Seattle Children's Hospital

Clinical Research Protocol

Phase 1 study of ABI-009 (*nab*-rapamycin) for Surgically-Refractory Epilepsy (RaSuRE)

Protocol Number:	RS-001
Version Date:	October 31, 2018
Investigational Product:	ABI-009 (*nab*-rapamycin, AADi Bioscience, Inc)
IND Number:	139135
Development Phase:	Phase 1
Sponsor-Investigator:	Jason S. Hauptman, MD PhD Seattle Children's Hospital 4800 Sand Point Way NE Seattle, WA 98105

Approval:

_____ _____

PI or Sponsor Signature (Name and Title) *Date*

This confidential information about an investigational product is provided for the exclusive use of investigators of this product and is subject to recall at any time. The information in this document may not be disclosed unless federal or state law or regulations require such disclosure. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information.

PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing the Sponsor-Investigator with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: RS-001

Protocol Title: Phase 1 study of ABI-009 (*nab*-rapamycin) for Surgically-Refractory Epilepsy (RaSuRE)

Protocol Date: 10/31/2018

_____ _____

Investigator Signature *Date*

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LIST OF ABBREVIATIONS AND ACRONYMS

AED	anti-epileptic drug
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical (Classification System)
AUC	area under the curve
BUN	blood urea nitrogen
CBC	complete blood cell (count)
CR	complete response
CRA	Clinical Research Associate
CRF	case report form
CSF	cerebral spinal fluid
CTCEA	Common Terminology Criteria for Adverse Events
CTMS	Clinical Trial Management System
DLT	dose limiting toxicity
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
FCBP	female of childbearing potential
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBeAg	Hepatitis B “e” antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HGB	hemoglobin
HIV	human immunodeficiency virus
ICH	International Conference on Harmonization
IND	investigational new drug application
IRB	Institutional Review Board
IV	intravenous
LDH	lactate dehydrogenase
LFT	liver function test
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI	National Cancer Institute
ORR	overall response rate
PEComa	perivascular epithelioid tumor
PO	*Per os* (by mouth, orally)
RBC	red blood cell (count)
SD	standard deviation
SGA	subependymal giant cell astrocytoma
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
TSC	Tuberous Sclerosis Complex

ULN	upper limit of normal
WBC	white blood cell (count)

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PROTOCOL SYNOPSIS

TITLE	Phase 1 study of ABI-009 (*nab*-rapamycin) for Surgically-Refractory Epilepsy (RaSuRE)
SPONSOR-INVESTIGATOR	Jason S. Hauptman, MD, PhD Seattle Children's Hospital 4800 Sand Point Way NE Seattle, WA 98105
FUNDING ORGANIZATION	AADi Bioscience, Inc. Seattle Children's Hospital
NUMBER OF SITES	1
RATIONALE	Seizures that are refractory to both medical and surgical therapy increase the risk of morbidity and mortality in children with epilepsy. At this point in time, options for these children are sparse and suboptimal. This hypothesis-driven phase 1 study aims to evaluate the use of a mammalian target of rapamycin [mTOR] inhibitor, ABI-009, in this subset of challenging participants. The underlying hypotheses being tested in trial are: (1) ABI-009 is a safe and well-tolerated medication in children who have medically-refractory epilepsy and have failed epilepsy surgery, and (2) The addition of ABI-009 therapy to the current clinical standard of continued antiepileptic medications results in improved epilepsy control. This is unique among trials of anti-epileptic medications in that it also studies mTOR inhibition in a non-Tuberous Sclerosis Complex (TSC) specific population for whom few additional effective therapies exist.
STUDY DESIGN	This is a prospective, single-center, phase 1 safety study to investigate the safety, tolerability, seizure control, and quality of life in participants with medically-refractory epilepsy who failed epilepsy surgery. These participants will have continued seizures despite being at least 3 months post-epilepsy surgery (resective surgery with an intent to cure). Upon enrollment, participants will be continued and observed on their pre-existing, clinically prescribed antiepileptic drug (AED) regimen for 1 month. At the 1-month mark, participants will receive weekly ABI-009 intravenously at different dose levels in cohorts of 3 participants each using the standard 3+3 dose-finding design. ABI-009 will be continued for a total of 3 weeks. ABI-009 will then be discontinued and the participants will be observed for an additional 3 months. We intend an expansion of the maximum tolerated dose (MTD) cohort to an estimated additional 6 participants for a maximum possible enrollment of 18 participants.
DURATION OF SUBJECT PARTICIPATION	Participants will be on study for about five months. Initial control period: 1 month Dosing period: 3 weeks

AND DURATION OF STUDY	Follow-up: 3 months
PRIMARY OBJECTIVE	**Safety and Tolerability**: ▪ Determine dose-limiting toxicities (DLTs) and MTD of ABI-009 in participants with surgically-refractory epilepsy ▪ Record the adverse events (AEs) and document their severity with ABI-009 dosing for medically intractable epilepsy that has failed surgical resection, administered in conjunction with their pre-existing AED regimen. Record the compliance of families with medication and record the number of participants that withdraw from study either voluntarily or by necessity secondary to AEs. ▪ Columbia-Suicide Severity Rating Scale (C-SSRS) serious suicidal ideation score stays below a 4 rating.
SECONDARY OBJECTIVES	**Efficacy and Quality of Life**: Efficacy is defined as the change from in seizure frequency between baseline and follow-up, expressed as **percentage reduction in seizure rate** (reduction in seizure frequency from baseline, calculated as (Baseline frequency [seizures/week] – frequency at follow-up [seizures/week])/Baseline frequency [seizures/week]) and **median percentage reduction** in seizure frequency. **Treatment response rate** will be defined as the proportion of participants achieving at least a 25% reduction in seizure frequency from baseline. **Seizure frequency** will be defined as the ratio between the number of seizures and the number of days on which seizure information was known within the same period of time (for either baseline or maintenance phase). Additional secondary endpoints will include **frequency of seizure-free days** during the maintenance period, **seizure-free rate** (participants remaining seizure free during the entire maintenance period), and rapamycin **blood level–response relationship** analysis. Evaluate quality of life indices for participants before, during, and after dosing with ABI-009 in conjunction with pre-existing AED regimens.
NUMBER OF PARTICIPANTS	Up to 18
PARTICIPANT SELECTION CRITERIA: Inclusion Criteria	<u>Inclusion Criteria:</u> 1. Written informed consent (and assent when applicable) obtained from participant or participant's legal representative 2. Willingness and able to adhere to the study visit schedule and other protocol requirements 3. Male or female ≥3 and ≤26 years of age at Visit 1 a. Because no dosing or adverse event data are currently available on the use of ABI-009 in participants <3 years of

age, these young children are excluded from this study.

4. Documentation of a diagnosis of medically intractable epilepsy as defined by the failure of at least 2 appropriately dosed and tolerated AEDs to eliminate all clinical seizures over a 6-month period prior to epilepsy surgery
5. Documentation of resective epilepsy surgery following appropriate presurgical evaluation
6. Documentation of continued clinical seizures that persist at least 3 months following resective epilepsy surgery. In order to proceed with enrollment and study drug initiation, participants must have had >8 seizures in the last 30 days without 2 consecutive weeks of seizure freedom.
7. Documentation that the participant is not a candidate for *or* refuses any additional resective epilepsy surgery
8. Participants must have adequate bone marrow function (ANC $\geq$1,000/mm^3, platelet count of $\geq$100,000/mm^3, and hemoglobin $\geq$ 9 gm/dL) before study drug dosing.
9. Participants must have adequate liver function (SGPT/ALT $\leq$ 5 times ULN and bilirubin $\leq$5 times ULN) before study drug dosing.
10. Participants must have adequate renal function before study drug dosing, defined as: Creatinine clearance or radioisotope GFR $\geq$70mL/min/1.73 m^2 **or** a serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	Female
3 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR utilizing child length and stature data published by the CDC.

11. Participants must have a fasting cholesterol level <350 mg/dL and triglycerides <400 mg/dL before starting study drug. In case one or both of these are exceeded, the participant can only be included after initiation of appropriate lipid lowering medication and documentation of cholesterol <350mg/dL and triglycerides <400mg/dl before study drug dosing.
12. Participants must have normal oxygen saturation before study drug dosing.
13. The effects of ABI-009 on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and

	because ABI-009 are known to be teratogenic, participants of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 6 months after the last dose of ABI-009. Should a female participant become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately. a. Participants of child-bearing potential must not be breastfeeding or pregnant as evidenced by a negative pregnancy test before enrollment and before initiating study drug dosing.
PARTICIPANT SELECTION CRITERIA: Exclusion Criteria	<u>Exclusion Criteria:</u> 1. For females of child bearing potential: a. Positive pregnancy test at any visit or b. Lactating or c. Unwilling to practice a medically acceptable form of contraception (acceptable forms of contraception: abstinence, hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent), unless surgically sterilized or postmenopausal during the study 2. Participant has any other condition that, in the opinion of the Site Investigator/designee, would preclude informed consent or assent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives. 3. Participant has received immunization with attenuated live vaccines within 1 week of study entry and/or is planning to receive immunization with attenuated live vaccines during study period. Examples of live vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines. Close contact with those who have received attenuated live vaccines should be avoided during dosing with ABI-009. 4. Participant tests positive for Hepatitis C antibodies or the Hepatitis B antigen. HBsAg and HCVAb blood test must be done at screening (HBsAg only needs to be screened in patients who have not received the full complement of Hepatitis B immunizations). Alternatively, if the patient has received the complement of Hepatitis B immunizations and documentation is provided, this would suffice. 5. A known history of HIV seropositivity. HIV-positive patients on combination antiretroviral therapy are ineligible because of the

<table>
<tr>
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<td>potential for pharmacokinetic interactions with ABI-009. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.
6. Participant is receiving chronic, systemic treatment with corticosteroids or another immunosuppressive agent. Note: Patients that are currently using inhaled, intranasal, ocular, topical or other non-oral or non-IV steroids are not necessarily excluded from the study but must be discussed with the study chair.
7. Participant has been previously treated with a systemic mTOR inhibitor for epilepsy. Skin cream use with rapamycin or everolimus, however, is permitted.
8. Participant has a known hypersensitivity to human albumin, ABI-009, or other rapamycins (e.g. sirolimus, everolimus, temsirolimus).
9. Participant is receiving any other concurrent anticancer or investigational therapy. Participants will be permitted to enroll in the study after a 30-day washout of previously used investigational drugs.
10. Participant has any clinically significant unrelated systemic illness that would compromise their ability to tolerate protocol procedures.
11. Participant is unable to return for treatment and follow-up visits to assess toxicity to the study drug.
12. Participant has an uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, active lung disease, or psychiatric illness/social situations that would limit compliance with study requirements.</td>
</tr>
<tr>
<td>TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION</td>
<td>For dose finding, ABI-009 will start at 5 mg/m^2/dose IV, once a week for three weeks, in cohorts of 3 participants each using the standard 3+3 dose-finding design.
<table>
<tr><th>Dose-levels</th><th>ABI-009 in mg/m^2</th></tr>
<tr><td>-2</td><td>1</td></tr>
<tr><td>-1</td><td>2.5</td></tr>
<tr><td>1</td><td>5</td></tr>
<tr><td>2</td><td>10</td></tr>
<tr><td>3</td><td>20</td></tr>
</table>
Escalation to the next dose level with a new cohort of 3 participants will occur after no DLT is observed. There will be no intra-participant dose escalation allowed. If a DLT occurs in a cohort, an additional 3 participants will be recruited to the cohort. If no further DLTs occur,</td>
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	then a new cohort of 3 participants at the next higher dose level can be enrolled. If 2/6 participants at dose level 1 experience a DLT, then that cohort will be closed to further enrollment and 3 participants will be enrolled at the next lower dose level, and so on. The MTD is the highest dose level in which ≤1 participant has a DLT. Once the MTD has been determined, the MTD cohort will be opened for an additional 6 participants. These participants will adhere to the identical protocol of the rest of their cohort. Product will be administered IV every seven days for three weeks.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	There is no control product for this study. Participants will serve as their own controls by continuing their pre-existing AED regimen for 1 month after enrollment prior to starting dosing with ABI-009. In order to proceed with drug administration participants will have to have had >8 seizures in 30 days without 2 consecutive weeks of seizure freedom.
CONCOMMITANT MEDICATIONS	**Allowed:** Standard therapy for epilepsy is allowed except for treatments noted in the prohibited medications section below. Pre-existing AEDs must not be titrated while participants are enrolled in the study, unless the dose change is solely to address subtherapeutic levels as determined by the treating neurologist. Rescue medication is allowed for prolonged seizure event or cluster of seizures. **Prohibited:** The following medication changes are prohibited during participation in the trial, and as such will be considered a protocol violation: • Any changes in dosage of AED regimen, unless solely to address subtherapeutic levels of AEDs as determined by the treating neurologist. • Any additions or removal of AEDs to the participant's AED regimen during participation unless medically necessary and discussed with investigator
PRIMARY ENDPOINT	**Safety:** DLT and MTD Incidence of adverse events and clinically significant abnormal lab values **Tolerability:** Number of participants withdrawn from study Adherence to prescribed ABI-009 regimen Columbia-Suicide Severity Rating Scale (C-SSRS) serious suicidal ideation score stays below a 4 rating.
SECONDARY	**Efficacy:**

ENDPOINTS	Seizure frequency Percentage reduction in seizure frequency Median percentage reduction in seizure frequency Response rate Frequency of seizure-free days Seizure-free rate Rapamycin blood level–response relationship **Other:** Quality of life and behavioral index Determine weekly whole blood rapamycin trough levels for three consecutive weeks
STATISTICS **Primary Analysis Plan**	**Analysis of Primary Endpoints** The number of SAEs and AEs will be summarized for each dosing group as follows: (i) The proportion of participants with at least one (S)AE, (ii) The average number of (S)AEs per participant, and (iii) The rate of (S)AEs per participant week of follow-up. Histograms showing the frequency of the number of (S)AEs in each dosing group will be included. Rates of (S)AEs by System Organ Class (SOC) will be presented by treatment group. Poisson regression modeling for count data will be used to derive rate ratios and 95% CIs for each SOC. Safety lab data at each study visit and changes from baseline will be summarized by dosing group. In addition, the following clinical laboratory summaries will be presented by dosing group: (i) the incidence of clinically significant abnormalities at each study visit; and (ii) tables summarizing the frequencies of participants below, within, and above the normal reference ranges at baseline and end of study; and (iii) tables displaying baseline to end of study shifts in each laboratory value (shifts between below, within or above normal range). The number of hospitalization events and proportion of participants hospitalized from baseline to final day of study will be summarized and compared by treatment group. Poisson regression modeling will be used to derive rate ratios and 95% CIs. Study compliance will be assessed via compliance with scheduled weekly infusions. **Analysis of Secondary Endpoints** Efficacy is defined as the change in seizure frequency between baseline and follow-up, expressed as **percentage reduction in seizure rate** (reduction in seizure frequency from baseline, calculated as (Baseline frequency [seizures/week] – frequency at follow-up

	[seizures/week])/Baseline frequency [seizures/week]) and **median percentage reduction** in seizure frequency. **Treatment response rate** will be defined as the proportion of participants achieving at least a 25% reduction in seizure frequency from baseline. **Seizure frequency** will be defined as the ratio between the number of seizures and the number of days on which seizure information was known within the same period of time (for either baseline or maintenance phase). Additional secondary endpoints will include **frequency of seizure-free days** during the maintenance period, **seizure-free rate** (participants remaining seizure free during the maintenance period), and rapamycin **blood level–response relationship** analysis. These endpoints will be analyzed using appropriate statistical tests, such as the test of proportions and time to event analyses. Quality of Life for Children with Epilepsy Parent Form (QOLCE) [1] and Nisonger Child Behavioral Rating Form (NCBRF) [2, 3] scores will be compared using appropriate statistical tests for ordinal data, including Wilcoxon signed rank tests, logistic regression, or contingency tables.
Rationale for Number of Participants	In this phase 1 study, it is estimated that a maximum of up to 18 participants will be required to achieve the MTD; however, MTD could be reached with as few as 9 participants, but also possible that the MTD is not reached in this study. With the MTD expansion cohort, it is estimated that additional 6 participants would bring the maximum number of participants enrolled to 18. A total of up to 18 participants should be enough for a safety and tolerability study in an orphan condition.

