRE (Autism Genetic Resource Exchange www.agre.org) and IMGSAC (International Molecular Genetics Study of Autism Consortium www.well.ox.ac.uk/monaco/autism/IMGSAC.shtml) studies.

Autism Diagnostic Interview – Revised (ADI-R): Item scores (ever/most abnormal)/ current

For children with mental age <18 months, clinical judgment should be used when determining the reliability/validity of an ADI-R administration. The ADI-R is a semi-structured diagnostic parent interview that probes for current behaviors and a developmental history consistent with autism symptomology. The ADI-R contains questions about an individual's early development, communication, social interaction and patterns of behavior.

Social Responsiveness Scale, Second Edition (SRS-2): Total T-score
Repetitive Behavior Scale – Revised (RBS-R): Total raw score

Improving Associated Behaviors

Behavior Rating Inventory of Executive Function (BRIEF): General executive composite and sub-scale scores
Adult/Child Behavior Checklist (CBCL ARF/CBCL): Total problems T score
Short Sensory Profile (SSP): Total Score

Improving Adaptive Behaviors

Vineland Adaptive Behavior Scales-III (VABS-III) Caregiver report: Composite and scale scores.

A measure of functional adaptive behavior, i.e. ability to cope with personal and social skills in everyday life that may be deficient in autism or developmental delays. Tester completes a questionnaire through interview with parent or caregiver. Any age. No parallel forms. *Outcome measures: raw scores converted to scaled scores, then a summary value for a number of domains.*

Table 11 Neuropsychological Assessments Timeline

Instrument	Information Source	Screening (Visit 1)	Baseline^ (Visit 2)	3-month# (Visit 5)	6-month# (Visit 8)
Primary Efficacy Outcome Neurocognitive Composite	Testing		X	X	X
Stanford-Binet Fifth Edition (SB-5) / Mullen Scales of Early Learning (MSEL)	Testing	X			X
Columbia-Suicide Severity Rating Scale	Interview	X		X	X
Stanford-Binet Working Memory Subscale Only	Testing		X	X	
Conners' Continuous Performance Test (CPT-3/K-CPT-2)	Testing	X	X	X	X
Wechsler Processing Speed Index (PSI)	Testing		X	X	X
Wide Range Assessment of Memory and	Testing		X	X	X

Learning-2 (WRAML-2)					
Peabody Picture Vocabulary Test – Fourth Edition (PPVT-4)	Testing		X	X	X
Expressive Vocabulary Test – Second Edition (EVT-2)	Testing		X	X	X
Purdue Pegboard (PP)	Testing	X	X	X	X
Autism Diagnostic Observation Schedule (ADOS-2)	Observational		X		X
Social Responsiveness Scale – Second Edition (SRS-2)	Parent/Caregiver/Informant or Self- Report		X	X	X
Repetitive Behavior Scale – Revised (RBS-R)	Parent/Caregiver/Informant or Self Report		X	X	X
Developmental Coordination Disorder Questionnaire (DCDQ)	Parent/Caregiver/Informant or Self Report		X	X	X
Behavior Rating Inventory of Executive Function (BRIEF)	Parent/Caregiver/Informant or Self Report		X	X	X
Adult/Child Behavior Checklist (CBCL ARF/CBCL)	Parent/Caregiver/Informant or Self Report		X	X	X
Short Sensory Profile (SSP)	Parent/Caregiver/Informant or Self Report		X	X	X
Vineland Adaptive Behavior Scales (VABS-III) – caregiver report	Parent/Caregiver/Informant or Self Report		X	X	X
Autism Diagnostic Interview (ADI-R)	Direct Interview with Parent/Caregiver/Informant		X		

Note: Individuals who are participating in the longitudinal study: Natural History of Individuals with Autism and Germline Heterozygous PTEN Mutations can leverage measures completed within +/- 6 month for this study at the discretion of the study psychologist.

^ *Procedures completed at the Double-Blind Month 6 visit will qualify as Open-Label baseline procedures, as applicable.*

Visits occur in both Double-Blind and Open-Label treatment phases, as applicable.

7.10 Dermoscopy Assessment

A dermoscopy exam will occur at baseline, 3-month and 6-month visits.

The dermoscopy assessment will be both non-invasive and objective. Exam will focus on the change in number, location, and size of skin manifestations commonly associated with PTEN mutations and will be performed by a physician at each site.

If feasible, de-identified photos of skin lesions will be taken and later evaluated by a blinded independent rater. The independent rater will evaluate change in skin lesions over time with respect to size, shape, etc.

7.11 Eye Tracking (Optional)

If parents/patients opt-in to eye tracking tasks, they will be conducted at baseline, 3-month and 6-month visits.

Eye tracking hardware. Gaze data will be collected using a remote eye tracker system available at all sites. Binocular gaze, 3D eye position, pupil, and timestamp data will be collected at a sampling rate of 60/120/250Hz. Gaze capture is automatically calibrated and provides position accuracy to 0.4° at a 60cm viewing distance

(spatial resolution 0.3°). Dwell time (proportion on target relative to total time on screen), time-to-first-fixation, and fixations (30 pixel area for 100ms) to regions-of-interest (ROIs) will be the primary measurements. Saccades between targets, revisiting between targets, and blink metrics will also be evaluated for potential validity.

Remote eye gaze acquisition and scoring. Eye gaze data will be captured during viewing of a brief battery (7 min) that consists of initial calibration and multiple stimuli from each of the following paradigms: dynamic individual faces, static side-by-side faces, joint attention bids, gaze following, reciprocal interactions, dynamic social versus dynamic geometric images, and passive viewing of social/object arrays. In each of the socially-focused stimuli, elements will be coded as social or non-social ROIs. Dwell times/fixations will be computed to each a priori target ROI. Separate social and non-social attention indices will be computed by averaging dwell times to social and non-social targets, respectively. An aggregate attention index (see preliminary data) will be computed by averaging dwell times to social and non-social targets.

Receptive language stimuli will also be shown at the end of the battery. These will include picture arrays with progressively larger numbers of stimuli (2, 4, 6, 8, 10 items per array) and more challenging stimuli (easy item: dog, hard item: tornado).[44,45] Pictures will include common objects or actions. Time-to-first fixation to the receptive language target within 2 seconds of the end of the directive to look at the target (e.g., "Look at the ball") will be computed. These times will be averaged across all receptive language stimuli to generate an average receptive language score.

For the whole battery, only individuals showing at least 50% total time on screen during the viewing period will be included in analyses, based on recent recommendations for eye tracking in children with autism.[46] For both the attention indices and receptive language score, fixation times will be manually inspected to detect outliers and distributions will be transformed, if needed. All stimuli will be presented using a standard package. Fixation/dwell times and time-to-first-fixation will be computed using available software systems. Data from the 3 sites will be transferred to Cleveland Clinic where it will be analyzed under the consultation of Thomas Frazier, PhD.

7.12 Resting State Encephalography and Auditory Evoked Potential (Optional)

Resting state encephalography (EEG) and auditory evoked potential (AEP) procedures are optional. Resting state encephalography (EEG) and auditory evoked potential (AEP) data collection will occur at baseline, 3-month, and 6-month visits if the parents/patients have opted in on the consent form.

To our knowledge, there have been no prior studies of quantitative EEG or AEP in individuals with PTEN mutations. Therefore, a key goal of including the EEG and AEP components in this trial is to offer the opportunity to collect and analyze such data. Given this knowledge gap, the choice of paradigms and proposed analyses were informed by (a) EEG findings in a mouse model of PTEN that offer translational potential, and (b) EEG and AEP findings in individuals with developmental synaptopathies and/or symptomatology related to PTEN.

In PTEN conditional knockout mice, loss of PTEN in cortical GABAergic neurons leads to a loss of somatostatin+ interneurons, an increased ratio of parvalbumin/somatostatin interneurons, and thus increased inhibition of glutamatergic cortical neurons. In these mice, baseline prefrontal gamma power (particularly in the high gamma range, 62-90Hz) is reduced compared to that of wild type mice, but is significantly increased (compared to wild type) during a social interaction task.[47] It is also worth noting, given the increased risk of autism in individuals with PTEN mutations, that individuals with autism spectrum disorder are often found to have increases in baseline gamma power on EEG.[48] Additionally, increased baseline gamma power has been seen in other genetic disorders on the P13K/AKT/mTOR and MAPK pathways, including tuberous sclerosis,[49] Fragile X,[50] and 16p11.2 deletions (preliminary data).

