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A Pilot Study to Evaluate the Effects of Everolimus on Brain mTOR activity and Cortical Hyperexcitability in Tuberous Sclerosis Complex (TSC) and Focal Cortical Dysplasia (FCD)

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List of Abbreviations

AED **Antiepileptic drug**
BMI **Body mass index**
BSA **Body surface area**
CTAE Common Terminology for Adverse Events
EIAED **Enzyme-inducing antiepileptic drug**
FCD Focal cortical dysplasia
mTOR Mammalian target of rampamycin
RMP Risk management plan
SAE Serious adverse event
SEGA Subependymal giant cell astrocytoma
TNF-α Tumor necrosis factor-alpha
TSC Tuberous sclerosis complex
TRE Treatment resistant epilepsy
NYULMC New York University Langone Medical Center
SUDEP Sudden unexpected death in epilepsy
SBMC St Barnabas Medical Center
INN Institute of Neurology and Neurosurgery at St Barnabas
NYU CEC New York University Comprehensive Epilepsy Center

Study Summary

Title	A Pilot Study to Evaluate the Effects of Everolimus on Brain mTOR activity and Cortical Hyperexcitability in Tuberous Sclerosis Complex (TSC) and Focal Cortical Dysplasia (FCD)
Short Title	Everolimus effects on brain mTOR and excitability
Protocol Number	Pending
Phase	2
Methodology	This is a multi center, open-label exploratory Phase II clinical trial of patients with treatment resistant epilepsy (TRE), ages 2 to 50 years old, with TSC or FCD who are scheduled for epilepsy surgery.
Study Duration	18 months of active recruitment
Study Center(s)	NYU Langone Medical Center;
Objectives	**Primary objectives:** A pilot study to assess the: 1) feasibility and safety of recruiting patients into a prospective study for patients with TSC and FCD treated for 7-21 days before epilepsy surgery and 2) test the hypothesis that everolimus therapy in TSC and FCD patients with TRE will reduce mTOR signaling (reduced S6 phosphorylation) as compared to concurrent controls with TSC and FCD who did not receive Everolimus. **Secondary Objectives**: to obtain preliminary data on the effect of everolimus therapy in TSC and FCD patients with TRE on mTOR signaling and cortical hyperexcitability by assessing 1) systemic mTOR signaling suppression (VEGF-D) pre- and post-treatment, 2) relation of S6 phosphorylation to specific genetic abnormalities, and 3) blood everolimus levels; and using historical controls to assess 4) markers of cortical hyperexcitability (glutamate and GABA receptors, levels of neuroinflammation (TNF-α), axonal abnormalities, myelination defects), and 5) electrocorticographic (ECoG) recordings
Number of Subjects	21 total subjects study wide; 9 treated subjects (NYU CEC) and 12 controls (@ NYU CEC;) This has been a very challenging study to recruit for and therefore our total number of enrolled patients is fewer than expected. This has to do with multiple factors, including a smaller number of eligible candidates, a smaller percentage of parents who have considered to participate, and the challenge imposed by the fact that many of the patients come from outside the NY Tristate area. Given this distance, enrollment of subjects 3-4 weeks before the surgery has not been possible, so we have shortened the length of the treatment phase.
Diagnosis and Main Inclusion Criteria	1. Female and male patients, age 2 year to 50 years 2. Diagnosis: treatment resistant epilepsy ($\geq$ 2 failed anti-epileptic drug [AED] treatment regimens) and either: • Tuberous Sclerosis Complex (TSC) • Focal Cortical Dysplasia (FCD) on MRI
Number of Subjects in comparison control group	12 subjects

Diagnosis and Main Inclusion Criteria	1. Female and male patients, age 2 year to 50 years 2. Diagnosis: treatment resistant epilepsy (> 2 failed anti-epileptic drug [AED] treatment regimens) undergoing routine surgery • Tuberous Sclerosis Complex (TSC) • Focal Cortical Dysplasia (FCD) on MRI
Study Product, Dose, Route, Regimen	Everolimus: • *Patients NOT taking EIAEDs*: The starting dose of everolimus for treatment patients will be 4.5 mg/m^2 once daily; adjust to attain trough concentrations of 5-15ng/ml. The dose will be adjusted for certain co-medications (see below). • *Patients TAKING* CYP3A4 and/or P-glycoprotein (PgP) Inhibitors Patients will be excluded if using strong CYP3A4 inhibitors. If the patient is receiving a co-administered drug with moderate CYP3A4 and/or PgP inhibitors, the everolimus dose will be reduced by approximately 50% to maintain trough concentrations of 5 to 10 ng/mL. No moderate or strong CYP3A4 and/or PgP inhibitor will be added or discontinued during the study period. • Patients TAKING one concomitant strong CYP3A4 inducer for seizure control (e.g., phenytoin, carbamazepine, phenobarbital) will be allowed in the study. Patients taking more than one strong CYP3A4 inducer will be excluded. For patients requiring a concomitant strong CYP3A4 inducer (an Enzyme-Inducing Antiepileptic Drug (EIAED), we will double the everolimus dose from that prescribed for patients not taking EIAEDs
Duration of administration	7 to 21 days
Reference therapy	Not applicable
Statistical Methodology	Sample size considerations. For this preliminary study, safety analyses are primary. Study termination will occur if, among the 9 treated subjects, there are two cases of wound healing problems or three cases of serious infection, including wound site infection. In the case of a wound site infection, any problems with wound healing will be considered secondary to the infection and NOT as a primary wound healing problem. These estimates are based on neurosurgical experience and reports with the frequency of wound healing problems of <2% and infection from 1.6 to 8.6% in published series, with a mean infection rate of 5.3%. For the analysis of S6 phosphorylation, based on a 2 sample 2 sided t- test, we can detect a difference of |1.4|- |1.5 |standard deviations between the comparison controls and the treated group with 9 treated subjects with alpha =0.05 and power of 80%. Preliminary data using quantitative Western blots obtained from 4 resected tubers demonstrated phospho-S6/S6 ratios of 257□42% of control. With 9 treated subjects, and 12 controls we would be able to detect a 64 – 65% difference in phosphor-S6/S6 levels between treated and untreated groups, which is likely a biologically meaningful difference.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted in accordance with US government research regulations, and applicable international standards of Good Clinical Practice, and institutional research policies and procedures.

1.1 Background

Early onset and treatment-resistant epilepsy (TRE) commonly complicates Tuberous Sclerosis Complex (TSC) and focal cortical dysplasia (FCD) and can contribute to or cause severe cognitive and behavioral impairments in these patients. In the case of TSC, the genetic loss of TSC1/TSC2 inhibition results in upregulation of the mTOR signaling pathway causing multiple structural and functional alterations in the brain that contribute to epilepsy, although the specific alterations that cause epilepsy remain unknown. Many patients with TSC and FCD who have TRE benefit from epilepsy surgery if seizures are localized to a single epileptogenic focus. However, a multicenter study of 70 patients undergoing epilepsy surgery for TSC found that only 53% became seizure-free after surgery [1]. Thus, nearly half of patients experience seizures after surgery while many others do not qualify for epilepsy surgery because of multifocal or widespread epileptic networks. TRE has a high morbidity, especially for TSC patients, in which autistic spectrum disorder and intellectual disability are associated with frequent seizures and early life onset epilepsy [2] [3]. Further, the risk of sudden unexpected death in epilepsy is significantly elevated among patients with tonic-clonic seizures during the past year, early-onset epilepsy, long-duration of epilepsy, and symptomatic epilepsy. In some studies, after adolescence, sudden unexpected death in epilepsy rates exceed 10% per decade for individuals with multiple risk factors [4], which are present in many TRE patients with TSC or FCD.

mTOR inhibition in animal models of TSC has anti-epileptic and anti-epileptogenic effects, and preliminary data suggest that mTOR inhibitors can reduce seizure frequency in some TSC patients. The rationale for studying everolimus in TSC is straightforward given the known effect of TSC1 and TSC2 mutations on mTOR activity, the effect of mTOR inhibition in animal models of TSC to reduce both the development (epileptogenesis) and occurrence (antiseizure) of seizures [5] [6, 7], and preliminary human data suggesting that everolimus can reduce seizures in TSC patients [8]. For FCD, studies from human brain tissue reveal that balloon cells exhibit robust phosphorylation of S6 protein in a pattern similar to giant cells in cortical tubers from patients with TSC [9-11]. Furthermore, brain tissue from FCD and TSC cases has similar cellular (e.g., balloon cells) and architectural (e.g., dyslamination) changes, further supporting shared pathogenetic mechanisms [12]. Additionally, FCD tissue expresses similar patterns of dysregulated excitatory and inhibitory neurotransmitter receptors as those observed in TSC [13, 14]. Together, these findings suggest that, as with TSC, in FCD, there is upregulation of mTOR activity in the brain and this may directly contribute to seizure activity. Given these brain molecular and structural parallels between TSC and FCD, and the significant morbidity (e.g., depression; psychosis; memory, language and other cognitive impairments) and mortality (e.g., sudden unexpected death in epilepsy, drowning, status epilepticus, suicide) associated with TRE, this pilot trial of an mTOR inhibitor has the potential to inform future studies in these FCD patients. Further, for the FCD group or individual patients, if this study finds evidence that an mTOR inhibitor can down-regulate excess mTOR activity and restore excitatory/inhibitory neurotransmission and reduce neuronal hyperexcitability, a subsequent trial of an mTOR inhibitor would have potential therapeutic benefit.

No randomized clinical trial has demonstrated efficacy of mTOR inhibitors in seizure suppression, and the potential mechanisms of action have not been investigated. The goal of this study is to obtain data to help plan an exploratory Phase II clinical trial to assess the efficacy of everolimus therapy to improve the molecular and electrophysiological abnormalities in the brains of TSC and FCD patients with TRE. Patients enrolled in this study will receive everolimus for a minimum of 7 days prior to planned epilepsy surgery. This short-term duration of treatment highlights the unique approach of our study, when compared to other investigations of the effect of mTOR inhibition in TSC patients. Other randomized studies gave patients everolimus for six months or longer in clinical trials of patients with TSC with subependymal giant cell astrocytomas (SEGAs) of the brain [8]. The effectiveness of everolimus in this setting was based on reduced SEGA volume. These authors noted that 16 patients for whom 24-hour

video electroencephalography data were available, seizure frequency for the 6-month study period decreased in 9, did not change in 6, and increased in 1 (p=0.02). However, a clinical benefit for epilepsy has not been definitively demonstrated thus far.

Our rationale for a short-term (7-21 days) treatment is based on prior work in animal models showing cellular changes in mTOR signaling (down-regulation) after only 24 hours of treatment [5]. This effect of mTOR inhibition on mTOR signaling and epileptiform activity in humans, however, was only demonstrated in patients with glioblastoma multiforme, a tumor associated with disruptions of the blood-brain barrier (see below) [15].

Despite lack of human "proof of concept" data for everolimus treatment in patients with TRE who have TSC or FCD, clinical trials of TSC patients have nevertheless been conducted. To date, no adequately powered, prospective studies have addressed the efficacy of everolimus in patients with TSC who have TRE. One small study is ongoing to assess the efficacy and safety of everolimus in TSC patients with TRE and we will monitor the results of this study as they become available. No studies have examined the effects of everolimus in TRE in patients with FCD. We believe that our study of brain tissue obtained at the time of epilepsy surgery in TSC and FCD patients, as well as ECoG data, will provide the first in vivo evidence of a biologic effect of everolimus in human epilepsy brain tissue. We have a unique opportunity to demonstrate this in human TSC and FCD patients undergoing epilepsy surgery by studying the degree of mTOR inhibition in individual patients. Defining the molecular and electrophysiological changes of mTOR inhibition in human brain tissue may provide critical insights into the efficacy, tolerability, and limitations of this therapy. A better understanding of the effects of mTOR inhibition on mTOR signaling, neurotransmission, and epileptogenicity in human cortical tissue is critical to develop more effective mTOR-targeted therapies in TSC and FCD patients, and for designing and a clinical trial. This study is a logical next step based for both pre-clinical studies and preliminary clinical data on the effect of everolimus on seizures in TSC patients. Our study may help to explain why some patients treated with mTOR inhibitors fail treatment, as well as help elucidate the effect of these agents on epileptogenesis in human TSC and FCD patients. Therefore, data obtained from the proposed studies may help guide future treatment with mTOR inhibitors.