1 BACKGROUND

1.1 Intractable epilepsy in children

Unlike adults, the primary objective of epilepsy surgery in the pediatric population is to avoid the adverse consequences of uncontrolled epilepsy, particularly with regards to cognitive and developmental outcomes [4]. Additionally, aggressive seizure control in young children is mandatory to avoid the development of the epileptic encephalopathy [5]. It is this desire to avoid the untoward sequelae of intractable epilepsy in developing children that underscores the necessity for early diagnosis and referral to a pediatric epilepsy surgery center.

A critical point is that the diagnosis of therapy-resistant (i.e., intractable) epilepsy can be made early without an exhaustive trial of antiepileptic medications. This concept has been borne out of epidemiological studies done early last decade. Among children in the general population, the incidence of epilepsy has been estimated at five cases per 10000 per year [6, 7]. Of these, from 23% to 33% will become pharmacoresistant [8-10]. Pharmacoresistant epilepsy is usually defined as one or more seizures per month that persist after trying more than two antiepilepsy drugs (AED) [11]. Pharmacoresistance is best predicted by the response to AEDs. Once a patient has failed two to four AEDs, the chances that additional medications will stop the seizures are less than 5% [12]. If there is an associated lesion on MRI, the chance that additional medications will stop the seizures is near zero [13, 14].

Epilepsy syndrome classification predicts response to medical therapy. Nearly all children with genetic/idiopathic epilepsy experience near-optimal seizure control with medication. Of these, approximately 59% experience remission at one year and 40% of children have less than one seizure a month. Thus, 99% of children with genetic epilepsy have near-complete seizure control. Approximately 92% of children with cryptogenic/unknown epilepsy achieve near-optimal seizure control at two years. Remission rates for metabolic/structural epilepsy, on the other hand, are not as high with medical therapy with about 50% uncontrolled on AEDs. Many children considered for epilepsy surgery have metabolic/structural seizures. Furthermore, the development of new AEDs has not significantly impacted seizure control in children of this latter group [15].

Risk factors, such as mental retardation, perinatal anoxia, a history of neonatal convulsions, a history of status epilepticus, and symptomatic etiology increase the risk of refractory epilepsy in children [16]. Younger age at seizure onset and frequent seizures (more than once per month) are also independent risk factors for the development of treatment resistance. Furthermore, high initial seizure frequency and focal EEG findings correlate with intractability.

Suboptimal seizure control in infants, children, and adolescents poses serious neurodevelopmental consequences, spanning cognitive, behavioral, and psychosocial domains. Intellectual dysfunction (IQ <79) can be detected in 57% of children with uncontrolled unilateral temporal lobe epilepsy [17]. Independent of etiology, intractable seizures during the first 24 months of life is a significant risk factor for mental retardation [18]. Intellectual deterioration is particularly rampant when seizures occur daily. When uncontrolled unilateral temporal lobe epilepsy begins in the first 12 months of life, the incidence of intellectual impairment is approximately 83%. Inadequately controlled infantile spasms may result in severe impairments of cognition, language, social skills, and communication abilities that appear to be clinically similar to autism [19].

Beyond cognition, suboptimal seizure control may result in poor psychosocial outcomes compared with patients whose epilepsy is controlled [20]. Psychosocial deficiencies manifest as lower rates of high school completion, employment, marriage, and overall socioeconomic productivity. Early surgical intervention for intractable seizures results in improved IQ scores and developmental indices [5, 21-25].

Early intervention to control seizure frequency is also critical to reducing seizure-related morbidity and mortality. Compared to children without disease, children with refractory epilepsy are at risk of higher rates of morbidity and death [26-28]. The mortality risk of uncontrolled epilepsy is approximately 0.5% per year and accumulates over the child's lifetime. Examples of the seizure-related mortality include sudden unexplained death due to epilepsy (SUDEP), aspiration pneumonia, trauma, accidents, drowning, and status epilepticus. Importantly, SUDEP risk does not change with medically controlled seizures, but is only reduced in the population made seizure-free.

In order to reduce the risk of developmental decline and decrease seizure-related morbidity and mortality, surgical therapy should be considered as a therapeutic option in the management of some therapy-resistant children. Furthermore, psychosocial function and quality of life indices may benefit greatly from seizure control.

1.2 Clinical characteristics of pediatric epilepsy surgery patients

The general clinical features of pediatric epilepsy surgery patients are those associated with a high risk of inducing an epileptic encephalopathy. This is best illustrated from the results of an international survey of pediatric epilepsy surgery centers for the calendar year 2004 [29]. For example, age at seizure onset is two years or less in 83% and seizures frequency is daily or greater in 65% of cases. Of note, fewer than 32% of children receive their epilepsy surgery within two years of onset. Thus, many children are exposed to an epilepsy duration that is associated with the poorest developmental outcomes.

Etiologies are often quite variable in patients undergoing pediatric epilepsy neurosurgery. Cortical dysplasia (CD), a congenital structural aberration consisting of cortical dyslamination and columnar disorganization, is the most commonly encountered substrate in pediatric epilepsy surgery. Cortical dysplasia can be graded as severe or mild [30, 31]. While milder forms of CD solely involve defects in lamination, severe cortical dysplasia consists of cortical dyslamination with the addition of abnormal cytomegalic neurons and balloon cells in the cortex and subcortical white matter [32, 33]. Severe CD is more likely to present at younger ages, have higher seizure frequencies, and be extratemporal [34]. Though CD is most often detected using conventional structural MRI, abnormalities may be subtle or undetectable. In addition, scalp EEG may be non-localized from 23% to 50% of cases. For this reason, adjunctive neuroimaging such as FDG-PET, MEG, and ictal-SPECT may be utilized. FDG-PET, in particular, is positive in 75 to 90% of CD cases [35]. Focal CD is most often found in the frontal and temporal lobes, though larger lesions may involve multiple lobes or a cerebral hemisphere. In our experience, children with early-onset epilepsy tend to have larger dysplastic lesions with more severe CD histopathology.

The second most common substrate found in pediatric epilepsy surgery patients is low-grade neoplastic tumors, such as gangliogliomas and dysembryoplastic neuroepithelial tumors (DNETs). Seizures are the first clinical presentation in about 80% of patients with low-grade

tumors [36, 37]. DNETs may also be found in association with regions of cortical dysplasia. Fortunately, high seizure remission rates result from complete resection of these tumors [38]. Other structural abnormalities leading to pediatric epilepsy surgery may result from perinatal infarcts or infections such as bacterial or viral encephalitides [39]. While hippocampal sclerosis is the most common etiology in adult epilepsy surgery patients, it is less frequent in children.

Other etiologies are much less frequent in pediatric epilepsy surgery patients. In Sturge-Weber, for example, suboptimal seizure control may lead to developmental delay and progressive hemiparesis. For this reason, children may require focal or hemispheric resections to prevent developmental decline [40]. Tuberous sclerosis complex (TSC), a phakomatosis that results in hamartomatous lesions throughout the body, is another fairly rare condition referred for epilepsy surgery consideration. In TSC patients, seizures may be focal or multifocal, are often treatment-resistant, and may impair neurocognitive development [41-43]. Even though in many of these cases there are several tubers as well as multifocal EEG abnormalities, optimal seizure control may result from resection of the epileptogenic cortical tuber(s) [44-46].

Rasmussen's syndrome, hypothalamic hamartoma, and hemimegaloencephaly are other less-frequently encountered substrates in pediatric epilepsy surgery. Rasmussen's syndrome is a disease of unknown cause where progressive involvement of one hemisphere leads to hemiparesis and intractable seizures [47]. While steroids, immunoglobulins, and plasmapheresis provide temporary relief, hemispherectomy is the only known effective treatment. Laundau-Kleffner syndrome, an epileptic aphasia that associated with severe developmental regression and intractable seizures, may respond to surgical intervention [48]. Hemimegalencephaly is a phenomenon where one hemisphere is hypertrophied and contains abnormal and dysplastic glioneuronal proliferation [49]. Children with hemimegalencephaly have unilateral or bilateral EEG abnormalities often with hypsarrhythmia or suppression bursts. These children also present with macrocrania, mental retardation, and contralateral paresis. Hemispherectomy provides the best chances for seizure control and normalization of psychomotor development velocity [50].

1.3 Perioperative considerations and surgical approaches

The particular surgical treatment and timing of surgery are customized to the individual child. Children may present with acute status epilepticus and urgent surgery may be needed to control life-threatening seizures [51]. Operations that may be offered include invasive recording using grid and depth electrodes, resection of the epileptic lesion, disconnection of the abnormal cortex, or palliation of seizure frequency. About 80% of operations performed are aimed at diagnosis and/or resection, while 20% are palliative. The most common types of surgery range from cerebral hemispherectomy to focal resections. Of the multiple techniques of hemispherectomy-hemispherotomy described, many have similar seizure control rates. These surgeries have similar efficacy compared to temporal lobectomy for complex partial limbic seizures.

The majority of palliative procedures in the United States are placement of vagus nerve stimulators, with corpus callosotomy performed in rare instances. Palliative surgery is offered to children with unresectable epilepsy, such as seen in Lennox-Gastaut syndrome [52, 53]. Radiosurgery is currently being investigated as a less invasive alternative, particularly for hypothalamic hamartoma and medial temporal lobe epilepsy [54-58]. To date, the use of radiosurgery to treat infants and children has not been adequately studied. Another surgical technology being investigated involves stereoendoscopy, particularly for treating hypothalamic

hamartomas [59, 60]. Resective and palliative surgeries continue to be the mainstay of treatment in a majority of these children.

1.4 Outcomes after epilepsy neurosurgery in children

Approximately 60% to 80% of children will become seizure free after epilepsy neurosurgery [15, 27, 61-71]. The two most consistent factors associated with the highest chance of seizure control are: (1) a lesion identified on MRI scan, and (2) complete excision of the lesion [72-75]. Seizure remission rates are higher in children who have temporal resections compared to extratemporal resections. The best predictor of long-term optimal seizure control is early seizure control after epilepsy surgery. If the patient has no seizures during the first 6 months after surgery, they have up to a 95% chance of remaining completely seizure-free over time [76]. Up to 30% to 50% of children will have AEDS withdrawn following successful epilepsy surgery [28, 77-79].

Focal resections may result in seizure remission rate that approaches 88% for hippocampal sclerosis, 81% for tumor, and 62% for cortical dysplasia [65]. Compared to focal or lobar resections, patients with more widespread and/or diffuse pathology (e.g. hemimegalencephaly) requiring hemispheric surgery or disconnection have lower seizure remission rates [80]. It is possible that these diffuse EEG patterns indicate an interface between the early lesion and the developing brain [81].

Surgical mortality is from 0.25% to 2% for procedures such as temporal lobectomy and hemispherectomy, while permanent surgical morbidity is reported to be less than 5% [82]. Temporal resections are most often complicated by visual field deficits, while extratemporal resections are most often complicated by transient hemiparesis. Infrequently, infarcts, permanent hemiparesis (temporal lobe resections), and language deficits may occur. In general, the risks of surgery are less than the risks associated with the natural history of treatment-resistant epilepsy [75]. Re-operations for a failed first epilepsy surgery occur in approximately 14% of pediatric patients [29]. Most re-operations involve extending the resection to include areas thought not to be involved with the epileptogenic process with the first operation.

Conclusion: In children with intractable epilepsy who are candidates for surgical resection, seizure freedom is by no means guaranteed. Depending on underlying pathology, seizures persist in 40% or more children. The options for these children, beyond palliative surgical procedures and continued medication trials, are non-existent.

1.5 mTOR

mTOR is a ubiquitous 289kDa serine/threonine kinase whose structure places it in the family of phosphatidylinositol 3-kinase (PI3K)-related kinases (PIKK) [83]. mTOR is dysregulated in a number of human diseases, including tuberous sclerosis and epilepsy. Inhibition of mTOR reduces cell proliferation, angiogenesis, and glucose uptake by cells in both in vivo and in vitro studies [84-87].

Activation of mTOR begins with the binding of a variety of extracellular ligands to receptor tyrosine kinases. These ligands include mitogens, trophic factors, and hormones [88]. In neurons, mTOR activation is also modulated by N-methyl-D-aspartate receptors (NMDAR), alpha-amino-3- hydroxy-5- methyl-4-isoxazolepropionic acid receptors (AMPARs), brain-derived neurotrophic factor (BDNF) and dopaminergic and metabotropic glutamate receptors (mGluRs) [89]. Calcium influx through NMDARs or voltage-gated calcium channels, which stimulates

both the ERK/MAPK and PI3K pathways through calcium/calmodulin, ras, and cAMP, is another important upstream activator of mTOR [90]. Receptor tyrosine kinase activation then leads to activation of PI3K, which in turn causes Akt to be recruited to the cell membrane and become phosphorylated [88]. This phosphorylation of Akt is catalyzed by two different enzymes: phosphoinositol-dependent protein kinase 1 (PDK1, which is dependent on PI3K activation) and mTORC2, a relatively rapamycin-resistant complex of mTOR. Akt is also negatively regulated by phosphatase and tensin homologue (Pten), the major antagonist of PI3K/Akt-dependent signaling [91]. Upon activation, Akt, also a serine/threonine kinase, proceeds to phosphorylate (and thereby inactivate) tuberous sclerosis protein 2 (Tsc2) [88]. Tsc2, along with its counterpart tuberous sclerosis protein 1 (Tsc1), form the Tsc1/Tsc2 complex. When activated, the Tsc1/Tsc2 complex acts as a GAP (GTPase-activating protein) for Rheb (Ras homolog enriched in brain protein), thus inhibiting it. When the Tsc1/Tsc2 complex is neutralized through Akt activation, however, resultant Rheb disinhibition leads to activation of mTOR and formation of mTORC1. Activated Rheb is not, however, capable of increasing mTORC2 activity. In fact, little is known about upstream activators of mTORC2.

mTOR complex 1 (mTORC1) consists of the proteins mTOR, raptor (regulatory associated protein of mTOR), Pras40, and mLST8 [83]. Raptor is critical for mTORC1 function (it is an adaptor protein and binds to and presents substrates to the catalytic region of mTOR). In particular, raptor binds S6K and 4EBP, allowing them to undergo phosphorylation by mTORC1. Raptor is also an area that itself undergoes phosphorylation to modulate mTOR activity by proteins such as AMPK, p90 ribosomal S6 kinase (RSK), and mTORC1 itself. Raptor also acts as a regulatory subunit of mTOR and can be phosphorylated at a variety of serine residues by enzymes such as AMPK and ribosomal S6 kinase, and Rheb to either promote or inhibit mTORC1 activity. The remaining mTORC1 proteins serve either a scaffolding or regulatory capacity. Deptor is a protein that is able to independently bind and negatively regulate mTORC1. mTORC1 is sensitive to rapamycin, which competes with raptor to bind the FRB domain of mTOR [89]. mTORC1 has a variety of important functions, many of which have been characterized by using rapamycin. Perhaps the two most notable and well-described substrates of mTORC1 are 4E-BP1 and p70S6K, both of which are phosphorylated by mTORC1 [83]. When unphosphorylated, 4E-BP1, a translation repressor, inhibits translation of 5' capped mRNAs by binding translation initiation factor 4E (eIF-4E). Upon phosphorylation by mTORC1, 4E-BP1 dissociates from eIF-4E and allows translation to occur. Phosphorylation of p70S6K, on the other hand, results in subsequent phosphorylation and activation of ribosomal protein S6 (a protein necessary for the translation of 5' terminal oligopyrimidine (TOP) mRNAs). Importantly, 4E-BP1 and p70S6K have been found to be the key effectors regulating cell growth and cell cycle progression [92, 93]. Additional targets of p70S6K include insulin receptor substrate-1 (IRS-1), glycogen synthase kinase 3 (GSK3), translation elongation factor 2 (eEF2) kinase, and Bad [83]. IRS-1 is important for activation of the IGF-1 receptor, which, in turn, leads to activation of the PI3K pathway. When IRS-1 is phosphorylated, its actions on IGF-1 are inhibited and thus the PI3K pathway is negatively regulated. GSK3 is an important effector protein that has roles in cytoskeletal structure, cell growth, metabolism, and cell cycle regulation [94, 95]. In the CNS, GSK3 has been shown to have a critical influence on synaptic plasticity [96]. Bad, a proapoptotic molecule, is phosphorylated and inactivated by p70S6K [97].