Altered temporal coordination of neural oscillations, as measured by altered intra-individual variability and altered habituation in responses to a repetitive stimuli, has also been seen in a number of disorders in which symptomatology overlaps with that frequently seen in individuals with PTEN mutations.[51] For example, in individuals with Fragile X, an auditory gating task reveals decreased habituation of the N1 event-related potential response between the first and second tone in a train of repetitive tones. Such altered gating has been linked to altered sensory processing,[52] and to behaviors associated with autism.[53] Additionally, individuals with

Fragile X and idiopathic autism demonstrate altered phase locking, a measure of intra-individual response variability to repeated stimuli.[54,55] Of note, it has been suggested that such findings may be related to the activity of parvalbumin+ and somatostatin+ interneurons.[54,56,57]

Taken together, these findings lead to a hypothesis that the altered relationship between excitation and inhibition seen in PTEN conditional knockout mice may result in abnormalities in baseline gamma power on EEG and altered response variability and habituation on an auditory gating paradigm. In turn, abnormal EEG and AEP activity may be associated with some of the neurobehavioral findings frequently seen in PTEN. The paradigms chosen in this study were chosen primarily for the likelihood that individuals with PTEN mutations may demonstrate abnormalities on these paradigms, and thus for the potential that in the future such paradigms could be used as a biomarker to measure change over time in response to treatment. In addition, while this particular study does not include a control group without PTEN mutations, the paradigms were chosen for their overlap with other ongoing studies, to potentially allow for comparisons of findings in the future. For example, the baseline EEG paradigm is currently being used for several other studies examining EEG findings in individuals with idiopathic autism, genetic disorders such as TSC, and typically developing controls. The AEP paradigm, in addition to having been used in Fragile X, is currently being used in a study of individuals with Phelan-McDermid syndrome, which is also part of the Developmental Synaptopathies Consortium.

Resting EEG Procedures: Resting state EEG has been used increasingly to quantitatively characterize and track outcomes in a range of neuropsychiatric populations, including idiopathic autism. EEG leads will be placed on the participant's head, and EEG data will be collected while participants are presented with non-social, abstract moving images in random order. The resting EEG paradigm will take about 5 minutes.

AEP Procedures: Auditory evoked potentials will be collected using the same EEG system as the resting EEG, with clicks as the auditory input. Subjects will passively listen to 150 sets of two 5 ms broadband noise bursts (65 db) separated by an inter-stimulus interval of 500 ms with inter-set intervals of 4,000 ms (total duration=~12 mins). Offline, data will be average referenced and filtered for time-frequency analyses.

EEG/AEP data from the three sites will be transferred and analyzed by April Levin, M.D. The data will be transferred from CCF and Stanford to Boston by using Children's Hospital Boston File Transfer (CHBFT). This system has been approved by the IRB for use of secure file transferring. Once transferred, data will be stored at BCH on a password-encrypted computer and only research staff will have access to the files.

Along with the EEG/AEP data, photographs will be taken of the electrode placements on participants' head. These photos will allow the research staff to view electrode placement and make informed decisions concerning poor data in relation to incorrect electrode placement. Participants will also be videotaped during the EEG and AEP procedures. By videotaping participants, researchers will be able to determine whether abnormal EEG data may be due to the participant's behavior. The photographs and videos will allow the research staff to ensure appropriate collection methods are used. Both the photographs and the videos that are collected will be sent to Dr. April Levin at Boston Children's Hospital via secure methods. This data will be stored on securely at the DMCC.

7.13 Safety Outcomes

The Dosage Record and Treatment Emergent Symptom Scale (DOTES): This is a general rating scale published by the Early Clinical Drug Evaluation Unit of the National Institute of Mental Health. The scale has been widely used clinically for both children and adults to assess many central nervous system side effects as well as some behavioral side effects. It will be completed at screening, baseline, and at each subsequent visit. In addition, patients will be interviewed using the C-SSRS to proactively assess patients for suicidality and mood disturbances. If the patient's and/or parents' responses are of concern, the investigator performing the interview may refer them to a mental health professional at the hospital. More detailed information about the C-SSRS can be found in section 4.6.5. Specific adverse events will also be classified and graded according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Use of CTCAE will allow direct comparison with past and current clinical trials with everolimus, including the neurocognitive trial described in previous sections (NCT01289912). Vital signs (blood pressure, pulse, and temperature), height and weight will be obtained during screening, baseline, and at each subsequent visit.

Table 12. Primary and Secondary Efficacy and Safety Outcome Measures.

	Domain	Measure
Primary Efficacy Outcome	Neurocognitive Composite	Average working memory (SB-5 working memory), processing speed (CPT mean reaction time) and fine motor (Purdue Pegboard-average of both hands) subtests weighted at 2/3 of the composite score and the average of all other available neurocognitive testing measures (receptive and expressive language, non-verbal ability, verbal learning, sustained attention, impulsivity, and visuomotor skills) weighted 1/3 of the score.
Secondary Efficacy Outcomes	Global Ability	Stanford-Binet-5 (SB-5): Full scale IQ, Verbal IQ, and Non-Verbal IQ or the Mullen Scales of Learning: Cognitive IQ
	Attention	Conners' Continuous Performance Test -3 (CPT-3/K-CPT-2): Discriminability (d') and Omissions
	Processing Speed	Conners' Continuous Performance Test -3 (CPT-3/K-CPT-2): Mean Reaction Time; in cases where an individual cannot complete the CPT we will administer the appropriate Wechsler processing speed index subtest.
	Impulsivity	Conners' Continuous Performance Test (CPT): Commissions and Bias
	Long-Term Memory	WRAML-2 Verbal Learning: Scaled score
	Language	Peabody Picture Vocabulary Test -4 (PPVT-4): Standard Score Expressive Vocabulary Test – 2 (EVT-2): Standard Score
	Motor Functioning	Purdue Pegboard (Pegs): Dominant and non-dominant hand standard scores
	Motor Coordination	Developmental Coordination Disorder Questionnaire (DCDQ): Total score
	Autism Symptoms	Autism Diagnostic Observation Schedule (ADOS): Calibrated severity score
		Social Responsiveness Scale – 2 (SRS-2): Total T-score
		Repetitive Behavior Scale – Revised (RBS-R): Total raw score
	Other Behavioral Symptoms	Behavior Rating Inventory of Executive Function (BRIEF) - Global Executive Composite: Standard Score
		Child Behavior Checklist - Total Problems: Standard Score
	Sensory Processing	Short Sensory Profile (SSP): Total Score
	Adaptive Behavior	Vineland Adaptive Behavior Scales (VABS-III): Composite Standard Score
	Global Severity and Improvement	Clinical Global Impressions-Severity (CGI-S) Clinical Global Impressions-Improvement (CGI-I)

7.14 Vital Signs

Pulse, respiration rate, blood pressure and temperature, height and weight will be measured as indicated in the Assessment schedule (Table 13). Blood pressure, pulse and respiration rate should be measured on patients after at least 3 minutes in the sitting position.

7.15 Body Surface Area

Body Surface Area (BSA) should be calculated before the baseline visit. The measurements taken at the screening visit should be used to perform this calculation. The same process will be used at the Month 1 visit (i.e. using the baseline height and weight for the calculation) to ensure the patient's dose has not changed. BSA (in m^2) should be calculated via the Cornell online BSA calculator: http://www.users.med.cornell.edu/~spon/picu/calc/bsacalc.htm, or by using the following formula where weight (W) is in *kilograms* and height (H) is in *centimeters* (Dubois and Dubois, 1916):

$$BSA = (W^{0.425} x\ H^{0.725}) \times 0.007184$$

The BSA will indicate the starting medication dose for all participants, which will be calculated for the baseline and Month 1 visits. After the Month 1 visit, dosing should be based on the dose adjustments determined by the unblinded physician, and/or the study physician, as described in Section 6.2. The 2.5mg tablets will be used to make the adjustments. Sites may substitute 5mg or 10mg tablets as described in Section 6.2.

7.16 Laboratory Evaluations

All standard clinical laboratory analyses described below are to be performed by the central laboratory (LabConnect and their subcontracted entities). PK samples and serology panels will be processed by ARUP Laboratories. Blood may be drawn at the site, physician office or by traveling phlebotomist as appropriate, according to the Visit Schedule outlined in Table 13. The volume of sample collection is listed in Table 14.

7.16.1 Hepatitis and HIV Screening

Patients who have a positive test for hepatitis B, hepatitis C, or HIV at screening must be excluded from the study unless the positive Hepatitis B antibody test resulted from prior vaccination. The hepatitis and HIV tests for antibodies will be done only at screening, at the principal investigator's discretion, based off the risk factor assessment (see Table 13).

7.16.2 Hematology

Hematology tests are to be performed at each scheduled onsite visit (± 14 days) as indicated in Table 13. These must include: hemoglobin, hematocrit, platelets, red blood cell count (RBC), total white blood cell count (WBC) absolute & differential including neutrophils, lymphocytes, monocytes, eosinophils, basophils). Absolute neutrophil count (ANC) will be calculated by the laboratory.

7.16.3 Coagulation

Prothrombin time will be determined at screening, 3 months and 6 months (end of treatment) will be reported as international normalized ratio (INR). In addition, fibrinogen and partial thromboplastin (PTT) will be determined at screening, 3 months and 6 months.

