UCSF Helen Diller Family Comprehensive Cancer Center

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Phase II Study of everolimus for recurrent or progressive low-grade gliomas in children

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Please refer to the PNOC SOP: Study Chair and Co-Chair Responsibilities for a description of PNOC Study Chair/Co-Chair responsibilities. This document can be found in the PNOC Members SharePoint website documents area

Please refer to the PNOC Constitution for a description of PNOC Investigator responsibilities. This document can be found in the PNOC Members SharePoint website documents area

Protocol Signature Page

Protocol No.: PNOC 001/ CC #120817 Version Date: 01-07-2019

I agree to follow this protocol version as approved by the UCSF Protocol Review Committee (PRC), Institutional Review Board (IRB), and Data Safety Monitoring Committee (DSMC).

I will conduct the study in accordance with applicable IRB requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.

I certify that I, and the study staff, have received the requisite training to conduct this research protocol.

I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572), and with local regulatory requirements. In accordance with the FDA Modernization Act, I will ensure the registration of the trial on the www.clinicaltrials.gov website.

I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

UCSF Principal Investigator / Study Chair

_____
Printed Name

_____ _____
Signature Date

Participating Site(s): Pacific Pediatric Neuro-Oncology Consortium Institutions

Principal Investigator	**Site**
Printed Name	
Institution	
Signature	Date

Abstract

Title	Phase II Study of everolimus for recurrent or progressive low-grade gliomas in children
Patient population	Children with recurrent or progressive low-grade gliomas
Rationale for Study	**This study is driven by two hypotheses**—first that mTOR inhibition will be efficacious for pediatric LGGs and second that PI3K/AKT activation will be associated with response to mTOR inhibition. This study is unique among all pediatric brain tumor trials in its hypothesis-driven biological approach and it fulfills an unmet need for a pediatric population for whom few other therapeutic options exist.
Primary Objective	**Efficacy:** Estimate the Progression Free Survival (PFS) rate at 6-months associated with everolimus therapy for symptomatic, progressive or recurrent pediatric low-grade glioma patients with measureable disease with the aim of determining whether everolimus warrants additional study only in patients with activated PI3K/Akt/mTOR pathway as measured by positivity of p-S6 status or in the entire population.
Secondary Objectives	• Estimate PFS and OS distributions as well as objective response (CR+PR) rates associated with everolimus treatment in recurrent pediatric LGGs. • Explore associations between activation of the PI3K pathway as measured by expression of phospho-AKT, phospho-PRAS40, and phospho-4EBP1 and outcome as measured by the 6-month disease stabilization rates (a dichotomous variable; see section 7.1.2.2) as well as PFS for progressive or recurrent pediatric low-grade glioma patients with measureable disease treated with everolimus. • Collect tissue from all enrolled patients and prospectively analyze key molecular features including activation of the PI3K, mTOR and MAPK pathways, aberrations in PTEN, p53, *PDGFRA* amplification, CDKN2A loss, and activating mutations in BRAF (*KIAA1549-BRAF* fusion and *BRAFV600E* missense BRAF mutation). In addition to the targeted approaches described above that examine previously reported alterations additional genomic profiling studies will be performed including targeted sequencing, RNA sequencing, and/or exome (plus) next generation sequencing if sufficient tissue is available. Such studies will include but are not limited to molecular analyses of DNA, RNA and protein in tumor biopsy specimens and blood samples. When possible and/or sufficient blood/tissue is provided, sample will be used to establish tumor cell cultures, cell lines and/or transplantation models. These studies will be carried out in collaboration with Dr. Adam Resnick, Children's Hospital of Philadelphia. • Explore MR quantitative measures of relative cerebral blood volume, permeability and apparent diffusion coefficient within the

	region of hyper-intensity on T2-weighted images as markers of disease response and/or progression in comparison to institutional evaluation of disease response and/or progression and quantitative measures of tumor response as determined by central review (based upon both area and volumetric measures.
Study Design	This is an open label study of everolimus in children with recurrent or progressive low-grade glioma. All patients will receive everolimus at a dose of 5 mg/m^2/dose daily. An adaptive Simon two-stage design for phase 2 studies of targeted therapies will be used to assess the efficacy primary objective. The proposed treatment with everolimus will be deemed not worthy of further investigation in this patient population if the true PFS at 6-months (PFS6) is less than 50%. If in the first stage, with a combined sample size of 25, there is preliminary evidence to suggest efficacy of everolimus is restricted to patients with PI3K/AKT/mTOR activation as measured by p-S6 positivity, a total of 45 patients will be enrolled and the design will have 81% statistical power to detect a true disease stabilization rate ≥70%. If in the first stage there is preliminary evidence to suggest efficacy of everolimus is independent of PI3K/AKT/mTOR activation, a total of 65 patients will be enrolled and the design will have >95% statistical power to detect a true disease stabilization rate ≥70%.
Number of patients	Up to 65 patients
Duration of Therapy	Patients may continue treatment for 24 cycles from the time of study entry.
Duration of Follow up	Patients will be followed after removal from protocol therapy for five years from initiating protocol therapy or until death, whichever occurs first.
Duration of study	The study will reach completion approximately 8 years from the time the study opens to collection of last data point.
Study Drugs	Everolimus
Safety Assessments	Analyses will be performed for all patients having received at least one dose of study drug. The study will use the NCI CTCAE v4.0.
Efficacy Assessments	The primary goal of this study is to evaluate efficacy as determined using 6-month progression free survival. Response will be determined by the bi-dimensional diameters. However, RECIST criteria will be collected and used for secondary evaluation. Patients will have brain MRI scans with and without gadolinium performed prior to therapy, before every odd cycle (3, 5, 7, 9, and 11) in the first year, and before every third cycle in the second year (13, 16,19, and 22), and at the End of Treatment visit (if not done within prior 3 months). Spine MRIs should be performed prior to therapy and at the same time points as standard brain MRIs if clinically indicated.

Unique Aspects of this Study	This study is unique in several critical respects: (i) the requirement for available tumor tissue as an eligibility criterion in order to accomplish the important objective of exploring associations between activation of specific signaling cascades and response to everolimus; (ii) the incorporation of metabolic and physiologic imaging analyses that may be critical to future assessments of tumor responses in trials that increasingly test inhibitors that are cytostatic rather than cytotoxic.

List of Abbreviations

AE	adverse event
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
CRC	Clinical Research Coordinator
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
ECOG	Eastern Cooperative Oncology Group
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
HDFCCC	Helen Diller Family Comprehensive Cancer Center
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

List of Abbreviations

MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI	National Cancer Institute
ORR	overall response rate
PD	disease progression
PO	*Per os* (by mouth, orally)
PR	partial response
PRC	Protocol Review Committee (UCSF)
RBC	red blood cell (count)
SD	stable disease
SD	standard deviation
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
ULN	upper limit of normal
WBC	white blood cell (count)

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1 Introduction

1.1 Pediatric Low Grade Gliomas (LGG)

Astrocytomas are the most common childhood central nervous system (CNS) tumor representing 40-50% of all pediatric tumors. The 5- and 10-year overall survival (OS) rates for children with low-grade gliomas (LGGs) are relatively high; however, progression free survival (PFS) after subtotal resection remains disappointing (1). Patients with high-risk features such as hypothalamic location and young age at diagnosis have overall survival rates as low as 60% at 10 years despite multimodality treatment (1). Therapies for inoperable, residual or recurrent disease remain controversial and may include observation, second surgery, radiation and/or chemotherapy. Since patients with low-grade gliomas have relatively long survival times these patients may be considered as having a chronic disease and treatment strategies should focus not only on cure but also on reducing treatment related morbidity (1).

1.2 Everolimus

Everolimus has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation and has obtained marketing authorization (Certican®) for prophylaxis of rejection in renal and cardiac transplantation in a number of countries, including the majority of the European Union. Everolimus has been in development for patients with various malignancies since 2002 (2). Everolimus 5 mg and 10 mg tablets were recently approved under the trade name Afinitor® for patients with advanced renal cell carcinoma (RCC) after failure of treatment with Sutent® (sunitinib) or Nexavar® (sorafenib) in the US, EU and several other countries and is undergoing registration in other regions worldwide (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012).

Everolimus is being investigated as an anticancer agent based on its potential to act:

Directly on the tumor cells by inhibiting tumor cell growth and proliferation (3);

Indirectly by inhibiting angiogenesis leading to reduced tumor vascularity (through potent inhibition of tumor cell HIF-1 activity, VEGF production and VEGF-induced proliferation of endothelial cells). The role of angiogenesis in the maintenance of solid tumor growth is well established, and the mTOR pathway has been implicated in the regulation of tumor production of pro-angiogenic factors as well as modulation of VEGFR signaling in endothelial cells (4-7).

1.2.1 mTOR pathway and mechanism of action

At cellular and molecular levels, everolimus acts as a signal transduction inhibitor. Everolimus selectively inhibits mTOR (mammalian target of rapamycin), a key and highly conservative serine-threonine kinase, which is present in all cells and is a central regulator of protein synthesis and ultimately cell growth, cell proliferation, angiogenesis and cell survival. mTOR is the only currently known target of everolimus [Reviewed in (3)].

mTOR signals downstream within the PI3K/AKT pathway, a pathway known to be dysregulated in a wide spectrum of human cancers (e.g. through loss/mutation of the PTEN negative regulator; PI3K mutation/amplification; AKT/PKB overexpression/overactivation; modulation of TSC1/TSC2 tumor suppressors). In addition, activation of the PI3K/AKT/mTOR pathway is frequently a characteristic of worsening prognosis through increased aggressiveness, resistance to treatment and progression (8).

The main known functions of mTOR include the following (3, 9):

- mTOR functions as a sensor of mitogens, growth factors and energy and nutrient levels, facilitating cell-cycle progression from G1 to S phase in appropriate growth conditions.
- The PI3K-mTOR pathway itself is frequently activated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors.
- Through inactivating eukaryotic initiation factor 4E binding proteins (4E-BP1) and activating the 40S ribosomal S6 kinases (i.e., p70S6K1), mTOR regulates translation of important messages, including those encoding the HIF-1 proteins, c-myc, ornithine decarboxylase, and cyclin D1, as well as ribosomal proteins themselves.
- The activation of mTOR pathway leads to increased production of pro-angiogenic factors (i.e., VEGF) in tumors and to cell growth and proliferation of tumor, endothelial, and smooth muscle cells.
- The regulation of mTOR signaling is complex and involves positive regulators, such as AKT that phosphorylate and inactivate negative regulators such as the Tuberous Sclerosis Complex (TSC1/TSC2).

mTOR is represented by two structurally and functionally distinct multiprotein signaling complexes, mTORC1 (mTOR complex 1, rapamycin sensitive) and mTORC2 (mTOR complex 2, rapamycin insensitive) (10).

mTORC1 is mainly activated via the PI3-kinase pathway through AKT (also known as PKB, protein kinase B) and the tuberous sclerosis complex (TSC1/TSC2) (9). Activated AKT phosphorylates TSC2, which leads to the dissociation of TSC1/TSC2 complex, thus inhibiting the ability of TSC2 to act as a GTPase activating protein. This allows Rheb, a small G-protein, to remain in a GTP bound state and to activate mTORC1. AKT can also activate mTORC1 by PRAS40 phosphorylation, thereby relieving the PRAS40-mediated inhibition of mTORC1 (11, 12).

mTORC2 (mTOR complex 2) is activated through a currently unknown mechanism, possibly by receptor tyrosine kinase (RTK) signaling (12). It has been suggested that mTORC2 phosphorylates and activates a different pool of AKT that is not upstream of mTORC1. PHLPP phosphatase plays a role of a negative regulator. mTORC2 is rapamycin insensitive and is required for the organization of the actin cytoskeleton (10).

mTORC1-mediated signaling is subject to modulation by the macrocyclic lactone rapamycin and its derivatives, such as everolimus. Once these agents bind to the 12 kDa cytosolic FK506-binding protein immunophilin FKBP12, the resulting rapamycin-FKBP12 complexes bind to a specific site near the catalytic domain of mTORC1 and inhibit phosphorylation of mTOR substrates. As a consequence, downstream signaling events involved in regulation of the G1 to S-phase transition are inhibited. This mechanism is thought to be responsible for the immunosuppressive effects of rapamycin as well as its putative antineoplastic activity [Reviewed in (13)]. As many cancers are characterized by dysregulation of G1 transit (for example, overexpression of cyclin or cyclin-dependent kinases), inhibition of mTOR becomes an intriguing target for inducing cytostasis (9).

1.2.2 **Preclinical studies**

Everolimus acts as an inhibitor of interleukin and growth-factor-dependent proliferation of cells. The only currently known target of everolimus is mTOR, a key regulatory protein affecting cell growth (3). Everolimus exerts its activity through high affinity interaction with an intracellular receptor protein, the immunophilin FKBP12. The FKBP12/everolimus complex subsequently interacts with the mTOR protein kinase, inhibiting downstream signaling events involved in regulation of the G1 to S-phase transition.

The main known functions of mTOR include:

• Function as a sensor of mitogens, growth factors, energy and nutrient levels, facilitating cell-cycle progression from G1 - S phase in appropriate growth conditions.

• Regulation of protein synthesis important for tumor cell proliferation and angiogenesis through inactivating eukaryotic initiation factor 4E binding proteins and activating the 40S ribosomal S6 kinases (e.g. p70S6K1). For example, activation of the mTOR pathway leads to a) increased production of pro-angiogenic factors (e.g. VEGF) in tumors b) tumor, endothelial and smooth muscle cell growth and proliferation.

The PI3K-mTOR pathway itself is frequently activated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors. The regulation of mTOR signaling is complex and involves positive regulators such as AKT that phosphorylate and inactivate negative regulators such as the Tuberous Sclerosis Complex (TSC1/TSC2). In summary, mTOR has pleiotropic functions; hence, the activities of everolimus may vary depending upon cell type.

The mTOR inhibitory activities presumably contribute to the antiproliferative activity of everolimus against tumor cell lines. However, everolimus may also exert an antitumor effect through the inhibition of angiogenesis. Indeed, both rapamycin and everolimus potently inhibit proliferation of endothelial cells (5, 7, 14) and have antiangiogenic activity *in vivo* (4-6, 15). Exactly which molecular determinants predict responsiveness of tumor cells to everolimus is still unclear. Currently, the activation status of the PI3K/AKT/mTOR/p70S6K pathway may be indicative of responsiveness to rapamycins. For example, preclinically, loss of PTEN or constitutive/hyper-activation of AKT has been suggested to sensitize tumors to the effects of inhibition of mTOR [Reviewed in (3, 6). Also, clinically, it has been suggested that high p70S6K activation in baseline GBM tumor samples may predict a patient population more likely to derive benefit from mTOR inhibition (16).

Everolimus is a highly specific inhibitor of mTOR, which is afforded by high-affinity binding to the protein FKBP-12 (IC50 of 5.3 nM) similar to that of rapamycin. Similar potency of rapamycin and everolimus was also demonstrated at forming the mTOR / FKBP-12 tertiary complex *in vitro*. Specificity was demonstrated by a lack of inhibitory activity against 10 other protein kinases at concentrations up to 10 μM.