While 4E-BP1 and p70S6K have been described as the canonical targets of mTORC1, additional effectors have been identified [98]. Mitochondrial metabolism directly correlates with mTORC1

activity. Another transcription factor that is activated by mTORC1 is STAT3. STAT3 is a focus of scientific interest with regards to oncogenesis, stem cell differentiation, immunity, and a variety of human diseases [99, 100]. Two additional effectors found downstream of mTORC1 include serum- and glucocorticoid-inducible kinase-1 (SGK1) and hypoxia-inducible factor (HIF). SGK-1 is thought to mediate a raptor-dependent effect on the cyclin-dependent kinase inhibitor, p27 (KIP1), thus exerting control over cell cycle progression. HIF is an important molecule that is involved in the expression of a variety of genes that modulate angiogenesis, erythropoiesis, and metabolism. As the name implies, HIF is activated under hypoxic conditions.

mTOR complex 2 (mTORC2) is only similar in structure to mTORC1 in that it contains mTOR and the protein mLST8. In addition to these two components, mTORC2 also contains rictor (rapamycin insensitive companion of mTOR), Sin1, and protor. Rictor is necessary for the assembly, stability, and activity of mTORC2 [101]. In particular, rictor is required for mTORC2 interaction with PKCa. Like mTORC1, deptor is able to bind mTORC2 and negatively regulate its kinase activity [102]. mTORC2 is unaffected by acute exposure to rapamycin [83]. Chronic exposure does inhibit mTORC2 activity through disruption of new mTORC2 complex assembly [103]. In fact, only rictor that is newly synthesized can be affected by rapamycin; thus, existing mTORC2 complexes appear to be all that is resistant to rapamycin therapy [89]. That said, chronic rapamycin treatment results in suppression of Akt and PKC signaling through a reduction in mTORC2.

As of yet, the upstream signaling pathway of mTORC2 is relatively unknown. There is some evidence that mTORC2 may be activated through either PI3K signaling or through crosstalk with mTORC1 via p70S6K [102]. Also, some data suggest that mTORC2 may be positively regulated by the Tsc1/Tsc2 complex in a fashion that is independent of its inhibitory activity on mTORC1 [102, 104]. When Tsc1 or Tsc2 are conditionally deleted, mTORC1 levels are elevated and mTORC2 levels are suppressed. This is particularly interesting because it may help to explain why lesions that are seen when Tsc1 or Tsc2 is lost (as in tuberous sclerosis) are benign and not cancerous. Because mTORC2 may be jointly regulated by Tsc1/Tsc2 (positively) and mTORC1/p70S6K (negatively), when mTORC1 is constitutively active (such as in tuberous sclerosis) these feedback loops aid in dampening Akt activation [102].

One target of mTORC2 is protein kinase C-alpha (PKC), which, through phosphorylation, has its activity, stability, and localization regulated [89]. Among the many known functions of PKC, perhaps the most notable with regards to neuronal activity is its regulation of synaptic plasticity. PKC also is involved in cell growth and actin cytoskeletal organization [105], regulation of ion channels [106], modulation of GABAA receptor function and trafficking [107, 108], and calcium-dependent vesicular signaling in astrocytes [109]. Regulation of cell size by mTORC2 has also been shown to occur through another downstream effector, serum- and glucocorticoid-induced protein kinase 1 (SGK1) [83]. Also, the family of SGKs are important in sodium transport regulation and in oncogenesis [102, 110]. Besides PKC and SGK1, perhaps mTORC2's most prominent downstream target is Akt. Through Akt, mTORC2 exerts control over cell size, cell survival, cytoskeletal arrangement, metabolic function, apoptosis, protein expression, cell motility, and oncogenic transformation [111].

1.6 mTOR and epilepsy

In models of pilocarpine-induced seizures, it has been shown that about 30 min after pilocarpine injection levels of phosphorylated S6K increase and peak at 1 h within the hippocampus and cortex [112]. This rise in phosphorylated S6K can be blocked by pre-treatment with systemic rapamycin (5 mg/kg/day) for three days prior to pilocarpine injection, though the pre-treatment does not affect the severity of the acute seizures. Pilocarpine-treated animals with recurrent spontaneous seizures who are treated with chronic systemic rapamycin (5 mg/kg/day for three days, then every other day for three weeks), on the other hand, demonstrate a reduction in seizure frequency and duration during treatment that gradually increases following withdrawal of rapamycin. In a model of pilocarpine-induced status epilepticus, continuous infusion of rapamycin into the dorsal hippocampus prevented mossy fiber sprouting in the molecular and granular layers that then emerged upon withdrawal of treatment [113]. Interestingly, when rapamycin was started after mossy fiber sprouting began (2 months after seizure onset), no effects were seen. This effect on mossy fiber sprouting has been confirmed by others [112].

In another model of temporal lobe epilepsy induced by kainate injection, elevation in phosphorylated S6K was noted at 1 h after kainate injection with a peak at 3-6 h and a return to baseline at 24 h in hippocampus and cortex [114]. An additional phase of rising phosphorylated S6K levels was noted in the hippocampus only, starting at 3 days after injection, peaking by 5 days, and returning to baseline by 5 weeks. Similar to the studies by Huang et al. [112] rapamycin was administered systemically (6 mg/kg/day) for three days before injection. The biphasic rise in phosphorylated S6K was blocked by this treatment; again, however, the severity of the acute seizures was not affected. Furthermore, rapamycin pre-treatment reduced kainate seizure-induced hippocampal cell death, kainate-seizure induced dentate granule cell neurogenesis, supragranular mossy fiber sprouting, and chronic recurrent kainate-induced spontaneous epilepsy. When rapamycin treatment was changed from a pre-treatment to a post-treatment paradigm (6 mg/kg/day for 6 days starting 24 h after onset of kainate status epilepticus and then every other day from that point forward), late phase mTOR activation, mossy fiber sprouting, and chronic kainate-induced spontaneous seizures were all reduced. There was no effect on cell death or neurogenesis.

Several studies have suggested that rapamycin may play an important role in the suppression of congenital and acquired epilepsy. In one model of tuberous sclerosis where Tsc1 is conditionally deleted from most cortical neurons, both rapamycin and RAD-001 increase survival, improve the histological phenotype (cortical organization, soma size and polarity, and myelination), and reduce seizures [115]. Additional work has shown that rapamycin completely reverses the elevated endoplasmic reticulum and oxidative stress that can lead to cell death in Tsc2-deficient hippocampal neurons and Tsc1 deficient brain lysates [116]. In another model of cortical dysgenesis in which PTEN is conditionally deleted from cortical neurons, rapamycin administration also improved the histological abnormalities (enlarged, disorganized neurons), reduced abnormal EEG activity, and suppressed the frequency and duration of spontaneous seizures [117]. In animal model of tuberous sclerosis in which Tsc1 is conditionally deleted in [primarily] glia, rapamycin had significant beneficial effects as well [118]. When rapamycin was given systemically starting at P14 (before the onset of seizures), astrogliosis was prevented, epilepsy did not develop, and animals did not die prematurely. When rapamycin was begun after the onset of epilepsy (at 6 weeks), seizure frequency was decreased, interictal EEG was improved, and survival was prolonged.

1.7 Overview of Clinical Effects of mTOR Inhibitors on Seizures

The effects of mTOR inhibitors on the reduction of seizures have been demonstrated in several clinical studies with rapamycin and everolimus.

In an open-label prospective study, 52 pediatric participants with the diagnosis of TSC complicated with epilepsy received rapamycin treatment for at least 24 weeks [119]. This study was conducted in China. The drug dosage was 1 mg/m^2/d, average 0.7 mg/d (0.35-1.20 mg/d). The median age at onset of epilepsy was 4.8 months (4 days-49 months), the median age for treatment with rapamycin was 27 months (4.5-172.5 months). Ten children had a family history of TSC. In 24 children with TSC gene detection, TSC1 mutation was detected in 4 cases and TSC2 mutation in 20. Before rapamycin therapy, 59.62% (31/52) participants took more than 3 antiepileptic drugs, of whom 10 cases even took more than 5 kinds of antiepileptic drugs. In participants received rapamycin treatment for 24, 48, 72, and 96 weeks, seizure free rate was 25% (13/52), 19% (6/31), 29% (5/17), and 25% (3/12) respectively. The authors report a "total effective rate" of 73% (38/52), 74% (23/31), 76% (13/17), and 75% (9/12) respectively, though it is unclear how exactly that terminology is being used. The use of antiepileptic drug types was reduced with reduction in seizure frequency. Before rapamycin therapy, the average frequency of seizures was 70.27 times/d and the average number of antiepileptic drugs was 1.30. After 24, 48, 72, and 96 weeks' treatment, the average seizure frequency was reduced to 1.94-2.80 times/d and the mean number of concomitant antiepileptic drugs were reduced to 0.83-0.97. On every visit during the follow-up, blood and urine routine tests, liver and kidney function test showed no abnormality. Blood concentrations of rapamycin remained below 10 μg/L (average 6.5 μg/L). In this study it is unclear how often these levels were measured (presumably whole blood trough but not certain). The main side effect was oral ulcer which happened in 23.08% of patients (12/52) and would generally disappear 2-3 days later. Of all patients, 17.31% (9/52 cases) had upper respiratory infection.

Everolimus (Novartis Pharmaceuticals) is a chemically modified rapamycin derivative that is currently approved for the treatment of pediatric and adult patients with TSC who have subependymal giant cell astrocytomas (SGA) that need to be treated but cannot be surgically resected. The first, largest study to be published evaluating everolimus in TSC was in 2010 [120]. This open label study enrolled 28 children with SGA treated with everolimus 3mg/m^2 to a blood trough concentration of 5-15ng/ml. In addition to a significant reduction in the volume of SGA, improvements in quality of life and reductions in seizure frequency were also observed. Overall, the medication had few grade 3 AEs and was well tolerated. The two-year open-label extension of the EXIST-1 trial, which looked at SGA and angiomyolipoma outcomes, supported longer-term use of everolimus, with a sustained efficacy in reducing the size of the SGAs [121, 122]. The safety and tolerability in this group was also reasonable, with a 5% dropout rate and 16% SAE rate. In the open-label EXIST-2, treatment was continued up to 4 years, with an 8.9% dropout rate [123].

The most relevant iteration of the everolimus trials for TSC was the EXIST-3, a double-blind placebo-controlled study evaluating everolimus as an adjunctive therapy for treatment-resistant focal-onset seizures in TSC [124]. These participants, all of which were already receiving 1-3 AEDs at the time of enrollment, had ≥16 seizures over an 8-week period to qualify for the study. Participants were assigned to placebo, low-dose everolimus (3-7ng/mL), or high-dose everolimus (9-15ng/mL). The primary endpoint in this study was change in seizure frequency from baseline,

with the response rate defined as the proportion of participants achieving ≥50% reduction in seizure frequency. In the study, the median percentage reduction in seizure frequency was 29% in the low dose group and 40% in the high-dose group, also with reasonable safety and tolerability results.

In a recent single-center, open prospective study of 15 pediatric participants with TSC and epilepsy, 80% of participants treated with everolimus responded with seizure reduction [125]. In the study, the safety and tolerability of treatment appeared favorable, with no participants withdrawn from treatment over the course of the trial. In another study out of Cincinnati Children's Hospital, 78% of children treated with everolimus reported ≥50% reduction in seizure frequency at 2 years [126]. Over 94% of AEs were mild or moderate. There are also smaller scattered reports in the literature also associating a reduction in seizure frequency in children with SGA treated with everolimus [127-129].

1.8 ABI-009

ABI-009 (*nab*-rapamycin) is a nanoparticle form of human albumin-bound rapamycin with a mean particle size of approximately 100 nm developed with a proprietary nanoparticle albumin-bound (*nab*®) technology. ABI-009 is freely dispersible in saline and is suitable for intravenous administration, and has produced both a favorable safety profile and evidence of efficacy in patients with metastatic solid tumors [130]. The *nab* technology may enhance tumor penetration and accumulation via the albumin receptor-mediated (gp60) endothelial transcytosis. Albumin is highly soluble, has long plasma half-life, broad binding affinity, making it an ideal candidate for drug delivery [131, 132]. Importantly, albumin has been shown to be able to penetrate the blood-brain barrier (BBB). In biodistribution studies in rats, ABI-009 showed significant brain uptake at 5 days (AADi internal data, see Section 0).

1.9 Preclinical Pharmacology, Pharmacokinetics, and Toxicity of ABI-009

Preclinical primary pharmacology studies in vivo demonstrated significant antitumor activity of ABI-009 as a single agent administered intravenously at 40 mg/kg, 3 times weekly for 4 weeks, across different tumor xenograft models in nude mice [133-137]. This dose level correlates to approximately 120 mg/m^2 in human. These findings are consistent with published information on rapamycin as an mTOR inhibitor and the role of mTOR in tumor growth [138].

Preclinical pharmacokinetic (PK) studies in rats showed that IV ABI-009 exhibited linear PK with respect to dose and large volume of distribution (Vz), due to efficient tissue extraction of rapamycin from the central blood compartment [135]. Shortly after dosing, tissue rapamycin level was 3-5 folds higher than that of blood, indicating efficient extraction. The terminal half-life of ABI-009 was long in rats, ranging from 13.4 - 25.8 hours and resulted in significant blood level at 48 hours (~10 ng/mL) and 120 hours (>1 ng/ml). Consistent with rapamycin literature [139], excretion of ABI-009 was primarily through the fecal route (68.57 - 69.99%) with minimum contribution from the renal route (7.73 - 8.84%).

The safety and toxicity of ABI-009 were evaluated in a series of preclinical studies. In a Good Laboratory Practice (GLP) repeat-dose toxicity study in male and female rats, ABI-009 administered IV was well tolerated at doses up to 90 mg/kg (equivalent to 540 mg/m^2 human dose) when delivered every 4 days for 3 cycles. Nonclinical toxicology studies of ABI-009 showed no new or unexpected toxicity compared to what is already known for rapamycin and

other rapalogs [140-142]. Refer to the Investigators' Brochure of ABI-009 for more detailed preclinical safety information.

1.10 Clinical Pharmacokinetics of ABI-009

Clinically, ABI-009 has unique characteristics amongst the available mTOR inhibitors. ABI-009 produced a fairly dose proportional increase of C_{max} and AUC across the dose range tested, and it significantly inhibited mTOR targets S6K and 4EBP1. The PK profile of rapamycin administered as ABI-009 is categorically different from those of the other oral or IV administered mTOR inhibitors. Intravenous infusion of ABI-009 from 45 to 150 mg/m^2 results in high C_{max} of rapamycin upon administration followed by a long half-life of approximately 60 hrs that allows once weekly dosing. Peak levels of rapamycin after ABI-009 are well above 1000 ng/mL range that may have significant tumor penetration effect. Oral mTOR inhibitors such as rapamycin or everolimus have poor absorption with a high inter- and intra-patient variability and a poor safety profile that requires low basal levels (low ng/mL level) to be maintained over the course of treatment. Temsirolimus on the other hand is a prodrug of sirolimus (rapamycin) and as a result, high levels of rapamycin are not achieved (< 100 ng/mL) [143]. Clinically, administration of ABI-009 at the maximum tolerated dose of 100 mg/m^2 IV weekly led to a much higher exposure to rapamycin (sirolimus) as compared with daily oral rapamycin at 5 mg/day (C_{max} 130-200 fold, AUC 16-27 fold, AUC/dose ~3-5 fold higher with ABI-009), temsirolimus weekly IV at 25 mg (C_{max} ~50 fold, AUC ~12 fold, AUC/dose ~2 fold for ABI-009), and daily oral everolimus at 4-10 mg/day (C_{max} 50-70 fold, AUC 12-29 fold, AUC/dose ~4-5 fold higher with ABI-009) [130, 143, 144]. Unlike with other mTOR inhibitors, micromolar concentrations are reached and maintained for several hours with ABI-009 which could potentially circumvent acquired resistance to mTOR inhibitors via retroactivation of AKT by TORC2 [145, 146].