7.16.4 Serum Pregnancy/Hormone Testing

Serum Pregnancy
All females of childbearing potential must have a negative serum pregnancy at screening. Urine pregnancy tests may be used for onsite visits (if deemed clinically necessary). A serum pregnancy test will be administered again at the final six-month visit.

Adequate contraception, excluding estrogen containing contraceptives, must be used on-study and for up to 8 weeks after ending treatment. Abstinence will be considered an acceptable contraceptive.

To ensure patient safety, each pregnancy in a patient on study drug must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the local Novartis Integrated Medical Safety ("IMI") Department. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the Novartis study drug of any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

Male Contraception
Sexually active males must use a condom during intercourse while taking the drug and for 8 weeks after stopping treatment and should not father a child during this period.

Pregnancy outcomes must be collected for the female partners of any males who took study drug in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

<u>Hormone Testing</u>
For all participants, female or male, over the age of 7 years, a hormone test will be done at the screening visit and again at the month 6 visit.

For all participants under the age of 7 years, a sample will be drawn for estradiol, LH, and FSH at the screening visit and again at the month 6 visit.

The potential for everolimus to cause infertility in male and female patients is unknown. However, menstrual irregularities, secondary amenorrhea and associated luteinizing hormone (LH)/follicle stimulating hormone (FSH) imbalance has been observed. Based on non-clinical findings, male and female fertility may be compromised by treatment with everolimus.

7.16.5 Biochemistry and Lipid Profile

The following tests will be performed at each scheduled onsite visit (Screening, Month1, Month 3, Month 6 visits) (± 14 days) and will include sodium, potassium, chloride, bicarbonate, creatinine, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid, BUN, calcium, magnesium, phosphate, total LDH, and fasting glucose. A sample will be drawn for T4 and TSH at the screening visit and again at the 6 month visit.

In order to assess renal function during the trial, an age appropriate measure, either the Cockcroft-Gault (1976) formula the Schwartz (2009) formula.

The Cockcroft-Gault formula, for patients 18 and older, is shown below, where creatinine clearance is "*x*" (in Ml/min), age is measured in years, weight in kg, creatinine in µmol/L, and the constant "K" is 1.23 for men and 1.04 for women (Cockcroft-Gault, 1976).

$$x = \frac{(140 - Age)\ x\ Weight\ x\ K}{Serum\ Creatinine}$$

The Schwartz formula is shown below, where Glomerular Filtration Rate (GFR) is "*x*" (in Ml/min/1.73 m^2), height is measured in cm and serum creatinine in mg/Dl (Schwartz et al, 2009).

$$x = \frac{0.41\ x\ Height}{Serum\ Creatinine}$$

A lipid profile (cholesterol, triglycerides, LDL, HDL) will be determined at screening, 3 months and 6 months (end of treatment). The patient must be in a fasting state at the time of blood sampling for this evaluation.

7.16.6 HbA1c

A HbA1c, hemoglobin A1c, test will be collected from patients at the screening visit. This test monitors a patient's average blood glucose over the last 2 to 3 months. The test will inform study staff about a patient's fasting glucose and/or diabetes mellitus, which is used when determining eligibility. Uncontrolled diabetes mellitus as defined by HbA1c >8% despite adequate therapy, will be exclusionary. Patients with a known history of impaired fasting glucose or diabetes mellitus (DM) may be included, however blood glucose and antidiabetic treatment must be monitored closely throughout the trial and adjusted as necessary;

7.16.7 Urinalysis

A standard urinalysis assessment (pH, protein, glucose, blood, ketones, and leukocytes) should be performed at each onsite visit. This must be supplemented with laboratory quantification of any potentially relevant abnormalities when dipstick test is abnormal.

7.16.8 Blood Sample for Everolimus (Everolimus Levels)

A maximum volume of 3 ml of blood will be drawn for a trough everolimus level. Blood draws will be timed to occur at 24 ± 4 hours after ingestion of the drug. Everolimus levels will be measured at ARUP Laboratories associated with LabConnect in Salt Lake City, UT. For families that do not live close to the study site, there are

two options for remote blood collection. A kit for remote collection of blood samples can be sent to the participant and returned per the shipping instruction by express mail, or a designated and trained remote health care provider can collect a PK sample and ship it per the shipping instruction by express mail. A central unblinded investigator will be responsible for dose adjustment in both the treatment and placebo arms. If the everolimus level falls outside the range of 5-15 ng/ml, the local research team and PI will be notified that a dose adjustment is needed, the dose adjustment will be implemented by the local investigator per protocol and unblinded investigator direction, and the blood level will be redrawn two weeks (+/- one week) later. The site team is blinded with respect to treatment assignment at all times. This process will be repeated until the everolimus level falls within the target range. Dose adjustments will be randomly pre-assigned to occur in the placebo arm of this trial. The DMCC will assign dose adjustments to placebo randomization codes and share this information with the unblinded physician who will facilitate a dose adjustment at the appropriate time-point for these placebo participants.

A pre-dose trough blood sample will be collected immediately prior to dosing at month 1 (visit 3). This sample should be collected prior to the patient's next dose of study drug ~24 ± 4 hours after the last dose. The central unblinded investigator will be responsible for dose adjustment, using the 2.5mg tablets, to achieve the target everolimus concentration range of 5-15 ng/ml. Sites may substitute 5mg or 10mg tablets as described in Section 6.2.

Additional pre-dose trough blood samples will be taken if a dose adjustment occurs due to toxicity or due to concomitant medication dose adjustment (See Section 7.6.3). Should clinically significant changes in the patient's height and/or weight occur at any time point during the study, the principal investigator may choose to have additional pre-dose trough blood samples taken.

Samples will be frozen as whole blood within 2 hours after collection at -80°C and transported to the central laboratory, LabConnect, for analysis. Everolimus concentrations will be analyzed in whole blood using a validated method.

7.16.9 Additional Blood Sample for PTEN Associated Proteins

An additional blood sample (3.5 ml) for future research studies will be allowed during the course of study participation with the subject's consent and authorization. Samples will be obtained at three study time points (screening or baseline, 3-month visit, 6-month visit) for serum, RNA and plasma for future biomarker research projects regarding PTEN associated proteins. When possible, blood draw will be combined with clinically-indicated venous blood tests to minimize the need for additional venipuncture and total blood volume for combined clinical and research studies will not exceed NIH recommended guidelines for subject age and weight. The consent form includes a checkbox for participants to decide if they want to provide an extra blood sample for future biomarker research projects. Samples will be de-identified and sent to the Genomic Medicine Institute Biorepository at Cleveland Clinic.

7.16.10 Additional Blood Sample for Insulin Like Growth Factor Binding Protein 2 (IGFBP-2)

An additional blood sample (3.5 ml) for IGFBP-2 analysis will be collected at the screening or baseline visit, 3 month, and the 6 month visit. IGFBP is a useful biomarker of response, as over expression of IGFBP-2 has been associated with cancers and overgrowth syndromes. In a short treatment trial, we may be able to capture changes in IGFBP expression due to everolimus that shows promising evidence of therapeutic value in patients with PTEN. This measure will serve as a surrogate biomarker of effect. Samples will be de-identified and sent to the Genomic Medicine Institute Biorepository at Cleveland Clinic for analysis.

7.16.11 Microbiome/Mycobiome Sample Collection

When possible, we will collect optional saliva, urine, and fecal samples from all patients at baseline, month 3, and month 6 of the double-blind treatment phase and during the open label phase. Patients will receive the kits at home from Cleveland Clinic. Coordinators will instruct the participant/family to collect the samples ±14 days from the onsite visit. Once the samples are collected, the kit should be shipped back to Cleveland Clinic. The kit will also contain these instructions, all materials needed to collect the samples, and a return label.

These samples will be sent for storage at the Genomic Medicine Biorepository (GMB). If funding becomes available, future correlative studies will investigate PTEN pathway protein extraction and quantification, RNASeq, proteomic, microbiomic, and mycobiomic analyses.

7.17 Data from Samples

Data obtained from samples may also be shared with publically accessible databases, such as the database of Genotypes and Phenotypes (dbGaP).

7.18 Medical Records

We will collect medical records and clinical reports (if available) for screening and confirmation of medical history.

All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) administered after the patient starts everolimus and for up to 56 days (8 weeks) after study drug discontinuation must be listed on the CRF. All AEs and SAEs, regardless of causality will be collected and recorded up until 56 days (8 weeks) after study drug has been discontinued. This information will be collected on the CRFs listed under the phone call that happens after 1-month (28-days) of stopping study medication and/or completing the trial.

7.19 Follow-Up Questionnaires

All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) administered after the patient starts everolimus must be listed on the CRF. All AEs and SAEs, regardless of causality will be collected and recorded until the study drug has been discontinued. This information will be collected by phone call at 28 days post-treatment termination and for 8 additional weeks after, as indicated.

At the follow-up time point we will collect information about behavior (CBCL) and motor skills (DCDQ) as well as overall neurocognitive outcome. Parental questionnaires will be sent home and completed by the family of the participant. The questions about overall neurocognitive outcome will be conducted over the phone.