The anti-proliferative effects of everolimus were investigated in a mixed panel of 48 different tumor cell lines (including breast, colon, epidermoid, glioblastoma, lung, melanoma, prostate and renal). The majority of tumor cell lines were highly sensitive to the anti-proliferative effects of everolimus while a few others appeared intrinsically insensitive, or ‘resistant’ (IC50 range 0.2 to 4125 nM) (17). The median IC50 value of the 48 cell lines was 0.5 nM. Similar findings have been observed for rapamycin (2). Everolimus was also shown to have activity in human pancreatic neuroendocrine cells, where induction of apoptosis was reported (18), as well as in acute myeloid leukemia cells (19), mantle cell lymphoma cells (20), adult T-cell leukemia cells (21), diffuse large B cell lymphoma cells (22), pancreatic tumor cells (23), ovarian cancer cells (6, 24) and hepatocellular carcinoma cells (25).

Everolimus was also evaluated in a clonogenic assay using cells derived from 81 patient derived tumor xenografts never cultured in vitro (11 human tumor types with 3 to 24 tumors each: bladder, colon, gastric, NSCLC, SCLC, breast, ovary, pancreatic, renal, melanoma, and pleuramesothelioma). Everolimus inhibited colony formation in a concentration-dependent manner (mean IC_{50}: 175 nM). In addition, normal hematopoetic stem cells were found to be relatively insensitive to everolimus, with an IC_{50} about 15 fold higher than the tumor lines

(RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012).

Everolimus was effective and well tolerated against subcutaneous tumors established from a variety of tumor cell lines of diverse histotypes (NSCLC, pancreatic, colon, melanoma, epidermoid), including a PgP170-overexpressing, multi-drug resistant tumor line. Typically, the antitumor activity of everolimus was that of reduction of tumor growth rates rather than producing regressions or stable disease although, in the case of A549 and NCI-H596 lung and ARJ42 pancreatic tumors, regressions could be obtained. These effects occurred within the dose range of 2.5 to 10 mg/kg, p.o., once per day. The change in tumor volume of the treated mice divided by the change in tumor volume of control mice (T/C) typically ranged from approximately 15 to 50% at optimal doses. A marked loss of antitumor activity occurred when tumor-bearing mice were treated with everolimus once per week, but improved moderately with twice per week dosing (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012). Antitumor activity of everolimus has also been demonstrated in mouse models of ovarian (6), breast (26, 27), and gastrointestinal stromal tumors (28).

1.2.3 **Preclinical safety**

In safety pharmacology studies, everolimus was devoid of relevant effects on vital organ functions including the cardiovascular, respiratory and nervous systems. Everolimus had no effects on QT interval. Furthermore, everolimus showed no antigenic potential. Although everolimus passes the blood-brain barrier, there was no indication of relevant changes in the behavior of rodents, even after single oral doses up to 2000 mg/kg or after repeated administration at up to 40 mg/kg/day (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012).

The preclinical safety profile of everolimus was assessed in mice, rats, minipigs, monkeys, and rabbits. The major target organs were male and female reproductive systems (testicular tubular degeneration, reduced sperm content in epididymides and uterine atrophy) in several species; lungs (increased alveolar macrophages) in rats and mice; and eyes (lenticular anterior suture line opacities) in rats only. Minor kidney changes were seen in the rat (exacerbation of age-related lipofuscin in tubular epithelium, increases in hydronephrosis) and mouse (exacerbation of background lesions). There was no indication of kidney toxicity in monkeys or minipigs (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012.

Genotoxicity studies covering relevant genotoxicity endpoints showed no evidence of clastogenic or mutagenic activity. Administration of everolimus for up to 2 years did not indicate any oncogenic potential in mice and rats up to the highest doses, corresponding respectively to 4.2 and 0.2 times the estimated clinical exposure. In reproduction studies, everolimus was toxic to the conceptus in rats and rabbits, and was considered potentially teratogenic in rats. It is therefore recommended that females of childbearing potential should use effective contraceptive measures during the entire treatment period and for 8 weeks thereafter.

More pre-clinical information is provided in the RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012.

1.2.4 **Everolimus pharmacokinetics**

Everolimus is rapidly absorbed with a median t_{max} of 1-2 hours. The steady-state AUC0-τ is dose-proportional over the dose range between 5 to 70 mg in the weekly regimen and 5 and 10 mg in the daily regimen. Steady state was achieved within two weeks with the daily dosing

regimen. Cmax is dose-proportional between 5 and 10 mg for both the weekly and daily regimens. At doses of 20 mg/week and higher, the increase in Cmax is less than dose proportional. In healthy subjects, high fat meals reduced systemic exposure to everolimus 10 mg (as measured by AUC) by 22% and the peak plasma concentration Cmax by 54%. Light fat meals reduced AUC by 32% and Cmax by 42%. Food, however, had no apparent effect on the post absorption phase concentration-time profile.

The blood-to-plasma ratio of everolimus, which is concentration-dependent over the range of 5 to 5,000 ng/mL, is 17% to 73%. The amount of everolimus confined to the plasma is approximately 20% at blood concentrations observed in cancer patients given everolimus 10 mg/day. Plasma protein binding is approximately 74% both in healthy subjects and in patients with moderate hepatic impairment.

Everolimus is a substrate of CYP3A4 and a substrate and moderate inhibitor of PgP. Following oral administration everolimus is the main circulating component in human blood and is considered to contribute the majority of the overall pharmacologic activity. No specific excretion studies have been undertaken in cancer patients; however, data available from the transplantation setting found the drug to be mainly eliminated through the feces. There was a significant correlation between AUC0-τ and pre-dose trough concentration at steady state on the daily regimen. The mean elimination half-life of everolimus is approximately 30 hours.

Please refer to Section 5.4.2 for more information on the concomitant use of CYP3A4 inhibitors/inducers and other medications.

More information on RAD001 pharmacokinetics is provided in the RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012.

1.2.5 **Everolimus pharmacodynamic studies**

Pharmacokinetic/pharmacodynamic modeling based on inhibition of the biomarker p70S6 kinase 1 in peripheral blood mononuclear cells (PBMC) suggests that 5-10 mg daily should be an adequate dose to produce a high-degree of sustained target inhibition (Study C2101 / Study 2102). In molecular changes in tumor were evaluated through serial biopsies before and during treatment. Biopsy on treatment took place at week 4 (pharmacokinetic steady-state). All patients underwent a 24-hour post-dose biopsy. Patients following the weekly regimen had a further biopsy on Day 4 or 5 of the same week. Molecular activity was measured by immunohistochemistry. In the absence of a reliable technique for measuring mTOR phosphorylation, the phosphorylation status of downstream markers S6 and eIF4G, for which reliable antibodies exist, was selected as reflecting the immediate pharmacodynamic effect of everolimus. Also measured were changes in the phosphorylation status of upstream AKT and the proliferation index Ki67. Fifty-five patients were treated and the results revealed a dose and schedule dependent inhibition of the mTOR pathway with a near complete inhibition of pS6 and peIF-4G at the 10 mg/day and 50 mg/wk schedules. In addition, pAKT was upregulated in 50% of the treated tumors. In the daily schedule, there was a correlation between everolimus plasma trough concentrations and inhibition of peIF4G and p4E-BP1. There was good concordance of mTOR pathway inhibition between skin and tumor [(Study C2107), (29)].

More information is provided in the RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012.

1.2.6 **Clinical Experience**

Everolimus has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation and was approved in Europe in 2003 under the trade name Certican®, for the prevention of organ rejection in patients with renal and cardiac transplantation. Approval was granted in the United States in 2010 under the trade name of Zortress® for the prevention of organ rejection in adults receiving kidney transplants who are at a low to moderate immunologic risk.

Additional non-oncologic indications currently being explored are wet age-related macular degeneration (AMD) and autosomal dominant polycystic kidney disease (ADPKD). Clinical experience of everolimus in the transplant indication is summarized in a separate Investigator's Brochure version 11.

In oncology, everolimus has been in clinical development since 2002 for patients with various hematologic and non-hematologic malignancies as a single agent or in combination with antitumor agents. Malignancies that are currently being evaluated in Novartis sponsored studies include the following: metastatic renal cell carcinoma (mRCC), breast cancer, gastroenteropancreatic neuroendocrine tumors (NET), mantle cell lymphoma (MCL) and diffuse large B cell lymphoma (DLBCL), hepatocellular cancer (HCC), gastric cancer, and lung cancer. In addition, treatment of patients with Tuberous Sclerosis Complex (TSC) associated subependymal giant cell astrocytoma (SEGA) and Angiomyolipoma are also being evaluated. Colorectal cancer (CRC) and lung cancer are no longer being evaluated. As of 30- Sep-2011, there are a total of 11 Phase III trials ongoing in mRCC (1), advanced NET (2), breast cancer (3), TSC (2), DLBCL (1), gastric cancer (1), and hepatocellular carcinoma (1) (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012).

Everolimus 5mg and 10mg tablets were recently approved under the trade name Afinitor® for patients with advanced renal cell carcinoma in the US, EU and several other countries and are undergoing registration in other regions worldwide. Everolimus has been evaluated as a single agent and in combination with other antitumor agents, including cytotoxic chemotherapeutic agents, targeted therapies, antibodies and hormonal agents. Phase I dose escalating studies, exploratory Phase I/II studies with everolimus as single agent or in combination with other anti-cancer agents, Phase II/III studies of everolimus in indications, and Phase III double-blind studies are contributing to the extensive database. Approximately 18,730 patients, (excluding those patients who received marketed Afinitor®/Votubia® have been treated with everolimus as of 30-Sep-2011:

- 9,528 patients in Novartis-sponsored clinical trials
- 2,559 patients in the individual patient supply program
- 6,638 in investigator-sponsored studies.

In addition, healthy volunteer subjects and non-oncology hepatically impaired subjects have participated in the clinical pharmacology studies.

Recent approvals of everolimus (Afinitor®) were based upon a Phase III, international, multicenter randomized, double-blind, placebo-controlled study [C2240] in patients with metastatic renal cell carcinoma (mRCC) whose disease had progressed despite prior treatment with VEGFR-TKI (vascular endothelial growth factor receptor tyrosine kinase inhibitor) therapy. Progression-free survival (PFS) assessed *via* a blinded, independent central review, was the primary endpoint. Secondary endpoints included safety, objective tumor response

In the pivotal, Phase III study [C2240], which included patients with advanced renal cell carcinoma, the most common adverse reactions (incidence ≥10%) were 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, including Everolimus and some of these cases have been severe and on rare occasions, fatal outcomes have been observed. Everolimus has 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 everolimus. 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%) were 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. Deaths due to acute respiratory failure (0.7%), infection (0.7%), and acute renal failure (0.4%) were observed on the everolimus arm. The rates of treatment-emergent adverse reactions resulting in permanent discontinuation were 7% and 0% for the everolimus and placebo treatment groups, respectively.

Overall, safety data available from completed, controlled and uncontrolled studies are consistent with the aforementioned findings of the Phase III trial. Everolimus is generally well tolerated at weekly and daily dose schedules. The safety profile is characterized by manageable adverse events (AEs). These AEs are generally reversible and non-cumulative.

Further detailed information regarding RAD001 clinical development, safety and efficacy is provided in the RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012.

1.3 Rationale for the Proposed Study

1.3.1 Rationale for targeting mTOR in low-grade gliomas

This study is driven by two hypotheses—first that mTOR inhibition will be efficacious for pediatric LGGs and second that PI3K/AKT activation will be associated with response to mTOR inhibition. This study is unique among all pediatric brain tumor trials in its hypothesis-driven biological approach and it fulfills an unmet need for a pediatric population for whom few other therapeutic options exist.

A compelling rationale exists for targeting mTOR in LGGs. Activation of PI3K/AKT leads to increased susceptibility of tumors to inhibition of mTOR in pre-clinical and clinical studies (30). And in a recent report we document that over half of adult LGGs display activation of the PI3K/AKT/mTOR pathway, likely due to methylation of the PTEN promoter (31). With this rationale as a foundation we initiated a phase II trial of everolimus (RAD001) for adults with recurrent LGGs. Of the 36 enrolled patients, 11 patients continue on active therapy, 15 patients had stable disease for over a year, and 4 had stable disease for over 2 years, all despite multiple prior recurrences. Although accrual continues towards the planned 60 patients, preliminary analyses of molecular parameters and imaging studies have yielded provocative results. Imaging studies in the first 17 accrued patients, demonstrated that compared to patients who progressed on everolimus, those who did not progress (n=13) demonstrated statistically

significant decreases in tumor vascular properties, as measured by size of contrast-enhancing lesions, capillary density, and vascular permeability. Perfusion parameters may thus represent an early marker of treatment response of LGGs to everolimus and to our knowledge this is the first demonstration of anti-angiogenic effects of an mTOR inhibitor in a clinical setting. We plan on incorporating these imaging parameters into our trial of everolimus for PLGGs in order to determine whether early changes in angiogenic and vascular properties will predict responses of PLGGs to everolimus. Preliminary analyses of molecular features of the first 23 patients on study have also yielded provocative results. Whereas in a cohort of newly diagnosed adult LGGs who were not treated with signaling inhibitors, PI3K/AKT/mTOR activation as measured by positive p-S6 and p-PRAS40 staining, was associated with worse progression-free survival (PFS), in our cohort of recurrent adult LGGs treated with everolimus PI3K/AKT/mTOR activation was associated with improved PFS. The “reversal” of the association of PI3K/AKT/mTOR activation with PFS when everolimus is administered supports our central hypothesis that PI3K/AKT/mTOR pathway activation acts as a predictive marker of response to mTOR inhibition. We plan on testing this hypothesis in the current PLGG trial.

We have extended our pre-clinical studies and biologic analyses to pediatric gliomas. Approximately half of pediatric LGGs displayed PI3K/AKT/mTOR activation and methylation of the PTEN promoter. Specifically, pediatric LGGs demonstrated PI3K/Akt/mTOR pathway activation in 14/32 (43.8%) tumors by phosphorylated-S6 and 16/32 (50%) tumors by phosphorylated-4EBP1. Over 50% of grade I (6/11) and almost all grade II tumors (6/7) showed PTEN promoter methylation. In addition, an inverse association was found between PTEN promoter methylation and PTEN protein expression (32). As for the adult study described above, these findings, in combination with the link between response to mTOR inhibition and PI3K/AKT activation provide the pre-clinical rationale for treating pediatric LGGs with an mTOR inhibitor. In this proposed study, molecular features, including PTEN status and activation of the PI3K/mTOR pathway, will be assessed prospectively to investigate possible associations between these molecular characteristics and response to mTOR inhibition.

Everolimus is an orally bioavailable derivative of the macrolide rapamycin. By inhibiting its intracellular target, mTOR, everolimus arrests cell proliferation, induces apoptosis in selected models, and inhibits angiogenesis, all with limited normal tissue toxicity. Cell lines derived from childhood tumors including glioblastoma demonstrate marked sensitivity to everolimus. The Pediatric Preclinical Testing Program (PPTP) has completed testing of pediatric solid tumors in *in vitro* and *in vivo* models and reported intermediate activity for 3 of 4 GBM xenografts. Studies investigating the determinants of sensitivity to mTOR inhibition suggest that tumor cells sensitive to mTOR inhibition are the tumors with aberrant signaling through PI3K/Akt such as those with activating mutations and/or amplification of growth factor receptors, PI3K, Akt, or through loss of PTEN or TSC1/2 (30).