1.11 Clinical Studies with ABI-009

ABI-009 has been tested in study CA401, a completed phase 1 study in participants with advanced nonhematological malignancies using weekly administration of ABI-009 [130]. Currently, there are several ongoing trials investigating the safety and efficacy of single-agent ABI-009 in various disease areas, including a trial in participants with malignant perivascular epithelioid tumors (PEComas), a rare type of soft-tissue tumors (NCT02494570), and a trial in by the Children's Oncology Group in pediatric oncology participants (ADVL1514, NCT02975882). In addition to studies in various cancer, ABI-009 is also being investigated in participants with severe pulmonary arterial hypertension (PAH, NCT NCT02587325).

Study CA401 (Advanced Nonhematological Malignancies)

In a phase 1 dose escalation, tolerability and pharmacokinetics study conducted at MD Anderson Cancer Center (Protocol CA-401), ABI-009 was well tolerated with evidence of responses and SD in various solid tumors including renal cell carcinoma and bladder cancer, both of which typically overexpress mTOR [130]. Twenty-six participants were treated with 45, 56.25, 100, 125, 150 mg/m^2 ABI-009 per week for 3 weeks, followed by a week of rest (28-day cycle). ABI-009 was administered intravenously. The MTD was established at 100 mg/m^2.

Nineteen participants were evaluable for efficacy. One participant in the 45 mg/m^2 (95 mg actual rapamycin dose) cohort diagnosed with adenocarcinoma of the kidney and with bone and

intrathoracic metastases had a confirmed PR. The target lesion of this participant was reduced by 35.1% and the duration of response lasted 183 days. Two (11%) participants (at doses 45 and 125 mg/m^2, with actual rapamycin doses of 88 mg and 193 mg, respectively) had an overall tumor evaluation of SD (confirmed): 1 participant with mesothelioma had SD for 365 days and 1 participant with a neuroendocrine tumor in the left axillary node had SD for 238 days.

For all cohorts and all grades, 25 of 26 (96%) participants experienced at least 1 treatment-related adverse event (TRAE). The most common nonhematologic TRAEs reported were mucosal inflammation (10 participants, 38%), fatigue (7 participants, 27%), rash (6 participants, 23%), diarrhea (6 participants, 23%), and nausea (5 participants, 19%). Most of these AEs were grade 1/2 events, with only three grade 3 nonhematologic AEs (2 elevated AST and 1 dyspnea). Specifically, at the MTD (100 mg/m^2), all 7 participants experienced at least 1 TRAE of any grades, and the most common AEs were thrombocytopenia, mucositis, and fatigue (5 participants, 71% each). A total of 7 participants (27%) had infections, including candidiasis, oral candidiasis, cellulitis, folliculitis, and urinary tract infection. All these events were grade 1 or 2.

Four (15%) participants experienced at least 1 treatment-related serious AE, including cardiac arrhythmia (grade 2) and mood alteration (grade 3) both in the 125 mg/m^2 cohort, vomiting (grade 3) in the 45 mg/m^2 cohort, and dyspnea (grade 3) in the 100 mg/m^2 cohort.

The most common hematologic TRAE, for all cohorts and grades, were thrombocytopenia (58%), followed by hypokalemia (23%), anemia and hypophosphatemia (19% each), and neutropenia (15%). Most of these events were grade 1/2, and only one grade 4 hematologic event occurred (thrombocytopenia in the 150 mg/m^2 arm). At the MTD, the only hematologic AE was a grade 3 anemia.

Study PEC-001 (Malignant PEComa)

Study PEC-001 is a phase 2, multi-center, single arm, open-label, multi-institutional study to determine the efficacy and safety profile of ABI-009 administered by IV infusion in participants with malignant PEComa. The primary objective of this study is to investigate the efficacy of the mTOR inhibitor ABI-009 in advanced malignant PEComa. The secondary objectives are to further investigate the efficacy and safety of intravenous (IV) ABI-009 100 mg/m^2 given weekly for 2 of 3 weeks in participants with advanced malignant PEComa. At least 30 evaluable participants will be enrolled in study PEC-001.

Study PAH-001 (Severe Pulmonary Arterial Hypertension)

This study is a prospective phase 1, single arm, open-label, multi-institutional study to determine the MTD, safety, and preliminary efficacy of IV ABI-009 in participants with severe PAH. ABI-009 is administered weekly for 16 weeks with a dose range of 1-10 mg/m^2.

More information is provided in the ABI-009 Investigator's Brochure.

1.12 Clinical Experience with ABI-009 in Pediatric Participants

ABI-009 is currently being investigated in a multicenter phase 1 study (ADVL1514) by the Children's Oncology Group (COG) in pediatric participants with recurrent or refractory solid tumors. The aims of the trial will be to establish the maximum tolerated pediatric dose of ABI-009 administered as an intravenous infusion over 30 minutes on Days 1 and 8 of a 21-day cycle, in combination with temozolomide and irinotecan (administered on Days 1-5), and to investigate

the toxicities, pharmacokinetics, and pharmacodynamics of ABI-009 in pediatric participants with recurrent or refractory solid tumors, including CNS tumors. The study uses the Rolling Six escalation/de-escalation design, and the starting dose of ABI-009 is 35 mg/m^2.

As of Jan 30, 2018, 6 participants have been enrolled and received treatment. Overall, the adverse events observed with ABI-009 in pediatric participants are consistent with those observed in previous clinical studies in adults. The main dose limiting toxicity of ABI-009, administered as a single agent and in combination with cytotoxic agents temozolomide and irinotecan, is grade 3 thrombocytopenia (2 out of 5 participants receiving ABI-009 at 35 mg/m^2). As per the protocol de-escalation guidelines, the new cohort of participants will receive an ABI-009 dose of 20 mg/m^2 in combination with temozolomide and irinotecan.

1.13 Comparison of Exposure of ABI-009 and Oral mTOR Inhibitors

Preclinical and clinical PK studies showed that ABI-009 administered IV exhibits an overall linear PK (C_{max} and AUC) with respect to dose. In contrast, oral rapamycin has poor bioavailability (14% with oral solution, 18% with tablet) and its uptake is affected by food [147]. The oral bioavailability is higher with everolimus at ~30% but is still heavily affected by food, which can reduce C_{max} by ~60% and AUC by ~16% [143]. Clinically, administration of ABI-009 at the maximum tolerated dose of 100 mg/m^2 IV weekly led to a much higher drug exposure as compared with daily oral rapamycin at 5 mg/day (C_{max} 130-200 fold, AUC 16-27 fold higher with ABI-009) and oral everolimus at 4-10 mg/day (C_{max} 50-70 fold, AUC 12-29 fold higher with ABI-009) [130, 143, 144]. The exposure normalized to dose (AUC/dose) is also significantly higher with ABI-009 compared with oral rapamycin (~3-5 fold) and oral everolimus (~4-5 fold) [130, 143, 144].

PK modeling and limited clinical data have also suggested that weekly IV administration of ABI-009 at doses similar to or lower than oral rapamycin and everolimus can achieve a weekly trough rapamycin blood level within the targeted therapeutic range of 5-15 ng/ml with oral mTOR inhibitors. In the current study, the starting dose of ABI-009 is 5 mg/m^2/week, with potential dose escalation to 10 and 20 mg/m^2/week or de-escalation to 2.5 and 1 mg/m^2/week. Linear modeling of rapamycin blood concentration one week after ABI-009 IV administration using phase 1 clinical PK data indicates that a weekly dose of ABI-009 at 5-20 mg/m^2 can still maintain a relevant therapeutic weekly whole blood rapamycin trough level (see table below) with doses below 5 mg/m^2 reaching somewhat lower levels.

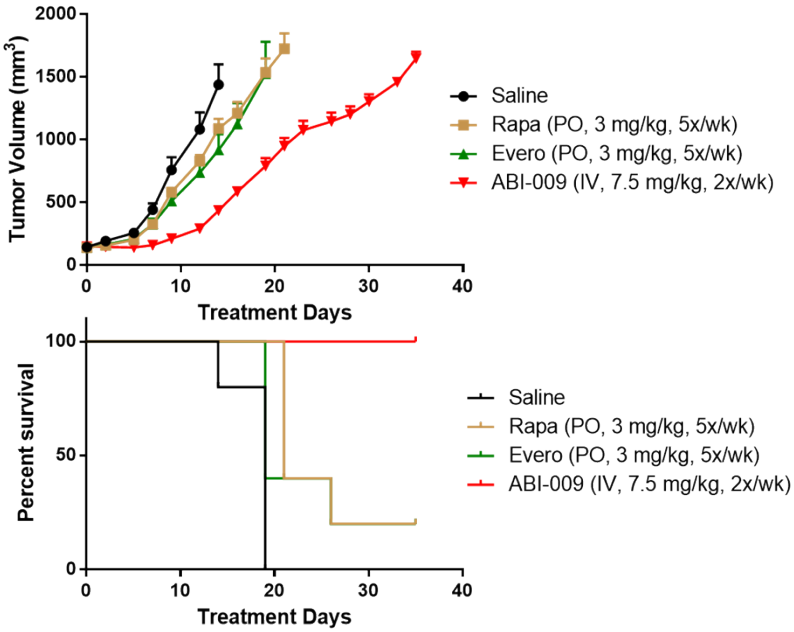
Table 1: Predicted Rapamycin Blood Levels after ABI-009 IV Administration

Dose (mg/m^2)	**Time Post-infusion (hr)**								
	0	**1**	**4**	**8**	**24**	**48**	**72**	**96**	**168**
1	28.44	9.45	7.40	5.20	3.64	2.43	1.42	1.39	0.71
2	56.88	18.89	14.80	10.39	7.27	4.86	2.84	2.79	1.42
3	85.32	28.34	22.20	15.59	10.91	7.29	4.26	4.18	2.13
5	142.20	47.23	37.00	25.99	18.18	12.15	7.10	6.97	3.56
10	284.40	94.46	73.99	51.97	36.35	24.30	14.20	13.94	7.11
20	568.80	188.92	147.98	103.94	72.70	48.60	28.40	27.88	14.22

In an ongoing phase 1 clinical study of ABI-009 in participants with severe pulmonary arterial hypertension (PAH-001), rapamycin blood levels one week after IV ABI-009 administration are available from 3 participants. The whole blood trough rapamycin levels are within the range of 4.3-9.1 ng/ml for the 5 mg/m^2/week ABI-009 dose and 7.3-17.5 ng/ml for the 10 mg/m^2/week dose, in general agreement with the PK modeling. Although there is no pediatric PK data with ABI-009 currently available, overall the current dose escalation scheme is justified based on existing and predicted adult clinical PK data.

The planned ABI-009 weekly starting dose levels in this study are lower than the previous dose of everolimus in similar clinical settings. In EXIST-3, a double-blind placebo-controlled study evaluating everolimus as an adjunctive therapy for treatment-resistant focal-onset seizures in TSC with mostly pediatric participants [124], the median dose received by participants in the everolimus low-exposure group was 5.2 mg/m^2/day (range 13–14.5) and the median whole blood rapamycin trough level (C_{min}) observed at the end of the core phase was 5.1 ng/mL (1.4–25.3), whereas in the high-exposure group the median dose was 7.5 mg/m^2/day (1.4–24.4) and the median C_{min} was 8.3 ng/mL (0.8–22.0).

Figure 1: Tumor Volume and Animal Survival following Treatment with IV ABI-009 and Oral mTOR Inhibitors



Due to its different PK profile, ABI-009 can achieve greater drug exposure than oral mTOR inhibitors at the same dose level. Consistent with this PK observation, preclinical study results also demonstrate significantly greater antitumor activity and prolonged survival with ABI-009 administered IV compared with equal weekly dosing of oral rapamycin and oral everolimus (AADi internal data). In athymic mice bearing UMUC3 human bladder cancer xenografts, ABI-009 was administered IV at 7.5 mg/kg, twice weekly (total weekly dose: 15 mg/kg), whereas rapamycin and everolimus were administered PO at 3 mg/kg/day, 5 days per week to achieve the same weekly total dose. The tumor growth inhibition (TGI) was 69.6% with ABI-009, significantly greater than oral rapamycin (TGI 24.3%; $p < 0.00001$ vs ABI-009, ANOVA) and

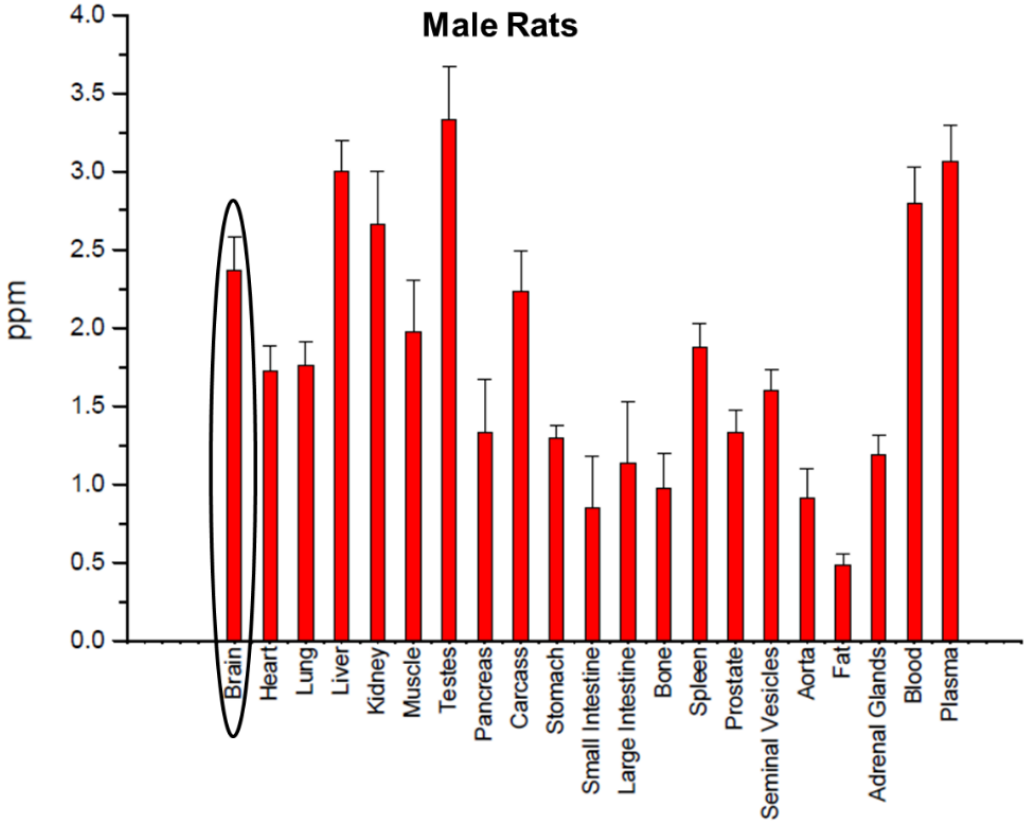
oral everolimus (TGI 36.2%; p = 0.0023 vs ABI-009, ANOVA). The median overall survival was also significantly longer with ABI-009 (not reached) compared with oral rapamycin (21 days; $p < 0.05$, log rank test) and oral everolimus (19 days; $p < 0.05$, log rank test). Results from this preclinical study clearly demonstrate superior therapeutic efficacy of ABI-009 to oral mTOR inhibitors.

1.14 Distribution of ABI-009 in the Brain

Albumin has been shown to be able to penetrate the blood-brain barrier (BBB) and distribute in the brain. Preclinical and clinical studies demonstrate the usefulness of albumin and albumin-bound drugs in the treatment of glioblastoma. In a clinical study, intravenous administration of 5-aminofluorescein-labeled albumin 0.5-4 days prior to surgery at 0.5-1 mg/kg in 13 participants resulted in the fluorescent visualization of malignant gliomas in 11 participants (84%), which facilitated the complete resection of glioblastoma in 9 participants (69%) [148]. In an orthotopic mouse GBM xenograft model, albumin-linked aldoxorubicin but not doxorubicin significantly delayed tumor growth and prolonged survival. In a Phase 2 study of participants with recurrent GBM, aldoxorubicin treatment resulted in 3 PR, 7 SD, and 11 PD in 21 participants [149].