Table 13A: Double-Blind Treatment Phase Study Timeline

Measurement	Performed by	Rationale	Screen (Visit 1)	Baseline[2] (Visit 2)	Month 1 (Visit 3)	Month 2* (Visit 4)	Month 3 (Visit 5)	Month 4* (Visit 6)	Month 5* (Visit 7)	Month 6 (Visit 8)	Follow-up	PRN
Visit Windows				Within 42d of Screen	+/-14d	+/-14d	+/-14d	+/-14d	+/-14d	+/-14d	28d after last visit +/-14d	
Medical History	Clinician	Eligibility	X	X	X**	X	X**	X	X	X**	X	
Clinical Interview	Clinician	Eligibility + Safety	X	X	X**	X	X**	X	X	X**		
Inclusion/ Exclusion criteria	Clinician	Eligibility	X	X								
CGI Scale: Severity & Improvement	IE	Efficacy		X[3]	X**	X[5]	X**	X[5]	X[5]	X**		
Vital Signs	Clinician	Safety	X	X	X		X			X		
Physical / Neurological Exam	Clinician	Safety	X							X**		X
Dermoscopy	Clinician	Efficacy		X			X**			X**		

Microbiome/ Mycobiome Sample Collection		Efficacy		X			X			X		
Tanner Staging	Clinician	Safety		X						X		
Side-effects (CTCAE v5.0 + DOTES)	Clinician	Safety	X	X	X**	X	X**	X	X	X**	X	
Laboratory tests[1]	Lab	Safety	X		X		X			X		X
Everolimus level (only done after M1 if needed)	Lab	Dose Adjust			X							X
PTEN-associated proteins	Lab	Efficacy	X[4]	X[4]			X			X		
Concomitant treatment log	Clinician	Safety+ Efficacy	X	X	X	X	X	X	X	X	X	
Autism Diagnostic Interview-Revised	Clinician	Sample Description		X								
Primary Efficacy Outcome Neurocognitive Composite	Clinician	Efficacy		X			X			X	X	
Columbia-Suicide Severity Rating Scale	Clinician	Safety	X		X**		X**			X**		
Conners' Continuous Performance Test (CPT-3/K-CPT-2)	Clinician	Efficacy	X	X			X			X		
Stanford-Binet Fifth Edition (SB-5) / Mullen Scales of Early Learning (MSEL)	Clinician	Efficacy	X							X		
Stanford-Binet Working Memory Subscale Only	Clinician	Efficacy		X			X					
Purdue Pegboard (PP)	Clinician	Efficacy	X	X			X			X		
Wechsler Processing Speed Index	Clinician			X			X			X		
Wide Range Assessment of Memory and Learning-2 (WRAML-2)	Clinician	Efficacy		X			X**			X**		
Peabody Picture Vocabulary Test – Fourth Edition (PPVT-4)	Clinician	Efficacy		X			X**			X**		
Expressive Vocabulary Test – Second Edition (EVT-2)	Clinician	Efficacy		X			X**			X**		
Autism Diagnostic Observation Schedule (ADOS-2)	Clinician	Efficacy		X						X		
Social Responsiveness Scale – Second Edition (SRS-2)	Parent Report	Efficacy		X			X			X		

Repetitive Behavior Scale – Revised (RBS-R)	Parent Report	Efficacy		X			X			X		
Developmental Coordination Disorder Questionnaire (DCDQ)	Parent Report	Efficacy		X			X			X	X	
Behavior Rating Inventory of Executive Function (BRIEF)	Parent Report	Efficacy		X			X			X		
Adult/Child Behavior Checklist	Parent Report	Efficacy		X			X			X	X	
Sensory Profile Questionnaire - Short Form (SPQ)	Parent Report	Efficacy		X			X			X		
Vineland Adaptive Behavior Scales (VABS-III) – caregiver report	Parent Report	Efficacy		X			X			X		
Eye Tracking (Optional)	Clinician	Efficacy		X			X			X		
Resting State EEG/Auditory Evoked Potentials (Optional)	Clinician	Efficacy		X			X			X		
Participant Unblinding										X**		X

[1]Coagulation testing will only be performed at screening, month 3 and month 6. Pregnancy testing, serum is done at screening and month 6 and urine may be used for onsite visits (if deemed clinically necessary) for women of childbearing potential.
[2]Monthly visit windows are calculated based on date of Baseline visit.
[3]At the baseline visit, only the CGI: Severity scale will be completed.
[4]PTEN-associated protein blood collections will be collected at either the screening or baseline visit (as well as at the 3 month and 6 month visits)
[5]CGI optional for phone interviews month 2, month 4, and month 5.

*CGI: Clinical Global Impressions Scale; IE: Independent Evaluator; CTCAE: Common Terminology Criteria for Adverse Events; DOTES: Dosage Record and Treatment Emergent Symptom Scale;**: primary outcome mesaure only.*
**= May be performed by phone or in person*
***= May be performed by phone/videoconference under extenuating circumstances (note: Physical / Neurological Exam can not be completed in full remotely, but a modified version can be performed)*

Table 13B: Open Label Treatment Phase Study Timeline

Measurement	Performed by	Rationale	Baseline[2] (Visit 9)	Month 1 (Visit 10)	Month 2* (Visit 11)	Month 3 (Visit 12)	Month 4* (Visit 13)	Month 5* (Visit 14)	Month 6 (Visit 15)	Follow-up	PRN
Visit Windows			Within 2 weeks of Phase A, Month 6	+/-14d	+/-14d	+/-14d	+/-14d	+/-14d	+/-14d	28d after last visit +/-14d	
Medical History	Clinician	Eligibility	#	X**	X	X**	X	X	X**	X	
Clinical Interview	Clinician	Eligibility + Safety	#	X**	X	X**	X	X	X**		
Inclusion/ Exclusion criteria	Clinician	Eligibility	X								
CGI Scale: Severity & Improvement	IE	Efficacy	X[3]**	X**	X[4]	X**	X[4]	X[4]	X**		
Vital Signs	Clinician	Safety		X		X			X		
Physical / Neurological Exam	Clinician	Safety							X**		X

Dermoscopy	Clinician	Efficacy				X**			X**		
Microbiome/ Mycobiome Sample Collection		Efficacy				X			X		
Tanner Staging	Clinician	Safety							X		
Side-effects (CTCAE v5.0 + DOTES)	Clinician	Safety	#	X**	X	X**	X	X	X**	X	
Columbia-Suicide Severity Rating Scale	Clinician	Safety		X**		X**			X**		
Laboratory tests[1]	Lab	Safety	#	X		X			X		X
Everolimus level (only done after M1 if needed)	Lab	Dose Adjust		X							X
PTEN-associated proteins	Lab	Efficacy				X			X		
Concomitant treatment log	Clinician	Safety+ Efficacy	#	X	X	X	X	X	X	X	
Primary Efficacy Outcome Neurocognitive Composite	Clinician	Efficacy	#			X			X	X	
Conners' Continuous Performance Test (CPT-3/K-CPT-2)	Clinician	Efficacy				X			X		
Stanford-Binet Fifth Edition (SB-5) / Mullen Scales of Early Learning (MSEL)	Clinician	Efficacy							X		
Stanford-Binet Working Memory Subscale Only	Clinician	Efficacy				X					
Purdue Pegboard (PP)	Clinician	Efficacy				X			X		
Wechsler Processing Speed Index	Clinician					X			X		
Wide Range Assessment of Memory and Learning-2 (WRAML-2)	Clinician	Efficacy				X**			X**		
Peabody Picture Vocabulary Test – Fourth Edition (PPVT-4)	Clinician	Efficacy				X**			X**		
Expressive Vocabulary Test – Second Edition (EVT-2)	Clinician	Efficacy				X**			X**		
Autism Diagnostic Observation Schedule (ADOS-2)	Clinician	Efficacy							X		
Social Responsiveness Scale – Second Edition (SRS-2)	Parent Report	Efficacy				X			X		
Repetitive Behavior Scale – Revised (RBS-R)	Parent Report	Efficacy				X			X		

Developmental Coordination Disorder Questionnaire (DCDQ)	Parent Report	Efficacy				X			X	X	
Behavior Rating Inventory of Executive Function (BRIEF)	Parent Report	Efficacy				X			X		
Adult/Child Behavior Checklist	Parent Report	Efficacy				X			X	X	
Sensory Profile Questionnaire - Short Form (SPQ)	Parent Report	Efficacy				X			X		
Vineland Adaptive Behavior Scales (VABS-III) – caregiver report	Parent Report	Efficacy				X			X		
Eye Tracking (Optional)	Clinician	Efficacy				X			X		
Resting State EEG/Auditory Evoked Potentials (Optional)	Clinician	Efficacy				X			X		

[1]Coagulation testing will only be performed at screening, month 3 and month 6. Pregnancy testing, serum is done at screening and month 6 and urine may be used for onsite visits (if deemed clinically necessary) for women of childbearing potential.
[2]Monthly visit windows are calculated based on date of Baseline visit.
[3]At the baseline visit, only the CGI: Severity scale will be completed.
[4]CGI optional for phone interviews month 2, month 4, and month 5.