This is an open label study of everolimus in children with recurrent or progressive low-grade glioma. All patients will receive everolimus at a dose of 5 mg/m^2/dose daily. An adaptive Simon two-stage design for phase 2 studies of targeted therapies will be used to assess the efficacy primary objective. The proposed treatment with everolimus will be deemed not worthy of further investigation in this patient population if the true PFS at 6-months (PFS6) is less than 50%. If in the first stage, with a combined sample size of 25, there is preliminary evidence to suggest efficacy of everolimus is restricted to patients with PI3K/AKT/mTOR activation, a total of 45 patients will be enrolled and the design will have 81% statistical power to detect a true disease stabilization rate ≥70%. If in the first stage there is preliminary evidence to suggest efficacy of everolimus is independent of PI3K/AKT/mTOR activation, a total of 65 patients will be enrolled and the design will have >95% statistical power to detect a true disease stabilization rate ≥70%.

This will be a multi-institutional study. The study will be carried out under the auspices of the newly formed Pacific Pediatric Neuro-Oncology Consortium (PNOC). It must be emphasized that there is a glaring lack of current studies for this patient population. Such a study within a multi-institutional environment provides children with recurrent LGGs access to a much-needed therapeutic treatment in a geographic region in which many children have no other options.

This study is unique in several critical respects: (i) the requirement for available tumor tissue as an eligibility criterion in order to accomplish the important objective of exploring associations between activation of specific signaling cascades and response to everolimus; (ii) the incorporation of metabolic and physiologic imaging analyses that may be critical to future assessments of tumor responses in trials that increasingly test inhibitors that are cytostatic rather than cytotoxic. Overall, the current proposed study is distinct from other pediatric studies in that it steps beyond response-driven approaches and attempts to define which patients are most likely to respond to a specific signaling inhibitor. Clearly, only a subgroup of pediatric LGGs is likely to respond to a given signaling inhibitor and as future studies attempt to design rational combinations of novel agents, it is critical to explore potential predictive molecular features of individual tumors. Future rationale approaches will include combining everolimus with a BRAFV600E-, a PDGFR-, or a MEK-inhibitor, among others. Completion of this trial will allow progression to the next generation of clinical trials for children with gliomas in which rational drug combinations are designed and guided by molecular features of individual tumors.

1.3.2 **Rationale for everolimus in pediatric central nervous system malignancies**

Everolimus (RAD001) is an orally bioavailable derivative of the macrolide rapamycin. It has the same intracellular target as rapamycin, mTOR (mammalian target of rapamycin) a serine threonine kinase implicated in the control of cellular proliferation of activated T-lymphocytes and neoplastic cells (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012). mTOR is a ubiquitous protein kinase implicated in cell cycle control and specifically in the progression of cells from G1 to S phase. mTOR function is modulated by numerous interleukins and growth factors.

Rapamycin inhibits interleukin and growth factor dependent proliferation of cells, exerting its activity through a high affinity interaction with an intracellular receptor protein, the immunophilin FKBP -12 (13). This complex inhibits downstream signaling pathways involved in the regulation of the G1 to S transition (33). The primary downstream targets of mTOR, eIF4E binding protein (4EBP1) (34, 35) and p70S6 kinase (36, 37), play key roles in the translation regulation of mRNA encoding proteins involved in G1 phase progression (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012). Thus, through inhibition of mTOR function rapamycin blocks these essential translational events resulting in inhibition of G1 progression.

Rapamycin also indirectly blocks phosphorylation and inactivation of pRb, a G1 suppressor protein (38), as well as rRNA synthesis (39, 40), PKCα phosphorylation (41) and the activity of steroid receptor-regulated genes (39). Finally, rapamycin is a potent inhibitor of vascular endothelial cells (7, 14) and has antiangiogenic activity *in vivo* (4) possibly through inhibiting translation of Hypoxia Inducible Factor (HIF)-1α mRNA. $P70^{s6k}$ mediated protein synthesis is important in endothelial cell proliferation (7).

In vitro data demonstrate that everolimus inhibits the proliferation of a range of human tumor cell lines, including lung, breast, colon, melanoma, and glioblastoma with IC_{50} values ranging from 0.7 nM to 4.1 μM. Relative sensitivity to everolimus *in vitro* often correlates with the degree of activation of the Akt/PKB protein kinase pathway (e.g. in glioblastoma). Treatment with 20 nM of everolimus for 30 minutes potently inhibits the mTOR signaling pathway for 96-120 hr. Rapamycin and its other analogue, CCI 779, have also shown *in vitro* activity in

medulloblastomas/PNET and glioblastoma cell lines. Georger *et al.* reported that rapamycin inhibits PNET/medulloblastoma cell lines at IC_{50} values <10ng/ml (42).

In vitro, everolimus exhibits antiangiogenic properties, inhibiting the proliferation of HUVECS with IC_{50} values in the picomolar range for VEGF (120 ± 22 pM) and βFGF (841 ± 396 pM). Tumor cell lines that were relatively sensitive to everolimus had higher phospho-AKT levels than lines that were more resistant to everolimus. PTEN status was not generally predictive of sensitivity to everolimus, although in glioblastoma PTEN-/- lines exhibited a trend towards increased sensitivity to everolimus (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012). Cell lines derived from childhood tumors including glioblastoma demonstrate marked sensitivity to everolimus (43).

Recently, the PPTP has completed testing of pediatric solid tumors in *in vitro* and *in vivo* models and reported intermediate activity for 3 of 4 GBM xenografts (44). Several studies have reported that PTEN loss correlates with rapamycin sensitivity; however, Houghton *et al.* noted a poor correlation between *in vitro* and *in vivo* sensitivity in solid tumor xenograft models and hypothesized that the activity of rapamycin *in vivo* for these xenografts was primarily due to anti-angiogenic effects (44). Thus, the fact that Pollack *et al*. have recently demonstrated the low incidence of PTEN loss in pediatric gliomas does not diminish the justification for the use of mTOR inhibitors in these tumors, as the primary mechanism of activity in high-grade glioma models is rapamycin's antiangiogenic function (45).

A further rationale for evaluating rapamycin and its analogues in pediatric high-grade gliomas is the potential for these agents to inhibit signaling downstream of the type I insulin-like growth factor receptor. These drugs may block the autocrine growth or paracrine stimulation found frequently in glioblastoma (46-48).

In one Phase II study of CCI779 in adults with recurrent high grade gliomas, Chang *et al.* demonstrated 17% objective response rate in patients not on EIACD (49). Galanis *et al.* reported no objective responses in another phase II trial in patients with recurrent GBM (16). Everolimus penetration into brain tissue has been evaluated in mice and rats (50). Non-tumor bearing BALB/c mice were administered everolimus once at 5 mg/kg orally or 1 mg/kg intravenously, and blood and tissues were obtained at specified times after drug administration. The extent of penetration (AUC_{brain}/AUC_{blood}) was 1.8% and 5.2% after oral and intravenous administration, respectively. Despite the relatively low penetration, everolimus brain tissue concentrations (69 ng/g after oral; 8 ng/g after intravenous) are within range of the IC_{50} values for glioblastomas for 2-9 hours (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012).

Phase I studies in children with recurrent solid tumors have established the MTD of everolimus at 5 mg/m^2 daily continuously with mucositis, diarrhea, and liver dysfunction as the major toxicities. Preliminary correlative studies revealed that target inhibition in peripheral mononuclear cells was achieved in patients at higher dose levels. Although no PRs or CRs were noted, 1 patient with medulloblastoma remained on therapy for 4 courses, 1 patient with ependymoma continued on therapy for 4 courses, 1 patient with AA continued on therapy for 4 courses and developed dose limiting toxicity and was removed from study, 1 patient with a BSG continued on therapy for 5 courses and a patient with gliomatosis cereberi continues on therapy after 12+ courses. One patient with osteosarcoma continues on therapy for 7+ courses. Serum concentrations achieved exceeded the IC_{50} values in *in vitro* studies (16).

Everolimus (RAD001) has been investigated as a component of multi-drug immunosuppression in solid organ transplantation since 1996. Because of its potent antiproliferative effects against a variety of tumor types and evidence that it synergizes with chemotherapy in pre-clinical models, more recently it has been investigated as an anti-tumor agent (47). Phase I clinical

trials in 147 advanced cancer patients, utilizing various dosing regimens and schedules have been investigated (including weekly schedule of 5-70 mg and daily schedules of 5-10 mg). Common toxicities included rash or erythema (46%), stomatitis/mucositis (40%), fatigue (30%), headache (14%), diarrhea (16%), nausea (16%), pruritis, infection and constipation (10% each) (46, 48). Efficacy has been noted in clinical trials at various doses on both the weekly and daily schedules in patients with non-small cell lung cancer, breast, renal, rectal cancer and mesothelioma (51).

The Pediatric Oncology Experimental Therapeutics Investigators' Consortium (POETIC) has initiated a Phase II study of everolimus (RAD001) for children with chemotherapy refractory and/or progressive or recurrent low-grade gliomas (Protocol 10-047). The stated objective of the study is to assess "the safety and effectiveness of everolimus (RAD001) in children with low-grade gliomas that have either not responded to treatment or have come back after treatment." This is a standard phase II response-driven trial in which the investigators also propose to examine mTOR inhibition in peripheral blood mononuclear cells (PBMCs) and limited pharmacokinetic studies as well as standard neuroimaging analyses. The study proposed herein is distinct from the POETIC trial in several critical respects: (i) the PNOC study requires the availability of tumor tissue as an eligibility criterion in order to accomplish the important Primary Objective of exploring associations between activation of specific signaling cascades and response to everolimus; (ii) the proposed PNOC study prospectively incorporates metabolic and physiologic imaging analyses that may be critical to future assessments of tumor responses in trials that increasingly test inhibitors that are cytostatic rather than cytotoxic. Overall, the current proposed study is distinct from the POETIC trial in that recent biological studies of pediatric LGGs have allowed our PNOC consortium to step beyond the response-driven approach of other pediatric trials and incorporate important Secondary Objectives that may shed light on which patients are most likely to respond to a specific signaling inhibitor. Clearly, only a subgroup of pediatric LGGs is likely to respond to a given signaling inhibitor and as future studies attempt to design rational combinations of novel agents, it is critical to explore potential predictive molecular features of individual tumors.

Specific hypothesis: mTOR inhibition will be efficacious for pediatric low-grade gliomas.

1.4 Correlative Studies

1.4.1 Rationale for additional radiographic imaging as an exploratory aim

Standard anatomic MRI in conjunction with clinical evaluation such as neurologic status and corticosteroid use remains the key determinant of response to therapy and the evaluation of tumor recurrence for LGG (52, 53). Increase in contrast enhancement, worsening cerebral edema, and mass effect are universal traits of malignant transformation. Acquiring tissue samples to confirm tumor upgrade, although considered the "gold standard" for determining the presence of viable tumor, can result in both false positives and negatives that relate to sampling error (54, 55). Recent studies using MR spectroscopic imaging (MRSI) suggest that *in vivo* levels of metabolites such as choline, creatine, N-acetylaspartate, lactate and lipids provide more-reliable measures of the presence of recurrent tumor. Perfusion-weighted imaging (PWI) and diffusion-weighted imaging (DWI) have been explored for patients with newly diagnosed glioma, but only on a limited basis for patients with recurrent LGG. Techniques to characterize the molecular morphology of pre-specified areas in addition to neuroimaging parameters may better define the biologic behavior of such lesions.

Many of the newer drugs being studied in neuro-oncology target specific aberrant pathways and are cytostatic rather than cytotoxic. Examples include anti-invasive and anti-angiogenic agents. These agents may cause stability of the tumor rather than regression, and surrogate biomarkers are important in the assessment of these types of agents. Pending funding availability, we will

prospectively incorporate metabolic and physiologic imaging to this trial to allow us to assess the ability of the key parameters to predict clinically relevant endpoints such as treatment related radiographic response, time to progression and survival.

We are eager to incorporate these imaging aims into this pediatric study because preliminary analysis of our institutional study of everolimus for adult LGGs has resulted in the first clinical demonstration of anti-angiogenic effects of an mTOR inhibitor and we are eager to extend these analyses to the pediatric population. Specifically, the phase II trial of everolimus (RAD001) for adults with recurrent LGGs includes a critical imaging objective that incorporates MR spectroscopy, perfusion-weighted, and diffusion-weighted imaging to predict clinically relevant endpoints. Early analyses reveal that capillary density and vascular permeability measured 4 and 6 months after start of everolimus decreased significantly in patients with stable disease but not in those with progressive disease, suggesting that decreased angiogenesis may represent an early marker of response to treatment.

1.4.2 **Rationale for molecular profiling studies**

Only patients for whom tumor tissue is available for molecular analyses will be eligible for this study. This will be the only pediatric LGG cohort for which tissue will be collected prospectively from every enrolled child, thus providing critical data on the prevalence of genetic aberrations among pediatric LGGs. These molecular analyses will be determined using tissue samples from prior surgeries.

The correlative studies aim to address the following specific goals:

(i) To explore associations between pS6 positivity and outcome as measured by the 6-month disease stabilization rates (a dichotomous variable, see section 7.1.2.2) as well as PFS for progressive or recurrent pediatric low-grade glioma patients with measureable disease treated with everolimus.

(ii) To collect tissue from all enrolled patients and prospectively analyze key molecular features including activation of the PI3K, mTOR and MAPK pathways, aberrations in PTEN, IDH1, and IDH2, and activating mutations in BRAF (KIAA1549-BRAF fusion and BRAFV600E missense BRAF mutation).

(iii) In addition to the targeted approaches described above that examine previously reported alterations additional genomic profiling studies will be performed including targeted sequencing, RNA sequencing, and/or exome(plus) next generation sequencing if sufficient tissue is available including fresh frozen tissue. Such studies will include but are not limited to molecular analyses of DNA, RNA and protein in tumor biopsy specimens using blood as control. When possible and/or sufficient blood/tissue is provided, sample will be used to establish tumor cell cultures, cell lines and/or transplantation models. These studies will be carried out in collaboration with Dr. Adam Resnick, Children's Hospital of Philadelphia.

2 Objectives of the Study

2.1 Primary

Efficacy: Estimate the Progression Free Survival (PFS) rate at 6-months associated with everolimus therapy for symptomatic, progressive or recurrent pediatric low-grade glioma patients with measureable disease with the aim of determining whether everolimus warrants additional study only in patients with activated PI3K/Akt/mTOR pathway or in the entire population.

2.2 Secondary

- Estimate PFS and OS distributions as well as objective response (CR+PR) rates associated with everolimus treatment in recurrent pediatric LGGs.
- Explore associations between pS6 positivity and outcome as measured by the 6-month disease stabilization rates (a dichotomous variable; see section 7.1.2.2) as well as PFS for progressive or recurrent pediatric low-grade glioma patients with measureable disease treated with everolimus.
- Collect tissue from all enrolled patients and prospectively analyze key molecular features including activation of the PI3K, mTOR and MAPK pathways, aberrations in PTEN, IDH1, and IDH2, and activating mutations in BRAF (KIAA1549-BRAF fusion and BRAFV600E missense BRAF mutation). In addition to the targeted approaches described above that examine previously reported alterations additional genomic profiling studies will be performed including targeted sequencing, RNA sequencing, and/or exome(plus) next generation sequencing if sufficient tissue including fresh frozen tissue is available. Such studies will include but are not limited to molecular analyses of DNA, RNA and protein in tumor biopsy specimens and blood samples. When possible and/or sufficient blood/tissue is provided, sample will be used to establish tumor cell cultures, cell lines and/or transplantation models. These studies will be carried out in collaboration with Dr. Adam Resnick, Children's Hospital of Philadelphia.
- Explore MR quantitative measures of relative cerebral blood volume, permeability and apparent diffusion coefficient within the region of hyper-intensity on T2-weighted images as markers of disease response and/or progression in comparison to institutional evaluation of disease response and/or progression and quantitative measures of tumor response as determined by central review (based upon both area and volumetric measures.