As an albumin-bound nanoparticle, ABI-009 is potentially a suitable treatment option for disease indications in the brain, including epilepsy. In a biodistribution study in rats, radiolabeled ABI-009 showed significant brain uptake at 5 days (AADi internal data). As shown in Figure 2, the radioactivity accumulation in the brain was comparable to all other major organs 5 days after a single IV dose of radiolabeled ABI-009.

Figure 2: Distribution of Radioactivity in Tissues in Male Rats



Further, in a recent PK/biodistribution study, female SD rats received a single intravenous dose of ABI-009 at 1.7, 9.5, and 17 mg/kg. The lowest dose of 1.7 mg/kg corresponded with 10 mg/m^2 human dose (Dose level 2) proposed in the current clinical protocol. The rapamycin concentrations in blood and brain at different time points between 2 to 120 hours were analyzed by LC-MS/MS. The data are in Table 2 below.

Table 2: Rapamycin Levels in Brain and Blood after ABI-009 IV Administration

ABI-009 (mg/kg)	Time (hr)	Brain (ng/g)		Blood (ng/ml)		Brain/Blood Ratio
		Mean	SD	Mean	SD	
1.7 (N = 3/group)	2	42.4	4.0	58.5	1.2	0.7
	8	38.2	3.6	24.9	5.1	1.5
	24	40.8	13.3	9.6	2.9	4.3
	72	31.8	8.6	2.7	0.5	11.9
	120	94.9	4.5	1.2	0.4	77.9
9.5 (N = 3/group)	2	215.3	33.5	560.3	106.7	0.4
	8	263.7	27.6	160.3	21.5	1.6
	24	328.0	39.7	15.1	5.6	21.7
	72	246.3	10.6	4.3	2.3	58.0
	120	225.3	54.2	1.1	0.3	201.5
17 (N = 3/group)	2	568.3	84.6	1510.0	115.3	0.4
	8	640.7	56.0	372.7	31.5	1.7
	24	884.0	86.8	34.5	15.1	25.6
	72	606.0	70.7	3.0	0.7	203.4
	120	522.3	53.5	2.1	0.5	243.7

At all times and all doses, tissue levels of rapamycin in the brain were well above the threshold of 5 ng/ml (or ng/g, assuming density of tissue is 1 g/ml) which is recognized as the threshold for therapeutic activity. The levels in the brain were stable over the 5-day study period suggesting accumulation over time despite clearance from the blood over the same time period. In summary, results from this study clearly demonstrated drug distribution to the brain with ABI-009 IV administration.

Everolimus is the 40-O-(2-hydroxyethyl) derivative of rapamycin and works similarly to rapamycin as an allosteric mTORC1 inhibitor by binding to FKBP12. The IC_{50} of rapamycin is ~0.1 nM in HEK293 cells [150], and the IC_{50} of everolimus is 1.6-2.4 nM in a cell-free assay [151]. As everolimus has been shown in multiple clinical studies to reduce seizure frequency [120, 124-126], ABI-009, which contains rapamycin and may have the advantage of CNS penetration due to albumin, is also likely to be effective as a treatment for epilepsy.

2 STUDY RATIONALE

The proposed participants in question pose a challenging clinical population. Given the 20-40% chance of failure with resective surgery, and the fact that once a child is intractable the chances of additional AEDs eliminating seizures ≤5%, options are limited. Aside from palliative surgical procedures such as corpus callosotomy, vagal nerve stimulation (VNS), or responsive neurostimulation (RNS), there are no other novel approaches. There is a strong suggestion in the

preclinical literature of an association between mTOR activity and epilepsy/epileptogenesis, particularly in malformations of cortical development. The clinical experience with mTOR inhibitors as related to epilepsy, however, has really been limited to the TSC population. Of course, this is intuitive given the defined genetic contribution and the characterization of the relevant pathways. However, there is a growing body of evidence supporting the dysregulation of PI3K/Akt/mTOR in a variety of pathologies encountered in pediatric epilepsy surgery, including focal cortical dysplasia (FCD) [152-154]. In fact, whole exome sequencing of children with FCD and hemimegaloencephaly from peripheral samples demonstrates an association between mTOR mutations and an entire spectrum of pediatric brain malformation/epilepsy phenotypes [154]. Given the lack of options for these children, animal model data with rapamycin and the emerging evidence that the mTOR inhibitor may play a role in treating epilepsy, it seems practical that the next step would be to treat these children with ABI-009 as a therapeutic endeavor.

2.1 Risk / Benefit Assessment

The mTOR inhibitors (rapamycin/sirolimus, temsirolimus and everolimus) as a class have similar side effects and safety profiles and one can refer to the package inserts of the marketed mTOR inhibitors for safety information. No new side effects were noted with ABI-009 (see investigator brochure).

The safety data available in the literature to date for all completed controlled and uncontrolled clinical studies suggest that: (a) mTOR inhibitors are generally well tolerated at both daily and weekly dose schedules, and (b) mTOR inhibitors give a favorable safety profile, with adverse events (AEs) being manageable, reversible, and non-cumulative. Most common adverse reactions (incidence ≥10%) associated with mTOR inhibitors are stomatitis, rash, fatigue, asthenia, diarrhea, anorexia, nausea, mucosal inflammation, vomiting, pneumonitis, cough, peripheral edema, infections, dry skin, epistaxis, pruritus, and dyspnea. The most common grade 3-4 adverse reactions (incidence ≥2%) were infections, stomatitis, fatigue, and pneumonitis. Non-infectious pneumonitis is a class effect of rapamycin derivatives, and some of these cases have been severe and on rare occasions, fatal outcomes have been observed.

mTOR inhibitors have immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoan 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 mTOR inhibitors. Some of these infections have been severe (e.g. leading to respiratory or hepatic failure) and occasionally have had a fatal outcome.

The most common laboratory abnormalities (incidence ≥50%) associated with mTOR inhibitors are anemia, hypercholesterolemia, hypertriglyceridemia, hyperglycemia, lymphopenia, and increased creatinine. The most common grade 3-4 laboratory abnormalities (incidence ≥3%) were lymphopenia, hyperglycemia, anemia, hypophosphatemia, and hypercholesterolemia.

Comprehensive clinical safety data and nonclinical toxicology information are available for long term dosing with rapamycin and other mTOR inhibitors, which have been included in previous regulatory submissions with ABI-009 (IND 125669 and Investigator's Brochure, version 5.0).

Long term clinical studies with rapamycin and everolimus in both adult and pediatric patient populations demonstrate acceptable safety and tolerability. Rapamycin is approved for the prophylaxis of organ rejection in patients aged 13 years or older receiving renal transplants based on results from 2 clinical studies, with study duration of 24 months and 36 months respectively at 2 or 5 mg/day or 14-35 mg/week (Rapamune® Package Insert). The safety and effectiveness of everolimus have been established in pediatric patients age 1 year and older with Tuberous Sclerosis Complex (TSC)-Associated Subependymal Giant Cell Astrocytoma (SEGA) and in pediatric patients aged 2 years and older with TSC-associated partial-onset seizures. Everolimus did not appear to adversely impact growth and pubertal development in the 115 pediatric SEGA patients treated with everolimus for a median duration of 4.1 years (Afinitor® Package Insert). Notably, the toxicological findings in the nonclinical toxicity studies (summarized below) with rapamycin were not considered to be real issues in terms of human safety, as most were not encountered in the long-term clinical studies in patients.

Importantly, the proposed doses in our current study with ABI-009 is significantly lower compared with the doses of rapamycin and everolimus used in previous long term clinical studies. The initial ABI-009 dose of 5 mg/m^2/week results in an estimated 5 mg/week dose depending on the age and body surface of the patient, compared with substantially higher rapamycin doses of 14 and 35 mg/week for 24 or 36 months in renal transplant patients, or estimated everolimus doses of 36.4 and 52.5 mg/week (median dose for low-exposure group: 5.2 mg/m^2/day, high-exposure group: 7.5 mg/m^2/day) over extensive period in pediatric patients with TSC-associated partial-onset seizures (French, 2016). Therefore, the proposed ABI-009 dose in this study should result in an acceptable safety profile that is similar to those observed in previous rapamycin and everolimus clinical studies, and there is sufficient clinical and nonclinical safety data with rapamycin and other mTOR inhibitors to support ABI-009 dosing for similar periods as rapamycin.

Extensive long term nonclinical studies have previously been conducted for rapamycin to support long term clinical administration, with repeated-dose daily PO studies in mice for up to 3 months, in rats for up to 1 year (dose range for 1-month study: 0.05-5 mg/kg/d; 3-month: 0.05-5 mg/kg/d; 0.5-2 mg/kg/d; 0.5-5 mg/kg/d; 6-month: 0.05-5 mg/kg/d; 1-year: 0.2-6 mg/kg/d), and in monkeys for up to 6 months (dose range for 1-month study: 0.05-15 mg/kg/d; 3 and 6-month: 0.05-10 mg/kg/d) (NDA 21-083, Pharmacologist's Review). In rats, the major changes and target organ toxicities included depression of body weight gain, lymphoid atrophy (spleen, lymph nodes, thymus), increased incidence of focal myocardial degeneration, pancreatic islet cell degeneration with associated hyperglycemia, elevated triglycerides and cholesterol, pulmonary phospholipidosis, testicular tubular atrophy, ovarian atrophy, increased hemosioderosis, increased fibrinogen. and a high-turnover form of osteopenia. In monkeys, the major changes and target organ toxicities included chronic diarrhea, colitis and typhlitis, increases in fibrinogen, lymphoid atrophy and testicular tubular atrophy. Toxic effects of rapamycin have been observed on the male reproductive system, including testicular toxicities. Rapamycin was also associated with embryo and fetal toxicity in rats (Rapamune® Package Insert). Notably, the toxicological findings in the nonclinical toxicity studies with rapamycin were not considered to be real issues in terms of human safety, as most were not encountered in the long-term clinical studies in patients.

There is indisputable neurological morbidity to uncontrolled seizures, including resultant deficits in cognitive, behavioral, and psychosocial function [17-20]. Intractable seizures also present a

high mortality risk [26-28]. The mortality risk of uncontrolled epilepsy is approximately 0.5% per year and accumulates over the child's lifetime. In this subset of challenging cases, the relatively low risk of significant morbidity associated with mTOR inhibitor treatment warrants an attempt to see if ABI-009 has potential as a safe and effective therapy.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of this study is to assess the **safety** and **tolerability** of ABI-009 over a range of doses and to determine the DLT and MTD in participants with surgically-refractory epilepsy.

Safety: Defined as the incidence of AEs and SAEs during the study period. We will assess adverse events according to the National Cancer Institute Common Toxicity Criteria for Adverse Events version 4.03. MTD will be determined based on safety assessment, including the Columbia-Suicide Severity Rating Scale (C-SSRS) which will be administered at baseline, after study drug, and during follow-up.

Tolerability: Defined as the rate of withdrawal from the study, either voluntary or as medically directed as well as rate of compliance with medication regimen.

Columbia-Suicide Severity Rating Scale (C-SSRS) serious suicidal ideation score stays below a 4

3.2 Secondary Objectives

The secondary objectives of this study are to:

- Determine **efficacy**. Efficacy is defined as the change in seizure frequency between baseline and follow-up, expressed as **percentage reduction in seizure rate** (reduction in seizure frequency from baseline, calculated as (Baseline frequency [seizures/month] – frequency at follow-up [seizures/month])/Baseline frequency [seizures/month]) and **median percentage reduction** in seizure frequency. **Treatment response rate** will be defined as the proportion of participants achieving at least a 25% reduction in seizure frequency from baseline. **Seizure frequency** will be defined as the ratio between the number of seizures and the number of days on which seizure information was known within the same period of time (for either baseline or maintenance phase). Additional secondary endpoints will include **frequency of seizure-free days** during the maintenance period, **seizure-free rate** (participants remaining seizure free during the maintenance period), and rapamycin **blood level–response relationship** analysis.
- Determine **quality of life** and **behavioral** changes. Quality of Life for Children with Epilepsy Parent Form (QOLCE) [1] and Nisonger Child Behavioral Rating Form (NCBRF) [2, 3] will be completed at baseline, at the completion of maintenance, and at the completion of the 3 month follow-up period.
- Determine weekly whole blood rapamycin trough levels for three consecutive weeks.

3.3 Exploratory Objectives

For children enrolled in the study who underwent epilepsy surgery at our institution, informed consent will be sought for us to use their previously acquired/banked surgical tissue (including fibroblast cultures) for the following exploratory aims:

- To explore associations between histopathologic diagnosis and seizure outcomes
- To explore associations between pS6 positivity and seizure outcomes
- To analyze key molecular features including activation of the PI3K, mTOR and MAPK pathways as they relate to seizure outcomes
- To possibly perform additional genomic studies or obtain previously performed genomic analyses including genomic profiling from peripheral samples and correlate to seizure outcomes
- Explore any relationships between safety, efficacy and whole blood rapamycin trough levels
- Explore any relationships between AED levels (acquired outside the study through clinical standard of care) and rapamycin levels during the study

None of the exploratory aims would require additional experimental procedures to the participants throughout the study beyond those explicitly discussed in the protocol. Tissue would only be tested on participants who have already consented to and have banked tissue within our institution from prior epilepsy-related procedures.

4 STUDY DESIGN

4.1 Study Overview

This is a prospective, single-center, non-randomized open-label phase 1 study to investigate multiple dose levels of ABI-009 for surgically-refractory epilepsy. Up to 18 participants are planned to be enrolled into the study.

The duration of participation for each participant is up to 18 weeks, including a 4-week baseline period to obtain baseline outcome rates, a 3-week active study period and a 12-week follow-up period (see Figure 1). After the baseline period, participants will be started on the assigned dose of ABI-009, given once weekly for a total of 3 weeks. Dose levels to be tested are below:

For dose finding, ABI-009 will be tested in cohorts of 3 participants each using the standard 3+3 dose-finding design. Initial starting dose will be at 5 mg/m^2/week.

Table 3: ABI-009 Cohort Dosing Schedule

Dose levels	ABI-009 in mg/m^2
-2	1
-1	2.5
1	**5**
2	10

3	20

Escalation to the next dose level with a new cohort of 3 participants will occur after no DLT was observed. There will be no intra-participant dose escalation allowed. If a DLT occurs in a cohort, an additional 3 participants will be recruited to the cohort. If no further DLTs occur, then a new cohort of 3 participants at the next higher dose level can be enrolled. If 2/6 participants at a dose level experience a DLT, then the cohort will be closed to further enrollment and 3 participants will be enrolled at the next lower dose level, and so on. The MTD is the highest dose level in which ≤1 participant has a DLT. There will be no intra-participant dose escalation allowed.

Once the MTD has been determined, the MTD cohort will be opened for an estimated additional 6 participants. These participants will adhere to the identical protocol of the rest of their cohort.

Product will be administered IV every 7 days for 3 weeks.

For a detailed review of visits, please see Appendix 1.

Total duration of the study is expected to be about one year.

5 CRITERIA FOR EVALUATION

5.1 Primary Endpoints

I. Safety

- DLT and MTD
- Incidence of adverse events and clinically significant abnormal lab values during dosing

II. Tolerability

- Voluntary or medically-determined withdrawal from study
- Adherence to medication regimen
- C-SSRS serious suicidal ideation score stays below a 4 rating

5.2 Secondary Endpoints

I. Efficacy

- Seizure frequency
- Percentage reduction in seizure frequency
- Median percentage reduction in seizure frequency
- Response rate
- Frequency of seizure-free days
- Seizure-free rate
- Rapamycin blood level–response relationship

II. Quality of life and behavioral changes

- Quality of Life for Children with Epilepsy Parent Form (QOLCE) [1] and

- Nisonger Child Behavioral Rating Form (NCBRF) [2,3]
- Both will be completed at baseline, at the completion of maintenance, and at the completion of the 3-month follow-up period.

III. Weekly whole blood rapamycin trough levels for three consecutive weeks.

6 PARTICIPANT SELECTION

6.1 Study Population

Participants with a diagnosis of medically intractable epilepsy who meet all of the inclusion and none of the exclusion criteria will be eligible for participation in this study. Participants must be enrolled before study procedures begin.