*CGI: Clinical Global Impressions Scale; IE: Independent Evaluator; CTCAE: Common Terminology Criteria for Adverse Events; DOTES: Dosage Record and Treatment Emergent Symptom Scale;**: primary outcome mesaure only.*
**= May be performed by phone or in person*
***= May be performed by phone/videoconference under extenuating circumstances (note: Physical / Neurological Exam can not be completed in full remotely, but a modified version can be performed)#=procedures may be carried over from the Double-Blind Month 6 visit, if within 2 weeks of the visit.*

Table 14: Blood Collection Amounts in mL

Visit Name	Screen (Visit 1)	Baseline[2] ^‡ (Visit 2)	Month 1‡ (Visit 3)	Month 2*‡ (Visit 4)#	Month 3‡ (Visit 5)	Month 4*‡ (Visit 6)#	Month 5‡ (Visit 7)#	Month 6‡ (Visit 8)	PRN‡
Time point		Within 42d of Screen	+/-14d	+/-14d	+/-14d	+/-14d	+/-14d	+/-14d	
Serum/RNA/plasma storage (optional)	3.5×	3.5×			3.5			3.5	
IGFBP-2 (optional)	3.5×	3.5×			3.5			3.5	
Serology Panel: Hepatitis B and C, or HIV (as needed)	9.0								

Hormone Testing/ Serum Pregnancy (if needed)*	5.0							5.0	
Chemistry Panel **	5.0		4.0		4.0			5.0	
Hematology	3.0		3.0		3.0			3.0	
HbA1c	3.0								
Coagulation Studies PT/PTT/INR	4.5				4.5			4.5	
Everolimus level***			3.0						3.0
Total Blood Drawn (mL)	36.5	7.0	10		18.5			24.5	

** For patients over the age of 7, hormone testing collected on all participants only at screening. For participants under the age of 7, estradiol, LH, and FSH will be tested. Serum Pregnancy collected at screening and Month 6 for female participants of childbearing potential.*

*** Fasting glucose will constitute 2.0 mL of this amount and be collected in a separate tube.*

****Additional everolimus levels may be drawn if a dose adjustment occurs due to toxicity or due to concomitant medication dose adjustment.*

 # Visits may occur in person or by phone, no blood collected.

^ Procedures completed at the Double-Blind Month 6 visit will qualify as Open-Label baseline procedures, as applicable.

‡ Visits occur in both Double-Blind and Open-Label treatment phases, as applicable.

× Optional blood samples can be collected at either the screening or baseline visit

7.20 Data Collection

All data outlined in schedule of events and in sections below will be collected on case report forms.

Screening data will be collected on screen-fails. Patients who complete the informed consent process and do **not** meet all entry criteria and therefore who do not receive everolimus or placebo will be considered screen failures. Screen failures should be documented as ineligible in the database.

All study data will be collected via systems created in collaboration with the RDCRN Data Management and Coordinating Center and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

7.21 Inclusion/Exclusion Criteria

Information regarding eligibility criteria will be entered on the Inclusion/Exclusion CRF, for both the double-blind and open-label phases. Patients who do not meet all entry criteria for a given phase of the study should not be entered into that phase.

7.22 Patient Demographics/Clinical Interview (Medical History)

Data will be collected on patient characteristics including demographic information (age, sex, race, weight) and other background or relevant medical history (disease history, family history of disease, psychiatric history, prior therapies, hepatitis and HIV history), and any other assessments that are done for the purpose of determining eligibility for inclusion in the study. For women of childbearing potential, serum pregnancy test will be required at screening for eligibility and again at the end of the study. Urine pregnancy test may be used for onsite visits (if deemed clinically necessary).

Physical examination must include a total body examination (general appearance, skin, neck, including thyroid, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities and a basic nervous system neurological exam). Physical examinations will occur at the screening and month 6 (visit 8). A physician will evaluate patient at each visit for significant changes and AEs per parent report. If noted, the physician will initiate a PRN physical exam as detailed above.

Significant findings made after the start of study drug that meet the definition of an Adverse Event must be recorded.

7.23 GCP Statement

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice and all applicable regulatory requirements.

8. Benefits

Treatment on this study may or may not improve the cognitive and behavioral symptoms of individuals with PTEN gene mutation. Information learned from this study may help patients with PTEN that are impacted by behavioral and cognitive dysfunctions by better describing the neurocognitive deficits and the safety and efficacy of available treatments.

9. Risks

Study Drug: This research study may involve unpredictable risks. Problems or side effects that are not now known could also occur. The participant will be given any new information that may affect their willingness to start or continue themselves or their child in the study.

One of the most common possible side effects of the study drug, everolimus, are mouth lining changes (stomatitis) manifested as redness, irritation, swelling in the mouth or mouth ulcers (Section 6.3).

Sample collection: there is a minor risk of pain, bleeding, bruising, or infection. Any additional biological specimens to be used in future genetic and molecular studies will be obtained from skin or left-over tissue obtained from a clinically-indicated procedure. Such procedures could include the minor risk of pain, bleeding, bruising, or infection

Loss of Confidentiality: minor risk for a loss of confidentiality. Strict patient confidentiality will be observed throughout all aspects of the study. While medical records will be reviewed by members of the research team, no individually identifiable patient data will be distributed to non-research or care-giving team members. All data collected as a part of this study, with the exception of photos/videos, will be de-identified prior to being uploaded into our consortium database, or sent to another site.

10. Study Records Retention

Data will be stored for at least two years following study completion.

11. Sample Destruction

All specimens and the derivatives from them will be stored indefinitely unless the participant withdraws from the study and requests the destruction of samples in writing.

12. Protocol Deviations

Any deviations from the protocol will be logged at each individual site and reported to the central IRB (or local IRB for non-reliant sites) at time of continuing review. This information will also be captured on the protocol deviation CRF in the database. Any significant deviations will be reported within 24 hours to the central IRB.

13. Adverse Event Criteria and Reporting Procedures

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate.

13.1 Definitions and Reporting

An adverse event is defined as the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) that occur after patient's signed informed consent has been obtained.
Abnormal laboratory values or test results occurring after informed consent constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, require therapy (e.g., hematologic abnormality that requires transfusion or hematological stem cell support), or require changes in study medication(s).

Conditions, or symptoms, that are present at the time of informed consent should be recorded on the Medical History CRF and be entered into the database. Adverse events that occur after informed consent should be recorded on the running AE log, as well as on the Adverse Events CRF and be entered into the database Adverse event monitoring should be continued for at least 28 days (or 5 half-lives, whichever is longer) following the last dose of study treatment. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event.

Adverse events will be assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, and life-threatening, corresponding to Grades 1 - 4, will be used.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. The severity grade (CTCAE Grade 1-4)
2. Its duration (Start and end dates or if continuing at the Safety Follow-up Visit)
3. Its relationship to the study treatment (Reasonable possibility that AE is related: No, Yes)
4. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, hospitalized, unknown, not applicable)
5. Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequela, fatal, unknown)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in 13.3.1.

All adverse events should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about the investigational drug can be found in the Investigators' Brochure (Appendix 1). This information should be included in the patient informed consent and should be discussed with the patient during the study as needed. Adverse event monitoring should be continued for at least 30 days following the last dose of study treatment.

13.2 Laboratory Test Abnormalities

Laboratory abnormalities that constitute an Adverse event in their own right (are considered clinically significant, induce clinical signs or symptoms, require concomitant therapy or require changes in study treatment), should be recorded on the Adverse Events CRF. Whenever possible, a diagnosis, rather than a symptom should be provided (e.g. anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event.

Laboratory abnormalities, that do not meet the definition of an adverse event, should not be reported as adverse events. A Grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious as defined below and/or as per investigator's discretion. A dose hold or medication for the lab abnormality may be required by the protocol and is still, by definition, an adverse event.

In the event of a laboratory abnormality that the investigator believes to be inaccurate, repeat testing may be done at a facility geographically close to the participant and then shared with the study team.

13.3 Serious Adverse Event Criteria and Reporting Procedures

13.3.1 Definitions

A serious adverse event (SAE) is defined as one which:

- Constitutes a congenital anomaly/birth defect
- Results in persistent or significant disability/incapacity
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
    - Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
    - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of an SAE given above and not resulting in hospital admission
    - Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Is fatal or life-threatening
- Is medically significant i.e. defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

Adverse events that are grade 1-2 and expected should be reported through the RDCRN database within 20 working days and logged appropriately. Determination of causality and relatedness should be made by the site PI.

Adverse events that are grade 1-2 and unexpected should be reported through the RDCRN database within 24 hours, to local IRBs, and to the sponsor only if the event suggests that there are greater risks to study subjects than previously suspected.