The goals of our pilot study is to obtain the necessary data to plan a prospective, multi-center Phase II study of everolimus for TRE in TSC and FCD. Our pilot study would provide critical data on patient enrollment and retention, assess safety, identify challenges to study methodology, dosing of everolimus (e.g., relation of dose (mg/m^2/day) to serum level, relation of serum and potentially brain levels if a reliable assay can be identified before this subsequent study) and cellular physiological effects of everolimus (e.g., inhibition of neuronal mTOR activity, changes in excitatory/inhibitory neurotransmission, reduction of cortical excitability), and the relation of changes in mTOR activity to different genetic abnormalities in brain tissue. The study may also provide initial data on the efficacy of everolimus in reducing epileptiform activity on electrocorticography (ECoG). The use of biomarkers – molecular signaling, cellular physiology, and cortical excitability – will provide sensitive indicators of the effects of everolimus on seizure foci. The data we acquire may also help to inform the use of everolimus to treat SEGAs and potential uses in cognitive and psychiatric disorders that complicate TSC and FCD.

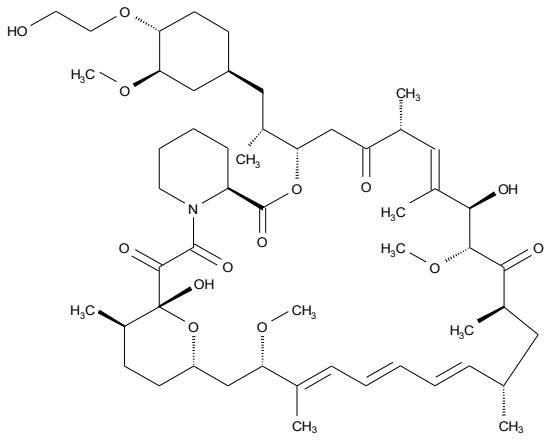
1.2 Overview of Everolimus

Everolimus is a derivative of rapamycin which acts as a signal transduction inhibitor (Table 1, Figure 1). 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 [37].

Table 1 Everolimus - 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

Figure 1 Chemical structure of Everolimus



Everolimus, an inhibitor of mammalian target of rapamycin (mTOR), is FDA-approved as an antineoplastic agent. The molecular formula is C53H83NO14 and the molecular weight is 958.2

mTOR is a serine-threonine kinase, downstream of the PI3K/AKT pathway. The mTOR pathway is dysregulated in several human cancers. Everolimus binds to an intracellular protein, FKBP-12, resulting in an inhibitory complex formation with mTOR Complex 1 (mTORC1) and thus inhibition of mTOR kinase activity. Everolimus reduces the activity of S6 ribosomal protein kinase (S6K1) and eukaryotic elongation factor 4E-binding protein (4E-BP1), downstream effectors of mTORC1, involved in protein synthesis. In addition, everolimus inhibits the expression of hypoxia-inducible factor (HIF-1) and reduces the expression of vascular endothelial growth factor (VEGF). Inhibition of mTORC1 by everolimus has been shown to reduce cell proliferation, angiogenesis, and glucose uptake in in vitro and/or in vivo studies.

Two regulators of mTORC1 signaling are the oncogene suppressors hamartin (TSC1) and tuberin (TSC2), which together form a complex to inhibit mTORC1. Loss or inactivation of either TSC1 or TSC2 leads to activation of downstream signaling. In Tuberous Sclerosis Complex (TSC), a genetic disorder, inactivating mutations in either the TSC1 or the TSC2 gene lead to hamartoma formation throughout the body.

1.3 Preclinical Data

There are no preclinical data for the use of everolimus as planned for this protocol.

1.4 Clinical Data Related to Study Drug Use

The potential surgical risks associated with 7- to 21-day trial of everolimus treatment prior to elective epilepsy surgery in TSC or FCD have never been documented. The optimal data for analyzing this risk would be the experience treating TSC or FCD patients receiving everolimus therapy who required neurosurgical intervention while on the medication. However, this clinical experience has not been

reported. Further, limited information is available regarding brain tumor patients receiving everolimus who undergo neurosurgery. Two studies in patients with glioblastomas are cited below. The large datasets available are with everolimus use in other surgical settings, typically in patients receiving organ transplantation (e.g., kidney, heart, pancreas). However, the transplant patients are often chronically ill and immunosuppressed from chronic illness and other immunosuppressive agents as part of their treatment protocol.

mTOR inhibition with rapamycin: Everolimus in glioblastoma multiforme

Two trials of mTOR inhibitors in the short term (~1 week) treatment of glioblastomas before brain surgery failed to identify any evidence of impaired wound healing, increased peri-operative infections, or bleeding [15,16]. Intratumoral rapamycin concentrations sufficient to inhibit mTOR in vitro occurred in all 15 patients, but the degree of mTOR inhibition in tumor cells, as measured by reduced ribosomal S6 protein phosphorylation, varied substantially [15]. Thus, although the dose of rapamycin ranged from 2 to 10 mg per day (5-fold range), the tumor levels of rapamycin ranged from 0.3 to 36.3 nM (>100-fold range). Further, rapamycin treatment led to Akt activation in seven patients, presumably due to loss of negative feedback, and this activation correlated with shorter time-to-progression during post-surgical maintenance rapamycin therapy ($p < 0.05$) [15]. In this trial, there were no grade 3 or 4 toxicities during the preoperative treatment or perioperatively. After recovery from surgery and resumption of therapy, 3 patients developed grade 3 toxicities that were potentially related to therapy: hypokalemia, hypercholesterolemia, and cytopenias. All were managed with supportive care and none led to discontinuation.

In a trial of everolimus for glioblastoma multiforme one week before surgery, 11 patients were randomized to treatment with 5 mg daily (n=5) or 10 mg daily (n=10) [17]. None of these 11 patients had peri-operative infections or impaired wound healing. Further, among those who continued on everolimus post-operatively, none developed infections. Notably, 63% of these patients were treated with dexamethasone and could have been immunocompromised as a result. Although dexamethasone may be used peri-operatively by neurosurgeons as standard care for epilepsy surgery, the dexamethasone therapy is associated only with the perioperative period whereas patients with glioblastoma multiforme are often on dexamethasone chronically, both before and after brain surgery. The glioblastoma patients are almost always on AEDs perioperatively and often chronically, and many took other drugs (e.g., warfarin) that could complicate their perioperative course.

CCI-779, an mTOR inhibitor, was used to treat 43 patients with recurrent gilioblastomas.[17] Unlike the prior studies, the drug was administered as an ongoing therapy. In this study, the drug was well tolerated by most subjects with lymphopenia, anemia, stomatitis, and elevated lipids as the only adverse effects. There was no reported increase in infections or wound healing problems.

Everolimus: effect on wound complications in organ transplantation

Several studies reviewed the surgical wound complications in patients receiving everolimus for organ transplantation, examining large, randomized, multicenter clinical trials. Some studies found an increased risk of wound complications in the everolimus-treated groups. Multivariate analyses reveal that this risk is higher in the presence of other well-known risk factors for surgical wound healing, most notably increased body mass index (BMI). This was most robust for patients undergoing heart transplantation, but a similar effect was found after renal and pancreas transplantation.

A systematic reviewed of 37 randomized control trials in solid organ transplants comparjng mTOR inhibitors with other therapies examined the incidence of wound complications and/or lymphocele formation.[18] The pooled analyses showed a higher incidence of wound complications (OR 1.77, CI 1.31–2.37) and lymphoceles (OR 2.07, CI 1.62–2.65) for kidney transplant recipients on mTOR inhibitors together with calcineurin inhibitors. There was also a higher incidence of wound complications (OR 3.00, CI 1.61–5.59) and lymphoceles (OR 2.13, CI 1.57–2.90) for kidney transplant recipients on mTOR inhibitors together with antimetabolites. A retrospective study of 513 kidney transplant patients found wound healing problems in 19% with elevated BMI as the greatest risk factor [25]. A retrospective

analysis of 97 liver transplant patients found no increase in poor wound healing events associated with mTOR inhibitory therapy [23].

Heart transplant patients receiving mTOR inhibitors together with calcineurin inhibitors also had more wound complications (OR 1.82, CI 1.15–2.87) [18]. A randomized study of 833 de novo kidney transplant recipients found a higher rate of wound healing complications in the two everolimus groups compared to a control group receiving mycophenolate sodium [19]. Among 634 de novo heart transplant patients, wound infection occurred in 6.7% of the 1.5 mg and 5.2% of the 3 mg everolimus groups; the rate was 3.3% in the group treated with azathioprine in combination with cyclosporine and corticosteroids [20]. A subsequent study of everolimus in conjunction with cyclosporine in 176 cardiac transplant recipients reported a 6.6% wound infection rate [21]. Wound healing complications relating to surgical intervention were reported as serious adverse events in 8.0% of patients receiving cyclosporine with everolimus and steroids in de novo heart transplant patients enrolled in a multicenter, randomized trial [22] Zuckerman et al [22] studied risk factors associated with wound complications among 711 everolimus-treated patients who underwent heart transplantation: 12.3% had an incision-related wound complication. Only BMI was significant in a multivariate analysis (12.9% increased odds for each 1 kg/m increase in BMI). They conclude that the overall risk for this complication is low, but increased in patients with increased BMI [22]. They suggest that if a patient is obese, diabetic, or needs surgical re-exploration, a higher surgical wound risk exists [22]. Similar findings have emerged from other solid organ transplant studies.[23-25]

Everolimus: experience in patients with TSC
Everolimus is a kinase inhibitor indicated with FDA approval for the treatment of:
• adults with renal angiomyolipoma and TSC, not requiring immediate surgery. The effectiveness of everolimus in the treatment of renal angiomyolipoma is based on an analysis of durable objective responses in patients treated for a median of 8.3 months. Further follow-up of patients is required to determine long-term outcomes.
• pediatric and adult patients with TSC who have subependymal giant cell astrocytoma (SEGA) that requires therapeutic intervention but cannot be curatively resected. The effectiveness is based on demonstration of durable objective response, as evidenced by reduction in SEGA tumor volume. Improvement in disease-related symptoms and overall survival in patients with SEGA and TSC has not been demonstrated.