3 Study Design

3.1 Characteristics

This is a nonrandomized, open label Phase 2 study evaluating the efficacy of everolimus therapy for symptomatic, progressive or recurrent pediatric low-grade glioma patients. Patients will receive everolimus once daily by mouth. Twenty-eight (28) days will constitute one cycle and subsequent cycles will immediately follow, with no break in the administration of the drug. Dosing is based on the BSA calculated at the beginning of each cycle of therapy. The dose prescribed should be rounded to the nearest deliverable dose based on the BSA adjustment and the available pill sizes. Dosing Tables that reflect this approach are available in Appendix 1. Patients will be provided with a drug diary for everolimus instructed in its use, and asked to bring the diary with them to each appointment. The total length on study is 24 cycles. If disease remains stable or improved and there are no significant side effects, the patient might be able receive the medication off study based on patient's preference, treating physician's recommendation and availability of everolimus (RAD001) outside the clinical trial. Monitoring should be guided per best clinical practice based on the treating physician's recommendation.

3.2 Number of Subjects

With the anticipated accrual numbers provided by the eight PNOC institutions, we anticipate that 25-30 patients/year may be available. If we assume that 50% of these would be eligible for the biology objective, i.e. would have measureable disease and would have tissue available from prior surgeries, we can expect that the interim analysis will take place within 2.5-3.5 years from

initiation of accrual, including the 6-month disease evaluation window. Hence at the time of the interim analysis we expect to have accrued 25 patients.

If preliminary evidence in the first stage (at the interim analysis) suggests efficacy of everolimus is limited to the patients with pathway activation, an additional 20 pathway activated patients will be recruited. Assuming 50% of the eligible patients have pathway activation, we can expect 6-7 patients per year for a full accrual in 5.5-6.5 years from initiation of accrual. If preliminary evidence in the first stage (interim analysis) suggests efficacy of everolimus is not limited to the patients with pathway activation, an additional 40 patients will be recruited regardless of their activation status. We can then expect 13-15 patients per year for a full accrual in 5.5-6.5 years from initiation of accrual.

3.3 Eligibility Criteria

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

3.3.1 Inclusion Criteria

- Patients must have progressive or recurrent confirmed low-grade glioma (WHO grade I or II) that was confirmed histologically.
- Tissue from the initial diagnosis or recurrence must be made available for correlative testing. Appendix 3 or an email from the pathology department may be used to confirm tissue availability at the time of enrollment.
- Depending on the stage of the protocol, pathway activation based on p-S6 will need to be done in real time to assess if patient is eligible. As of April 2017, pathway activation does not need to be performed in real time to access eligibility.
- Patients must have measurable disease, defined as at least one lesion that can be accurately measured in at least two dimensions on MRI. See Section 7.1.2.2 for the evaluation of measurable disease.
- Patients may have had treatment (chemotherapy and/or radiotherapy) or no treatment for any number of relapses prior to this recurrence.
    - Patients must have received their last dose of myelosuppressive anticancer chemotherapy at least three (3) weeks prior to study registration or at least six (6) weeks of nitrosourea.
    - Patients must have received their last dose of other investigational or biological agent > 7 days prior to study entry.
        - For agents that have known adverse events occurring beyond 7 days after administration, this period should be extended beyond the time during which adverse events are known to occur. This should be discussed with the study chair.
    - If patients received prior monoclonal antibody treatment, at least three half-lives must be elapsed by the time of treatment initiation. These patients should also be discussed with the study chair.

  - Patients must have received their last fraction of craniospinal or focal radiation to primary tumor or other sites >12 weeks (3 months) prior to registration.

- Age ≥3 and ≤21 years.
  - Because no dosing or adverse event data are currently available on the use of everolimus in patients <3 years of age, these young children are excluded from this study.

- Patient must have a Karnofsky (if ≥ 16 years of age) or Lansky Performance score (if ≤ 15 years of age) of ≥50 by the time of registration. Patients who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing the performance score.

- Patients must have adequate bone marrow function (ANC ≥ 1,000/mm^3, platelet count of ≥ 100,000/mm^3, and hemoglobin ≥ 9 gm/dL) before starting therapy. Eligibility level for hemoglobin may be reached by transfusion.

- Patients must have adequate liver function (SGPT/ALT ≤ 2.5 times ULN and bilirubin ≤ 1.5 times ULN) before starting therapy.

- Patients must have adequate renal function, defined as:
  - Creatinine clearance or radioisotope GFR ≥ 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.

- Patients must have cholesterol level <350 mg/dL and triglycerides < 400 mg/dL before starting therapy. In case one or both of these are exceeded, the patient can only be included after initiation of appropriate lipid lowering medication and documentation of cholesterol < 350mg/dL and triglycerides < 400mg/dl before start of therapy.

- Patients must have normal pulmonary function testing for age based on pulse oximetry.

- The effects of everolimus on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because everolimus are known to be teratogenic, female patients 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 patient become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

- Female patients of child bearing potential must not be breastfeeding or pregnant as evidenced by a negative pregnancy test.

3.3.2 **Exclusion Criteria**

- Patients with primary spinal cord tumors
- Patients receiving concomitant medication that may interfere with study outcome. For example, patients cannot be on enzyme inducing anticonvulsants like phenytoin. For a complete list please refer to section 5.4.2.
- Patients should not receive immunization with attenuated live vaccines within one week of study entry or during study period. Close contact with those who have received attenuated live vaccines should be avoided during treatment with everolimus. Examples of live vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines.
- HBsAg and HCVAb blood test must be done at screening for all patients. Patients who test positive for Hepatitis C antibodies or the Hepatitis B antigen are ineligible.
- A known history of HIV seropositivity. HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with everolimus. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.
- Patients 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 need to be discussed with the study chair.
- Patients may not have therapy for this recurrence (including radiation).
- Patients who do not have measurable disease on MRI.
- Patients who have been previously treated with an mTOR inhibitor.
- Patients with a known hypersensitivity to everolimus or other rapamycins (e.g. sirolimus, temsirolimus).
- Patients receiving any other concurrent anticancer or investigational therapy.
- Patients with any clinically significant unrelated systemic illness that would compromise the patient's ability to tolerate protocol therapy.
- Impairment of gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of everolimus (e.g., ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome or small bowel resection.
- Patients with inability to return for follow-up visits to assess toxicity to therapy.
- Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

- Patients with a history of any other cancer (except non-melanoma skin cancer or carcinoma *in situ* of the cervix), unless in complete remission and off of all therapy for that disease for a minimum of 3 years.

Important note: The eligibility criteria listed above are interpreted literally and cannot be waived.

3.4 Registration Procedures

3.4.1 General Guidelines

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

Registration materials will be submitted to the PNOC operations office as described below. The PNOC operations office will check the registration materials for completeness and contact the site with any discrepancies.

The PNOC operations office will forward eligibility checklist including source documentation to the Study Chair or Co-Chair as well as the project leader or co-project leader of PNOC for review of eligibility and sign off.

Eligible patients will be registered using the UCSF OnCore® database that is used for all PNOC trials. Treatment on protocol therapy cannot be initiated prior to receiving registration confirmation email from the PNOC operations office.

3.4.2 Reservation and Registration Process

The wait-list for study slots will be maintained by the PNOC Operations Office. Investigators can view updated information about slot availability on the PNOC Member's SharePoint website using their secure login and password, or by emailing a request to [redacted].

To place a patient on the waitlist, please send an email to [redacted] and include the following information in the email: Study name, Patient initials, Patient age, Consent signed date (or estimate), and Expected start date (if found eligible).

To register a patient for the study, the patient demographics along with a signed consent form and HIPAA authorization should be emailed to the PNOC Operations Office at [redacted] The patient will be given the status of consented in OnCore®.

When the eligibility checklist has been completed the member institution PI and/or Coordinator will upload the completed eligibility checklist along with copies of any supporting documents into the patient's OnCore® record.

Once the necessary documents have been received and the patient eligibility has been confirmed, the PNOC Operations Office will send a confirmation e-mail to the site PI(s) and research coordinator(s) with the patient's study ID and dose information.

Detailed patient screening and registration instructions can also be found on the PNOC Member's SharePoint Wiki.

To allow non-English speaking patients to participate in this study, bilingual health services will be provided in the appropriate language when feasible.

Patients must begin treatment within 10 business days of study registration. Registration and informed consent must be completed prior to beginning of everolimus (RAD001).

3.5 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue for 24 cycles or until a "off treatment" criterion is met (see section 5.6)

Note – after 24 cycles of therapy, if patients continue to show benefit from therapy, treatment may be extended "off study" at the investigator's discretion but will not be supported by Novartis.

3.6 Duration of Follow Up

Patients who are off protocol therapy must be followed for up to five years from the start of therapy or until an "Off Study Criterion" is met, whichever occurs first (see section 5.7). Any patient removed from protocol therapy for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

3.7 Study Timeline

3.7.1 Interim Analysis

Based on the expected accrual as outlined above the first analysis will take place 2.5-3.5 years from initiation of accrual, including the 6-month disease evaluation window. Hence at the time of the interim analysis we expect to have accrued 25 patients.

3.7.2 Primary Completion

If preliminary evidence in the first stage (at the interim analysis) suggests efficacy of everolimus is limited to the patients with pathway activation, an additional 20 pathway activated patients will be recruited. Assuming 50% of the eligible patients have pathway activation, we can expect 6-7 patients per year for a full accrual in 5.5-6.5 years from initiation of accrual. If preliminary evidence in the first stage (interim analysis) suggests efficacy of everolimus is not limited to the patients with pathway activation, an additional 40 patients will be recruited regardless of their activation status. We can then expect 13-15 patients per year for a full accrual in 5.5-6.5 years from initiation of accrual.

3.7.3 Study Completion

The study will reach completion approximately 8 years from the time the study opens to accrual to the final date on which all data are expected to be collected.

4 Study Drugs

4.1 Description, Supply and Storage of Everolimus

[Refer to the package insert(s) for complete information].

Everolimus (RAD001, Afinitor®) is an investigational agent and will be supplied by Novartis Pharmaceuticals Corporation.

Formulation

Everolimus is formulated as tablets of 2.5 mg, 5 mg, and 10 mg strength.

Storage
Everolimus is blister-packed under aluminum foil in units of 10 tablets that should be opened only at the time of administration as drug is both hygroscopic and light sensitive. Storage must be at temperatures below 30°C and in the original blister packs. Shelf life will be described on the medication label.

Administration

Everolimus may be taken with or without food. The tablets should be swallowed whole with a glass of water and should not be chewed or crushed. For patients unable to swallow tablets, the tablet(s) should be dispersed completely in a glass of water (containing approximately 30 mL) by gently stirring, immediately prior to drinking. The glass should be rinsed with the same volume of water and the rinse completely swallowed to ensure the entire dose is administered.

Side Effects
See section 5.3.2. Complete and updated adverse event information is available in the Investigational Drug Brochure and/or product package insert.

4.2 Drug Accountability

Novartis will directly distribute study drug to PNOC institutions. Each PNOC institution will be responsible for reconciling the drug supply at their site.

4.3 Drug Ordering

Each PNOC institution will be responsible for requesting their own drug supply directly from Novartis. The PNOC001 drug request form can be found on the SharePoint member website under the documents tab.

4.4 Packaging and Labeling of Study Drugs

Drugs will be packaged and labeled per institutional standards, adhering to applicable local and federal laws.

5 Study Procedures and Observations

The study-specific assessments are outlined in Table 5.1 and are detailed below. Imaging evaluations necessary to establish eligibility for study entry must be done within 21 days prior to the start of therapy. All other evaluations except laboratory parameters necessary to establish

eligibility for study entry must be done within 14 days prior to the start of therapy. Patients must start therapy within 10 business days of registration. If a test that is repeated after registration and prior to therapy is outside the limits for eligibility, it must be rechecked 48 hours prior to the start of therapy. If the recheck is still outside the limits for eligibility, the patient may not receive protocol therapy. All patients must meet the following inclusion and exclusion criteria.

All on-study visit procedures are allowed a window of ± 3 days unless otherwise noted.

A written, signed, informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A copy of the consent form will be given to the subject and filed in the medical record. A copy of the consent must also be uploaded into OnCore® as part of the registration process. The original will be kept on file with the study records.

All patients who are consented will be registered in OnCore®, the UCSF Helen Diller Family Comprehensive Cancer Center Clinical Trial Management System (CTMS). The system is password protected and meets HIPAA requirements.

Refer to section 3.4 for registration procedures.

Table 5.1 Schedule of Assessments**

All on-study visit procedures are allowed a window of ±3 days unless otherwise noted.

Period/ Procedure	Screening	Cycle 1	Cycle 1	Cycle 2	Cycle 2	Cycle 3, 5, 7, 9,11,13,16,19, 22	Cycle 4, 6, 8, 10, 12, 14, 15, 17, 18, 20, 21, 23, 24			
Study Day/Visit Day	-14 to 0	Day 1	Day 15	Day 1	Day 15	Day 1	Day 1	End of Treatment visit[1]	30 Day Tox Check[2]	Follow up[3]
Informed consent	X									
AE assessment		X		X		X		X	X	
Concomitant medications	X	X		X		X		X	X	
Tumor Tissue Collection	X									
Treatment Administration										
Everolimus		Daily, oral, self-administration								
Clinical procedures										
Physical exam	X	X		X		X		X		
Vital signs	X	X		X		X		X		
Medical history	X									
Performance Status	X	X		X		X		X		
Neurologic exam	X	X		X		X		X		
Pulse oximetry	X	X		X		X		X		
Survival										X
Laboratory procedures										
CBC w/ Diff	X	X	X	X	X	X	X	X		
Blood chemistry [4]	X	X	X	X	X	X	X	X		
Coagulation	X	X						X		
Hepatitis	X									
Pregnancy test (HCG)[5]	X	X		X		X	X	X		
Correlative Studies		X								
Imaging procedures										

Period/ Procedure	Screening	Cycle 1	Cycle 1	Cycle 2	Cycle 2	Cycle 3, 5, 7, 9,11,13,16,19, 22	Cycle 4, 6, 8, 10, 12, 14, 15, 17, 18, 20, 21, 23, 24			
Study Day/Visit Day	-14 to 0	Day 1	Day 15	Day 1	Day 15	Day 1	Day 1	End of Treatment visit[1]	30 Day Tox Check[2]	Follow up[3]
Imaging (MRI) [6]	X	X				X		X		
Chest X-Ray [7]	X	X		X		X		X		
ECG/EKG[8]	X									

****Please refer to sections 5.1 and 5.2 for complete information about screening assessments and study procedures**

[1] To be completed when one of the off-treatment criteria is met.