Adverse events that are grade 3-4 should be considered serious if they fit into one of the categories listed above (as defined by the FDA). If the event is classified as an SAE then the appropriate reporting guidelines must be followed. All SAEs will be further classified as serious adverse events or serious adverse drug reactions based on the events relation to the study drug. For SAEs, the final determination of causality is made by the Medical Review Officer. If the event is unrelated to the drug (probably not related or definitely not related) then it is defined as a Serious Adverse Event. If the event is related to the drug (definitely related, probably related, or possibly related) then it is defined as a serious adverse drug reaction.

The principal investigator has the obligation to report all serious adverse events to the FDA, IRB, RDCRN, and Novartis Pharmaceuticals Drug Safety and Epidemiology Department (DS&E).

All SAEs deemed reportable by the medical review officer, will be submitted to the FDA by the investigator utilizing the Form FDA 3500A (MedWatch Form).

13.3.2 Adverse Event Reporting

All adverse events will be recorded in the RDCRN database for reporting to respective IRBs, FDA, industry sponsors, and for data analysis. The DSC will review all AEs at regularly scheduled meetings.

- Within **24 hours** (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:
  - Is considered life-threatening/disabling or results in death of subject
    -OR-
  - Is Unexpected/Unanticipated
- Investigators must report all other reportable SAEs to the RDCRN database within **5 working days** (of learning of the event)
- All other (suspected) reportable AEs must be reported to the RDCRN within 20 working days of the notification of the event or of the site becoming aware of the event.

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided informed consent and until at least 28 days after the patient has stopped study treatment/participation, after protocol-specified procedures begin (e.g., placebo run-in, washout period, double-blind treatment, etc.) and 28 days after the patient has stopped study treatment, after the start of any period in which the study protocol interferes with the standard medical treatment given to a patient (e.g., treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication) and until 28 days after the patient has stopped study treatment.

All SAEs must be reported to Novartis within 24 hours of learning of its occurrence. Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form; all applicable sections of the form must be completed in order to provide a clinically thorough report. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and **send the completed, signed form by fax to (fax: 877-778-9739) within 24 hours to the oncology Novartis DS&E department**. Should the designated SAE Fax number be non-functional please send SAEs to the designated SAE mailbox: clinicalsafetyop.phuseh@novartis.com. A Novartis SAE Coversheet must be attached to all SAE Submissions. This includes serious, related, not related, labeled (expected) and, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within24 hours.

Any SAEs experienced after this 28 days period should only be reported to Novartis if the investigator suspects a causal relationship to the study drug. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. A SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. The end date of the first event must be provided.

The original copy of the SAE Report and the fax confirmation sheet must be kept within the Trial Master File at the study site.

Follow-up information is sent to the same fax number as the original SAE Report Form was sent, using a new fax cover sheet, stating that this is a follow-up to the previously reported SAE, and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not (if applicable), and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Everolimus Investigator Brochure or Package Insert (new occurrence) and is thought to be related to the Novartis study drug, a DS&E associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

13.4 Adverse Event Data Management

Upon entry of a serious adverse event, the DMCC created Adverse Event Data Management Form will immediately notify the Study Chair, site PIs, the Medical Review Officer, and any additional agencies (if applicable - industry sponsor, CTEP, etc.) of any reported adverse events via email.

Serious adverse events: The site physician will determine initial causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event when submitting the report in the DMCC database. The final determination of causality will be made by the study appointed medical monitor, David Franz, M.D.. The medical monitor [and, if applicable, sponsor] may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the medical monitor beyond a specified period of time are forwarded to a secondary reviewer. The Adverse Event Data Management Form maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his/her research team will be submitted to the DMCC in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the medical monitor and Safety Monitoring Committee on a bi-annual basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DMCC will post aggregate reports of all reported adverse events for site investigators and IRBs.

13.5 Unanticipated Problem Reporting

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research (“possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Per the definition, only a subset of adverse events would be characterized as unanticipated problems. There are other types of incidents, experiences, and outcomes that are not considered adverse events, but are characterized as unanticipated problems (e.g., breach of confidentiality or other incidents involving social or economic harm).

Incidents or events that meet the OHRP criteria for unanticipated problems are to be reported to the IRB, per local institutional reporting requirements. Local institutional reporting requirements to IRBs, any GCRC oversight committee and the FDA, if appropriate, remain the responsibility of the treating physician and the Study Chair.

13.6 Pregnancies

Any pregnancy that occurs during study participation should be reported by the site. To ensure patient safety, each pregnancy must also be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details or birth, and the presence or absence of any birth defects, congenital abnormalities or maternal and newborn complications.

Pregnancy should be recorded on a Clinical Study Pregnancy Form and reported by the investigator to the Novartis Drug Safety and Epidemiology (DS&E) department. Pregnancy follow-up should be recorded on the

same form and should include an assessment of the possible relationship to the Novartis study treatment of any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

14. Study Oversight

The study protocol will be reviewed and approved by the National Institutes of Health (NIH) before submission to individual center IRBs for approval. Participant enrollment may only begin with IRB approved consent forms.

Auditing procedures will be followed to ensure that the study is conducted, documented, and reported in accordance with the IRB approved protocol, Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements of collaborating sites. Verification of eligibility and appropriate documentation of informed consent/assent will be performed for a sample of subjects enrolled in the study. The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, and the results of any other tests or assessments. All information on CRFs must be traceable to these source documents in the patient's file. The investigator must also keep the original informed consent form signed by patient (a copy is given to the patient).

15. Data and Safety Monitoring Committee (DSMC)

The Study Chair has primary oversight responsibility of this clinical trial. The appointed Data and Safety Monitoring Committee (DSMC) has oversight responsibility of the Data and Safety Monitoring Plan (DSMP) for this clinical trial. Each site's Principal Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying adverse events. Aggregate report - detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available from the DMCC for site review. Adverse events will be reviewed by the research team at time of occurrence and reviewed at monthly team meetings.

The DSMC is an internally appointed group of individuals who will monitor and evaluate the safety of the proposed clinical trial. The DSMC's primary role is to ensure the safety of patients in the study. The DSMC will follow the NIH's published guidelines regarding its function and may provide input such as the recommendation to stop the trial for safety reasons. Due to the signal-seeking nature and the small sample size in this study, there is no formal efficacy interim analysis planned. However, the DSMC will review adverse events and serious adverse events and provide a report to the group bi-annually. The DSMC will include individuals familiar with everolimus administration and clinical trial design and will be comprised of Phillip Pearl, M.D. (pediatric neurologist from Boston Children's Hospital), Brenda Porter, M.D. (pediatric neurologist at Stanford University, Chairperson), and Eric Youngstrom, Ph.D. (biostatistician from the University of North Carolina).

David Franz, M.D. (Cincinnati Children's Hospital Medical Center) will be the independent medical review officer for this trial. He is an expert in the use of mTOR inhibitors in children and adults with TSC. Dr. Franz will review any adverse events/serious adverse events throughout and will also determine if unblinding and reporting to the FDA and DSMC is required. If Dr. Franz is unable to fulfill his duty at any point during the study, Sarah Spence, M.D. (Boston Children's Hospital) will serve as the back-up medical review officer until Dr. Franz returns to his duties.

16. Data Management and Coordinating Center and Database

All study data will be collected via systems created in collaboration with the Rare Diseases Clinical Research Network Data Management Coordinating Center (RDCRN DMCC) and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

16.1 Registration

Registration of participants on this protocol will employ an interactive data system in which the clinical site will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. IRB approval for the protocol must be on file at the DMCC before accrual can occur from the clinical site.

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject. When the participant is registered to participate in the study, using the DMCC provided web-based registration system; site personnel will insert the unique ID generated from the National Database for Autism Research (NDAR) Global Unique Identifier (GUID) software program, explained in the following section 16.2, as the participant ID number. Thus each participant will have two codes: the local one that can be used by the registering site to obtain personal identifiers and a second code designated by the NDAR GUID. For all data transfers to the DMCC both numbers will be required to uniquely identify the subject. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a participant for data entry since the numbers should match to properly identify the participant. The only personal identifier accessible to the DMCC is participants' date of birth, as well as the date of onset for all recorded medical events. The database will not collect any other components of a participant's protected health information.

If the subject is participating in more than one DSC study, such as this study and the DSC "Natural History Study of Individuals with Autism and Germline Heterozygous PTEN Mutations" study, data from those studies can be linked.

16.2 Data Elements

Every individual who provides consent will be assigned a Global Unique Identifier (GUID). The GUID is a unique universal subject ID that allows researchers to share data specific to a study participant without exposing personally identifiable information. A member of the research team will enter private health information (PHI) collected from each study participant into the National Database for Autism Research (NDAR) GUID software program. The software performs a one-way encryption of these data, de-identifying and randomizing the data to produce a digital "fingerprint". The GUID program at NIH converts this digital fingerprint into a final GUID that is completely devoid of PHI. The final GUID is returned to the research team as a unique identifier for the participant.