There is considerable experience with everolimus use in TSC patients. In a prospective, open-label trial of everolimus for SEGAs in 28 patients, everolimus was associated with few serious side effects during the initial 6 months [8]. Self-limited upper respiratory tract infections and stomatitis were most frequent. Four patients had serious adverse events during the 6-month trial but they were not clearly drug related: hospitalization for respiratory infection (n=2; one had a prior history of reactive airway disease; one also had grade 3 vomiting) and convulsion (n=2). Twenty-five of these patients continued on everolimus for an average of 3 years and no new serious adverse events occurred [26]. A Phase III, prospective, double blind, randomized, multi-center study compared everolimus (n=78) and placebo (n=39) in TSC patients with radiologically enlarging SEGAs [27]. Everolimus was given at 4.5 mg/m2/day and titrated to a level of 5-15 ng/ml; MRI scans were performed at 3, 6, and 12 months after initiating treatment. The investigators found that 27 of 78 patients (34.6%) receiving everolimus, versus none of the placebo group, had a significant response of > 50% reduction in tumor volume ($p<0.0001$) [27]. Everolimus was generally well tolerated. A subsequent long-term followup study of 28 patients enrolled, 25 were still under treatment at the time of analysis.[28] Median dose was 5.3 mg/m(2)/day and median treatment duration was 34.2 months (range 4.7-47.1). At all time points (18, 24, 30, and 36 months), primary SEGA volume was reduced by ≥30% from baseline (treatment response) in 65%-79% of patients. All patients reported ≥1 adverse event (AE), mostly grade 1/2 in severity, consistent with that previously reported, and none led to everolimus discontinuation. The most commonly reported drug-related AEs were upper respiratory infections (85.7%), stomatitis (85.7%), sinusitis (46.4%), and otitis media (35.7%). No drug-related grade 4 or 5 events occurred.[27a] In a larger, international multicenter study, 117 patients were randomly assigned to everolimus (n=78) or placebo (n=39). 27 (35%) patients in the everolimus group had at least 50% reduction in the volume of SEGAs versus none in the placebo group (difference 35%, 95% CI 15-52; $p<0{\cdot}0001$).[29] Adverse events were mostly grade 1 or 2; no patients discontinued treatment because of adverse events. The most common adverse events were mouth ulceration (25 [32%] in the everolimus

group vs two [5%] in the placebo group), stomatitis (24 [31%] vs eight [21%]), convulsion (18 [23%] vs ten [26%]), and pyrexia (17 [22%] vs six [15%]).[29]

Other studies have used rapamycin to treat angiomyolipomas over 12 months with good efficacy and safety [30]. Everolimus was approved to treat renal angiomyolipomas in TSC. The most common AE during the 48-week trial was stomatitis. The most common Grade 3-4 adverse reactions (incidence >= 2%) were stomatitis, amenorrhea and convulsion. The most common laboratory abnormalities (incidence >= 50%) were hypercholesterolemia, hypertriglyceridemia and anemia. (Novartis data on file. http://www.pharma.us.novartis.com/product/pi/pdf/afinitor.pdf. Accessed April 2012)

1.5 Dose Rationale and Risk/Benefits

The starting dose of everolimus for treatment patients will be 4.5 mg/m^2 once daily; adjust to attain trough concentrations of 5-15ng/ml. The dose will be adjusted for co-medications as outlined in section 5.1. However, these are initial doses often need to be titrated up to therapeutic everolimus serum levels (5 – 15 ng/mL). Since our everolimus treatment duration is 7-21 days and the half-life is 30 hours, the time to equilibration will be approximately 150 hours (6.25 days) and will not allow the investigators to adjust dose to achieve therapeutic levels in the initial groups of patients who will be treated for one to two weeks. We will double the dose in our study for patients who are taking an enzyme-inducing antiepileptic drug (EIAED) since serum everolimus levels will be lower due to increased hepatic metabolism.

After a minimum of three patients are treated for one week, if there is no evidence of serious adverse effects and other dose-level tolerability and safety issues, we will extend the treatment duration by a maximum of one week. Again, after a minimum of three patients treated for two weeks without evidence of serious adverse effects and other dose-level tolerability and safety issues, we will extend the treatment duration to three weeks. . For patients treated longer than two weeks, an everolimus blood level will be obtained after 10-12 days and used to adjust dose to maintain a serum level of 5-10 ug/ml.

The most common adverse reactions (>30%) to everolimus treatment in TSC patients include stomatitis in patients with angiomyolipomas and stomatitis and respiratory tract infections in patients with SEGAs. Potential risks associated with 7-28 days of everolimus treatment include problems with wound healing and infections. Published data in patients with glioblastoma multiforme who underwent brain surgery, in a similar protocol to our study found no evidence of problems with wound healing or infection. There appears to increase the risk of infection and problems with wound healing in some patients who have received everolimus treatment and undergone organ transplantation but the risk of serious infection over control rates is not documented in patients with brain tumors or TSC.

For patients in the cardiac and renal transplant groups, much of the risk of infection and impaired wound healing is associated with obesity, age over 40 years old, and other factors. Further, the transplant groups in which wound healing issues have been identified suffer from chronic illness and treatment with other immunosuppressive agents. To reduce the potential risk to patients with TSC in our study, we will exclude patients who are 1) obese, 2) over age 50, 3) have a history of chronic medical illness, or 4) treatment with an immunosuppressant (other than everolimus during the 7-day trial). These criteria should minimize the potential for peri-operative infection or impaired wound healing. However, we will be vigilant with the Data Safety Monitoring Plan to closely assess all patients postoperatively and record all complications, including infection and impaired wound healing. Further, we will institute a special surveillance system to monitor for these potential side effects.

The following are possible benefits that could occur through this research study:

1) Elimination of need for epilepsy surgery if there is a significant reduction in seizure activity;
2) Improvement in seizure control, as well as reduction of cognitive, behavioral, and physical injury, and sudden death risks associated with ongoing seizures;
3) Improvement in cognitive or behavioral function; and
4) Reduction in signs and symptoms of TSC (e.g., renal angiomyolipomas, lymphangiomyelotmatosis, facial angiofibromas).
5) Close medical follow-up care

For patients with FCD, the potential benefits are likely less, but could include 1 through 3 above since increased mTOR signaling is documented in FCD and it remains uncertain if Everolimus therapy may provide clinical benefits.

The potential risks of everolimus must be weighed against the potential benefits, which for the patients with TSC include: 1) elimination of need for epilepsy surgery if there is a significant reduction in seizure activity, 2) improvement in seizure control and reduction of cognitive, behavioral, physical injury, and sudden death risks associated with ongoing seizures, 3) improvement in cognitive or behavioral function, and 4) reduction in systemic signs and symptoms of TSC (e.g., renal angiomyolipomas, lymphangiomyelotmatosis, facial angiofibromas, etc.). For patients with FCD, the potential benefits are likely less, but could include 1 through 3 above since abnormalities of mTORC1 signaling are documented in FCD and it remains uncertain if therapy may provide clinical benefits.

2 Study Objectives

2.1 Primary Objective:

The primary objectives of this pilot study are to 1) test the feasibility and safety of recruiting patients into a prospective study before epilepsy surgery in patients with TRE and either TSC or FCD, and 2) test the hypothesis that everolimus therapy in TSC and FCD patients with TRE will reduce mTOR signaling (reduced S6 phosphorylation) as compared to TSC and FCD who did not receive everolimus (control group).

2.2. Secondary Objectives:

The secondary objectives include
1) systemic mTOR signaling suppression (VEGF-D) pre-and post-treatment,
2) genetic analysis of resected brain tissue,
3) correlate blood everolimus levels with brain measures of S6 phosphorylation,
4) measure markers of cortical hyperexcitability (glutamate and GABA receptors, levels of neuroinflammation, axonal abnormalities, myelination defects) as compared to historical controls and
5) electrocorticographic (ECoG) recordings as compared to historical controls

3 Study Design

3.1 General Design

This is a multi center open-label pilot clinical trial of patients with TRE, ages 2 to 50 years old, with TSC or FCD who are scheduled for epilepsy surgery. Patients will be treated with everolimus for 7 to 21 days prior to epilepsy surgery with extension of time from 7 to 21 days in successive cohorts of patients. The initial cohort of at least three patients will be treated for 7 days and after the safety of therapy is assured for this group, there will be an extension of the treatment to 14 days for at least three patients. This will be extended at one week intervals/three patient groups to a maximum treatment duration of 21 days. Resected brain tissue will be analyzed for activation of mTORC1 and mTORC2 signaling pathways, glutamatergic and GABA-ergic neurotransmission using histochemistry, genetic analysis, as well as extracellular field recordings in acute ex-vivo brain slices from surgery. A blood sample, collected at the time of surgery, will be analyzed for everolimus levels and VEGF-D. All patients will undergo standardized intra-operative ECoG recordings over the primary epileptogenic region and reviewed blindly.

Subjects will be in the study for approximately 10 weeks. We will study variables listed in specific aims 1 and 2 in TSC and FCD patients treated with 7 to 21 days of everolimus and compare these to untreated control patients with TRE and TSC or FCD. A concurrent comparison group of 12 subjects will also be enrolled. They will all be undergoing routine surgery for the diagnosis of TRE with TSC or FCD.

All study procedures will be performed at either the Comprehensive Epilepsy Center (CEC) with the exception of the surgery, which will be performed at Tisch Hospital

3.2 Primary Outcome Measures

The primary outcome measures for this study are to 1) test the feasibility and safety of recruiting patients into a prospective study before epilepsy surgery in patients with TRE and either TSC or FCD, and 2) estimate S6 phosphorylation status as a measure of downstream mTOR activity in patients treated with Everolimus as compared to 12 control subjects' brain tissue (FCD and TSC patients who had epilepsy surgery) who had not received Everolimus.

3.3 Secondary Outcome Measures

- Other mTOR downstream/upstream markers in the brain (S6 phosophorylation) and peripheral VEGF-D levels.
- Blood and brain levels of cytokines (and TNF-α) and inflammatory proteins (HMGB1)at the time of surgery
- Brain glutamate and GABA receptor expression at the time of surgery
- Blood levels of everolimus at the time of surgery

- **Electrocorticography** of minimum of 10 minutes. Site of expected maximal epileptogenicity will be recorded with grid or strip array of at least 12 contact points. **Extracellular field and recordings in acute ex-vivo brain slice**: After resection, one brain sample will be immediately placed, under sterile **conditions, into cold, oxygenated artificial cerebrospinal fluid (ACSF).** Tissue will be transported to the lab and, within 5-10 min from resection, acute brain slices will be prepared. Extracellular field recordings, from visually identified neurons will be employed to assess overall cortical excitability, intrinsic neuronal firing properties and basic synaptic function.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria (Active Group)

1. Female and male patients, age 2 years to 50 years.
2. Diagnosis: treatment resistant epilepsy (> 2 failed anti-epileptic drug [AED] treatment regimens) and either:
   - Tuberous Sclerosis Complex
   - Focal Cortical Dysplasia on MRI or neuropathology from prior brain surgery
3. Diagnosis of TSC confirmed by genetic testing and/or clinically definite diagnosis of TSC according to the modified Gomez criteria [31]. Clinically definite diagnosis is defined as:
   - Two Major Features, or
   - One Major Feature plus two Minor Features
4. Adequate bone marrow function as shown by: ANC ≥1.5 x 10^9/L, Platelets ≥100 x 10^9/L, Hb >9 g/dL;
5. . Adequate liver function as shown by
   a. Total serum bilirubin ≤2.0 mg/dL,
   b. ALT and AST ≤2.5x ULN (≤5x ULN in patients with liver metastases),
   c.INR ≤2; if applicable
6. Adequate renal function: serum creatinine ≤1.5x ULN;
7. Fasting serum cholesterol ≤300 mg/dL OR ≤7.75 mmol/L AND fasting triglycerides ≤2.5x ULN.if applicable NOTE: In case one or both of these thresholds are exceeded, the patient can only be included after initiation of appropriate lipid lowering medication;
8. Signed informed consent obtained prior to any screening procedures.

4.1.1 Inclusion Criteria (Concurent Comparison Group)

1. Female and male patients, age 2 year to 50 years. Matched for age (+/- 7 years) and sex of subjects in the treatment group.
2. Diagnosis: treatment resistant epilepsy (≥ 2 failed anti-epileptic drug [AED] treatment regimens) due to TSC or FCD. Matched for diagnosis of TSC and FCD.
3. Brain surgery for seizure control in which tissue is banked for research utilizing an existing IRB-approved study.