[2] The toxicity check can be done via phone at 30 days after the last dose (+7 days). All study drug related adverse events must continue to be followed until resolution or return to baseline. Review of concomitant medications.

[3] Patients who are off-treatment will be followed for up to 5 years after discontinuing drug or until one of the off-study criteria is met, to collect required follow-up information (see Long Term/Survival Follow-up Procedures).

[4] Including: Sodium, potassium, chloride, bicarbonate, calcium, fasting glucose, albumin, total protein, BUN, serum creatinine, total bilirubin, AST, ALT, alkaline phosphatase, uric acid, phosphorus, and fasting serum lipid profile.

[5]For females of childbearing age; pregnancy test should be done.

[6] Brain MRI scans are due prior to therapy, before every odd cycle (3, 5, 7, 9, and 11) in the first year (+/- 7 days), and before every third cycle in the second year (13, 16, 19, and 22) (+/- 7 days). Spine MRIs should be performed if clinically indicated.

[7] Chest X-Ray to be performed as clinically indicated.

[8] EKG to be performed at screening and then as clinically indicated.

5.1 Pre-Treatment Period

5.1.1 Screening Assessments

The Screening procedures and assessments must be completed within 14 days of Cycle 1, Day 1 visit except brain MRI.

- Physical examination
- Vital signs (Height, pulse, blood pressure, respiration rate, temperature and weight)
- Complete medical history, including current medications and baseline conditions
- Performance status (Karnofsky Performance Status or Lansky Performance score)
- Neurologic exam
- Pulse oximetry
- Laboratory assessments
  - Complete Blood Count (CBC) with differential and platelet count
  - Blood chemistry assessment, including:
    - 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)
  - Coagulation assessment, including prothrombin time, partial thromboplastin time, international normalized ratio (PT/PTT/INR)
  - Hepatitis B and C screening (including HBsAg and HCVAb). Should be done at screening and then as clinically indicated.
  - Serum or urine pregnancy test - Only if participant is a female of child bearing age; pregnancy test should be done at screening and monthly thereafter (± 3 days within Day 1 of each cycle).
- Imaging (Magnetic Resonance Imaging) of brain with gadolinium and echoplanar diffusion for tumor/lesion assessment. Spine MRIs should be performed if clinically indicated. Screening scans may be done within 21 days of beginning study treatment. See Imaging Guidelines (Appendix 4) for specifics.
- Electrocardiogram (ECG) – to be performed at screening and then as clinically indicated
- Chest X-Ray - If clinically indicated. If pulse oximetry is less than 93% on room air patient should get a CXR monthly until pulse oximetry is >93. May be done within 21 days of beginning study treatment
- Archival tumor tissue collection –tumor samples from a prior surgery will be used to test for molecular analyses. Appendix 3 or an email from the pathology department may be used to confirm tissue availability at the time of enrollment. The tissue itself must be submitted within 90 days of registration (see appendix 8).

5.2 Treatment Period

Clinical assessments will be required after the first and second cycle, and then every 2 cycles through cycle 13, and finally every 3 cycles until end of treatment unless more frequent monitoring is clinically indicated. After 4 weeks of treatment, a toxicity check will be performed as outlined in Table 5.1. Whenever possible, all assessments at each scheduled time-point will

be completed in a single clinic visit. Evaluations can be obtained within ± 3 days from a scheduled time point unless otherwise noted.

All relevant information regarding drug doses, concomitant medications and doses, evaluable lesions with measurements, tumor response, laboratory examinations, and treatment-related toxicities shall be documented in the patient's medical record and flow sheets. Patients will maintain a treatment diary while on therapy. The Treatment Diary will be initiated on Day 1 of therapy and continue throughout treatment. Treatment Diaries will be collected from the patient during clinical follow up per study calendar until the patient discontinues therapy.

5.2.1 Study Procedures, Cycle 1, Day 1

Day 1 procedures do not need to be repeated if done within 14 days prior during screening assessments. MRI does not need to be repeated if done within 21 days prior.

- Physical examination
- Evaluation of adverse events
- Concomitant medications
- Vital signs
- Performance status
- Neurologic exam
- Laboratory assessments
  - Complete Blood Count (CBC) with differential and platelet count
  - Blood chemistry assessment (see Screening Assessments for complete list)
  - Coagulation assessment
  - Pregnancy test- if a female of childbearing age
- Pulse oximetry
- Chest X-ray – if clinically indicated
- Brain MRI –does not need to be repeated if done within 21 days prior during screening
- Spinal MRI – if clinically indicated
- Research blood (required) and Fresh Frozen Tissue (optional, if available) for genomic DNA isolation within (+90) days of registration for genomic profiling studies (see Appendix 8).

5.2.2 Study Procedures, Cycle 1, Day 15

- Laboratory assessments
  - Complete Blood Count (CBC) with differential and platelet count
  - Blood chemistry assessment (see Screening Assessments for complete list)

5.2.3 Study Procedures, Cycle 2, Day 1

- Physical examination
- Evaluation of adverse events
- Concomitant medications

- Vital signs
- Performance status
- Neurologic exam
- Laboratory assessments
  - Complete Blood Count (CBC) with differential and platelet count
  - Blood chemistry assessment (see Screening Assessments for complete list)
  - Pregnancy test – if a female of childbearing age
- Pulse oximetry
- Chest X-ray – if clinically indicated

5.2.4 **Study Procedures, Cycle 2, Day 15**

- Laboratory assessments
  - Complete Blood Count (CBC) with differential and platelet count
  - Blood chemistry assessment (see Screening Assessments for complete list)

5.2.5 **Study Procedures, Cycle 3, 5, 7, 9, 11, 13, 16, 19, 22, Day 1**

- Physical examination
- Evaluation of adverse events
- Concomitant medications
- Vital signs
- Performance status
- Neurologic exam
- Laboratory assessments
  - Complete Blood Count (CBC) with differential and platelet count
  - Blood chemistry assessment (see Screening Assessments for complete list)
  - Pregnancy test – if a female of childbearing age
- Pulse oximetry
- Chest X-ray – if clinically indicated
- Brain MRI (+/- 7 days)
- Spinal MRI – if clinically indicated

5.2.6 **Study Procedures, Cycles 4, 6, 8, 10, 12, 14, 15, 17, 18, 20, 21, 23, and 24, Day 1**

- Laboratory assessments
  - Complete Blood Count (CBC) with differential and platelet count
  - Blood chemistry assessment (see Screening Assessments for complete list)
  - Pregnancy test – if a female of childbearing age

5.2.7 **Study Procedures, End-of-Treatment**

After 24 cycles of treatment with everolimus, patients will have completed the protocol-defined treatment period and will be considered “off-treatment”. Patients may continue on treatment with everolimus, based on the recommendation of the treating physician; however, Novartis will not sponsor these efforts.

The following assessments must be performed within 30 days after the last dose unless these have been evaluated within 14 days prior to the last dose.

- Physical examination
- Evaluation of adverse events
- Concomitant medications
- Vital signs
- Performance Status
- Neurologic exam
- Laboratory procedures -
  - Complete Blood Count (CBC) with differential and platelet count
  - Blood chemistry assessment (see Screening Assessments for complete list)
  - Coagulation assessment (see Screening Assessments for complete list)
  - Pregnancy test – if a female of childbearing age
- Pulse oximetry
- Chest X-ray – if clinically indicated
- MRI if not performed within the past 12 weeks.

In addition to assessments due at the End of Treatment, a separate 30-day Toxicity Check is required for all patients. The toxicity check can be done via phone at 30 days after the last dose (+7 days).

- Evaluation of adverse events (All study drug related adverse events must continue to be followed until resolution or return to baseline).
- Review of concomitant medications

Patients removed from protocol therapy for unacceptable adverse events while on treatment and/or within 30 days of the last administration of study drug will be followed until resolution or return to baseline of the adverse event.

5.2.8 **Study Procedures, Long Term/Survival Follow-up Procedures**

Patients off protocol therapy will continue to be followed for PFS, OS and start of new treatment for up to five years after initiation of protocol therapy or patient death, whichever is earlier.
All patients will be followed until resolution (or return to baseline) of all adverse events occurring while on treatment and/or within 30 days of the last administration of study drug. Toxicities that are related to study therapy and are ongoing at the end of day 30 after last treatment date will be followed until resolution or return to baseline. Patients who are Off-treatment will be followed to collect any adverse events that are possibly, probably or definitely related to the study drug

as well as the date of progression, date of last contact and date of death. Post treatment the patient's status is to be reported every follow-up visit for neuroimaging and/or clinical evaluation. The requested follow-up data is to be collected every three months (+/- 30 days) from the date the patient went off treatment. Please refer to Appendix 7 for submission deadlines.

The following elements are captured during the long-term follow-up:

- Patient Status
- Date of progression and associated disease assessments
- Date of last contact
- Date of death

5.3 Interruption or discontinuation of treatment

For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to keep the patient on study drug. If administration of everolimus must be interrupted because of unacceptable toxicity, drug dosing will be interrupted or modified according to rules described in Table 5.3. Toxicity will be assessed using the NIH-NCI Common Terminology Criteria for Adverse Events, version 4.0 (CTCAEv4.0, If a patient requires a dose delay of ≥ 3 weeks from the intended day of the next scheduled dose, then the patient must be discontinued from treatment.

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

It will be documented within OnCore® whether or not each patient completed the clinical study. If for any patient either study treatment or observations were discontinued the reason will be recorded in OnCore® and a deviation report will be submitted if applicable.

Table 5.2 Everolimus dose level modification guidelines

Dose level	Dose and schedule
0 (starting dose)	5 mg/m^2 daily
-1	2.5 mg/m^2 daily
-2	2.5 mg/m^2 mg every other day

Table 5.3 Criteria for dose-modification in case of suspected everolimus toxicity and re-initiation of everolimus treatment

Toxicity	Actions
Non-hematological toxicity	
Grade 2 (except pneumonitis – refer to Table 5.4)	If the toxicity is tolerable to the patient, maintain the same dose. If the toxicity is intolerable to patient, interrupt everolimus until recovery to grade ≤1. Then reintroduce everolimus at same dose. If event returns to intolerable grade 2, then interrupt everolimus until recovery

	to grade ≤1. Then reintroduce everolimus at the lower dose level.
Grade 3 (except hyperlipidemia*) (except pneumonitis – refer to Table 5.4)	Interrupt everolimus until recovery to grade ≤1. Then reintroduce everolimus at the lower dose level. For pneumonitis consider the use of a short course of corticosteroids.
Grade 4	Discontinue everolimus.
Hematological toxicity	
Grade 2 Thrombocytopenia (platelets <75, ≥ 50x10^9/L)	Interrupt everolimus until recovery to grade ≤1 (>75 x10^9/L). Then reintroduce everolimus at initial dose. If thrombocytopenia again returns to grade 2, interrupt everolimus until recovery to grade ≤1. Then reintroduce everolimus at the lower dose level.
Grade 3 Thrombocytopenia (platelets <50, ≥ 25 x10^9/L)	Interrupt everolimus until recovery to grade ≤1 (platelets ≥ 75 x10^9/L). Then resume everolimus at one dose level lower. If grade 3 thrombocytopenia recurs, discontinue everolimus.
Grade 4 Thrombocytopenia (platelets < 25 x10^9/L)	Discontinue everolimus.
Grade 3 Neutropenia (neutrophils <1, ≥0.5 x10^9/L)	Interrupt everolimus until recovery to grade ≤1 (neutrophils ≥ 1.5 x 10^9/L). Then resume everolimus at the initial dose. If ANC again returns to Grade 3, hold everolimus until the ANC ≥ 1.5 x 10^9/L. Then resume everolimus dosing at the lower dose level. Discontinue patient from study therapy for a third episode of grade 3 neutropenia.
Grade 4 Neutropenia (neutrophils < 0.5 x10^9/L)	Interrupt everolimus until recovery to grade ≤ 1 (neutrophils ≥ 1.5 x 10^9/L). Then resume everolimus at the lower dose level. If grade 3 or grade 4 neutropenia occurs despite this dose reduction, discontinue everolimus.
Grade 3 febrile neutropenia (not life-threatening)	Interrupt everolimus until resolution of fever and neutropenia to grade ≤ 1. Hold further everolimus until the ANC ≥ 1,500/mm^3 and fever has resolved. Then resume everolimus at the lower dose

	level. If febrile neutropenia recurs, discontinue everolimus.
Grade 4 febrile neutropenia (life-threatening)	Discontinue everolimus.
Any hematological or non-hematological toxicity requiring interruption for ≥ 3 weeks	Discontinue everolimus

*Grade 3 hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) should be managed using medical therapies (see Sec. 5.3.5).

5.3.1 **Monitoring of everolimus suspected toxicities**

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

5.3.2 **Known Undesirable Side Effects of everolimus**

The data described below reflect exposure to everolimus (n=274) and placebo (n=137) in a randomized phase III study for the treatment of metastatic renal cell carcinoma. In total, 165 patients were exposed to everolimus 10 mg/day for ≥4 months. The median age of patients was 61 years (range 27 to 85). The most common adverse reactions (incidence ≥10%) were stomatitis, rash, fatigue, asthenia, diarrhea, anorexia, nausea, mucosal inflammation, vomiting, 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. Amenorrhea has been observed in two phase 3 studies of patients with TSC, Amenorrhea occurred in 17% of AFINITOR-treated females aged 10 to 55 years (3 of 18) and none of the females in the placebo group in one trial. For this same group of AFINITOR-treated females, the following menstrual abnormalities were reported: dysmenorrhea (6%), menorrhagia (6%), metrorrhagia (6%), and unspecified menstrual irregularity (6%). In a second trial, Amenorrhea occurred in 15% of AFINITOR-treated females (8 of 52) and 4% (1 of 26) of females in the placebo group. Other adverse reactions involving the female reproductive system were menorrhagia (10%), menstrual irregularities (10%), and vaginal hemorrhage (8%). (RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012).

The median duration of blinded study treatment was 141 days (range 19 to 451) for patients receiving everolimus and 60 days (range 21 to 295) for those receiving placebo. The rates of treatment-emergent adverse reactions resulting in permanent discontinuation were 7% and 0% for the everolimus and placebo treatment groups, respectively. Most treatment-emergent adverse reactions were grade 1 or 2 in severity. Grade 3 or 4 treatment-emergent adverse reactions were reported in 39% versus 7% of patients receiving everolimus and placebo, respectively. Deaths due to acute respiratory failure (0.7%), infection (0.7%), and acute renal failure (0.4%) were observed on the everolimus arm.

Everolimus has 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 everolimus. Some of these infections have been severe (e.g. leading to respiratory or hepatic failure) and occasionally have had a fatal outcome.

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

Reactivation of Hepatitis B (HBV) has been observed in patients with cancer receiving chemotherapy (56). Sporadic cases of Hepatitis B reactivation have also been seen in this setting with everolimus. Use of antivirals during anti-cancer therapy has been shown to reduce the risk of Hepatitis B virus reactivation and associated morbidity and mortality (57). A detailed assessment of Hepatitis B/C medical history and risk factors must be done for all patients at screening, with testing performed prior to the first dose of everolimus. Testing includes HBsAg and HCVAb at screening and then as clinically indicated.