6.2 Inclusion Criteria

1. Written informed consent (and assent when applicable) obtained from participant or participant's legal representative
2. Be willing and able to adhere to the study visit schedule and other protocol requirements
3. Male or female ≥3 and ≤26 years of age at Visit 1
    b. Because no dosing or adverse event data are currently available on the use of ABI-009 or other mTOR inhibitors in participants <3 years of age, these young children are excluded from this study.
4. Documentation of a diagnosis of medically intractable epilepsy as defined by the failure of at least 2 appropriately dosed and tolerated AEDs to eliminate all clinical seizures over a 6-month period, prior to epilepsy surgery
5. Documentation of resective epilepsy surgery following appropriate pre-surgical evaluation
6. Documentation of continued clinical seizures that persist at least 3 months following resective epilepsy surgery. In order to proceed with enrollment and study drug initiation, participants will have to have had >8 seizures in the last 30 days without 2 consecutive weeks of seizure freedom, as noted by a daily seizure diary.
7. Documentation that the participant is not a candidate for *or* refuses any additional resective epilepsy surgery
8. Participants must have adequate bone marrow function (ANC ≥1,000/mm^3, platelet count of ≥100,000/mm^3, and hemoglobin ≥9 gm/dL) before study drug dosing.
9. Participants must have adequate liver function (SGPT/ALT ≤5 times ULN and bilirubin ≤5 times ULN) before study drug dosing.
10. Participants must have adequate renal function, defined as: Creatinine clearance or radioisotope GFR ≥70mL/min/1.73 m2 **or** a serum creatinine before study drug dosing based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	Female
3 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this table were derived from the Schwartz formula for estimating GFR utilizing child length and stature data published by the CDC.

11. Participants must have a fasting cholesterol level <350 mg/dL and triglycerides <400 mg/dL before starting study drug. In case one or both of these are exceeded, the participant can only be included after initiation of appropriate lipid lowering medication and documentation of cholesterol <350mg/dL and triglycerides <400mg/dl before study drug dosing.
12. Participants must have normal oxygen saturation before study drug dosing.
13. The effects of ABI-009 on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because rapamycin is known to be teratogenic, female participants of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a female participant become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.
    a. Participants of child bearing potential must not be breastfeeding or pregnant as evidenced by a negative pregnancy test at enrollment and before study drug initiation.

6.3 Exclusion Criteria

1. For females of child bearing potential:
    a. Positive pregnancy test at Visit 1, or
    b. Lactating, or
    c. Unwilling to practice a medically acceptable form of contraception (acceptable forms of contraception: abstinence, hormonal birth control, intrauterine device, or barrier method plus a spermicidal agent), unless surgically sterilized or postmenopausal during the study.
2. Participant has any other condition that, in the opinion of the Site Investigator/designee, would preclude informed consent or assent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives.
3. Participants has received immunization with attenuated live vaccines within one week of study entry and/or is planning to receive immunization with attenuated live vaccines during study period. Examples of live vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines. Close contact

with those who have received attenuated live vaccines should be avoided during the ABI-009 dosing period.

4. Participant tests positive for Hepatitis C antibodies or the Hepatitis B antigen. HBsAg and HCVAb blood test must be done at screening (HBsAg only needs to be screened in patients who have not received the full complement of Hepatitis B immunizations). Alternatively, if the patient has received the complement of Hepatitis B immunizations and documentation is provided, this would suffice.
5. A known history of HIV seropositivity. HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with ABI-009. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.
6. Participant is receiving chronic, systemic treatment with corticosteroids or another immunosuppressive agent. Note: Patients that are currently using inhaled, intranasal, ocular, topical or other non-oral or non-IV steroids are not necessarily excluded from the study but must be discussed with the study chair.
7. Participant has been previously treated with a systemic mTOR inhibitor for epilepsy. Skin cream use with rapamycin or everolimus, however, is permitted.
8. Participant has a known hypersensitivity to human albumin, ABI-009 or other rapamycins (e.g. sirolimus, everolimus, temsirolimus).
9. Participant is receiving any other concurrent anticancer or investigational therapy. Participants will be permitted to enroll in the study after a 30-day washout of previously used investigational drugs.
10. Participant has any clinically significant unrelated systemic illness that would compromise a participant's ability to tolerate protocol procedures.
11. Participant is unable to return for dosing and follow-up visits to assess toxicity to the study drug.
12. Participant has uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, active lung disease, or psychiatric illness/social situations that would limit compliance with study requirements. Study Specific Tolerance for Inclusion/Exclusion Criteria

Patients who fail to meet one or more of the inclusion criteria or who meet any of the exclusion criteria will not be enrolled in this study. Waivers of any of the above study entry criteria will not be granted.

7 CONCURRENT MEDICATIONS

All participants should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new chronic therapies.

7.1 Allowed Medications and Treatments

Standard therapy for epilepsy and related conditions is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below. A stable therapeutic regimen between screening and Visit 1 is the goal, as well as through the baseline period. Once enrolled and started into the baseline observation period, changes in AED medication regimen are not permitted unless it is solely for dose adjustments secondary to sub- or super-therapeutic serum levels. Participants that have been on a chronic AED regimen will be mandated to continue them throughout the entire study period. Treatment for prolonged seizures (>5 minutes) or clusters of seizures can be used per participant's previous rescue prescription.

7.2 Prohibited Medications and Treatments

Administration of immunosuppressant or chemotherapeutic drugs, IV/PO corticosteroids prescribed outside the context of the investigational protocol, and attenuated live vaccines, as well as any investigational drugs or biologics, is prohibited while the participant is enrolled in the study.

8 STUDY DRUG DOSING

8.1 Method of Assigning Participants to Dosing Groups

Up to 18 eligible participants will be enrolled to receive the study drug (ABI-009). There will be no randomization, and this will be an open-label trial. Participants will be assigned according to a standard 3+3 dose escalation design, in the order that they are enrolled.

Participants must meet all inclusion criteria and no exclusion criteria should apply. The participant must have signed and dated an approved, current version of all applicable consent forms. To allow non-English speaking participants to participate in this study, bilingual health services will be provided in the appropriate language when feasible.

8.2 Blinding

This is an open label study; no blinding to dosing assignment will be performed.

8.3 Formulation of Test and Control Products

8.3.1 Formulation and Administration of Test Product

Refer to the Pharmacy Manual for complete information.

ABI-009 is provided as a lyophilized powder containing 100 mg rapamycin/vial. Each vial of study drug will be labeled per standard requirements.

8.3.2 Formulation of Control Product

No control product will be used in this study.

8.4 Supply of Study Drug to the Site

AADi will ship the study drug to the investigational site. The initial study drug shipment will be shipped after site approval to enroll (i.e., all required regulatory documentation has been received by AADi and a contract has been executed). Subsequent study drug shipments will be made as needed.

8.4.1 Dosage/Dosage Regimen

All participants receive study drug every 5-9 days for 3 weeks.

8.4.2 Study Drug Preparation and Dispensing

Study drug will be prepared by the site's investigational pharmacist (or designee) per the Pharmacy Manual provided by AADi.

8.4.3 Administration Instructions

Refer to Pharmacy Manual. The rate of IV administration should not exceed 3.3 mg/m^2/min. The rate is based on clinical experience with adult participants, as ABI-009 at 100 mg/m^2 can be safely administered with 30-min IV infusion.

8.4.4 Storage

Storage for the study drug is at 2-8^{o}C in a secure area under restricted access. If the temperature of study drug storage exceeds or falls below this range, this should be reported to AADi and Sponsor and captured as a deviation.

8.5 Dose-Limiting Toxicities

Definition of Dose Limiting Toxicity (DLT):

Any grade 3 or higher toxicity and designated by the principle investigator as definitely or probably related (level of attribution) to the study drug, and occurring within 30 days of study drug dosing, or as specified per toxicity in the protocol.

8.6 Dose Modification or Discontinuation of Study Drug

For participants who are unable to tolerate the protocol-specified dosing schedule secondary to DLT, dose adjustments are permitted in order to keep the participant on study drug. If administration of ABI-009 must be interrupted due to DLT, drug dosing will be interrupted or modified according to rules described in the table below. Toxicity will be assessed using the NIH/NCI Common Terminology Criteria for Adverse Events, version 5.0. If a participant requires a dose delay of ≥3 weeks from the intended day of the next scheduled dose, then the participant must be discontinued from the study or may resume only with the approval of the Principle Investigator.

Table 4: Hematological and non-hematological toxicities and ABI-009 dosing adjustments

TOXICITY	**ACTIONS**
Non-hematological toxicity	
Grade 2	If the toxicity is tolerable to the participant, maintain the same dose. If

(pneumonitis management detailed separately in 8.7.4)	the toxicity is intolerable to participant, interrupt ABI-009 until recovery to grade 1 or better. Then reintroduce ABI-009 at same dose. If event returns to intolerable grade 2, then interrupt ABI-009 until recovery to grade 1 or better. Then reintroduce ABI-009 at the lower dose level (refer to Table 3). Reintroduction of ABI-009 may occur once.
Grade 3 (except hyperlipidemia*) (pneumonitis management detailed separately in 8.7.4)	Interrupt ABI-009 until recovery to grade 1 or better. Then reintroduce ABI-009 at the lower dose level. For pneumonitis consider the use of a short course of corticosteroids. Reintroduction of ABI-009 may occur once.
Grade 4	Discontinue ABI-009.
Hematological toxicity	
Grade 2 Thrombocytopenia (platelets <75, ⩾50x10^9/L)	Interrupt ABI-009 until recovery to grade 1 or better (>75x10^9/L). Then reintroduce ABI-009 at initial dose. If thrombocytopenia again returns to grade 2, interrupt ABI-009 until recovery to grade 1 or better. Then reintroduce ABI-009 at the lower dose level (refer to Table 3). Reintroduction of ABI-009 may occur once.
Grade 3 Thrombocytopenia (platelets <50, ⩾25 x10^9/L)	Interrupt ABI-009 until recovery to grade 1 or better (platelets ≥75x10^9/L). Then resume ABI-009 at one dose level lower. If grade 3 thrombocytopenia recurs, discontinue ABI-009.
Grade 4 Thrombocytopenia (platelets <25x10^9/L)	Discontinue ABI-009.
Grade 3 Neutropenia (neutrophils <1, ⩾0.5x10^9/L)	Interrupt ABI-009 until recovery to grade 1 or better (neutrophils ≥1.5x109/L). Then resume ABI-009 at the initial dose. If ANC again returns to Grade 3, hold ABI-009 until the ANC ≥1.5x10^9/L. Then resume ABI-009 dosing at the lower dose level (refer to Table 3). Discontinue participant from study drug for a third episode of grade 3 neutropenia.
Grade 4 Neutropenia (neutrophils <0.5x10^9/L)	Interrupt ABI-009 until recovery to grade 1 or better (neutrophils ≥1.5x10^9/L). Then resume ABI-009 at the lower dose level (refer to Table 3). If grade 3 or grade 4 neutropenia occurs despite this dose reduction, discontinue ABI-009.
Grade 3 febrile neutropenia (not life-threatening)	Interrupt ABI-009 until resolution of fever and neutropenia to grade 1 or better. Hold further ABI-009 until the ANC ≥1,500/mm^3 and fever has resolved. Then resume ABI-009 at the lower dose level (refer to Table 3). If febrile neutropenia recurs, discontinue ABI-009.
Grade 4 febrile neutropenia (life-threatening)	Discontinue ABI-009.
Any hematological or non-hematological toxicity requiring interruption for ⩾3 weeks	Discontinue ABI-009.

*Grade 3 hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) should be managed using medical therapies.

All interruptions or changes to study drug administration must be recorded.

8.6.1 Dose Modification for Participants on inducers of CYP3A4 and/or PgP

Co-administration with strong inducers of CYP3A4 or P-glycoprotein (PgP) should be avoided when possible. Co-administration with moderate CYP3A4 or PgP inducers should be used with caution. That said, there may be participants on CNS agents known to be inducers of CYP3A4 (barbiturates, carbamazepine, oxcarbazepine, phenobarbital, phenytoin, valproic acid, benzodiazepines) that need to be continued on their medication regimen. No dosage adjustment of ABI-009 will be made for these participants; they will be dosed at the current stage of dose finding based on Table 3.

8.6.2 Dose Modification for Participants on inhibitors of CYP3A4 and/or PgP

Co-administration with strong inhibitors of CYP3A4 or P-glycoprotein (PgP) should also be avoided when possible. Co-administration with moderate CYP3A4 inhibitors (e.g., erythromycin, fluconazole) or PgP inhibitors should be used with caution. If the participant requires co-administration of moderate CYP3A4 inhibitors or PgP inhibitors, ABI-009 dose will be reduced to the next lower dose according to Table 3. (Additional dose reductions to every other day may be required to manage toxicities. If the inhibitor is discontinued the ABI-009 dose should be returned to the dose used prior to initiation of the moderate CYP3A4/PgP inhibitor after a washout period of 2 to 3 days. Seville orange, star fruit, grapefruit and their juices affect P450 and PgP activity. Concomitant use should be avoided.

8.6.3 Missed Study Drug Doses

Missed doses of study drug should be recorded. Our intention is to tightly adhere to the weekly schedule of medication administration.

8.7 Management of known adverse events associated with mTOR inhibitors

8.7.1 Management of Hepatitis reactivation/flare

Monitoring and prophylactic treatment for hepatitis B reactivation should occur based on the institutional standard for pediatric participants and in consultation with a pediatric gastrointestinal specialist and/or pediatric infectious disease.

8.7.2 Management of stomatitis/oral mucositis/mouth ulcers

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

1. For mild toxicity (Grade 1), conservative measures such as non-alcoholic mouth wash or salt water (0.9%) mouthwash several times a day until resolution is recommended

2. For more severe toxicity (Grade 2 in which case participants have pain but are able to maintain adequate oral alimentation, or Grade 3 in which case participants cannot maintain adequate oral alimentation), the suggested treatments are topical analgesic mouth treatments (i.e., local anesthetics such as benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or

phenol) with or without topical corticosteroids, such as triamcinolone oral paste 0.1% (Kenalog in Orabase®).

3. Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.

4. Antifungal agents must be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (ketoconazole, fluconazole, itraconazole, etc.) should be avoided in all participants due to their strong inhibition of mTOR inhibitor metabolism, thereby leading to higher mTOR inhibitor exposures. Therefore, topical antifungal agents are preferred if an infection is diagnosed. Similarly, antiviral agents such as acyclovir should be avoided unless a viral infection is diagnosed.

Note: Stomatitis/oral mucositis should be appropriately graded using the functional grading given on the NCI-CTCAE for adverse events, v5.

8.7.3 Management of hyperlipidemia and hyperglycemia

Treatment of hyperlipidemia should take into account the pre-treatment status and dietary habits. Blood tests to monitor hyperlipidemia must be taken in the fasting state. Hyperlipidemia and hypertriglyceridemia should be treated according to local best clinical practice. Participants should be monitored clinically and through serum chemistry for the development of rhabdomyolysis and other adverse events as required in the product label/data sheets for HMGCoA reductase inhibitors. Hyperglycemia has been reported in clinical trials. Monitoring of fasting serum glucose is recommended prior to the start of mTOR inhibitor dosing and periodically thereafter. Optimal glycemic control should be achieved before starting study drug dosing.

8.7.4 Management of non-infectious pneumonitis

Non-infectious pneumonitis is a class effect of rapamycin derivatives. Cases of non-infectious pneumonitis (including interstitial lung disease) have also been described in participants taking mTOR inhibitors. Some of these have been severe and on rare occasions, a fatal outcome was observed.