4.2 Exclusion Criteria (Active Group)

1. Treatment with an mTOR inhibitor (everolimus, sirolimus) during the past four weeks.
2. Patients taking more than one strong CYP3A4 inducer will be excluded.
3. Known hypersensitivity to an mTOR inhibitor (everolimus, sirolimus)
4. Body mass index (BMI) > 95% for age in individuals 17 years old or younger; BMI > 30 kg/m^2 in individuals > 17 years old
5. Failure to establish diagnosis of treatment resistant epilepsy (i.e., adequate trials of two appropriately-chosen, tolerated and adequate trials of antiepileptic drugs) [32].
6. Presence of mesial temporal sclerosis or other brain structural lesion than those identified in TSC and FCD, other than incidental abnormalities (e.g., pineal cyst)
7. Exposure to any investigational agent in the month prior to study entry.
8. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during dosing of study treatment. Highly effective contraception methods include combination of any two of the following (a+b or a+c or b+c):
    a. Use of oral, injected or implanted hormonal methods of contraception or;
    b. Placement of an intrauterine device (IUD) or intrauterine system (IUS);
    c. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository;
    d. Total abstinence or;
    e. 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.
9. Male patients whose sexual partner(s) are women of child-bearing potential who are not willing to use adequate contraception, during the study and for 8 weeks after the end of treatment
10. Positive pregnancy test
11. Patients will be excluded if using strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir, voriconazole).
12. History of malignancy patients who are receiving anti-cancer treatments, such as radiation therapy and/or chemotherapy.
13. Patients with severe and/or uncontrolled medical conditions, including but not limited to cardiac disease, cancer, disorders requiring immunosuppressive therapy, systemic autoimmune disorders, or uncontrolled diabetes mellitus.
14. Patients on chronic corticosteroid therapy

15. A history of HIV seropositivity
16. Known drug or alcohol use or dependence
17. 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;
18. Known impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of oral everolimus;
19. 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;
20. Patients who have any severe and/or uncontrolled medical 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 chronic hepatitis (i.e. quantifiable HBV-DNA and/or positive HbsAg, quantifiable HCV-RNA),

4.2.1 Exclusion Criteria (Concurrent Comparison Group)

1. Treatment with an mTOR inhibitor (everolimus, sirolimus) during the past four weeks.
2. Known hypersensitivity to an mTOR inhibitor (everolimus, sirolimus)
3. Body mass index (BMI) > 95% for age in individuals 17 years old or younger; BMI > 30 kg/m^2 in individuals > 17 years old
4. Failure to establish diagnosis of treatment resistant epilepsy (i.e., adequate trials of two appropriately-chosen, tolerated and adequate trials of antiepileptic drugs) [31].
5. Presence of mesial temporal sclerosis or other brain structural lesion than those identified in TSC and FCD, other than incidental abnormalities (e.g., pineal cyst)
6. Exposure to any investigational agent in the month prior to study entry.
7. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, unless they are using highly effective methods of contraception during dosing of study treatment. Highly effective contraception methods include combination of any two of the following (a+b or a+c or b+c):
    a. Use of oral, injected or implanted hormonal methods of contraception or;
    b. Placement of an intrauterine device or intrauterine system;
    c. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository;
    d. Total abstinence or;
    e. 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.

8. Male patients whose sexual partner(s) are women of child-bearing potential who are not willing to use adequate contraception, during the study and for 8 weeks after the end of treatment
9. Positive pregnancy test
10. Patients will be excluded if using strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir, voriconazole).
11. History of malignancy patients who are receiving anti-cancer treatments, such as radiation therapy and/or chemotherapy.
12. Patients with severe and/or uncontrolled medical conditions, including but not limited to cardiac disease, cancer, disorders requiring immunosuppressive therapy, systemic autoimmune disorders, or uncontrolled diabetes mellitus.
13. Patients on chronic corticosteroid therapy
14. A history of HIV seropositivity
15. Known drug or alcohol use or dependence
16. 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;
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. Patients who have any severe and/or uncontrolled medical 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 chronic hepatitis (i.e. quantifiable HBV-DNA and/or positive HbsAg, quantifiable HCV-RNA),

4.3 Subject Recruitment, Screening, and Consenting

All patients will be recruited, consented and enrolled at the NYU Comprehensive Epilepsy Center (CEC). The NYU CEC is the ideal centers for recruitment since they already has a high influx of epilepsy patients that concomitantly have TSC or FCD. All eligible subjects have treatment resistant epilepsy with TSC or FCD and are planning to have their surgery in the next few months. All patients who are identified as potential candidates for the study will be entered into a study screening log which will document the individual who referred the subject to the study or if they were self-referred and if the subject/parents/guardian did not participate why they declined or were not eligible, and for those who were ineligible, the specific reason why they were ineligible.

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The physician will explain the study during the routine visit and inform the subject and surrogate that a capacity assessment will be performed to determine whether the subject is capable of providing an informed decision to participate in the study. At this time, a capacity assessment will be conducted if the subject does not refuse the assessment. If there is already documentation of lack of capacity, no assessment will be needed. Patients who are eligible and are lacking in capacity will be allowed to participate by receiving consent from a parent/legal guardian/health care proxy. This protocol is

specifically for patients with drug resistant epilepsy. If we were to only include patients with drug resistant epilepsy who have capacity to consent, we would have very few participants in this study, as one of the common co-morbidities of drug resistant epilepsy is global impairment.

. If the subject/parent/legal guardian/health care proxy agrees, they will be given the informed consent document and study personnel will be present to explain the study in further details the objectives and study procedures, risks and benefits. We will review the consent form documents with them and answer any questions they have. They will be given an opportunity to ask questions regarding the study and consent form. After all parties have signed off on the consent, we will give them a copy of the informed consent documents.

As the majority of our surgical patients reside outside of the NYC area, there may be circumstances in which patients are unable to be present at the study site for consenting due to distance and travel issues. In such cases, the informed consent will be faxed or emailed to the patient. The principal investigator will then review and obtain consent over the phone. The principal investigator will explain the study in full detail and ensure that all questions are answered. The patient will then sign and date the informed consent and return it to the principal investigator via fax or email. Once received the principal investigator will sign and date the informed consent. A copy of this signed and dated informed consent will then be faxed or emailed to the patient before beginning any study procedures. The patient will also bring the original signed informed consent to the study site at the first visit to again be signed by the principal investigator. Both the mailed informed consent and the original will be kept on file and copies will be given to the patient. All phone correspondence and mailings will be documented in the study file. Telephone consent is necessary for these long distance subjects who will be coming in for a combined visit 1 and 2 as certain study procedures need to be completed prior to their combined visit. Particularly, in order to meet the inclusion criteria, patients who have not had certain bloodwork need to have it completed in order to be eligible for the study. Therefore, in certain cases we need to obtain consent prior to combined Visit 1 and 2 so that these patients can obtain bloodwork and confirm eligibility prior to this combined initial visit.

Consent by subject
If the subject is 18 years old and it has been determined that he/she has capacity, he/she will review and sign an informed consent. The study personnel will be present to explain the study in further details and answer questions as needed. After all parties have signed off on consent, a copy of the informed consent documents will be given to the subject.

Assent by subject
If the subject is 7-17 years old and it has been determined that he/she has capacity, he/she will review and sign an assent form. In addition, if the parent/legal guardian/health care proxy agrees, they will be given the informed consent document and study personnel will be present to explain in further details the study the objectives and study procedures, risks and benefits. After all parties have signed off on assent and consent, a copy of the informed assent and consent documents will be given to the subject and the subject's parent/legal guardian/health care proxy.

Consent by surrogate (for subjects lacking capacity)
If the subject assents, and the parent/legally authorized representative agrees, they will be given the informed consent document and study personnel will be present to explain in further details the study the objectives and study procedures, risks and benefits. Legally authorized representative will be identified as per New York state law. The consent form will be reviewed and any questions they may have regarding the study and consent form will be answered. After all parties have signed off on the consent, a copy of the informed consent documents will be given to them.

<u>Assessment</u>
A capacity assessment will be conducted by Dr. Manisha Holmes, (NYU CEC) who is independent of the study to ensure the patient's level of capacity. She is delegated only to perform capacity assessment throughout the study. See is board certified Neurologist as well as a Epileptologist

experienced in dealing with patients with drug resistant epilepsy. Capacity will be assessed through neurological examinations and neuropsychology reports conducted by the physician as well as learning and/or development delays that have been documented.

For long distance subjects, Dr. Holmes will interview the parent and child via a speaker phone if the child is verbal. If the child is nonverbal they will be considered incapable of capacity to consent. For those that are verbal, Dr. Holmes will determine if the child is able to:
a) communicate clearly in English (or another language with translation services),
b) grasp the fundamental meaning of information that is communicated,
c) acknowledge medical condition and likely consequences of involvement in the study,
and d) engage in a rational process of manipulating relevant information.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

A subject may withdraw from the study due to:

1) Improvement in seizure activity to an extent that the patient and/or parents/legal guardians elect to forego epilepsy surgery for treatment with everolimus,
2) Difficult complying with protocol requirements or
3) A decision to withdrawal for personal reasons or adverse effects that are less severe than those that lead to mandatory discontinuation of the treatment phase.

A subject will be withdrawn from the study if any of the following are present:

1) The subject develops a systemic bacterial or fungal infection.
2) The subject develops a CTAE grade 3 or 4 (severe) event.
3) The patient and study will be terminated if he or she is the second case of impaired surgical wound healing or the third case of serious infection, including infection of the wound site, among the 18 everolimus-treated patients.

Given the short duration of treatment, there are no known or postulated safety concerns related to abrupt discontinuation after this duration and dosage of therapy with everolimus. Any subject who elects to withdraw from the study will be recommended to discontinue the everolimus therapy, but if they desire a tapering schedule, one will be provided with exception for those patients going forward with surgery, when everolimus will be completely discontinued prior to surgery.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

For any subject who withdraws from the study, we will obtain serum levels of everolimus on the last date they take the medication (or as close). We will also collect all safety data and seizure activity calendars. Since these patients will be entered into the study only 7 to 21 days before brain surgery, they typically have close contact with the neurosurgeon, epileptologist, and clinical staff of the epilepsy center. We will actively pursue with phone calls to patient, parents, and legal guardians to determine the cause for withdrawal and obtain as complete data as possible regarding study data elements during the baseline and treatment phase. We will utilize up to 5 phone calls and 2 certified letters to any patients who withdraw but for whom we do not have complete study records.

5 Study Drug

Everolimus will be supplied by Novartis at no charge.

5.1 Description & Packaging

Everolimus (RAD001) is an inhibitor of mTOR, and is FDA-approved as an antineoplastic agent. The chemical name of everolimus is (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[30.3.1.04,9]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone. The molecular formula is C53H83NO14 and the molecular weight is 958.2.

For this study, everolimus will be supplied as dispersal tablets of 2.0, 3.0, 5.0 mg strength. The drug will be supplied by Novartis at no charge for study enrollment to patients enrolled in treatment group at NYU CEC

The starting dose of everolimus for treatment patients will be 4.5 mg/m^2 once daily; adjust to attain trough concentrations of 5-15ng/ml. The dose will be adjusted for co-medications as outlined in the section immediately below.

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CYP3A4 and/or P-glycoprotein (PgP) Inhibitors

Patients will be excluded if using strong CYP3A4 inhibitors (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir, voriconazole). If the patient is receiving a co-administered drug with moderate CYP3A4 and/or PgP inhibitors (e.g., amprenavir, fosamprenavir, aprepitant, erythromycin, fluconazole, verapamil, diltiazem), the everolimus dose will be reduced by approximately 50% to maintain trough concentrations of 5 to 10 ng/mL. If dose reduction is required for patients receiving 2.5 mg daily, alternate day dosing will be used. Subsequent dosing will be individualized based on therapeutic drug monitoring in subject on everolimus for sufficient duration. No moderate or strong CYP3A4 and/or PgP inhibitor will be added or discontinued during the study period.

Patients taking one concomitant strong CYP3A4 inducer for seizure control (e.g., phenytoin, carbamazepine, phenobarbital) will be allowed in the study. Patients taking more than one r strong CYP3A4 inducer will be excluded. For patients requiring a concomitant strong CYP3A4 inducer (an Enzyme-Inducing Antiepileptic Drug (EIAED), we will double the everolimus dose from that prescribed in Table 2. Subsequent dosing should be individualized based on therapeutic drug monitoring if the patient is treated with everolimus for sufficient duration. No EIAED will be discontinued during the study.

Preparation of the everolimus dispersal:

The patient will receive everolimus tablets for oral suspension as a suspension only. The everolimus tablets for oral suspension will be administered orally once daily at the same time every day. It will be given either consistently with food or consistently without food. The suspension will be administered immediately after preparation. The suspension will be discarded if not administered within 60 minutes after preparation.

Prepare suspension in water only.

Using an oral syringe:

• Place the prescribed dose of everolimus tablets for oral suspension into a 10-mL syringe. Do not exceed a total of 10 mg per syringe.

If higher doses are required, prepare an additional syringe. Do not break or crush tablets.

• Draw approximately 5 mL of water and 4 mL of air into the syringe.

• Place the filled syringe into a container (tip up) for 3 minutes, until the everolimus tablets for oral suspension are in suspension.

• Gently invert the syringe 5 times immediately prior to administration.

• After administration of the prepared suspension, draw approximately 5 mL of water and 4 mL of air into the same syringe, and swirl the contents to suspend remaining particles. Administer the entire contents of the syringe.