16.3 Data Entry

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, online forms will be developed that contain the requisite data fields. The DMCC, in collaboration with DSC, is also responsible for data sharing with the NIH National Database for Autism Research (NDAR) and the database of Genotypes and Phenotypes (dbGaP).

All dosing data will be entered into the DMCC database within 24 business hours of the study visit to ensure the most up-to-date information is available for dose modification and participant safety decisions.

16.4 Laboratory Data Flow

Samples collected as a part of this study will be tracked utilizing the corresponding data collection sheets. These forms will be kept both locally and in the database to ensure that samples are sent to the appropriate laboratories. Samples results will be reported electronically to study sites directly from LabConnect. Additionally, the DMCC will data transfer from LabConnect electronically on a regular basis. All transactions are logged and validated for both methods.

16.5 Data Quality and Monitoring Measures

As much as possible data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

- Data Monitoring: The RDCRN DMCC will utilize the Data Quality Module within REDCap to identify missing or unclear data. Study sites will be able to generate queries and resolve for their site.

- Data Delinquency Tracking: REDCap will allow the consortium sites to monitor data delinquency on an as needed basis.

16.6 Quality Control Method

Standardized data collection and management procedures will be followed to ensure data quality control. Data will be scored, reduced, and managed on an ongoing basis. The PIs will assume responsibility for uniformity across sites and will coordinate on a weekly basis (or more frequently if needed) with the data collection and management team. After data have been collected, it will be entered into the RDCRN DMCC database. As much as possible, data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

All variables will be checked for out-of-range values and the marking of missing values.

17. Data Analysis Plan, Statistical Power and Sample Considerations

This is an exploratory study to evaluate the short-term safety and preliminary efficacy of everolimus in patients with a PTEN mutation. The analyses will be predominantly descriptive with the intent of generating plausible hypotheses to be tested in a Phase III confirmatory trial. Efficacy will be assessed using recognized measures of intellectual ability and neurobehavioral processes.

In objective 1, we will examine the safety and tolerability of everolimus by comparing the 1) dropout rates due to side effects, 2) dropout rates for any reason, and the 3) frequency of specific side effects in those receiving everolimus versus placebo. Based on the published literature and our experience in the tuberous sclerosis trial, we predict that the dropout rates due to side effects will be very similar across everolimus and placebo groups (<10% difference). However, we predict that everolimus will cause higher rates of medication-related side effects (≥10% difference) when compared to placebo. We will use chi-square statistics (or Fisher's exact test depending on cell counts) to compare dropout and rates of specific side effects. Statistical significance will be determined using a one-tailed test Type 1 error rate of α=.05, as only increases with everolimus treatment are expected. No false discovery rate correction will be applied because the concern is maximizing sensitivity to any increase in side effect prevalence with everolimus treatment. For dropout rates, we will also compute Kaplan-Meier survival analyses with discontinuation due to side effects or any reason as separate status endpoints. Number of days from baseline to medication discontinuation will be the time variable. Survival analyses are expected to be under-powered due to the generally low dropout rates expected, but these analyses can be useful for describing temporal trends. To ensure any observed group differences are not influenced by other variables or randomization imbalance, cox and logistic regression analysis will also be conducted with and without conditioning on relevant baseline covariates (e.g., demographics, language and cognitive ability, symptom severity).

In objective 2, we will examine whether the everolimus group will show more improvement, compared to the placebo group, on the main neurocognitive efficacy composite outcome. Randomization Group (everolimus versus placebo) differences on the neurocognitive composite will be analyzed using mixed effects (growth curve) modeling, fully utilizing repeated measurements collected at three points (Time: baseline, 3-month follow-up, and 6-month follow-up). A significant interaction between Time and Randomization Group, with the everolimus group showing a more favorable outcome trajectory, will support the primary efficacy hypothesis. Maximum likelihood estimation will be implemented in the Mplus program version 7.2 or above.

Mixed effects (growth curve) model analyses will be conducted following the intention-to-treat principle. Data points that are missing due to subject attrition will be handled assuming that data are missing at random (MAR) conditional on observed information, which is less restrictive than missing completely at random (MCAR) assumed in fixed effects analyses such as ANCOVA and regression analysis. In this procedure, all available cases including the ones with missing information will be included in the analyses. By including every subject who completed at least one assessment, we are not only more likely to conserve power, but also less likely to produce biased effect estimates. In mixed effects analyses, the slope of the outcome will be modeled as the key

dependent variable predicted by randomization group. We will conduct the analysis with and without conditioning on relevant baseline covariates. The results of these longitudinal analyses can be easily converted to a cross-sectional group effect at each assessment time point to describe the magnitude and significance of group differences. Of particular interest is the group difference at end of treatment (6-month assessment). The mixed effects analyses will be repeated using a model where the baseline scores on each outcome are treated as a baseline covariate to control for (instead of as a part of) the repeated measures. This model can be useful if there is a considerable heterogeneity between before and after treatment processes. We will also analyze the data using conventional ANCOVA treating the end point neurocognitive composite as the dependent variable and controlling for the baseline neurocognitive composite. Further analyses will be conducted using the same analysis models but with transformed data (e.g., log transformation) to check the sensitivity of the results to deviation from outcome normality. Statistical significance of all main effects and interaction terms as well as cross-sectional group differences will be determined using a one-tailed Type 1 error rate of α=.05. The same analysis strategy will be employed for secondary efficacy outcomes and a false discovery rate correction will be implemented to control inflation of Type 1 error.

If the exploratory biosample objectives are pursued, we will explore whether baseline peripheral blood levels of PTEN-associated pathway molecules (PI3K/AKT, mTOR, MAPK protein levels) change with treatment and if changes in peripheral pathway molecules correlate with clinical improvement. To determine whether PTEN-associated pathway molecules change with treatment, we will use a similar mixed effect (growth curve) modeling approach to that described above for Secondary Objective with screening, 3-month, and 6-month pathway measurements as outcome measures. The Time by Randomization Group interaction will test whether the everolimus treatment group shows improvement/normalization of PTEN-pathway molecule levels relative to placebo. This component will be dependent on securing additional funds.

In analyzing the resting state EEG and AEP data we will focus on the following:

1. Baseline EEG gamma power
2. AEP N1 habituation
3. AEP variability (phase-locking factor, also called intertrial phase coherence)

EEG and AEP analysis will occur via the Boston EEG Automated Processing Pipeline (BEAPP), created at Boston Children's Hospital by April Levin MD. BEAPP is MATLAB-based software designed to facilitate batch processing of EEG files for artifact removal and signal processing in an automated manner. Users have the option to alter multiple settings, to customize processing to their needs. The pipeline is freely available and covered under a General Public License, which allows other groups to replicate or alter analyses for their own data if they wish.

To prepare EEG and AEP data for analysis, the pipeline imports EEG from various acquisition formats and electrode layouts into a standardized format. Data will first be processed via PREP, which removes 60 Hz line noise, identifies and interpolates bad channels, and references to average.[58] Data will then be high pass filtered at 1 Hz, and will undergo artifact removal via MARA, an automated independent component-based supervised machine learning algorithm that handles artifacts including ocular artifact, muscle artifact, and loose electrodes.

For analysis of resting EEG data, segments of high-amplitude artifact (>150 uV) will be removed, and the remaining data will be segmented into 2-second epochs. Data will undergo a Laplacian transform, as this has been shown to reduce sensitivity of the EEG signal to contamination by myogenic activity, which is otherwise particularly prominent in the gamma band.[59,60] Spectral analysis will be performed on each epoch with multitaper methods using 3 tapers, in order to determine the average power spectrum across all epochs. This will allow for determination of power in the gamma frequency band, particularly the 62-90Hz range.

For analysis of AEP data, data will be epoched into 2000 ms trials (-500 to 1500 ms), and baseline corrected using the 500 ms period prior to the first auditory stimulus in each trial. Any trial with amplitude exceeding 150 uV will be removed. N1 will be defined as the most negative-going waveform deflection between 50 and 150ms post-stimulus, over the central region. (If N1 cannot be adequately identified in this population using these criteria, independent components analysis will be used to identify the N1 component instead). Habituation of the N1 amplitude will be quantified as percent change from the first to second auditory click in each trial. Intertrial phase coherence (ITPC) will be calculated using the function *newtimef*, and reported as the maximum absolute value ITPC in the 50-150ms timeframe, in the alpha frequency band (8-12Hz).

Two approaches will be used to explore whether changes in PTEN-pathway molecules are correlated with changes in clinical improvement. First, we will use the mixed effects modeling framework to examine the correlation between the longitudinal changes in the neurocognitive composite score and changes in PTEN-associated pathway molecules. In this framework, the relationship between slopes representing individual clinical changes and individual PTEN-pathway molecule changes are estimated. We will also examine whether this correlation differs by treatment group. Second, we will estimate a structural model with cross-lagged relationships estimated between screening/3-month PTEN-pathway levels and 3-month/6-month efficacy measurements. Rather than evaluating the relationship between changes over time, the cross-lagged structural model examines whether pathway molecule levels at earlier time points predict efficacy measurements at later time points. Each of these methods will use all available data following the intent-to-treat principle. As a result, they are less likely to produced biased estimates than a bivariate correlation approach using changes from baseline because the latter method can only be computed for individuals with complete data.