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

Hyperglycemia has been reported in clinical trials. Monitoring of fasting serum glucose is recommended prior to the start of everolimus therapy and periodically thereafter. Optimal glycemic control should be achieved before starting a patient on everolimus. Mouth ulcers, stomatitis and oral mucositis have been seen in patients treated with everolimus. In such cases topical treatments are recommended, but alcohol- or peroxide-containing mouthwashes should be avoided as they may exacerbate the condition. Antifungal agents should not be used unless fungal infection has been diagnosed.

Elevations of serum creatinine, usually mild, have been reported in clinical trials. Monitoring of renal function, including measurement of blood urea nitrogen (BUN) or serum creatinine, is recommended prior to the start of everolimus therapy and periodically thereafter.

Decreased hemoglobin, lymphocytes, platelets and neutrophils have been reported in clinical trials. Monitoring of complete blood count is recommended prior to the start of everolimus therapy and periodically thereafter.

Everolimus is not recommended in patients with severe hepatic impairment, (Child-Pugh class C).

The use of live vaccines and close contact with those who have received live vaccines should be avoided during treatment with everolimus.

Hypophosphatemia, hypomagnesemia, hyponatremia and hypocalcemia have been reported as serious adverse reactions. Electrolytes should be monitored in patients treated with everolimus.

Table 5.4 provides general recommendations for the management of patients, with suspected drug toxicities while on treatment with everolimus as single-agent therapy.

More detailed information regarding everolimus reported suspected toxicities and individual cases is provided in the RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012.

5.3.3 **Management of Hepatitis reactivation/flare**

In cancer patients with hepatitis B, whether carriers or in chronic state, use of antivirals during anticancer therapy has been shown to reduce the risk of hepatitis B virus (HBV) reactivation and associated HBV morbidity and mortality (57).

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

5.3.4 Management of stomatitis/oral mucositis/mouth ulcers

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

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

Note: Stomatitis/oral mucositis should be appropriately graded using the functional grading given on the NCI-CTCAE for adverse events, version 4.0.

5.3.5 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. Patients 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 HMG-CoA reductase inhibitors.

Hyperglycemia has been reported in clinical trials. Monitoring of fasting serum glucose is recommended prior to the start of everolimus therapy and periodically thereafter. Optimal glycemic control should be achieved before starting trial therapy.

5.3.6 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 patients taking everolimus (see Section 5.3.2 Adverse drug reactions). 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 patients presenting with non-specific 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. Patients should be advised to report promptly any new or worsening respiratory symptoms.

Patients who develop radiological changes suggestive of non-infectious pneumonitis and have few or no symptoms may continue everolimus therapy without dose alteration. If symptoms are moderate (Grade 2), consideration should be given to interruption of therapy until symptoms improve. The use of corticosteroids may be indicated. Everolimus may be reintroduced at a reduced dose until recovery to Grade 1 or better.

For cases where symptoms of non-infectious pneumonitis are severe (Grade 3), everolimus therapy should be discontinued and the use of corticosteroids may be indicated until clinical symptoms resolve. Therapy with everolimus may be re-initiated at a reduced dose depending on the individual clinical circumstances.

More detailed information regarding everolimus reported suspected toxicities and individual cases is provided in the RAD001/Everolimus Investigator's Brochure. Novartis Pharma AG. Edition 11, release date 15-Nov-2012.

Table 5.4 Management of non-infectious pneumonitis

Worst Grade Pneumonitis	Required Investigations	Management of Pneumonitis	Everolimus 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 is required	Administer 100% of everolimus dose.
Grade 2	CT scan with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent Cycle until return to baseline. Consider bronchoscopy *	Symptomatic only. Prescribe corticosteroids if cough is troublesome.	Reduce everolimus dose until recovery to ≤ Grade 1. Everolimus may also be interrupted if symptoms are troublesome. Patients will be withdrawn from the study if they fail to recover to ≤ Grade 1 within 3 weeks.
Grade 3	CT scan with lung windows and pulmonary function testing including: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent Cycle until return to baseline. Bronchoscopy is recommended *	Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.	Hold treatment until recovery to ≤ Grade 1. May restart protocol treatment within 2 weeks at a reduced dose (by one level) if evidence of clinical benefit. Patients will be withdrawn from the study if they fail to recover to ≤ Grade 1 within 2 weeks.
Grade 4	CT scan with lung windows and required pulmonary function testing includes: spirometry, DLCO, and room air O_2 saturation at rest. Repeat each subsequent Cycle until return to baseline. Bronchoscopy is recommended *.	Prescribe corticosteroids if infective origin is ruled out. Taper as medically indicated.	Discontinue treatment.

*A bronchoscopy with biopsy and/or bronchoalveolar lavage is recommended.

5.3.7 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.

5.3.8 **Diarrhea**

Follow the loperamide dosing guidelines in Table 5.5 for patients experiencing diarrhea. Prophylactic administration is NOT recommended. Patients are advised to call with first signs of poorly formed stools or an increased frequency of bowel movements.

Table 5.5 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

5.3.9 **Neurosurgical Procedures**

If a neurosurgical procedure is required for a reason other than tumor progression (i.e. the onset of hydrocephalus), these procedures should be documented, but will not constitute criteria for declaring the patient “off therapy”. Everolimus should be held until the patient is clinically stable and has recovered from the acute effects of surgery.

5.4 Treatment Plan

5.4.1 **Everolimus Administration**

The study drug everolimus will be self-administered (by the patients themselves). The investigator will instruct the patient to take the study drug exactly as specified in the protocol. Everolimus should be administered orally once daily, preferably in the morning, at the same time every day with or without food.

The tablets should be swallowed whole with a glass of water and should not be chewed or crushed. For patients unable to swallow tablets, the tablet(s) should be dispersed completely in a glass of water (containing approximately 30 mL) by gently stirring, immediately prior to drinking. The glass should be rinsed with the same volume of water and the rinse completely swallowed to ensure the entire dose is administered.

Everolimus will be administered orally as once daily continuously from study day 1 until progression of disease or unacceptable toxicity.

If vomiting occurs, no attempt should be made to replace the vomited dose.

All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded.

Medication labels will comply with US legal requirements and be printed in English. They will supply no information about the patient. The storage conditions for study drug will be described on the medication label.

5.4.2 Concomitant therapy

Patients will be instructed not to take any additional medications (including over-the-counter products) during the course of the study without prior consultation with the investigator. At each visit, the investigator will ask the patient about any new medications he/she is or has taken after the start of the study drug.

All Concomitant medications/Significant non-drug therapies taken ≤ 30 days prior to start and after start of study drug, including physical therapy and blood transfusions, should be recorded.

The following restrictions apply during the entire duration of the study:

- No other investigational therapy should be given to patients.
- No anticancer agents other than the study medication should be given to patients. If such agents are required for a patient then the patient must first be withdrawn from the study.
- Oral contraceptives in preclinical and clinical data have shown everolimus to have CYP3A4 inhibitory activity rather than induction activity, induction of metabolism of contraceptive hormones by everolimus is unlikely. Consequently, administration of everolimus should not reduce the efficacy of oral contraceptives.

Inhibitors of CYP3A4 and/or PgP

Co-administration with strong inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole, ritonavir) or P-glycoprotein (PgP) should be avoided.

Co-administration with moderate CYP3A4 inhibitors (e.g., erythromycin, fluconazole) or PgP inhibitors should be used with caution. If patient requires co-administration of moderate CYP3A4 inhibitors or PgP inhibitors, reduce the dose of everolimus to 2.5 mg daily. Additional dose reductions to every other day may be required to manage toxicities. If the inhibitor is discontinued the everolimus 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.

Inducers of CYP3A4 and/or PgP

Avoid the use of strong CYP3A4 inducers.

Table 5.6 Clinically relevant drug interactions: substrates, inducers, and inhibitors of isoenzyme CYP3A

SUBSTRATES	
Antibiotics: Clarithromycin, erythromycin, telithromycin	**Calcium channel blockers:** Amlodipine, diltiazem, felodipine, lercanidipine, nifedipine, nisoldipine, nitrendipine, verapamil

<table>
<tr><td>Anti-arrhythmics:
Quinidine</td><td>HMG CoA reductase inhibitors:
Cerivastatin, lovastatin, simvastatin</td></tr>
<tr><td>Benzodiazepines:
Alprazolam, diazepam, midazolam, triazolam</td><td>Steroid 6beta-OH:
estradiol, hydrocortisone, progesterone, testosterone</td></tr>
<tr><td>Immune modulators:
Cyclosporine, tacrolimus (FK506)</td><td rowspan="4">Miscellaneous:
Alfentanil, aprepitant, aripirazole, buspirone, cafergot, caffeine, cilostazol, cocaine, codeine-N-demethylation, dapsone, dexamethasone, dextromethorphan, docetaxel domperidone, eplerenone, fentanyl, finasteride, Gleevec/imatinib, haloperidol, irinotecan, LAAM, lidocaine, methadone, nateglinide, ondansetron, pimozide, propranolol, quetiapine, quinine, risperidone, salmeterol, sildenafil, sirolimus, sorafenib, sunitinib, tamoxifen, taxol, terfenadine, torisel, trazodone, vincristine, zaleplon, ziprasidone, zolpidem</td></tr>
<tr><td>HIV Antivirals:
Indinavir, nelfinavir, ritonavir, saquinavir</td></tr>
<tr><td>Prokinetic:
Cisapride</td></tr>
<tr><td>Antihistamines:
Astemizole, chlorpheniramine, terfenadine</td></tr>
<tr><td colspan="2">INDUCERS</td></tr>
<tr><td colspan="2">Barbiturates, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, phenytoin, pioglitazone, rifabutin, rifampin, St. John’s wort, troglitazone, efavirenz, nevirapine</td></tr>
<tr><td colspan="2">INHIBITORS</td></tr>
<tr><td colspan="2">Strong inhibitors:
indinavir, nelfinavir, ritonavir, clarithromycin, itraconazole, ketoconazole, nefazodone, saquinavir, telithromycin,
Posaconazole (58)

Moderate inhibitors:
aprepitant, diltiazem, erythromycin, fluconazole, grapefruit juice, verapamil,

Weak inhibitors:
Cimetidine,
Seville orange (59)

Unclassified as per the Indiana University DDI listing:
Ciprofloxacin, delaviridine, troleandamycin, mibefradil, amiodarone, chloramphenicol, diethyldithiocarbamate, fluvoxamine, starfruit, gestodene, imatinib, mifepristone, norfloxacin, norfluoxetine, voriconazole*,</td></tr>
</table>

Based on http://medicine.iupui.edu/clinpharm/ddis/table.asp as of December 01, 2009
* Voriconazole (unclassified as per the Indiana University DDI table)
Strong inhibitor according to the following reference:
(http://www.nature.com/clpt/journal/v80/n5/pdf/clpt2006438a.pdf)

Table 5.7 Clinically relevant drug interactions mediated by PgP

PgP Substrates	PgP Inhibitors in vivo	PgP Inducers
digoxin, fexofenadine, indinavir, vincristine, colchicine, topotecan, paclitaxel	amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, elacridar, erythromycin, felodipine, (GF120918), itraconazole, ketocoanzole, lopinavir, (LY335979), mibefradil, nifedipine, nitrendipine, (PSC833), quinidine, ranolazine, ritonavir, talinolol, valspodar, verapamil	rifampin, St John's wort

Reference:

Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated Dec. 2, 2009, which summarizes DDI data from three sources including the FDA's "Guidance for Industry, Drug Interaction Studies, the University of Washington's Drug Interaction Database, and Indiana University School of Medicine's Drug Interaction Table. "

NOTES:http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072101.pdf

****This list of clinically relevant drug interactions is updated as of December 02, 2009****

- Seville orange, star fruit, grapefruit and their juices affect P450 and PgP activity. Concomitant use should be avoided
- No chronic treatment with systemic steroids or other immunosuppressive agents. Topical or inhaled corticosteroids are allowed.
- The use of live vaccines and close contact with those who have received live vaccines should be avoided during treatment with everolimus. Examples of live vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines.

Oral anticoagulants such as warfarin are CYP2C9 substrates and, as such, no interaction with everolimus is expected. However, drug-drug interaction studies between macrolide antibiotics and warfarin have produced mixed outcomes and the disparity in these findings has led to the conclusion that multiple factors may alter the clearance of warfarin. The co-administration of everolimus and oral anticoagulants is possible but should be subject to verification of coagulation (INR) once steady state is reached (after one week's treatment).

Examples are provided in Table 5.7 (CYP3A4 inhibitors/inducers) and Table 5.8 (Drug interactions mediated by P-glycoprotein). A comprehensive list of cytochrome P450 isoenzymes and CYP3A4 inhibitors, inducers, and substrates can be found at

http://medicine.iupui.edu/flockhart. This website is continually revised and should be checked frequently for updates.

5.5 Reasons to Withdraw a Subject

The Investigator will withdraw a patient whenever continued participation is no longer in the patient's best interests. Reasons for withdrawing a patient include, but are not limited to, disease progression, the occurrence of an adverse event or a concurrent illness, a patient's request to end participation, or simply significant uncertainty on the part of the Investigator that continued participation is prudent. There may also be administrative reasons to terminate participation, such as concern about a patient's compliance with the prescribed treatment regimen.

5.6 Off Treatment Criteria

The "Off Treatment Date" and reason for discontinuation must be documented by the attending investigator in the medical record and recorded in two places within OnCore®, in the 'Follow-Up' section of OnCore® as well as in the 'PNOC End of Treatment eCRF'

The "Off Arm Date" must be documented in the 'Treatment' section of OnCore®. The 'Off Arm Date" should correspond with the "Off Treatment Date" and is the date the patient was discontinued from protocol treatment.

The "Last Treatment Date" is recorded in two places within OnCore®, in the 'Follow-Up section of OnCore®' as well as in the 'PNOC End of Treatment eCRF'. "Last Treatment Date" is defined as the last date that the patient received protocol based therapy.

Patients will be considered Off Treatment for the following reasons:

- Development of unacceptable toxicity
- Progressive disease (PD).
- Development of a medical or psychiatric illness, that in the investigator's judgment renders the patient incapable of further therapy on this protocol
- The patient, parent or legal guardian refuses further treatment on this protocol
- Completion of all protocol defined treatment
- Pregnancy

Patients who are off protocol therapy must be followed until an "Off Study Criterion" is met. All patients will be followed until resolution (or return to baseline) of all adverse events occurring while on treatment and/or within 30 days of the last administration of study drug. Toxicities that are related to study therapy and are ongoing at the end of day 30 of last treatment date will be followed until resolution or return to baseline. Patients who are Off-treatment will be followed to collect any adverse events that are possibly, probably or definitely related to the study drug as well as the date of progression, date of last contact and date of death. The requested follow-up data is to be submitted quarterly from the date the patient went off treatment.

5.7 Off Study Criteria

The date and reason for the patient coming off study must be documented in the 'Follow-Up' section of OnCore® as well as the 'PNOC End of Treatment eCRF'.

Patients will be considered Off Study for the following reasons:

- Patient determined to be ineligible.
- Parent, patient, or guardian withdraws consent for continued participation.
- Patient death while on study.
- Completion of protocol specific follow up period

No data will be collected documenting treatment or reporting events or disease status that occur subsequent to the official "off study" date.

6 Correlative Studies and Pathology Review

Pathology will be reviewed in the UCSF pathology department (Dr. Joanna Phillips and Dr. Arie Perry, Department of Neuropathology, UCSF).