Using a small drinking glass:

• Place the prescribed dose of everolimus tablets for oral suspension into a small drinking glass (maximum size 100 mL) containing

approximately 25 mL of water. Do not exceed a total of 10 mg of everolimus tablets for oral suspension per glass. If higher doses are required, prepare an additional glass. Do not break or crush tablets.

• Allow 3 minutes for suspension to occur.

• Stir the contents gently with a spoon, immediately prior to drinking.

• After administration of the prepared suspension, add 25 mL of water

Everolimus tablets for oral suspension should NOT be taken with grapefruit or Seville orange juice or with other effectors of CYP3A4 (other than known AEDs or other medications identified at Visit 1 as one of their baseline medications).

NOTE: Everolimus should be opened only at the time of administration as drug is both hygroscopic and light-sensitive.

5.2 Treatment Regimen

Everolimus will be self-administered (by the patient or parent at home during the 7-21 days prior to neurosurgery). The investigator will instruct the patient to take the study drug exactly as specified in the protocol.

Patients who are eligible for the active treatment group with TSC or FCD only will receive everolimus. After a minimum of three patients are treated for 7 days (Table 2), if there is no evidence of serious adverse effects or other dose-level tolerability and safety issues, we will extend the treatment duration by a maximum of one week. Again, after a minimum of three patients are treated for two weeks without evidence of serious adverse effects or other dose-level tolerability and safety issues, we will extend the treatment duration to three weeks. . For patients treated longer than two weeks, an everolimus blood level will be obtained after 10-12 days and used to adjust dose to maintain a serum level of 5-10 ug/ml. to revisit escalation and numbers If one patient at a dose duration has a serious adverse effect during a treatment cohort period (e.g., 7 days), then up to 6 patients are treated with that dose duration. If there are 2 or more serious adverse events among the 6 patients, then the duration is dropped back to the prior level. If there are no events in 3 patients or 1 event in 6 patients, then the dose duration is escalated to the next level. At the 3 week dose duration, 6 patients will be treated even if there are no events among the first 3 patients.

Table 2: Dosing Escalation for Serial Cohorts

Serial Cohort Number	Minimum # of patients if no Grade 3 or 4 adverse event or serious adverse events	Minimum # of patients if one Grade 3 or 4 adverse event or serious adverse events^	Duration of everolimus therapy	Result of one serious adverse events including wound healing problem and serious infection	Result of two serious adverse events including wound healing problem and serious infection
1	3	9	7 days		
2	3	6	14 days	Add additional patients to Cohort 1	Return to cohort one, no potential for longer treatment duration
3	1-3	6	21 days	Add additional patients to Cohort 2	Return to cohort two, no potential for longer treatment duration

^ Adverse events as defined by CTAE v.4.0

Dosing:

- **Patients NOT taking EIAEDs or other drugs know to inhibit or induce CYP34A or PgP**: The dose will be administered according to the criteria in Section 5.1.
- **Patients taking EIAEDs:** The dose will be administered according to criteria in Section 5.1.
- **Patients taking moderate CYP3A4 and/or PgP inhibitors**: The dose will be administered according to criteria in Section 5.1.

Dose adjustments will be permitted based on safety findings or serum everolimus levels for patients on treatment for 3 or 4 weeks. A detailed explanation of permitted dose adjustments and the process that will be implemented are found in Section 5.3.1 Dose Adjustments.

5.3 Study Drug Administration

Patients will be instructed to take the prescribed dose of everolimus (or no treatment in the comparison group) orally with a glass of water at regular intervals, and each taken dose should be at the same time in the morning after a light, nonfat breakfast. Body Surface Area (in m2) should be calculated 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 H0.725) x 0.007184. The dose will be 4.5 mg/m^2 once daily; adjust to attain trough concentrations of 5-15ng/ml and exceptions for patients taking EIAEDs based on section 5.1.

Subjects will take the medication as specified in Section 5.1.

Dietary habits around the time of everolimus intake should be as consistent as possible throughout the study, and in particular, on the day when sample is being taken for pharmacokinetic analysis.

NOTE: Everolimus should NOT be taken with grapefruit or Seville orange juice. Patients with taking drugs that inhibit or induce CYP3A4 /450 or P-glycoprotein (P-gp) will be identified and doses adjusted for those who are taking EIAEDs.

Patients will receive treatment with study drug for 7 to 21days or until the occurrence of unacceptable toxicity as defined by the patient or guardian, or if a Grade 3 or 4 Adverse Event occurs as defined by Common Terminology for Adverse Events (CTAE) v.4.0 occurs, or the investigator or patient decides that continuation is not in the best interest of the patient. Interruption for toxicity will lead to discontinuation of the medication.

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

5.3.1 Management of specific toxicities:

Overall, safety data available from completed, controlled and uncontrolled studies indicate that everolimus is generally well tolerated at weekly or daily dose schedules. 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 rash, stomatitis/oral mucositis, non-infectious pneumonitis, fatigue, headache, anorexia, nausea, vomiting, diarrhea, and infections. Overall, the most frequently observed laboratory abnormalities include neutropenia, thrombocytopenia, hypercholesterolemia, and/or hypertriglyceridemia. The majority of these AEs have been of mild to moderate severity (NCI CTC grade 1-2). Recommendations for dose adjustments for non-hematologic and hematologic adverse events are summarized in Tables 3 and 4.

Table 3: Dosing guidelines for Everolimus-related non-hematologic toxicities

Toxicity	Action
AST or ALT elevation Grade 1 (> ULN - 3.0 x ULN) Grade 2 (> 3.0 - 5.0 x ULN)	Maintain current dose level
AST or ALT elevation Grade 3 (> 5.0 - 20.0 ULN)*	Interrupt Everolimus administration until resolution to ≤ grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤ 7 days, Everolimus should be re-started at the dose level prior to interruption. If resolution takes > 7 days, or if event recurs within 21 days, hold Everolimus until recovery to ≤ grade 1 or baseline grade/ value and reintroduce Everolimus at one dose level lower, if available.
AST or ALT elevation Grade 4 (> 20 x ULN)*	Interrupt Everolimus administration until resolution to ≤ grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤ 7 days, Everolimus should be re-started at one dose level lower. If resolution takes > 7 days, discontinue Everolimus.
Recurrence of grade 4 after dose reduction or toxicity requiring Everolimus interruption for > 21 days	Discontinue Everolimus.
Intolerable grade 2 mucositis, or grade 3 AE, except hyperglycemia or hypertriglyceridemia or hypercholesterolemia	Interrupt Everolimus administration until resolution to ≤ grade 1 or baseline grade / value. If resolution occurs within ≤ 7 days, Everolimus should be re-started at the dose level prior to interruption. If resolution takes > 7 days, or if event recurs within 21 days, hold Everolimus until recovery to ≤ grade 1 or baseline grade/ value and reintroduce Everolimus at one dose level lower, if available. Patients will be withdrawn from the study if they fail to recover to □ grade 1 or baseline grade / value within 21 days.
Any other grade 4	Hold Everolimus until recovery to grade □ 1 or baseline value Reintroduce Everolimus at one dose level lower, if available.
Grade 3 or 4 clinical liver failure (asterixis or encephalopathy/coma)	Discontinue Everolimus
Recurrence of intolerable grade 2 mucositis or grade 3 event after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of Everolimus is 2.5 mg daily. Below this level, Everolimus must be discontinued.
Recurrence of grade 4 after dose reduction	Discontinue Everolimus
Any non-hematologic toxicity requiring Everolimus interruption for > 21 days	Discontinue Everolimus

Table 4: Dosing guidelines for Everolimus-related hematologic toxicities

Toxicity	Action
Grade 1 thrombocytopenia (platelets 0-75, ≥ 75 x109/L)	No action.
Grade 2 thrombocytopenia (platelets <75, ≥ 50x109/L)	~~Temporary dose interruption until recovery to Grade ≤1.~~ Re-initate treatment at the same dose.
Grade 3 thrombocytopenia (platelets <50,) **OR** Grade 4 thrombocytopenia (platelets < 25 x109/L)	Interrupt Everolimus until resolution to grade ≤1 Re-initate treatment at a lower dose.
Grade 3 neutropenia or anemia (neutrophil <1, ≥0.5 x109/L)	Interrupt Everolimus until recovery to grade ≤2. Then reintroduce Everolimus at the same dose.
Grade 4 neutropenia or anemia	Interrupt Everolimus until recovery to grade ≤2. Then reintroduce Everolimus at a lower dose.
Febrile neutropenia	Interrupt Everolimus until resolution to grade 1 (or baseline value) and no fever. Reintroduce Everolimus at one dose level lower, if available.*
Recurrence of grade 3 toxicity after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of Everolimus is 5 mg every other day (2.5 mg daily). Below this level, Everolimus must be discontinued.
***Recurrence of grade 4 toxicity (including febrile neutropenia) after dose reduction**	**Discontinue Everolimus**
***Any hematologic toxicity requiring Everolimus interruption for > 21 days**	**Discontinue Everolimus**

5.3.1.1. Management of stomatitis / oral mucositis / mouth ulcers

Patients with a clinical history of stomatitis/mucositis/mouth ulcers and those with gastrointestinal morbidity associated with mouth/dental infections, irritation of esophageal mucosa e.g. gastroesophageal reflux disease (GERD) and pre-existing stomatitis/mucositis must be monitored even more closely. Patients should be instructed to report the first onset of buccal mucosa irritation/reddening to their study physician immediately.

Stomatitis/oral mucositis/mouth ulcers due to everolimus should be treated using local 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. We will 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 (see Table 5), manage with 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®). Temporarily interrupt dose until recovery to Grade ≤1. Re-initiate Afinitor® at the same dose. If stomatitis recurs at Grade 2, interrupt dose until recovery to Grade ≤1. Re-initiate Afinitor® at a lower dose.
3. For grade 3 in which case patients cannot maintain adequate oral alimenation, manage with 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®). Temporarily interrupt dose until recovery to Grade ≤1. Re-initiate Afinitor® at a lower dose.
4. For grade 4 in which symptoms are associated with life-threatening consequences, discontinue Afinitor and treat with appropriate medical therapy.
5. Agents containing alcohol, hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.

Table 5: Treatment for Patients with Intolerable Grade 2 Mucositis

Intolerable grade 2 mucositis	Interrupt everolimus administration until resolution to ≤ grade 1 or baseline grade / value. If resolution occurs within ≤ 7 days, Everolimus should be re-started at the dose level prior to interruption. If resolution takes > 7 days, or if event recurs within 21 days, hold everolimus until recovery to ≤ grade 1 or baseline grade / value and reintroduce everolimus at one dose level lower, if available. Patients will be withdrawn from the study if they fail to recover to □ grade 1 or baseline grade / value within 21 days.

5.3.1.2. 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.

5.3.1.3. Management of diarrhea

Appearance of grade 1-2 diarrhea attributed to study drug toxicity may be treated with supportive care such as loperamide, initiated at the earliest onset (for example 4 mg orally followed by 2 mg orally every 2 hours until resolution of diarrhea).

5.3.1.4. Management of hyperlipidemia and hyperglycemia

Treatment of hyperlipidemia should take into account the pre-treatment status and dietary habits of the patient. Grade 2 or higher hypercholesterolemia (>300 mg/dL or 7.75 mmol/L) or grade 2 hypertriglyceridemia or higher (>2.5x upper normal limit) should be treated with a 3-hydroxy-3-methyl- glutaryl (HMG)-CoA reductase inhibitor (e.g. atorvastatin, pravastatin, fluvastatin) or appropriate triglyceride-lowering medication, in addition to diet.

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.

Hyperglycemia has been reported in clinical trials. Monitoring of fasting serum glucose is recommended prior to the start of everolimus and periodically thereafter. Patients with diabetes mellitus will be excluded from the study.

5.3.1.5 Management of infections

Everolimus has immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoal infections, including infections with opportunistic pathogens. Localized and systemic infections, including pneumonia, other bacterial infections, invasive fungal infections, such as aspergillosis or candidiasis and viral infections including reactivation of hepatitis B virus, have been described in patients taking everolimus. Some of these infections have been severe (e.g. leading to respiratory or hepatic failure) and occasionally have had a fatal outcome.