A diagnosis of non-infectious pneumonitis should be considered in participants presenting with nonspecific respiratory signs and symptoms such as hypoxia, pleural effusion, cough or dyspnea, and in whom infectious, neoplastic and other non-medicinal causes have been excluded by means of appropriate investigations. Participants should be advised to report promptly any new or worsening respiratory symptoms. Participants who develop radiological changes suggestive of non-infectious pneumonitis and have few or no symptoms may continue mTOR inhibitor dosing without dose alteration. If symptoms are moderate (Grade 2), consideration should be given to interruption of dosing until symptoms improve. The use of corticosteroids may be indicated. mTOR inhibitor may be reintroduced once at a reduced dose until recovery to Grade 1 or better. For cases where symptoms of non-infectious pneumonitis are severe (Grade 3), mTOR inhibitor dosing should be discontinued and the use of corticosteroids may be indicated until clinical symptoms resolve. Dosing with mTOR inhibitors may be re-initiated at a reduced dose depending on individual clinical circumstances.

Table 5. Pneumonitis Management and ABI-009 Dosing

Pneumonitis Grade	Required Investigations	Management of Pneumonitis	ABI-009 Dose Adjustment
Grade 1	CT scans with lung windows and pulmonary function testing including spirometry, DLCO, and room air O_2 saturation at rest. Repeat chest x-ray/CT scan every 2 cycles until return to baseline.	No specific therapy required.	Administer 100% of ABI-009 dose
Grade 2	CT scans with lung windows and pulmonary function testing including spirometry, DLCO, and room air O_2 saturation at rest. Repeat chest x-ray/CT scan every cycle until return to baseline. Consider bronchoscopy.	Symptomatic therapy. If cough is troublesome prescribe corticosteroids.	Reduce ABI-009 dose until recovery to ≤Grade 1. ABI-009 may also be interrupted is symptoms are troublesome. Participants will be withdrawn from the study if they fail to recover to ≤Grade 1 within 3 weeks.
Grade 3	CT scans with lung windows and pulmonary function testing including spirometry, DLCO, and room air O_2 saturation at rest. Repeat chest x-ray/CT scan every cycle until return to baseline. Bronchoscopy recommended.	Prescribe corticosteroids if infection is ruled out. Taper as medically indicated.	Hold ABI-009 until recovery to ≤Grade 1. ABI-009 may also be May restart protocol treatment within 3 weeks at a reduced dose (by one level) if evidence of clinical benefit. Participants will be withdrawn from the study if they fail to recover to ≤Grade 1 within 3 weeks.
Grade 4	CT scans with lung windows and pulmonary function testing including spirometry, DLCO, and room air O_2 saturation at rest. Repeat chest x-ray/CT scan every cycle until return to baseline. Bronchoscopy recommended.	Prescribe corticosteroids if infection is ruled out. Taper as medically indicated.	Discontinue ABI-009

8.7.5 Management of Febrile neutropenia

Febrile neutropenia should be managed according to the local institutional guidelines. Measures include laboratory testing, blood and urine cultures, and institution of broad spectrum antibiotics.

8.7.6 Management of Diarrhea

Following the loperamide dosing guidelines in Table 8 for participants experiencing diarrhea is recommended. Prophylactic administration is NOT recommended. Participants are advised to call with first signs of poorly formed stools or an increased frequency of bowel movements.

Table 6. Weight Specific Guidelines for Therapeutic Use of Loperamide

Weight (kg)	Initial (Loading) Loperamide dose (mg)	Subsequent daytime loperamide dose	Subsequent nighttime loperamide dose
8-10	1	0.5 mg q 3h	0.75 mg q4h
10-20	1	1 mg q 3h	1mg q 4h
20-30	2	1mg q 3h	2mg q 4h
30-43	2	1mg q 2h	2 mg q 4h
>42	4	2mg q 2h	4 mg q4h

8.8 Study Drug Accountability

An accurate and current accounting of the dispensing of study drug for each participant will be maintained on an ongoing basis by a member of the study site staff. The amount of study drug dispensed will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

9 STUDY PROCEDURES AND GUIDELINES

The procedures described below will be performed at the visits noted in the Schedule of Events (Appendix 1) and in Section 9.

Prior to conducting any study-related activities, written informed consent and the Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the participant or participant's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented as noted in the Schedule of Events. Dose, route, unit, frequency of administration, indication for administration and dates of medication will be captured.

9.1.2 Demographics

Demographic information (date of birth, sex, race) will be recorded.

9.1.3 Medical History

Relevant medical history, including history of current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded.

9.1.4 Epilepsy history

Epilepsy history will be recorded, including seizure types, proposed etiology, age of diagnosis, history of AED treatments and reasons for discontinuation/failure, history of pre-surgical investigations, history of epilepsy surgery including surgery type, date, postsurgical course, and relevant pathology.

9.1.5 Physical Examination

A complete or abbreviated physical examination will be performed by a licensed professional (MD, NP, RN, PA) as noted in the Schedule of Events. The abbreviated exam includes neurological, respiratory, cardiovascular, and abdominal assessments.

After screening, new abnormal physical exam findings must be documented as adverse events (AEs).

9.1.6 Weight and Height

Weight will be measured on the same scale and recorded as noted in the Schedule of Events. Adults and children may remain in clothes (without shoes). A standing height will be measured and recorded as noted in the Schedule of Events.

9.1.7 Vital Signs

Resting (preferably a minimum of 5 minutes) measurements of body temperature, blood pressure, pulse and respirations will be performed and recorded as noted in the Schedule of Events.

9.1.8 Oximetry

A resting (preferably a minimum of 5 minutes) measurement of oximetry will be measured on room air and recorded at as noted in the Schedule of Events.

9.1.9 Participant Questionnaire: Quality of Life for Children with Epilepsy Parent Form (QOLCE)

The QOLCE is a parental questionnaire used to probe the quality of life of children with epilepsy. It is a 4-page survey where parents are asked to rate their child and takes less than 10 minutes to complete. The questionnaire will be given at the end of the 1-month baseline period, at the end of the 3-week dosing period, and again at the end of the 3-month follow-up period.

9.1.10 Participant Questionnaire: Nisonger Child Behavioral Rating Form (NCBRF)

The NCBRF is a 66-item parental questionnaire regarding their child's behavior. It should take about 5-10 minutes to complete. The questionnaire will be given at the end of the 1-month baseline phase, at the end of the 3-week treatment phase, and again at the end of the 3-month follow-up period.

9.1.11 Epilepsy Diary

Participants and their parents/guardians will be requested to complete a daily diary to record any new symptoms in between visits, and most importantly to record seizures. This diary will be mandatory during enrollment in the study. The diary will include the numbers and types of seizure events that occur daily and will be requested to be completed daily to avoid memory bias. The diary will be reviewed as noted in the Schedule of Events and adverse events will be recorded in the case report form (CRF), as applicable.

9.1.12 Investigator Opinion

As specified in evaluations by visit, at the start of the visit the investigator will opine on the status of the seizures recorded – whether they seem to be the same, worse, somewhat better, or better when compared to the prior visit. This will be labeled and treated as an opinion, and will be based on the recorded seizure frequencies, the descriptions of the seizures as provided by participants and their families, and expert experience.

9.1.13 Columbia-Suicide Severity Rating Scale (C-SSRS)

The C-SSRS is a suicidal ideation and behavior rating scale to identify risk in participants. The questionnaire will be given at the end of the 1-month baseline phase, at the end of the 3-week treatment phase, and again at the end of the 3-month follow-up period. Participant serious suicidal ideation scores must remain below a 4 in order to stay on study.

9.1.14 Adverse Events

Information regarding occurrence of adverse events will be captured throughout each participant's study participation, starting at enrollment and ending once the participant has terminated from the study. Duration (start and end dates), grade, seriousness, outcome, treatment and relation to study drug will be recorded on the CRF.

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

Blood will be obtained as noted in the Schedule of Events and sent to each site's clinical hematology lab for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count). If values are found to be abnormal, a sample will be collected and these parameters re-evaluated at the next study visit. If blood sample is unusable for testing, a second sample will be requested from the patient provided blood volume total is within safety range.

9.2.2 Blood Chemistry Profile

Blood will be obtained as noted in the Schedule of Events and sent to each site's clinical chemistry lab for determination of sodium, potassium, chloride, bicarbonate, calcium, fasting glucose, albumin, total protein, BUN, serum creatinine, total bilirubin, AST, ALT, alkaline phosphatase, uric acid, phosphorus, fasting serum lipid profile (total cholesterol, triglycerides, HDL and LDL). If values are abnormal, a sample will be collected and these parameters re-evaluated at the next study visit. If blood sample is unusable for testing, a second sample will be requested from the patient provided blood volume total is within safety range.

9.2.3 Pregnancy Test

Urine or blood (approximately 2 cc) will be collected from females who are of childbearing age and capable of becoming pregnant for a pregnancy test as noted in the Schedule of Events and tested in clinic according to site standard procedures. If urine test is positive, confirmation with appropriate blood testing is to be performed.

9.3 Pharmacokinetic Measurements

No pharmacokinetic measurements will be attained for this study.

9.4 Research Laboratory Measurements

Whole blood rapamycin trough levels will be drawn for three consecutive weeks. If blood sample is unusable for testing, a second sample will be requested from the patient provided blood volume total is within safety range.

9.5 Specimens for Long-Term Biorepository Storage

No additional specimens designated for this study will be kept for long-term bio-repository storage.

10 EVALUATIONS BY VISIT

10.1 Screening Assessments (Day -14 to 0)

1. Review the study with the participant (participant's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Review eligibility (including epilepsy history)
3. Perform extended physical examination
4. Record demographics data
5. Record medical history, including a history of epilepsy, diagnosis date, and prior epilepsy treatments.
6. Vital signs (Height, pulse, blood pressure, respiration rate, temperature and weight)
7. Current medications and baseline conditions
8. Neurologic exam
9. Pulse oximetry (must be >93)
10. Laboratory assessments as required per the schedule of evaluations (need to be from within the last 30 days)
    a. Complete Blood Count (CBC) with differential and platelet count
    b. Blood chemistry assessment, including:
        i. Sodium, potassium, chloride, bicarbonate, calcium, fasting glucose, albumin
        ii. Total protein, BUN, serum creatinine
        iii. Total bilirubin, AST, ALT, alkaline phosphatase, uric acid, phosphorus
        iv. Fasting serum lipid profile (total cholesterol, triglycerides, HDL and LDL)
    c. Coagulation assessment, including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR)

d. Hepatitis B and C screening. Hepatitis B screening can be deferred in participants who have a history of having received the entire Hepatitis B immunization series and documentation is provided.

e. Serum or urine pregnancy test: If participant is of child-bearing potential, pregnancy test should be done at screening and monthly thereafter. If urine test is positive, confirmatory blood testing will be performed.

f. HIV seropositivity screening, if necessary.

11. If available and consent to archive has been given: Archival brain tissue tumor samples from a prior surgery will be used to test for molecular analyses. An email from the pathology/genetics department(s) may be used to confirm tissue availability at the time of enrollment.

10.2 Visit 1 – may also be Screening visit (Day 0)

1. Review the study with the participant/participant's legal representative and obtain written informed consent and HIPAA authorization and assent, if appropriate.

2. Assign the participant a unique screening number.

3. Record concomitant medications.

4. Perform abbreviated physical exam

5. Measure and record height and weight.

6. Obtain and record vital signs.

7. Initiate epilepsy diary

10.3 Visit 2 – End of Baseline (Day 30 +5/-2 days)

1. Review participant diary for compliance and seizure frequency.

2. Ensure participant has had >8 seizures in the last 30 days without 2 weeks of seizure freedom to continue onto next phase.

3. Collect baseline AEs

4. Administer C-SSRS, QOLCE and NCBRF

5. Record changes to concomitant medications.

6. Perform abbreviated physical exam

7. Obtain and record vital signs. Pulse oximetry must be >93 to continue.

8. Collect blood for clinical laboratory tests (chemistry, hematology).

9. Collect urine for pregnancy test (if child-bearing potential).

    a. If positive confirm with blood test

10. Laboratory assessments as required per the schedule of evaluations

    a. Complete Blood Count (CBC) with differential and platelet count.

    b. Blood chemistry assessment, including:

i. Sodium, potassium, chloride, bicarbonate, calcium, fasting glucose, albumin,
ii. Total protein, BUN, serum creatinine.
iii. Total bilirubin, AST, ALT, alkaline phosphatase, uric acid, phosphorus,
iv. Fasting serum lipid profile (total cholesterol, triglycerides, HDL and LDL)

11. Coagulation assessment, including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR).

10.4 Visits 3, 4, & 5: ABI-009 Doses 1-3 (+/-2 days)

1. Review adverse events.
2. Review epilepsy diary.
3. Investigator opinion regarding seizures recorded: same, worse, somewhat better, better.
4. Record changes to concomitant medications.
5. Perform abbreviated physical exam
6. Measure and record height and weight.
    a. BSA calculation (Calculated ONLY on Dose 1 visit; to be recalculated only if the weight changes by > 10% in subsequent cycles)
7. Obtain and record vital signs. Pulse oximetry must be >93 to continue.
8. Collect blood for clinical laboratory tests (chemistry, hematology).
9. Laboratory assessments as required per the schedule of evaluations
    a. Complete Blood Count (CBC) with differential and platelet count
    b. Blood chemistry assessment (required for Visit 4), including:
        i. Sodium, potassium, chloride, bicarbonate, calcium, fasting glucose, albumin,
        ii. Total protein, BUN, serum creatinine.
        iii. Total bilirubin, AST, ALT, alkaline phosphatase, uric acid, phosphorus,
        iv. Fasting serum lipid profile (total cholesterol, triglycerides, HDL and LDL)
10. Coagulation assessment, including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR)
11. Whole blood rapamycin trough levels
12. Dose modification as appropriate (only in the case of toxicities mandating a dose change as per section 8.6)
13. Administer study drug

10.5 Visit 6: Post-Dosing One-Week Assessment (+/-2 days)

1. Review adverse events.
2. Review epilepsy diary.

3. Investigator opinion regarding seizures recorded: same, worse, somewhat better, better.
4. Record changes to concomitant medications.
5. Perform abbreviated physical examination.
6. Obtain and record vital signs.
7. Collect urine for pregnancy test (if child-bearing potential).
    a. If positive confirm with blood test
8. Collect blood for clinical laboratory tests (chemistry, hematology).
9. Laboratory assessments as required per the schedule of evaluations
    c. Complete Blood Count (CBC) with differential and platelet count
    d. Blood chemistry assessment, including:
        i. Sodium, potassium, chloride, bicarbonate, calcium, fasting glucose, albumin,
        ii. Total protein, BUN, serum creatinine.
        iii. Total bilirubin, AST, ALT, alkaline phosphatase, uric acid, phosphorus,
        iv. Fasting serum lipid profile (total cholesterol, triglycerides, HDL and LDL)
10. Coagulation assessment, including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR)
11. Whole blood rapamycin trough level
12. Administer C-SSRS, QOLCE, and NCBRF

10.6 Follow-up Visits 7, 8, & 9: 30, 60, and 90 Days Post-Dosing (+/-7 days)

1. Review participant diary for compliance and seizure frequency.
2. Safety follow-up labs at Visit 7 per PI discretion following Visit 6 labs.
3. Investigator opinion regarding seizures recorded: same, worse, somewhat better, better
4. Record changes to concomitant medications.
5. Perform abbreviated physical exam (30 days post-doing only).
6. Measure and record height and weight (at final study visit).
7. Obtain and record vital signs (30 days post-dosing only).
8. On final visit (90 days post-dosing) administer C-SSRS, QOLCE and NCBRF

10.7 Early Permanent Discontinuation of Study Dosing

1. Administer C-SSRS, QOLCE, and NCBRF.
2. Record any adverse events.
3. Record changes to concomitant medications.
4. Perform abbreviated physical exam.

5. Measure and record height and weight.
6. Obtain and record vital signs.
7. Collect blood for clinical laboratory tests (chemistry, hematology).
8. Collect urine for pregnancy test (if child-bearing potential).

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events

An AE is any untoward medical occurrence in a clinical investigation of a participant administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the participant, for the occurrence of AEs during each participant visit and record the information in the site's source documents. All AEs are to be collected, from enrollment through end of the subject's study participation, and recorded in the participant CRF. Attribution will be unrelated, unlikely, possible, probably, or definite.

Taking into account the targeted population and the secondary endpoints of the study, Grade 1 and 2 seizures as defined by the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) v5 will not be tracked as adverse events and will not be included in the baseline patient assessment.