6.1 Obtaining tissue and blood samples

Please refer to Appendix 8 for more information.

6.2 Preparation of tissue samples

Please refer to Appendix 8 for more information.

6.3 Review of FFPE tissue samples

Pathology Materials Required for Review:

- A copy of the Pathology Report
- A PNOC Specimen Submission Form
- Tissue

A UCSF neuropathologist will review all H&E slides and will determine if the histology corresponds to the report and if it is appropriate for the study.

If applicable, the neuropathologist will also choose the blocks from which to prepare unstained slides for additional analysis.

In general, one to two representative H&E stained slides from a pre-registration biopsy and when available from the original surgery will be reviewed.

6.4 Immunohistochemical and molecular assays

All tumor tissue will be analyzed to assess the activation status of the PI3K/Akt/mTOR signaling pathway. In addition, all tumors will be molecularly profiled based upon known alterations in pediatric astrocytomas. First, tumors will be stratified into one of two categories, high PI3K/Akt/mTOR pathway activity and low PI3K/Akt/mTOR pathway activity, based upon the results of immunohistochemistry for phosphorylated-S6 ribosomal protein (Ser240/244) as described below. Second, all tumor tissue samples will be further characterized by immunohistochemistry, tumor-DNA sequencing, and FISH, as described below.

For immunohistochemistry, all antigens to be assayed in this protocol are cytoplasmic, nuclear, or membranous and will be scored using a semi-quantitative scale as described herein. The designated neuropathologists will identify areas of tumor on the glass slide that are appropriately stained with the antibody, and will review the positive and negative control samples. The neuropathologist will then determine the ratio of tumor cells staining positive to

those staining negative for the particular antibody. This interpretation does not consider intensity of staining per cell, but defines as "positive" all cells staining with a similar pattern to positive control cells. In general, immunohistochemical assays will be scored using a four-tiered system based on the extent of positive tumor cells staining positive: 0, no positive staining; 1, positive staining in less than or equal to 25% of the tumor cells; 2, positive staining in greater than 25% but less than or equal to 75% of the tumor cells; 3, positive staining in greater than 75% of the tumor cells.

Visual estimation may be aided by dividing the interpretable areas of the tissue on the slide into four quadrants using a tissue marker. This scoring system has been previously used and representative images are illustrated (32). Based on our experience assessing activity of the PI3K/Akt/mTOR pathway in formalin fixed paraffin-embedded tumor sections, we will stratify patients into two categories based upon the results of immunohistochemistry for phosphorylated-S6 ribosomal protein (Ser240/244). PI3K/AKT/mTOR activated tumors will be those with a score of 2 or higher.

In addition to phosphorylated-S6 ribosomal protein (Ser240/244) we will also analyze the expression and phosphorylation of other proteins in the pathway by immunohistochemistry with the antibodies listed in Table 6.1 and score them as described above.

Table 6.1 Summary of Assays to evaluate activation of the PI3K/AKT/mTOR pathway

Molecular feature	Assay (reagents)
PTEN expression	IHC: Cell Signaling #9559 (Rabbit)
Phosphorylated S6 235/236	IHC: Cell Signaling #2211 (Rabbit)
Phosphorylated S6 240/244	IHC: Cell Signaling #2215 (Rabbit)
Phosphorylated PRAS40 (pT246)	IHC: Cell Signaling #2997 (Rabbit)
Phosphorylated 4EBP1	IHC: Cell Signaling #2855 (Rabbit)
Phosphorylated Erk1/2	IHC: Zymed #18-2389 (Rabbit)
PTEN promoter methylation	Methylation-specific primers as described

To further characterize the paired tumor tissue samples we will perform additional assays if not already available including: immunohistochemistry to determine p53 expression and MIB-1 labeling index; sequencing of tumor DNA to detect the BRAFV600E mutation; and FISH on formalin fixed paraffin-embedded samples to determine *PDGFRA* amplification, CDKN2A loss, and KIAA1549-BRAF gene fusion.

All immunohistochemistry assays will be performed on the Ventana Medical Systems Benchmark XT on a research basis but in a CLIA certified laboratory. Positive controls will include paraffin tissue sections from glioblastomas that have been previously confirmed to overexpress the phospho-epitopes by Western blotting, and confirmed on immunohistochemistry with serial dilutions of the antibody. In addition, a subependymal giant cell astrocytoma from a tuberous sclerosis patient in which p-S6 is present due to mutation in TSC1/TSC2, will also be used as a positive control. For PTEN, endothelial cells serve as an internal positive control and glioblastomas with PTEN expression confirmed by Western blot are used as external positive controls.

To examine whether the *PTEN* promoter is methylated in glioma specimens, we will use methylation-specific primers that had previously been used to demonstrate methylation of the *PTEN* promoter in a subset of non-small-cell-lung cancer samples. These primers amplify a 181

base pair region of the *PTEN* promoter that starts 2477 nucleotides from the translation start site. The methylation-specific PCR (MSP) assay is sensitive to approximately 5% methylated product (31).

In addition to the targeted approaches described above that examine previously reported alterations additional genomic profiling studies will be performed including targeted sequencing, RNA sequencing, and/or exome (plus) next generation sequencing if sufficient tissue is available. Such studies will include but are not limited to molecular analyses of DNA, RNA and protein in tumor biopsy specimens including fresh frozen tissue and blood as control. When possible and/or sufficient blood/tissue is provided sample will be used to establish tumor cell cultures, cell lines and/or transplantation models.

DNA and RNA will be isolated and stored in the laboratory of Sabine Mueller, University of San Francisco [redacted] using the QIAamp DNA kit and the QIAamp RNA blood mini kit or similar available DNA and RNA isolation kits. DNA and RNA from tissue and blood will be stored at -80 degrees indefinitely for potential future genetic studies to further characterize pediatric low-grade gliomas. These studies will be carried out in collaboration with Dr. Adam Resnick, Children's Hospital of Philadelphia (CHOP). The samples (DNA and RNA) sent to CHOP will be de-identified. Patients may contact the study Co-Chair, Dr. Sabine Mueller [redacted] at any time to request any remaining, identifiable samples be returned.

7 Reporting and Documentation of Results

7.1 Evaluation of Efficacy (or Activity)

The primary goal of this study is to evaluate efficacy as determined using 6-month progression free survival. Standard anatomic MRI in conjunction with clinical evaluation such as neurologic status and corticosteroid use remains the key determinant of response to therapy and the evaluation of tumor recurrence for low-grade glioma. Increase in contrast enhancement, worsening cerebral edema, and mass effect are traits of malignant transformation. Acquiring tissue samples to confirm tumor upgrade, although considered the "gold standard" for determining the presence of viable tumor, can result in both false positives and negatives that relate to sampling error. Evaluating response in low-grade glioma by radiographic imaging is an accepted means of determining response and is the standard by which NIH grant-funded brain tumor consortiums operate. Response will be determined by the bi-dimensional diameters. However, RECIST criteria will be collected and used for secondary evaluation. Patients will have brain MRI scans with and without gadolinium performed prior to therapy, and before cycles 3, 5, 7, 9, 11, 13, 16, 19, and 22. Spine MRIs should be performed prior to therapy and at the same time points as standard brain MRIs if clinically indicated.

7.1.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with the study drug.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable.)

7.1.2 Disease Parameters

7.1.2.1 Methods for Evaluation of Measurable Disease

All measurements will be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations will be performed as closely as possible to the beginning of treatment and never more than 21 days before the beginning of the treatment.

The same method of assessment and the same technique will be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Pathology Review

Pathology will be reviewed in the UCSF pathology department (Dr. Joanna Phillips and Dr. Arie Perry, Department of Neuropathology, UCSF). This will not be part of the eligibility process but part of the final analysis upon study completion.

Radiology Review

All objective responses must be reviewed by the neuro-oncology tumor board of UCSF. Exploratory analyses of perfusion and diffusion parameters will be assessed as predictive biomarkers of response. Images will be viewed on PACS system and comparison will be made between the most recent MRI image and the previous MRI image. Measurements and responses are judged based on criteria outlined below.

For detailed imaging guidelines and transfer of images please see appendix 4.

7.1.2.2 Response Criteria

Response Criteria for PNOC Trials

Measurable disease

Measurable disease is defined as lesions that can be accurately measured in two dimensions (longest diameter to be recorded) with a minimum size of no less than double the slice thickness.

All tumor measurements will be recorded in millimeters or decimal fractions of centimeters. Previously irradiated lesions are considered non-measurable except in cases of documented progression of the lesion since the completion of radiation therapy.

Non-measurable disease

Non-measurable disease is all other lesions (or sites of disease), including small lesions. Leptomeningeal disease is non-measurable.

Target and Non-target lesion

Tumor dimensions are determined by measurement of the longest tumor dimension and its perpendicular for each target lesion. For most CNS tumors, only one lesion/mass is present and therefore is considered a “target” for measurement/follow up to assess for tumor progression/response. If multiple measurable lesions are present, up to 3 can be selected as “target” lesions. Target lesions should be selected on the basis of size and suitability for accurate repeated measurements. All other lesions will be followed as non-target lesions

(including CSF positive for tumor cells). The lower size limit of the target lesion(s) should be at least twice the thickness of the slices showing the tumor to decrease the partial volume effect.

Tumor Measurements

Regarding MRI imaging, the sequence that best highlights the tumor (T1 enhanced or T2 weighted or FLAIR images) will be chosen to determine response criteria. The same sequence should be used for serial measurements. Response determination will be based on a comparison of an area [**W** (longest diameter of the target lesion) **x T** (transverse measurement, perpendicular to W)] between the baseline assessment and the study date designated in the follow-up Report Form. Reports for the follow-up exams should reiterate the measurements obtained at baseline for each target lesion. Nontarget lesions or newly occurring lesions should also be enumerated in these reports, and changes in non-target lesions should be described.

1. For MRI imaging (preferred), the longest diameter can be measured from the axial plane or the plane in which the tumor is best seen or measured. The longest measurement of the tumor is referred to as the width (W).
2. The perpendicular measurements should be determined - transverse (T) measurement, perpendicular to the width in the selected plane.
3. The cystic or necrotic components of a tumor are not considered in tumor measurements. Therefore only the solid component of cystic/necrotic tumors should be measured. If cysts/necrosis composes the majority of the lesion, the lesion may not be “measurable”.

Options:

- if the cyst/necrosis is eccentric, the W and T of the solid portion should be measured, the cyst/necrosis will be excluded from measurement
- if the cyst/necrosis is central but represents a small portion of the tumor (< 25%), disregard and measure the whole lesion
- if the cyst/necrosis is central but represents a large portion of the tumor, identify a solid aspect of the mass that can be reproducibly measured
- Leptomeningeal tumor spread is usually not a target lesion, and usually cannot be measured accurately. Presence and location of leptomeningeal tumor spread should be noted and change in extent/thickness assessed on follow up studies.

Overall Response Assessment:

The overall response assessment takes into account response in both the target and non-target lesion, and the appearance of new lesions, where applicable, according to the criteria described in the table below. The overall response assessment is shown in the last column, and depends on the assessments of target, non-target, and new lesions in the preceding columns. The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesion(s)	Non Target Lesion (s)	New Lesions	Overall Response	Best Response for this Category Also Requires
CR	CR	No	CR	> 8 weeks confirmation
CR	IR/SD	No	PR	> 8 weeks confirmation

PR	CR/IR/SD	No	PR	
SD	CR/IR/SD	No	SD	documented at least > 4 weeks from baseline
PD	Any	Yes or No	PD	
Any	PD	Yes or No	PD	
Any	Any	Yes	PD	

CR – Complete Response; PR – Partial Response; SD – Stable Disease; PD – Progressive Disease; IR – Incomplete Response

Response Criteria for Target/Non-Target Lesions:

Response criteria are assessed in 2 dimensions – the product of W x T.
To assess response/progression, the ratio is calculated: **W x T (current scan) divided by W x T (reference scan).** Development of new disease or progression in any established lesions is considered progressive disease, regardless of response in other lesions – e.g. when multiple lesions show opposite responses, the progressive disease takes precedence.

For purposes of this study, response criteria for target lesions are:

Complete Response (CR): Complete disappearance of all known disease for at least 8 weeks. Complete response is dated from the time all lesions have disappeared on a stable or decreasing dose of corticosteroids.
Partial response (PR): A reduction of at least 50% in the size of all measurable tumor as quantitated by the sum of the products of the largest diameters of measurable lesions and maintained for at least 8 weeks on a stable or decreasing dose of corticosteroids. Partial response is dated from the time of first observation. In addition, there can be no appearance of new lesions or progression of any lesion.
Stable Disease (SD): A decrease of <50% or an increase of <25% in the sum of the products of the largest diameters of measurable lesions and no evidence of new lesions for at least 4 weeks on a stable or decreasing dose of corticosteroids.
Progressive Disease (PD): ≥ 25% increase in the sum of the products of the largest diameters of the measurable lesions or the appearance of one or more new lesions.

Progression free survival (PFS): PFS will be calculated at all times during follow-up, with particular interest in the 6-month time point. Progression free survival will be calculated from date of first treatment to the date of first observation of progressive disease, non-reversible neurological progression or increasing steroid requirements (applies to stable disease only), death due to any cause, or early discontinuation of treatment.

Overall survival (OS): Overall survival will be calculated from date of original diagnoses to death and also from the date of study registration to death. The latter will be an endpoint for assessment of benefit of this therapy.

7.2 Definitions of Adverse Events

7.2.1 Adverse Event

An adverse event (also known as an adverse experience) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. More specifically, an adverse event (can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

7.2.2 Adverse reaction

An adverse reaction is defined as any adverse event caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

7.2.2.1 Suspected

A suspected adverse reaction is defined as any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" indicates that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

7.2.2.2 Unexpected

An adverse event or suspected adverse reaction is considered *unexpected* if it is not listed in the investigator brochure or package insert(s), or is not listed at the specificity or severity that has been observed, or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected," as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse events that would be anticipated to occur as part of the disease process are considered *unexpected* for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure.

Some adverse events are listed in the Investigator Brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered *unexpected* until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class effect, the first case of angioedema observed with the drug under investigation should be considered *unexpected* for reporting purposes.

7.2.2.3 Serious

An adverse event or suspected adverse reaction is considered *serious* if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death,
- Life-threatening adverse experience (See section 7.2.2.4 below),
- Inpatient hospitalization or prolongation of existing hospitalization,
- Persistent or significant disability/incapacity,
- Congenital anomaly/birth defect, or cancer,
- Any other experience that suggests a significant hazard, contraindication, side effect or precaution that may require medical or surgical intervention to prevent one of the outcomes listed above, or
- Event that changes the risk/benefit ratio of the study.

Important medical events that may not result in death, are life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.2.2.4 Life-threatening

An adverse event or suspected adverse reaction is considered *life threatening* if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

7.3 Recording of an Adverse Event

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

All Grade 3-5 adverse events will be entered into OnCore®, regardless of relationship. Data about these events and their severity will be recorded using the NCI CTCAE v4.0.