Physicians and patients should be aware of the increased risk of infection with everolimus. Treat pre-existing infections prior to starting treatment with everolimus. While taking everolimus, be vigilant for symptoms and signs of infection; if a diagnosis of infection is made, institute appropriate treatment promptly and consider interruption or discontinuation of everolimus.

If a diagnosis of invasive systemic fungal infection is made, discontinue everolimus and treat with appropriate antifungal therapy.

5.3.2 Dose Adjustments

There only planned dose adjustments will be in patients taking the drug for 3. Patients will be started on a set dose of everolimus based on their BSA (m^2) and whether or not they are taking an EIAED. For those subjects taking everolimus for 3 weeks, a serum level will be obtained between days 10-12. If the result is in desired range (5-10 ng/ml) for patient receiving study medication, the recommendation will be no change. If the value is between 3-5 ng/ml, the dose will be increased by 2.0 mg/m^2/day and if the value is less than 3 ng/ml, the recommendation will be dose will be increased by 3.0 mg/ m^2/day. If the value is between 10-13 ng/ml, the dose will be decreased by 2.0 mg/m^2/day. If the value is above 13 ng/ml, the dose will be decreased by 3.0 mg/m^2/day.

5.3.3 Serum drug levels

The target range for everolimus serum levels in this study is between 5-10 mg/ml. For patients taking everolimus for 1 or 2 weeks, the only time an everolimus level will be assessed is on the morning of surgery from the reference laboratory. For those subjects taking everolimus for 3 weeks, a serum level will be obtained between days 10-12. All labwork will be performed at Tisch hospital, 560 First Avenue New York, NY 10016 or St Barnabas Medical Center 94 Old Short Hills Rd.Livingston, NJ 07039 and the subjects taking everolimus for 3 weeks, the bloodwork will be done at the outpatient lab at Tisch Hospital as St Barnabas will only enroll controls

5.4 Method for Assigning Subjects to Treatment Groups

All eligible patients will have TSC and FCD and will tentatively be scheduled or planning to have surgery. These eligible patients will be decided by Dr. Orrin Devinsky, or Dr. Doyle. Dr. Devinsky, Dr Yaun, Dr Harter or Dr.Doyle will also review their patient's charts to ensure they meet the inclusion criteria (review section 4.1 for all inclusion criteria). Once an eligible patient is decided upon, it will be presented to the patient if they would like to participate in this study. Anyone who declines at the NYU CEC will be asked to be take part in the control part of the study. Therefore it is up the patient to choose whether or not they will participate in the study and if they would like to be a part of the intervention or the control group.

The objective of this study is to show that everolimus can suppress mTOR signaling in patients. A control group is necessary for this study to compare the mTOR with patients who do not take the everolimus.

Subject Compliance Monitoring

Patients should be requested to bring their unused study drug and packaging to the clinic at each visit. Compliance should be verified by the investigator's staff through counting the number of tablets consumed between at the end of the study period.

5.4 Prior and Concomitant Therapy

Data will be collected on detailed information on all concomitant medical and over-the-counter medications taken by the patients. The dosage and time(s) of administration will be recorded at the start of the study and throughout the study period. For patients taking an EIAED or moderate CYP3A4 and/or PgP inhibitors, the dose of everolimus will be adjusted as specified in section 5-2.

Patients are permitted to take their regular medications for epilepsy, including rescue medications (e.g., rectal Diastat, oral clonazepam) during the study period. Patients are also permitted to take medications for medical conditions that are not exclusionary (e.g., propranolol for migraine will be permitted but prednisone for systemic lupus erythematosus will NOT be allowed). Patients are not permitted to take immunosuppressive drugs (other than everolimus as a blinded medication) during the study period.

Patients will be asked to avoid drugs known to affect coagulation or platelet adhesion for 14 days before surgery.

5.5 Blinding of Study Drug

Not applicable.

5.6 Receiving, Storage, Dispensing and Return

5.6.1 Receipt of Drug Supplies

Upon receipt of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. The designated study staff will count and verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

5.6.2 Storage & Handling

Study drug must be stored at 25°C (77°F); excursions permitted between 15°–30°C (59°–86°F); protect from light and moisture. Procedures for proper handling and disposal of anticancer drugs should be considered.

5.6.3 Dispensing of Study Drug

After a subject with FCD or TSC is consented and entered into the study, they will be instructed on the dosage and administration times of the study medication. All subjects will receive a specific quantity of tablets and they will be asked to record the time that each dose is given. At each follow-up visit, they will be asked to bring back the medication that was not used so it can be reconciled with expected number of tablets to be used. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team

5.6.4 Return or Destruction of Study Drug

The site will maintain an overall drug accountability log for the study, as well as individual accountability records for each patient. The dose, amount dispensed, amount received, and amount remaining unused must be recorded in the source document. The patient will return all unused study drug at each dispensing visit and at the end of the study

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

5.7 Visit 1 (Active/Control Patients)

NOTE: This visit will occur within 6 weeks of Visit 2, which will be when patient will be entered into the study.

After Informed Consent is obtained and study consent form is signed, Study Visit 1 will include the following, from interaction with the subject, and/or as applicable the subject's parents or guardian:

- A detailed review of the research subject's medical records and interview to ensure that the subject meets inclusion criteria and does NOT meet any of the exclusionary criteria.
- History of current seizure types and frequencies, concomitant medical or psychiatric disorders, and all prescribed and over-the-counter medications taken.
- Complete review of systems.
- Subject physical examination, including recording of vital signs, including height and weight to compute body mass index (BMI; see above) and body surface area (BSA), and neurological examination.
- Subject will be instructed to begin a daily seizure diary today that will be maintained and collected at the next visit.

5.8 Visit 2 – (Active patients- 'NOTE: This visit will occur 7-21 days before surgery; patients/caretakers will be given Everolimus.)

The following activities will be conducted and research subject information will be collected by interaction with the subject, and/or as applicable the subject's parents or guardian:

- Recent history will be reviewed for any change in their medical status or change in intake of prescribed or over-the-counter medication.
- Vital signs, physical and neurological examination.
- Collect and review seizure diary kept since Visit 1 and give new diary to be recorded until date of surgery.The protocol for study medication administration will be reviewed with the subject, and/or parent/guardian as applicable.
- Explanation of potential adverse events will be reviewed, as will the reporting of adverse events.
- Blood work: CBC and C-reactive protein (CRP). If not done prior to visit as part of PST (pre surgical testing)
- Review of the specific protocol for medication administration. (Active Patients Only at NYU CEC)
    - They will be instructed on the dosage and administration times of the study medication. All subjects will receive a specific quantity of tablets and they will be asked to record the time that each dose is given. They will be asked to bring back the medication that was not used so it can be reconciled with expected number of tablets to be used. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team

6.2a Visit 2a – For patients taking the Everolimus for 3 weeks, at NYU CEC, they will have serum levels of everolimus drawn after 10-12 days on the medication. A trough level will be obtained. The study nurse or doctor will contact the patient with the results when available and will adjust the dose as described above. The nurse or doctor will also ask the patient to report any adverse events.

For active patients who reside outside of the NYC area and are unable to arrive at NYU CEC due to distance and travel issues prior to pre-admission testing, Visits 1 and 2 will be combined as one visit to be completed at least 7 days prior to Visit 3. For these patients with a combined Visit 1 and 2, this could mean a treatment window of 7-21 days depending both on which cohort they are in and how soon they can begin treatment.

Pre-surgical evaluation: Visit 2- Control Group

- Recent history will be reviewed for any change in medical status or change in intake of prescribed or over-the-counter medication.

- Vital signs, physical and neurological examination
- A detailed seizure history during the prior week.
- Medication reconciliation.

Blood work: (pre surgical eval) CBC and C-reactive protein (CRP).

For control patients who reside outside of the NYC area and are unable to arrive at the study site due to distance and travel issues prior to pre-admission testing, Visits 1 and 2 will be combined as one visit and can be completed one day prior to Visit 3 if not earlier.

5.9 Visit 3 (Active/Control patients)– Day of Surgery NYU CEC

The following activities will be conducted and research subject information will be collected:

Study drug dosing: (ACTIVE PATIENTS ONLY)

- On the day of surgery, the patient will take their last dose of everolimus with a 30 cc or less of water shortly after awakening and at least 3 hours before surgery

- A detailed seizure history during the prior week. A blood sample will be used for investigation per the IRB-approved protocol. This will include everolimus serum levels, systemic cytokines and mTOR activity levels. These samples will be batched and stored in -80 freezer until requested for analysis.)

During surgery: (NYU Tisch Hospital)

- Intraoperative electrocorticography, performed in accordance with a standard protocol (see below).
- Resected tissue will be processed by the Pathology Departments at NYU Medical Center and a sample will be used for investigation per the IRB-approved protocol. This tissue will be used for primary analysis of biochemical, molecular, electrophysiological, and genetic measures.

Corticography Protocol:

Equipment Needed:

1. Nicolet EEG Portable Acquisition unit
2. **C64** Amplifier
3. 2 x sterile Grass monopolar needle electrodes (1 – ground, 1 – reference)
4. 2 x Touch-proof adapters for needle electrodes
5. 64-lead sterile Ad-tech electrode cable
6. 4 x sterile 16-contact Ad-tech connector blocks
7. 64-contact (8x8) Ad-tech electrode; spacing as follows:
    a. If lesion is <=2 cm in maximal dimension, use grid with 5mm spacing
    b. If lesion is 2.1-3 cm in maximal dimension, use grid with 7.5mm spacing
    c. If lesion >3cm in maximal dimension, use grid with 10mm spacing

Anesthesia:

Remifentanil/propofol. If using volatile anesthesia, turn off 15 minutes prior to beginning recording.

Procedure:

1. Wheel portable EEG station into room, turn on and enter patient demographics as per routine
2. Configure 64-electrode montage in NicOne
3. Neurosurgeon to place ground and reference electrodes in scalp muscle adjacent to craniotomy
4. Neurosurgeon to photograph lesion

5. Neurosurgeon to place selected subdural grid electrode with center of grid overlying approximate center of the lesion
6. Neurosurgeon to connect and pass 64-contact Ad-tech adapter to neurophysiologist who will connect it to C64 amplifier
7. Record ECoG for a minimum duration of 10 minutes using the following settings: High Pass Filter 1 Hz, Low Pass Filter 70 Hz, Gain 70uV/mm, notch filter off.
8. Neurosurgeon to place paper markers on 4 corners of grid then remove the grid and photograph the brain again.
9. Save ECoG file as anonymized file without video to external hard drive using subject number as file name.
10. Obtain intraoperative photos from neurosurgeon and relabel by subject number, XXXX_pregrid, XXXX_grid and XXXX_postgrid. Save files to external hard drive

5.10 Visit 4 (and all days with invasive electrodes) NYU Tisch Hospital

The day after surgery, and every day thereafter if invasive electrodes are utilized:

- The research subject will be seen by a board-certified neurosurgeon or study designee and evaluated for evidence of infection and abnormal wound healing as per the protocol (including digital photographs of the surgical site dressing) in both treated and untreated comparison groups.
    - We will take photographs of the surgical site dressing and subject's face will not be in the photo. All photos will be de-identified in the same manner all of their clinical information for this study will be de-identified. Consent for the photographs are part of the consent form. The photographs will not be shared with anyone outside of the research team or anyone who is not a part of the patient's medical care.
- A board-certified neurologist/epileptologist will also evaluate the subject daily while invasive electrodes are used.
    - All seizures and subclinical electrographic seizure discharges will be reviewed and the type (e.g., simple v. complex partial v. subclinical electrographic discharge), onset region(s), spread zone(s), and duration.
    - Each day a standard portion of the awake and sleep EEG records and all seizures, including the period for one minute before seizure onset and 5 minutes after seizure offset will be stored and available for subsequent analysis.

The neurologist/epileptologist or study designee will record vital signs and perform a physical and neurological examination and inquire about potential adverse events.