AE Severity

The CTCAE v5 should be used to assess and grade AE severity, including laboratory abnormalities. If the experience is not covered in the modified criteria, the guidelines shown in Table 3 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 7. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Moderate (2)	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate Instrumental activities of daily living (e.g., preparing meals, using the telephone, managing money)
Severe (3)	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (e.g., bathing, dressing, feeding self, using toilet, taking medications)

Life-threatening (4)	Life-threatening consequences; urgent intervention indicated
Death (5)	Death related to AE

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the participant or require intervention to prevent one of the outcomes listed above.

11.2.1 Serious Adverse Experience Reporting

The study site will document all SAEs that occur (whether or not related to study drug) on an SAE Report Form. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

Reporting of an SAE via SAE Report Form must be (1) sent to the site investigator, (2) reviewed by the site investigator and (3) sent to the study chair and scientific director of AADi **within one business day** of the site learning of the event. The study site will send the SAE report by email (electronic or scanned copy). When available or requested, follow-up information shall be reported via the SAE Report Form and sent to the site investigator and to the study chair and scientific director of AADi.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB) and federal regulations, the site investigator will report SAEs to the IRB.

11.3 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. 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. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

11.4 Monitoring suspected toxicities for discontinued participants

Participants whose treatment is permanently discontinued due to an adverse event or abnormal laboratory value suspected to be related to ABI-009 must be followed at least weekly until the adverse event or abnormal laboratory resolves or returns to grade 1. If a participant requires a dose delay of ≥3 weeks from the intended day of the next scheduled dose, then the participant must be discontinued from the study.

12 DISCONTINUATION AND REPLACEMENT OF PARTICIPANTS

12.1 Early Permanent Discontinuation of ABI-009 Dosing

A participant may be permanently discontinued from ABI-009 dosing at any time if the participant, the investigator, or the Sponsor feels that it is not in the participant's best interest to continue. The following is a list of possible reasons for early permanent discontinuation of study treatment:

- Participant or participant's legal representative decision
- Protocol violation
- C-SSRS serious suicidal ideation score is increased to a 4 rating
- Death
- Pregnancy
- Refusal to complete patient diary, QOLCE, NCBRF, or C-SSRS questionnaires

If a participant is permanently discontinued from dosing early due to an adverse event, the participant will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All participants who permanently discontinue study dosing early should come in for an early permanent discontinuation of study treatment visit as soon as possible and complete all remaining scheduled visits and procedures.

12.2 Screen Fail Criteria

Any consented participant who is excluded from the study before enrollment is considered a screen failure. All screen failures must be documented with the reason for the screen failure adequately stated. If a participant screen fails prior to enrollment, they can be rescreened up to 3 times if the site staff feels they meet eligibility criteria. Rescreened participants will have to complete all screening procedures (i.e., data from previous screenings cannot be used). Lab test screening may be done twice per screening.

12.3 Early Withdrawal of Participants from the Study

All participants are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. This may include participants who withdraw from study treatment early and who decline to continue to come in for remaining follow-up visits or it may include participants who completed treatment and decline to come in for remaining follow up visits.

Reasonable attempts will be made by the investigator to provide a reason for early participant withdrawals. The reason for the participant's early withdrawal from the study will be specified in the participant's source documents. Participants who withdraw early from the study should be encouraged to come in for a final early study withdrawal visit (and the procedures to be followed would include those for their next scheduled visit).

12.4 Replacement of Participants

Participants who withdraw from the study after having been both enrolled and having received at least 1 dose of ABI-009 will not be replaced. Participants may be replaced if they have been enrolled but withdraw prior to receiving any study drug or fail minimum seizure frequency required to receive study drug (≥8 seizures over the past 30 days with ≤2 weeks without seizures).

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the participant, investigator, study staff or the Sponsor fails to adhere to the protocol requirements. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Inappropriate administration of study drug
- Failure of adherence to study schedule

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The principle investigator will determine if a protocol violation should result in early permanent discontinuation of ABI-009 dosing for a participant.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by the Investigator. A copy of the form will be filed in the site's regulatory binder. The site will report the violation to their IRB in accordance with their IRB reporting requirements.

14 DATA SAFETY MONITORING

An independent Data Safety Monitoring Board consisting of at least three members will convene as frequently as necessary to review all participants enrolled on study. The DSMB will convene at minimum before the opening of each dose escalation cohort, and before opening MTD cohort expansion. All AEs experienced by participants on study will be summarized for review by the DSMB regardless of suspected relationship to the study drug. SAEs which are determined to be possibly, probably or definitely related to study drug will be reported to the DSMB within 5 days of occurrence, and any death of a participant that occurs while they are actively on study, regardless of relatedness to study drug, will be reviewed by the DSMB prior to enrollment of subsequent participants on study.

15 STATISTICAL METHODS AND CONSIDERATIONS

15.1 General Considerations

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written, describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

For the purposes of analyses, baseline period will be defined as Days 0-30. Except where otherwise noted, comparisons of continuous variables between pre and post treatment periods will utilize t-tests or linear regression, test of proportions and analysis of count data using Poisson regression. Except where otherwise noted, all tests will be two-sided and statistical significance will be determined at the 0.05 level. Missing data methods for the primary and key secondary endpoints will be described in the SAP. No adjustment for multiple comparisons will be made.

15.1.1 Data Sets Analyzed

All analyses will be performed using a pre-/post-treatment comparison within participants. The primary safety/tolerability analysis and key secondary endpoints will be repeated in the per-protocol population, which is defined as participants having completed >80% of study drug doses and have incurred no major protocol violations. The per-protocol analyses will analyze participants according to the treatment they received.

15.2 Demographic and Baseline Characteristics

Baseline demographic and clinical characteristics of the study participants such as age, gender, race, ethnicity, height, weight, body mass index, tissue diagnosis at the time of their epilepsy surgery (when available), use of concomitant medications (AEDs) will be summarized using means and standard deviations for continuous variables, and counts and proportions for categorical variables.

15.3 Analysis of Primary Endpoint - Safety Endpoints

All reported treatment emergent SAEs and AEs will be coded using MedDRA and grouped by body system. Treatment emergent AEs are defined as AEs presenting post study treatment initiation. SAEs and AEs will be tabulated using standard coding terms sorted by body system. The incidence of AEs in each treatment arm will be tabulated by seriousness, severity, and relationship to study drug. If an AE is reported more than once during the study period for a given participant, the greatest severity and the worst-case relationship will be presented in tables.

The number of SAEs and AEs will be summarized as follows: (i) The proportion of participants with at least one (S)AE, (ii) The average number of (S)AEs per participant, and (iii) The rate of (S)AEs per participant week of follow-up. Histograms showing the frequency of the number of (S)AEs in each treatment group will be included. Rates of (S)AEs by System Organ Class (SOC) will be presented by treatment group. Poisson regression modeling will be used to derive rate ratios and 95% CIs for each SOC.

Safety lab data at each study visit and changes from baseline will be summarized by treatment group. In addition, the following clinical laboratory summaries will be presented by treatment group: (i) the incidence of clinically significant abnormalities at each study visit; and (ii) tables summarizing the frequencies of participants below, within, and above the normal reference ranges at baseline and end of study; and (iii) tables displaying baseline to end of study shifts in each laboratory value (shifts between below, within or above normal range).

The number of hospitalization events and proportion of participants hospitalized will be summarized and compared by treatment group. Poisson regression modeling will be used to derive rate ratios and 95% CIs to compare hospitalization rates between treatment groups.

15.4 Analysis of Primary Endpoint – Tolerability (Study Drug Discontinuation and Compliance)

The proportion of participants permanently discontinuing study drug will be tabulated by treatment group. Drug discontinuation events will be categorized as: (1) Permanently discontinued study drug and (2) Permanently discontinued study drug and withdrew from study. The reason for permanent drug discontinuation will be summarized.

Treatment compliance will be assessed via compliance with scheduled weekly infusions.

15.5 Analysis of Secondary Endpoints

Efficacy is defined as the change in seizure frequency between baseline and follow-up expressed as **percentage reduction in seizure rate** (reduction in seizure frequency from baseline, calculated as (Baseline frequency [seizures/week] – frequency at follow-up [seizures/week])/Baseline frequency [seizures/week]) and **median percentage reduction** in seizure frequency. **Treatment response rate** will be defined as the proportion of participants achieving at least a 25% reduction in seizure frequency from baseline. **Seizure frequency** will be defined as the ratio between the number of seizures and the number of days on which seizure information was known within the same period of time (for either baseline or maintenance phase). Additional secondary endpoints will include **frequency of seizure-free days** during the maintenance period, **seizure-free rate** (participants remaining seizure free during the maintenance period), and rapamycin **blood level–response relationship** analysis. These endpoints will be analyzed using appropriate statistical tests, such as the t-test and ANOVA.

Quality of Life for Children with Epilepsy Parent Form (QOLCE) [1] and Nisonger Child Behavioral Rating Form (NCBRF) [2,3] scores will be compared using appropriate statistical tests for ordinal data, including Wilcoxon signed rank tests, logistic regression, or contingency tables.

15.6 Sample Size

This is intended to be small phase 1 study of an orphan disease focusing on safety and tolerability as primary endpoints. We anticipate recruiting up to 18 patients for studying an orphan disease, taking into account recruitment feasibility and the 3+3 dose escalation paradigm with an MTD cohort expansion.

16 DATA COLLECTION, RETENTION AND CLINICAL MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each participant who signs informed consent.

Study personnel at Seattle Children's will enter data from source documents corresponding to a participant’s visit into the protocol-specific electronic CRF when the information corresponding to that visit is available. This will be done according to the study timeline detailed in the protocol. The epilepsy diary, in particular, will be submitted directly by the parents on a daily basis electronically through REDCap to reduce memory bias. Participants will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a site number, participant number and initials.

If a correction is required for a CRF, the time and date stamp tracks the person entering or updating CRF data and create an electronic audit trail.

The Investigator is responsible for all information collected on participants enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. At the completion of the study, a copy of the CRF data will be archived and retained by the site Investigator.

Data Management Procedures

SCH utilizes REDCap for their EDC studies. The REDCap EDC system is designed to be US Code of Federal Regulations (CFR) 21 Part 11 compliant, with a robust audit trail system and electronic signature capabilities. Study personnel will enter data from a participant's visit onto electronic CRF screens via a web browser. Study participants will not be identified by name in the study database or on any data capture screens but will be identified by initials and a unique participant identification number. The Biostatistics and Clinical Data Management group will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed. All procedures for the handling and analysis of data will be conducted using good computing practices for the handling and analysis of data for clinical trials.

Data Quality Control and Reporting

After data have been entered into the study database, data validation checks will be applied on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented in an audit trail.

Security and Archival of Data

The EDC system is hosted by REDCap. Data are regularly backed up.

REDCap maintains 21 CFR Part 11-compliant electronic systems, with procedures in place to safeguard against unauthorized acquisition of data. Any authorized communication with the REDCap is conducted via SSL (128-bit) encryption. Robust password procedures, consistent with 21 Part 11, are in place. Robust physical security procedures are in place at the Data Center to prevent unauthorized personnel physical access to the server rooms. EDC account access is maintained and monitored by the Biostatistics and Clinical Data Management.

Other databases will be stored on Seattle Children's servers and are safeguarded against unauthorized access by established security procedures. Network accounts are password protected and maintained and monitored by Seattle Children's. Data is backed up regularly according to the Information Services group's procedures.

16.2 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB, or Regulatory Agency (e.g., FDA) inspectors upon request. A file for each participant must be maintained that includes the signed Informed Consent, HIPAA Authorization, Assent Form (if applicable) and copies of all source documentation related to that participant. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (e.g., participant files, signed informed consent forms, copies of CRFs, Essential Document and Study Reference Binders) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued or for a non-IND study for five years after database lock. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

16.3 Monitoring

By signing this protocol, the Investigator grants permission to the appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation. Monitoring visits will be conducted according to the U.S. CFR 21 Part 312 and ICH Guidelines for GCP (E6) and to ensure investigator compliance to 21 CFR Parts 50, 56 and 312 and to GCP.

16.4 Participant Confidentiality

In order to maintain participant confidentiality, only a site number, participant number and participant initials will identify all study participants on CRFs and other documentation. Additional participant confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Protocol amendments cannot be implemented without prior written IRB approval except as necessary to eliminate immediate safety hazards to participants. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Board

The protocol and consent form will be reviewed and approved by the IRB prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB in accordance with the standard operating procedures and policies of the IRB, and the Investigator will keep the IRB informed as to the progress of the study. The Investigator will obtain assurance of IRB compliance with regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning participant

recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB's unconditional approval statement will be transmitted by the Investigator to AADi prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB approval except when necessary to eliminate immediate hazards to the participants or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB must be informed of revisions to other documents originally submitted for review, serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB, new information that may affect adversely the safety of the participants of the conduct of the study, an annual update and/or request for re-approval, and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonisation and will also comply with local regulations. The Investigator will send an IRB-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each participant prior to entering the participant into the trial. Information should be given in both oral and written form and participants (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the participant will also be obtained. If a participant is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the participant. A copy of the signed consent form (and assent) will be given to the participant or legal representative of the participant and the original will be maintained with the participant's records.

During the course of the study, if modifications are made to the consent form that impact the participant, the participant will be re-consented as described above.

17.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply

with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.5 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the participants/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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APPENDIX 1. SCHEDULE OF EVENTS

	Screening Day -14 to Day 0*	Visit 1: Day 0	Visit 2: Day 30**	Visit 3: Dose 1	Visits 4&5: Doses 2 and 3	Visit 6: one week post-dosing	Visits 7&8: 30 and 60 days post-dosing	Visit 9: 90 days post-dosing	Early Permanent Discontinuation
Informed Consent Process	X								
Enrollment Eligibility Checklist	X								
Demographics	X								
Extended Physical Exam	X								
Neurological Exam	X								
Hepatitis C, and Hepatitis B, HIV if necessary [A]	X								
Epilepsy and Medical History	X								
Height, Weight, and BSA	X			X				X	X
Vitals	X		X	X	X	X			
Pulse Oximetry[F]	X		X	X	X				
CBC with Differential [C]	X		X	X	X	X	X[B] (visit 7 only)		X
Serum Chemistries with Coagulation Assessment [E]	X		X	X	X[B] (visit 4 required)	X[B]	X[B] (visit 7 only)		X
Urine Pregnancy [G]	X		X						
Study Continuation Checklist			X	X	X	X	X		
Abbreviated Physical Exam		X	X	X	X	X	X[D]		X
Concomitant Medication Review		X	X	X	X	X	X	X	X
Dispense Seizure Diary and Glossary		X							
Administer QOLCE, NCBRF, & C-SSRS			X			X		X	X
Review Seizure Diary			X	X	X	X	X		X
Investigator Opinion			X	X	X	X	X		
Dosing Checklist				X	X				
Study Drug Administration				X	X				
Adverse Events			X	X	X	X	X	X	X
Whole Blood Rapamycin Level [H]					X	X			X

*Screening may be combined with Visit 1

**Visit 2 may also be Visit 3 Dose 1

[A] Hepatitis C (and if necessary, Hepatitis B) test: 2 mL SST gold top tube. HIV seropositivity test, if necessary: 2 mL gold top tube. If testing for all together, can use one 4 mL SST gold top tube.

[B] Only if previous chemistries or coagulation profiles are abnormal or are otherwise clinically indicated.

[C] CBC with differential: 1 mL EDTA lavender top tubes if testing with blood trough rapamycin levels. If testing alone can collect only 0.5 mL in EDTA tube.

[D] Only at 30 days post-treatment

[E] 2 x 4 mL SST gold top tubes. Serum chemistry: Na, K, Cl, CO2, Ca, fasting glucose, BUN, creatinine, total protein, albumin, total bilirubin, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, uric acid, Ph, fasting serum lipid profile. Coagulation assessment:1.8 mL Sodium Citrate light blue top tubes. Coagulation assessment including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR).

[F] Must be greater than 93% on room air to continue with study procedures

[G] Only participants of child-bearing potential, and only monthly at most. If serum test is needed to confirm positive urine test, 1 mL SST gold top tube

[H] 0.5 mL EDTA lavender top tube if testing alone. Refrigerate if drawing on a Friday or when there may be a delay in lab processing.