17.1 Power Estimation

This is the first study to evaluate the efficacy of everolimus in patients with a PTEN mutation and ASD/DD. As such, analyses will be predominantly descriptive with the intent of generating plausible hypotheses to be tested in a Phase III confirmatory trial. The planned recruitment sample size of 40 was based on three considerations: 1) the expected recruitment potential of the three sites, 2) a desire to ensure sensitivity to any enrichment in side effects or problems with tolerability in everolimus treated patients, and 3) the need for adequate statistical power in examining primary and secondary efficacy outcomes. Assuming an overall dropout rate of approximately 10%, 44 patients will need to be enrolled to get 40 patients with complete trial data. However, following intent to treat procedures, all patients who are randomized, dosed, and for whom baseline data is collected will be included in statistical analyses. Statistical power considerations for each aim/hypothesis are described below. Except for safety analyses, where only everolimus-treated patients would be expected to show enrichment of dropout or side effects, statistical power estimates are based on one-tailed significance tests with Type 1 error rate of α=.05.

Primary Objective- Safety. The planned sample size of 40 is underpowered to detect small differences in dropout rates or side effects between everolimus versus placebo-treated groups. Statistical power will only be adequate if at least a 35% difference in the proportion of dropout/side effects is observed (1-$\beta \geq .81$), assuming a one-tailed Type 1 error rate of α=.05. This is equivalent to 1/20 patients experiencing an adverse event/side effect in the placebo arm and 8/20 in the treated arm. Similarly, statistical power of survival analyses will only be fair (1-$\beta \geq .57$) if a hazard ratio of 7.5 is observed, equivalent to a 5% dropout rate in the placebo group versus a 30% dropout rate in the everolimus group. However, because the goal is to maintain sensitivity to any potential enrichment in dropout or side effects, any indicator showing a difference of 10% or greater will be considered a meaningful difference and will be reported.

Secondary Objective – Efficacy. For power estimation purposes, the variability in PTEN patients treated with an mTOR inhibitor is expected to be similar to the untreated sample. Preliminary data indicates that the measures which comprise the dominant portion of the neurocognitive composite index (processing speed working memory, and fine motor) are highly sensitive to the brain dysfunction seen in PTEN patients with ASD. The effect sizes are approximately 1 SD or larger. In a comparison between mTOR treated vs. untreated PTEN patients, an effect size of at least 0.65 SD would be of interest and represent clinically- meaningful improvement. For example, an effect size of at least 0.65 SD on cognitive measures would translate to a 10-point standard score improvement in the everolimus-treated group relative to placebo. An improvement of 10 or more points is outside the standard error of measurement for most cognitive measures and is typically considered a reliable change when disordered populations are re-tested.

A sample size of 40 (20 per arm) patients will permit detection of endpoint (6-month follow-up) differences of at least 0.80 SD at the 5% significance level (one-sided alpha = 0.05) with 80% power (1-β). Statistical power will be weaker (1-β=.65) if smaller cross-sectional differences are observed (0.65 SD). However, statistical power is very strong, even for smaller differences (Cohen's $d \geq .50$ equivalent to $\geq .50$ SD difference) when the full repeated measures nature of the design is considered – baseline, 3-month follow-up, 6-month follow-up. Assuming even modest correlations between repeated measurements of outcome variables ($r \geq .30$), power to detect clinically meaningful differences of .50 SD is very good (1-β=.83; one-sided α=.05). If the primary outcome measure (neurocognitive composite) meets nominal statistical significance in the expected direction (one-tailed $p<.05$) or a secondary outcome measure meets a more stringent false discovery rate-corrected

significance level in the expected direction, these outcomes will be considered candidates for Phase III confirmatory trial evaluation. To determine if a secondary outcome meets the more stringent false discovery rate-corrected significance level, a Benjamini-Hochberg false discovery rate correction will be applied with adjustment for all secondary outcome measures. This adjustment involves sorting the list of obtained p-values in descending order. The smallest p-value is then compared to the most stringent alpha, the next smallest is compared to the next most stringent alpha, and so on. Only those secondary outcomes meeting FDR-corrected alpha level will be considered candidates for Phase III evaluation.

An interim analysis will be conducted after half the sample has been collected (10 patients in each arm) and will mainly focus on safety. Efficacy interim analysis may be under-powered, but will focus on whether the effects are in the expected direction and whether effect sizes (both the endpoint effect size and the interaction fully utilizing repeated measures) are approaching expected levels (at least Cohen's d≥.30). The goal of the interim analysis for efficacy will be to get a signal that will allow us to design more targeted trials in the future. As such, we will check for a signal in any domain and, if needed, include additional endpoints before the end of the study to expand the endpoint and optimize outcome.

Additional considerations. This is the first clinical trial in patients with PTEN mutations and ASD/DD. For this reason, and to facilitate recruitment, all types of PTEN mutations will be permitted within the randomized patients. However, post-hoc analyses will be completed to examine the existence of any differential effect of treatment based on the type of PTEN mutation (missense vs. other mutation types) or mutation location (exon number, location in 3-D PTEN structure).

18. Protocol Amendments or Changes in Study Conduct

Any change or addition (excluding administrative) to this protocol requires a written protocol amendment that must be reviewed by the central IRB at Boston Children's Hospital (Boston and Stanford only), Cleveland Clinic IRB the NIH, and Novartis as applicable. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study require additional approval by the IRB at each study center. A copy of the written approval of the IRB must be provided to Novartis.

Examples of amendments requiring such approval are:
1. increases in drug dose or duration of exposure of subjects,
2. significant changes in the study design (e.g. addition or deletion of a control group),
3. increases in the number of invasive procedures,
4. addition or deletions of a test procedure required for monitoring of safety.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by Novartis in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons Novartis must be notified and the IRB at the center must be informed immediately. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB approval include:
1. changes in the staff used to monitor trials
2. minor changes in the packaging or labeling of study drug.

19. Compensation

As a part of this study we may be able to provide travel reimbursement (i.e. airfare, hotel, and mileage costs) and/ or parking and meal vouchers for participants. The reimbursement process at Boston Children's Hospital and Stanford University will go through the National Organization for Rare Disorders (NORD). NORD will provide reimbursements in the form of a check to families after they have completed the study visit. The study team at Cleveland Clinic will reimburse their participants directly. Participants/families can receive up to $1,500 per completed study visit.

20. Privacy Provisions

To maintain the privacy of potential subjects, they will only be approached regarding study enrollment as part of their routine clinical care or via a mailed letter that they may choose to response to. Individuals will not be approached by someone they do not know or anticipate in an unknown or unwanted environment. Data and identifying information collected as part of this study will only be released to those listed in the consent/assent forms.

21. Confidentiality Provisions

Subjects will be provided with a unique ID code and only the study coordinator, Principal Investigator and study monitor will be able to view the link between participants' names and the unique ID. The link to all codes will be maintained in a secure, HIPAA compliant database in the study coordinator's office. The unique patient number will be used on all research documents, including case report forms, as well as on labels on samples collected. All information from this study will be kept in the same research binder in the study coordinator's private and locked office. This will include all paperwork with identifying information, including tracking sheets, orders, consents, etc.

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/guardian/subject 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. 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.

In addition, data will be placed into one or more publicly-accessible scientific databases. For example, the National Institutes of Health maintained databases called the database of Genotypes and Phenotypes (dbGaP) and the National Database for Autism Research (NDAR).

Data from this study will be submitted to the National Database for Autism Research (NDAR). NDAR is a computer system run by the National Institutes of Health that allows researchers studying autism to collect and share information with each other. With an easier way to share, researchers hope to learn new and important things about autism more quickly than before.

During and after the study, the researchers will send information obtained about the subject's health, behavior and genetics information to NDAR and/or submit to other publicly-accessible scientific databases. However, before the information is sent, the researchers will remove information such as name, address, and phone number, and replace that information with a code number. Other researchers nationwide can then file an application with the National Institutes of Health to obtain access to the study data for research purposes. Experts at the National Institutes of Health who know how to protect health and science information will look at every request carefully to minimize risks to subject privacy.

21.1 Certificate of Confidentiality

This research is covered by a Certificate of Confidentiality from the National Institutes of Health. The researchers cannot be forced to disclose information that may identify a study participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify a participant, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

Even with the Certificate of Confidentiality, the investigators continue to have ethical obligations to report child abuse or neglect and to prevent an individual from carrying out any threats to do serious harm to themselves or others. If keeping information private would immediately put the study participant or someone else in danger, the investigators would release information to protect the participant or another person.

Department of Health and Human Services (DHHS) personnel may request identifying information for purposes of performing audits, carrying out investigations of DHHS grant recipients, or evaluating DHHS funded research projects.

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