5.11 Visit 5a (Active and concurrent comparison group) (if a second surgery is done at the end of invasive recordings) NYU Tisch Hospital

Resected tissue at the time of surgery will be distributed by the Pathology Departments at the local institutions and samples will be used for investigation per the IRB-approved protocol. This tissue will be used for research analysis of biochemical, molecular, electrophysiological, and genetic measures.

6.5a Resected brain tissue will be distributed at the Pathology Department of the NYU Medical Center then stored in the Alexandria Center for Life Sciences, located at 450 East 29th Street on 9th floor a -80 freezer until requested to be analyzed..

The samples will be de identified and coded so that Dr. Devinsky will have access to the key. The samples will be stored and used only for analysis for this study. All tissue remaining after this study will be kept in our bank or destroyed per individual patient voluntary consent.

Samples will be stored secure labs in the above addresses. Our 3 NYU Pathologists-internal collaborators, Dr. Kasthuri, Dr. Leitner, and Dr. Snuderl) will perform analysis of brain resection specimens from patients on this study, and will be used for preparation of DNA/RNA assays. They will be looking for mutations in mTOR pathway genes. The samples will be studied using next gen sequencing, proteomics and/or Micro RNA assays, to search for mutations which may have caused the tuber or cortical dysplasia (CD). This analysis will focus on genes known to be involved in tuber/CD development including TSC1, TSC2, AKT1, AKT2, AKT3, MTOR, PTEN. Any variants that are found will be compared

with those found in blood DNA samples from the same subjects. If subjects have indicated an interest in learning of the genetic findings, the findings will be provided to them. This information will be offered to the patients and their families through a physician or genetics counselor experienced with tuberous sclerosis. As this is not a clinical grade (CLIA-certified) analysis, the findings will be presented as research findings, which must be confirmed by a CLIA lab if any clinical action were considered based upon the information. The results of the genetic analysis will not be shared with anyone outside of the research team. The use of samples for this study is required.

Our 3 pathologists mentioned above will be assaying mTOR pathway activation using immunochemistry, western blotting analysis, and capture ELISA and other biochemical and molecular biology techniques o], in order to obtain such results. He will be looking at levels of mTOR signaling proteins in lymphocytes of human blood specimens. 2 mls in purple cap EDTA tube will suffice. For all Biochemical assays we will utilize fresh frozen tissue, For Immunohistochemistry fixed frozen tissue in 4% PFA and sucrose sank 48hrs and frozen using OCT blocks can be used. The panel of Abs used for detection will be a panel of set-fixed 6 to 10 antibodies. Up to 5mg of tissue will be adequate for MTA purposes and research result solidity.

They will be looking for biomarkers of inflammation, and cytokines. She will measure the changes in serum HMBG1 with brain tissue expression. She will also measure miRNAs in serum using additional 100-200 microliters of serum from each patient, as well as brain tissue from specified post – resection pathological TSC, or / and Epilepsy patients, and collected under an Approved ICF and under the supervision of the BRC of NYULMC (in order to distribute),

If subjects wish to withdraw from this portion of the study, they will need to write a formal letter to the Principal Investigator stating why they would like to withdraw and we will withdraw them after that, having their sample destroyed. The withdrawal procedures will be explained to them when they are signing the consent form.

5.12 Visit 5b Active and concurrent comparison group) (postoperative day 2 and all days until discharge) NYU Tisch Hospital

Each postoperative day while the research subject is in the hospital, they will

- Be seen by a board-certified neurosurgeon or study designee and evaluated for evidence of infection and abnormal wound healing as per the protocol (including digital photographs of the surgical site dressing).
- Be seen by a board-certified neurologist/epileptologist or study designee who will also evaluate the subject daily each day to inquire about potential adverse events.

5.13 Visit 6 (Active and concurrent comparison group) NYU CEC

3 to 10 days after discharge, the research subject will:

- Be examined by a board-certified neurosurgeon or study designee and evaluated for evidence of infection and abnormal wound healing as per the protocol (including digital photographs of the surgical site dressing).
- Be examined by a board-certified neurologist/epileptologist or study designee to review current medications, any adverse events, and perform a physical and neurological examination.

6 Statistical Plan

6.1 Sample Size Determination

Statistical Methods

Distributions of patient and disease characteristics will be summarized descriptively at study entry by dose duration cohort and across cohorts in the everolimus group and in the comparison group. Frequency distributions and cross tabulations will be presented for categorical variables. For continuous variables, summary statistics (means, medians, interquartile ranges, standard deviations, etc) will be provided along with graphical displays (e.g., boxplots) by treatment group.

Safety will be summarized within and across dose duration cohorts and compared with the events in the comparison group.

S6 phosphorylation levels in brain tissue sample at surgery will be summarized descriptively for each dose duration cohort and across cohorts in the treated group and in the comparison group. Further, levels will be compared between the treated and comparison group using 2 sample 2 sided t-tests (with appropriate transformations of levels if required to meet the assumptions of the method.
Similar analyses will be carried out for all additional continuous endpoints of interest. No adjustments for multiplicity will be made in this small study. Bivariate scatterplots and correlation coefficients will also be provided to examine the associations between the multiple endpoints.

7.3 Subject Population(s) for Analysis

All patients who receive one or more doses of drug will be included in the analyses of safety. Those patients who undergo surgery will be included in the analyses of S6 phosphorylation and other measurements obtained at the time of surgery. Additional analyses will be conducted to compare those subjects who did not complete treatment and surgery with respect to disease and patient characteristics to identify any potential differences between these groups of patients.

7. Sample size considerations. For this preliminary study, safety analyses are primary. Study termination will occur if, among the 9 treated subjects, there are two cases of wound healing problems or three cases of serious infection, including wound site infection. In the case of a wound site infection, any problems with wound healing will be considered secondary to the infection and NOT as a primary wound healing problem. These estimates are based on neurosurgical experience and reports with the frequency of wound healing problems of <2% and infection from 1.6 to 8.6% in six published series, with a mean infection rate of 5.3%.[33-38]

For the analysis of S6 phophorylation, based on a 2 sample 2 sided t- test, we can detect a difference of $|1.4| - |1.5|$ standard deviations between the comparison controls and the treated group with 9 treated subjects with alpha =0.05 and power of 80%. Preliminary data using quantitative Western blots obtained from 4 resected tubers demonstrated phospho-S6/S6 ratios of 257□42% of control. With 9 treated subjects, and 12 controls, we would be able to detect a 62 – 65% difference in phosphor-S6/S6 levels between treated and untreated groups, which is likely a biologically meaningful difference. Prior studies in murine models of TSC treated with rapamycin have demonstrated 90% reduction in phospho-S6 levels [ref]

Safety monitoring and reporting

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.

6.2 Adverse events

6.2.1 Definitions and reporting

Adverse events that begin or worsen after informed consent should be recorded in the Adverse Events CRF. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF. Adverse event monitoring should be continued for at least 30 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.

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 (CTAE 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 sequalae, fatal, unknown)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in Section 8.2.1

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]. 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

6.2.2 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 CTAE 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.

6.2.3 Adverse events of special interest *(optional)*

In clinical trials, everolimus has been associated with serious cases of hepatitis B reactivation, including fatal outcome. Reactivation of infections is an expected event during periods of immunosuppression.

In clinical trials and post-marketing spontaneous reports, everolimus has been associated with renal failure events (including fatal ones) and proteinuria. Renal function will be monitored throughout the study. In clinical trials everolimus has been associated with certain adverse events (Table 6).

Table 6. Specific Adverse Events

Clinically Notable AE grouping	**Risk Definition in Risk Management Plan (RMP)**	**Comment**
Amenorrhea	Amenorrhea	Included under potential risk in RMP
Cytopenia	N/A	Included under clinically notable AEs
Hemorrhages	Hemorrhages	Included as important identified risk in the RMP
Hyperglycemia/ new onset of diabetes mellitus	N/A	Included under clinically notable AEs
Hypersensitivity reactions (anaphylactic reaction)	N/A	Included under clinically notable AEs
Infections and infestations	N/A	Included under clinically notable AEs
Intestinal obstruction/ileus	Intestinal obstruction/ileus	Included under potential risk
N/A	Cardiac Failure	Not reported in the MAP. (important identified risk- RMP specific
N/A	Increased creatinine	Please note that this RMP specific identified risk is based on lab data and is defined as newly occurring or worsening increase to CTC grade 3 or 4
Non infectious pneumonitis	Non infectious pneumonitis	Name changed from pulmonary events to non infectious pneumonitis in the MAP. Included under important identified risk in RMP
Rash and similar events	N/A	Not included in the RMP
Renal events	Renal failure/proteinuria	The names in RMP and MAP are different but the search terms are the same. It is included important identified risk in RMP

Stomatitis/oral mucositis/ulcers	N/A	Included under clinically notable AEs
Thromboembolism	Thromboembolism	Included as important identified risk in the RMP

6.3 Serious Adverse Events

6.3.1 Definitions

A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- 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 (specify what this includes)
- elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
- treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a 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 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

6.3.2 Reporting

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

All events reported to the FDA by the investigator are to be filed utilizing the Form FDA 3500A (MedWatch Form).

To ensure patient safety, every SAE, regardless of suspected causality, occurring

- after the patient has provided informed consent and until atl east 30 days after the patient has stopped study treatment/participation
- after protocol-specified procedures begin and 30 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 30 days after the patient has stopped study treatment

must be reported to Novartis within 24 hours of learning of its occurrence **(fax: 877-778-9739)**. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

Any SAEs experienced after this 30 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, 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.

6.3.3 Investigator reporting: notifying the NYULMC IRB

This section describes the requirements for safety reporting by investigators who are NYULMC faculty, affiliated with a NYULMC research site, or otherwise responsible for safety reporting to the NYULMC IRB. The NYU Langone Medical Center IRB (NYULMC IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The NYULMC IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The NYULMC IRB requires researchers to submit reports of the following problems within 5 working days from the time the investigator becomes aware of the event:

A reportable event is an event that occurs at any time during or after the research study, which in the opinion of the principal investigator is:

- **Unexpected:** An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.

  AND

- **Related to the research procedures:** An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.

  AND

- **Harmful**: Harmful includes causing harm to participants or others, or placing them at increased risk of harm**.**

Reporting Process
Unanticipated problems posing risks to subjects or others as noted above will be reported to the NYULMC IRB or St Barnabas Medical Center IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the NYULMCIRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks, or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

6.3.4 Investigator reporting: Notifying a non-NYULMC/ IRB

Not Applicable

6.3.5 Sponsor reporting: Notifying the FDA

The study IND sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days*** *(via telephone or facsimile report)*
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected,
  - fatal or life-threatening

- ***Within 15 calendar days*** *(via written report)*
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening

    -or-
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:

– suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events may be submitted on FDA Form 3500A or in a narrative format. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3. The contact information for submitting IND safety reports is noted below:

6.3.6 Sponsor reporting: Notifying participating investigators

Not Applicable

6.4 Unblinding Procedures

Not applicable.

6.5 Stopping Rules

A Data Safety Monitoring Program (DSMP) will be established to monitor adverse events and study safety. Prior to commencement of the study, the rate of infection and impaired wound healing associated with routine two-stage epilepsy surgeries as will be performed in this study over the past 5 years will be determined for each epilepsy surgeon (Amanda Yaun and Werner Doyle). A statistician from the NYU School of Medicine Biostatistics Department will provide blinded reports to the Data Safety Monitoring Committee (DSMC). The PI, Dr. Orrin Devinsky, Dr. Amanda Yaun, David Harter and Dr. Doyle (co-investigators) will aid in reviewing all patients during the treatment period and throughout the post-treatment monitoring period. They will review all cases and if it is deemed that the serious adverse event(s) are related to study drug, the study will be terminated for that subject. If two patients among the 9 treated with everolimus develop a) impaired surgical wound healing or if three patients among the 9 develop b) serious infection, including wound site infection. Any case of wound site infection complicated by a wound healing problem will NOT be considered a wound healing problem.

Medical Monitoring
It is the responsibility of the Principal Investigator to oversee research subject safety. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10. Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events..
It is the responsibility of the IND Sponsor to assess research subject safety.

Data Safety Monitoring Program (DSMP), Data Safety Monitoring Committee (DSMP): The investigators will review all safety data after each three subject treatment cohort has completed the trial. All safety data will be sent to the DSMB after every 3 patients have completed the protocol or if there is any serious adverse event that may possibly be related to the study.

For this pilot, open-label exploratory clinical trial, given the potential risk that may be associated with a 7-21 day trial of everolimus treatment prior to elective epilepsy surgery in TSC and FCD patients, a DSMP will be established. Monitoring will be performed on a regular basis throughout the participant accrual, treatment, and follow-up. Dose Limiting Toxicity is not anticipated to be an issue since the doses used are standard in prior research studies and now FDA approved for patients with TSC. However, all adverse events will be recorded and reviewed.

Any adverse event occurring during, or after 30 days of last treatment will be documented by the epilepsy neurologist member of the DSMP, including fatigue, rash, anorexia, mucositis, edema, cough, thromboembolism, nausea, vomiting, stomatitis, sinusitis, bronchitis, pneumonia, abscess, leukopenia, or hyperlipidemia.

Because of the specific concern regarding potential wound healing problems and wound infection resulting from treatment with everolimus prior to elective epilepsy surgery, wound status will be carefully monitored, specifically by a neurosurgeon.

The site of the planned surgical incision will be inspected by both of the DSMP members 5 days after initiating therapy (prior to surgery), and then at weekly intervals, beginning one week after surgery until six weeks after surgery. The neurosurgeon will document wound status with digital photography.

Blood test surveillance for possible infection will be performed at the same time intervals (7-10 days before starting everolimus, 7-21 days after starting everolimus, and 3-10 days after discharge from the hospital, and then 3 and 6 weeks after surgery if any evidence of infection is identified on examination or the post-hospital discharge blood test) consisting of a complete blood count (CBC) with differential and C-reactive protein (CRP).

Any abnormality will be documented for review by the DSMP. Since no ideal objective scoring system exists for assessing surgical site adverse events [39,40], any wound breakdown, dehiscence or infection will be considered a serious adverse event, and will be reported to the participating site IRB and to the NINDS, and the decision will be made as to whether the study should be terminated.

Individual subject participation in the study will be terminated if the DSMC finds any of the following unexpected new findings in a subject:

1) Documented wound infection requiring surgery
2) Documented wound dehiscence requiring surgery
3) Persistent fever above 101 degrees F and abnormally elevated serum wbc count and ESR and CRP in the absence of a known cause
4) Documented bacterial meningitis

6.6 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

6.7 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded in a digital RedCap database system. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

6.8 Data Management

Data Management
The data for this study will be entered into the REDCap, a research data management system. The reports, record locking at case report form (CRF) and subject level and the paperless query features allow for the cleaning of data throughout the clinical study. The system has extensive security features and all data transmitted into or out of the REDCap database will be encrypted.

Study Data Management staff will conduct data management and data quality assurance work activities for the A Pilot Study to Evaluate the Effects of Everolimus on Brain mTOR activity and Cortical Hyperexcitability in Tuberous Sclerosis Complex (TSC) and Focal Cortical Dysplasia (FCD) study. Data Management staff will be responsible for the integrity of all study data. They will review and monitor the completeness and accuracy of data thought out the duration of the study. Final data clean up will be completed shortly after the last subject visit and the study database will be locked and provided to the study statistician for analyses.

A CRF will be completed for each subject. This CRF has proven successful in previous studies. It is the investigator's responsibility to ensure the accuracy, completeness, clarity, and timelines of the data reported in the subject's CRF. Source documentation supporting the CRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, adverse events, other observations, and subject status.

The investigator, or designated representative, should complete the CRF as soon as possible after information is collected. An explanation should be provided for all missing data.

The PI, Dr. Devinsky, will provide the study site with CRF training sufficient to permit site personnel to enter or correct information in the CRFs for the subjects for which they are responsible.

6.8.1 Data Collection Forms: To be submitted by study coordinator

Within 48 hours of registration completion: On-study CRF, Signed, Dated Consent, On-Study Lab Reports, Medical History CRF, Eligibility Checklist; Within two weeks of registration: Pre-Operative Evaluation CRF, Completed Subject Diaries, Complete Lab test results; Within two weeks post surgery: Post-Op evaluation CRF, Off-Study Evaluation CRF; Within two weeks post-surgery, within 30 days for all other AE's: Toxicity CRFs; Within 24 hours of event occurrence: SAE's Hospitalization Admission/Discharge Summaries

6.8.2 Subject Identifiers

Each patient will be given a unique identifier (study number) in the order of enrollment. Each blood and tissue sample will be placed in an individual cryotube labeled with study number, date of surgery, and time of collection.

6.8.3 Confidentiality

Each subject enrolled will, from that point forward, be identified by a unique identifier (study number). This study number will also be used for research specimens collected and shipped to collaborating laboratories. Clinical information will be accessed, according to HIPAA requirements, by study personnel to complete study documents, as needed. Only the PI and the study coordinator will have access to the log linking the study number to the patient name, birth date, and medical record number. The log will be kept in a secure file.

Medical and study records will be kept in accordance with state and federal laws concerning the privacy and confidentiality of medical information. The confidentiality of each subject's medical record is also protected by federal privacy regulations.

Subjects will be informed of all protected health information used in this study. The consent form will seek written permission from the patient to use and disclose information for this research. The patient or parent is required to sign this form. This Authorization will not expire unless the patient withdraws it in writing. The volunteer has the right to withdraw authorization at any time, except to the extent that NYU has already relied upon it or must continue to use the information to complete data analysis or to report data for this study.

6.8.4 Disposition of Data

Research findings will be stored in a password protected computer. Written and electronic records, reports, and data resulting from this clinical trial will be kept in the volunteer's research record for at least six years or until the study is completed, whichever is longer. At that time, either the research information not already in the medical record will be destroyed or information identifying the patient will be removed from such study results at NYU. Any research information in the medical record will be kept indefinitely.

6.8.5 Sharing Study Results

We plan to make all data and resources generated under this grant available to the academic community immediately after we report them for the first time in a peer-reviewed publication. Results used in publications will not be identifiable or able to be traced back to enrolled subjects.

6.9 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 6 years after the study is completed and the database is locked for analysis.

7 Study Monitoring, Auditing, and Inspecting

7.1 Study Monitoring Plan

The Principal Investigator (Dr. Orrin Devinsky) will follow the data safety monitoring plan below to ensure that all safety measures are taking place in the best interest of the subjects.

Data Safety Monitoring Plan (DSMP)

Who will do the data monitoring for this study:

- The PI, Orrin Devinsky, MD.
- The sub investigator, Amanda Yaun, MD
- The independent monitor, Dimitrio Arkiloas, MD

Explain how data safety will be monitored:

- Drug tolerability and safety will be monitored during clinical evaluations and phone visits by investigator/study team member that will seek information on adverse events by specific questioning and, as appropriate, by examination.
- .

- Dr. Devinsky, Dr. Yaun and Dr. Doyle will review all patients during the treatment period and throughout the post-treatment period. They will review all cases and if it is deemed that the serious adverse event(s) are related to study drug, the study will be terminated for that subject. If two patients among the 9 treated with everolimus develop a) impaired surgical wound healing or if three patients among the 9 develop b) serious infection, including wound site infection, study termination will occur. Any case of wound site infection complicated by a wound healing, the problem will NOT be considered a wound healing problem, but secondary to the infection.

- There is a risk of PHI dissemination. All personal health information (on paper) will be stored at NYU Comprehensive Epilepsy Center located at 223 East 34th Street, New York, NY in a locked filing cabinet. Patient information may also be stored in REDCap, a secure research data management system. Only personnel listed on the study will have access to PHI.

Describe any potential conflict of interest the DSM contact may have:
None.

Data Safety Monitor Details:
The enrolled subjects will be monitored:
Drug

- Patients will be routinely monitored for adverse events via clinical evaluations and phone visits
- Subjects will have the research coordinator's phone number to call in case of need to report side effects on days not scheduled for clinical evaluation or phone visit.

PHI

- All PHI will be kept in locked filing cabinet at NYU Comprehensive Epilepsy Center and/or entered into REDCap (NYU secure data management system) with access given only to study personnel

Dropouts will be monitored:

- Dropouts will have a final visit to complete their enrollment and receive instructions for tapering off of Everolimus.
- .

Primary and secondary efficacy endpoints will be monitored:
The primary study endpoints of the safety and tolerability of everolimus in children and young adults and the establishment of optimal dose will be monitored via:

- Side Effects in children and subjects where caregiver reports and in adults that are able to self report.
- Evaluation of seizure diary at clinical evaluations and phone visits

The secondary study endpoint of change in frequency from baseline will be monitored via:

- Evaluation of seizure diary at clinical evaluations and phone visits

Reportable events will be monitored using an accepted scale:
No, there will be no accepted scale for monitoring reportable events. All reportable events will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. If the reportable event is still ongoing at the end of the study, it will be followed until final outcome has been reached.

Describe the frequency of monitoring:
The Independent monitor will evaluate every 12 months after the first patient receives the initial dosage of everolimus

Interim analysis will take place:
No, interim analysis will not take place unless requested by the IRB.

Reportable events
This protocol will adhere to NYU SOM IRB policy for reportable events reporting: Yes, we will adhere to the policy for reportable events. We will also be reporting to Novartis Pharmaceuticals Corporation.

Description of Data Safety Monitoring
1. Types of data and events that will be captured under the DSMP
The only type of event that we anticipate to capture is from subjects who have adverse side effects from drug or whose PHI was accidentally disseminated.

2. Responsibilities and roles for gathering, evaluating and monitoring data
Dr. Devinsky, Dr.Yaun, and Dr. Arkiloas (the independent monitor) will be responsible for evaluating and monitoring data. They will oversee the DSMP and DSMR.

3. Information about the monitoring entity
The independent monitor, Dr. Dimitrio Arkiloas, is the monitoring entity.

4. Reportable events that will be reported to the monitoring entity and when the events will be reported
Yes, all reportable events will be immediately reported to the PI by the independent monitor, study personnel, co-investigators or anyone who is aware of a reportable event. Reportable events may be reported by email or by phone to the PI.

5. Assessment for the frequency of monitoring
We will submit a Data Safety Monitoring Report every 12 months, at the same time as the annual renewal. If there is a reportable event, the independent monitor or other study personnel will report it to the IRB immediately.

6. Stopping rules and criteria for withdrawal of subjects
Subjects may withdraw from the study at the request of the parent or legal guardian for side effects that impair function or quality-of-life, assuming they do not want to lower the dose to a tolerable range. Subjects will also have drug discontinued by investigator if any serious adverse effect is identified and considered as a possible or definite result of the study medication. These include rash, other signs of hypersensitivity, or liver function tests that exceed baseline or normal measures by 50%. Clinical judgment will be used when determining need for everolimus therapy to be terminated. Other reasons for withdrawal include non-compliance with study procedures, seizure exacerbation, or interaction with current concomitant AED therapy. The method termination of everolimus therapy will be determined based on type of reaction and/or reason for withdrawal.

7. Communication and dissemination of information
If any accidental dissemination of PHI occurs, the independent monitor, Dr. Arkiloas, will communicate with the IRB immediately.

7.2 Auditing and Inspecting

The Investigator will permit study-related monitoring, audits, and inspections by the IRB, government funding and regulatory bodies, and NYULMC compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable NYULMC compliance and quality assurance offices.

8 Ethical Considerations

This study is to be conducted accordance with applicable: US government regulations, international standards of Good Clinical Practice, and institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. Assent will not be required since all of the children in this study will be cognitively impaired. All consent will be coming from their legal guardians/parents/health care proxy. A consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB/EC-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

9 Study Finances

9.1 Funding Source

The collection of preliminary data for this study at NYU Langone Medical Center will be funded through a grant from Novartis. Study drug and funding will be supplied by Novartis For the randomized study, funding will be requested from the National Institutes of Health using an RO1 grant application.

9.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must report this to the study IND Sponsor and have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMCinvestigators will follow the applicable University conflict of interest policies.

9.3 Subject Stipends or Payments

Subjects will not be compensated for travel for any study-related expenses.

10 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor.

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