The Investigator will assign attribution of the possible association of the event with use of the investigational drug, and this information will be entered into OnCore® using the classification system listed below:

Relationship	Attribution	Description
Unrelated to investigational drug/intervention	Unrelated	The AE *is clearly NOT related* to the intervention
	Unlikely	The AE *is doubtfully related* to the intervention
Related to investigational drug/intervention	Possible	The AE *may be related* to the intervention
	Probable	The AE *is likely related* to the intervention
	Definite	The AE *is clearly related* to the intervention

Signs or symptoms reported as adverse events will be graded and recorded by the Investigator according to the CTCAE. When specific adverse events are not listed in the CTCAE they will be graded by the Investigator as *none*, *mild*, *moderate* or *severe* according to the following grades and definitions:

Grade 0	No AE (or within normal limits)
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)
Grade 3:	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4:	Life-threatening consequences; urgent intervention indicated
Grade 5:	Death related to AE

7.4 Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management until resolved or return to baseline. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. For selected adverse events for which administration of the investigational drug was stopped, a re-challenge of the subject with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

7.5 Adverse Events Monitoring

The Investigator will assess all adverse events and follow reporting requirements to their institutional Data and Safety Monitoring Committee (DSMC) and Institutional Review Board (IRB).

All Adverse Events entered into OnCore® will be reviewed on a monthly basis by the PNOC Operations Office. The PNOC Operations Office will discuss the toxicity, grade, and relationship to study intervention for all AEs in question.

In addition, all adverse events and suspected adverse reactions considered “serious,” entered into OnCore® will be reviewed and monitored by the Data and Safety Monitoring Committee on

an ongoing basis, discussed at DSMC meetings which take place every six (6) weeks. For a detailed description of the Data and Safety Monitoring Plan please refer to Appendix 5.

Study Chair Reporting to Novartis

The study chair, co-chair or designee has the obligation to report all serious adverse events to the FDA, IRB, and Novartis Pharmaceuticals Drug Safety and Epidemiology Department (DS&E).

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

All events must be reported, [redacted] to Novartis Pharmaceuticals DS&E Department within 24 hours of learning of its occurrence. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

Any serious adverse event occurring after the patient has provided informed consent and until 4 weeks after the patient has stopped study participation must be reported. This includes the period in which the study protocol interferes with the standard medical treatment given to a patient (e.g. treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication).

Serious adverse events occurring more than 4 weeks after study discontinuation need only be reported if a relationship to the Novartis study drug (or therapy) is suspected.

For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer by the investigator.

The date the SAE was sent to all required reporting agencies will be documented in the UCSF Clinical Trials Management System.

7.6 Expedited Reporting

Participating Sites Reporting to Sponsor-Investigator (PNOC)

In addition to complying with all applicable regulatory reporting laws and regulations, each site must report the following:

- All SAEs, regardless of relationship must be entered into the Subject Console in OnCore® (https://oncore.ucsf.edu/) within one business day of first PI awareness, even if the SAE is ongoing. The SAE must be followed until resolution, and the OnCore® SAE record should be updated immediately as new information becomes available.

Email notification to the PNOC Operations Office [redacted] within one business day of first PI awareness:

- Reports of pregnancy exposure (pregnancy encompasses the entire cycle of pregnancy and delivery, perinatal and neonatal outcomes, even if there were no abnormal findings; both maternal and paternal exposure is collected)
- Reports of lactation exposure
- Overdose (with or without an SAE)
- Abuse (use for non-clinical reasons with or without an SAE)
- Inadvertent or accidental exposure

Sponsor-Investigator (PNOC) Reporting to the Data and Safety Monitoring Committee

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the PNOC Operations Office will notify the DSMC Chair (or qualified alternate) within 1 business day of knowledge of the event. The contact may be by phone or e-mail. Each participating site will follow their institutional reporting guidelines to institutional DSMC.

Sponsor-Investigator (PNOC) Reporting to UCSF Institutional Review Board (IRB)

The PNOC Operations Office must report events meeting the UCSF IRB definition of "Unanticipated Problem" (UP) within 10 business days of awareness of the event.

Each participating site will follow their institutional reporting guidelines to the IRB.

Sponsor-Investigator (PNOC) Reporting to Novartis

Investigators must report all SAEs to PNOC Operations Office within one business day of the Investigator's first awareness of occurrence: PNOC will submit the completed safety report along with an Interventional Clinical Trial SAE Fax Cover Sheet to Novartis Pharmaceuticals within the timelines described below:

All events, regardless of relationship, must be reported, by Email [redacted] OR Fax [redacted], to Novartis Pharmaceuticals DS&E Department within 24 hours of learning of their occurrence. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

Any serious adverse event occurring after the patient has provided informed consent and until 4 weeks after the patient has stopped study participation must be reported. This includes the period in which the study protocol interferes with the standard medical treatment given to a patient (e.g. treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication).

Serious adverse events occurring more than 4 weeks after study discontinuation need only be reported if a relationship to the Novartis study drug (or therapy) is suspected.

For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer by the investigator.

The date the SAE was sent to all required reporting agencies will be documented in the UCSF Clinical Trials Management System

8 Statistical Considerations and Evaluation of Results

8.1 Study Endpoints

The primary objective of the proposed study is to estimate the efficacy of the proposed regimen based on the 6-month progression free survival rate (PFS6). More specifically, the primary aim is to determine whether the true, unknown, response observed prior to classical disease progression warrants additional study of everolimus in only those patients with activation of the PI3K/AKT/mTOR pathway measured by p-S6 positivity or the entire population of patients with symptomatic, progressive or recurrent pediatric low-grade glioma.

In this setting of 2-staged phase II trials, the secondary objectives are considered exploratory.

- PFS and OS distributions as well as objective response (CR+PR) rates associated with everolimus treatment in recurrent pediatric LGGs.
- Associations between activation of the PI3K pathway as measured by expression of phospho-AKT, phospho-PRAS40, and phospho-4EBP1 and outcome as measured by the 6-month disease stabilization rates as well as PFS for progressive or recurrent pediatric low-grade glioma patients with measureable disease treated with everolimus.
- Analyses of key molecular features including activation of the PI3K, mTOR and MAPK pathways, aberrations in PTEN, p53, *PDGFRA* amplification, CDKN2A loss, and activating mutations in BRAF (KIAA1549-BRAF fusion and BRAFV600E missense BRAF mutation). In addition to the targeted approaches described above that examine previously reported alterations additional genomic profiling studies will be performed including targeted sequencing, RNA sequencing, and/or exome(plus) next generation sequencing if sufficient tissue is available. Such studies will include but are not limited to molecular analyses of DNA, RNA and protein in tumor biopsy specimens and blood samples. When possible and/or sufficient blood/tissue is provided, sample will be used to establish tumor cell cultures, cell lines and/or transplantation models. These studies will be carried out in collaboration with Dr. Adam Resnick, Children's Hospital of Philadelphia.
- MR quantitative measures of relative cerebral blood volume, permeability and apparent diffusion coefficient within the region of hyper-intensity on T2-weighted images as markers of disease response and/or progression in comparison to institutional evaluation of disease response and/or progression and quantitative measures of tumor response as determined by central review (based upon both area and volumetric measures).

8.2 Determination of Sample Size and Accrual Rate

8.2.1 Sample Size and Power Estimate

To estimate the efficacy of everolimus we will employ an adaptive Simon two-stage design for Phase II studies of targeted therapies (60, 61) and depicted in Figure 1. In the first stage of the adaptive two-stage Phase II trial design, two parallel studies are initiated, one for non-activated PI3K/AKT/mTOR pathway ($Path^{not\text{-}act}$) patients (enrolling 18 patients) and one for activated pathway ($Path^{act}$) patients (enrolling 7). Pathway activation is based on p-S6 positivity. Based on the results of the first stage, enrollment in the second stage will either be limited to $Path^{act}$ patients or open to all subjects regardless of PI3K/AKT/mTOR activation status.

If preliminary evidence in the first stage suggests efficacy of everolimus in Pathnot-act patients, enrollment in the second stage will be open to all patients regardless of activation status and a total of 65 patients will be enrolled. As detailed below, with α = 0.0512, the power for concluding efficacy when PFS6 is independent of PI3K/AKT/mTOR activation status is > 95%. If preliminary evidence in the first stage suggests efficacy of everolimus is limited to the Pathact patients, enrollment for the second stage will be restricted to only those patients and a total of 27 Pathact patients will be enrolled. As detailed below, with α = 0.0512, the power for concluding efficacy in the Pathact sub-population is 81%.

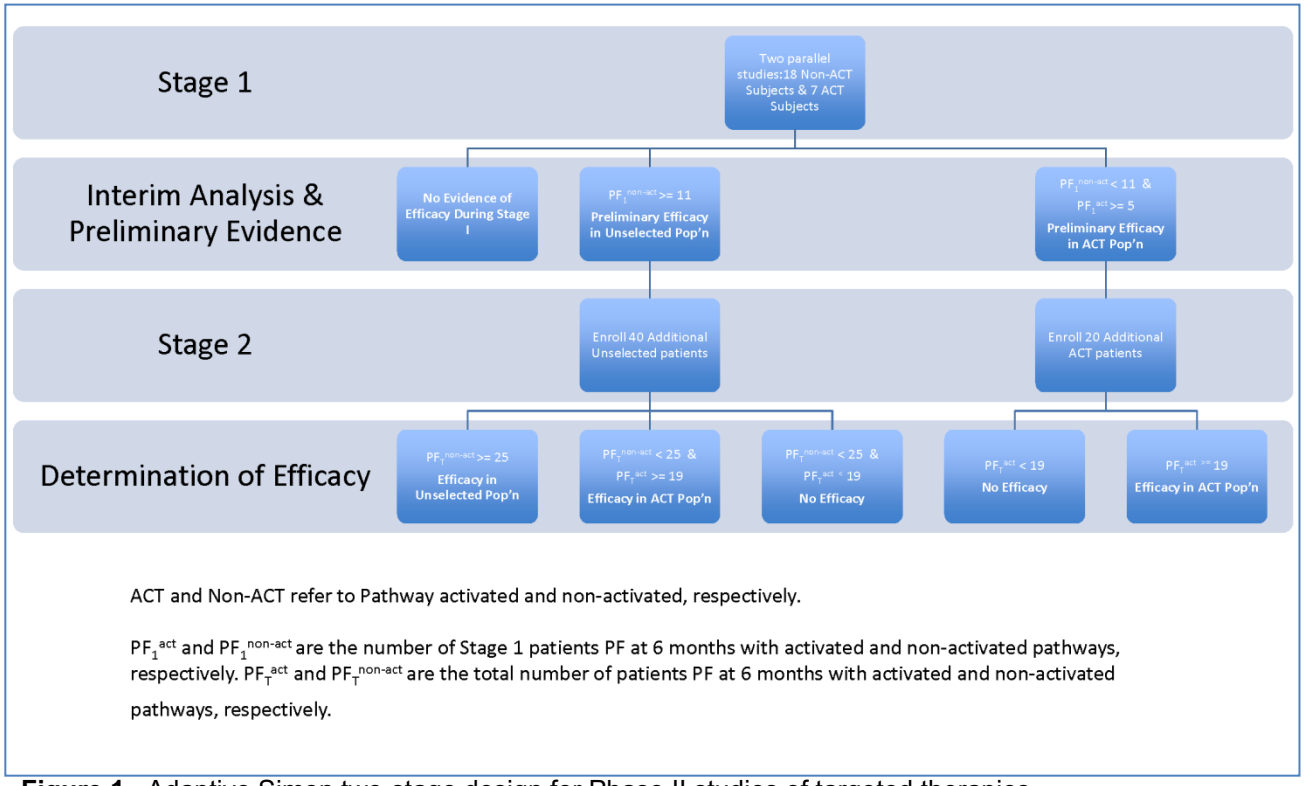

Figure 1. Adaptive Simon two-stage design for Phase II studies of targeted therapies

8.2.2 **Accrual estimates**

With the anticipated accrual numbers provided by the eight PNOC institutions, we anticipate that 25-30 patients/year may be available. If we assume that 50% of these would be eligible for the biology objective i.e. would have measureable disease and would have tissue available from prior surgeries, we can expect that the interim analysis will take place within 2.5-3.5 years from initiation of accrual, including the 6-month disease evaluation window. Hence at the time of the interim analysis we expect to have accrued 25 patients.

If preliminary evidence in the first stage (at the interim analysis) suggests efficacy of everolimus is limited to the patients with pathway activation, an additional 20 pathway activated patients will be recruited. Assuming 50% of the eligible patients have pathway activation, we can expect 6-7 patients per year for a full accrual in 5.5-6.5 years from initiation of accrual. If preliminary evidence in the first stage (interim analysis) suggests efficacy of everolimus is not limited to the patients with pathway activation, an additional 40 patients will be recruited regardless of their activation status. We can then expect 13-15 patients per year for a full accrual in 5.5-6.5 years from initiation of accrual.

8.3 Interim Analyses and Stopping Rules

The interim analysis will occur at or near the end of the first stage of the adaptive Simon 2-stage design for Phase II studies of targeted therapies. The first stage of this study design entails two parallel studies where accrual will stop, i.e. the trial will terminate early if evidence accumulates that the efficacy is lower than the acceptable levels. The probability of type I and type II errors will be set at 10%. The chosen values of these error probabilities reflect equal concerns for falsely continuing a regimen that is not effective and rejecting a regimen that has sufficient activity.

Accrual will continue until the first stage sample sizes are reached; however if the accrual is slow and current data with adequate follow-up indicates that the trial cannot result in expansion of the cohort beyond the first stage even if all future patients achieve PF status at 6 months, then the accrual will be stopped short of the planned sample size for the interim analysis. Similarly if this cohort is expanded beyond the interim analysis, i.e. beyond the first stage of the adaptive Simon 2-stage design and into the second stage, the accrual will continue until the final sample size is attained; however if the accrual is slow and current data with adequate follow-up indicates that the trial cannot result in a positive outcome even if all future patients achieve PF status at 6 months, then the accrual will be stopped short of the planned final sample size.

8.4 Analysis Plans

8.4.1 Analysis Population

Our primary analysis will be based on an intention to treat principle whereby all patients enrolled into the trial will be considered evaluable. All patients included in the study must be assessed for PFS6, even if there are major protocol treatment deviations or if they are ineligible. Patients who die or are lost to follow-up prior to reaching the 6-month time point will be considered as failures for the PFS6 endpoint.

All conclusions should be based on all eligible patients. Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

8.4.2 Primary Analysis (or Analysis of Primary Endpoints)

The primary objective of this study is to evaluate whether everolimus is active in only those patients with PI3K/AKT/mTOR pathway activation based on p-S6 positivity or in the population as a whole. To achieve this goal we will employ an adaptive Simon two-stage design for Phase II studies of targeted therapies (61).

In the first stage of the adaptive two-stage Phase II trial design, two parallel studies are initiated, one for PI3K/AKT/mTOR non-activated ($Path^{not\text{-}act}$) patients and one for PI3K/AKT/mTOR activated ($Path^{act}$) patients (see Figure 1). We expect that similar to the adult LGG population, approximately 50% of the patients will have PI3K/AKT/mTOR activation. Based on the results of the first stage, enrollment in the second stage will either be limited to $Path^{act}$ patients or open to all subjects regardless of PI3K/AKT/mTOR activation status.

First Stage: The two parallel first stage studies are identical to the first stage of Simon's two-stage optimal design. For both, everolimus will be deemed not worthy of further investigation, if the true PFS6 rate is less than 50%. To achieve an overall level α test of 0.05 the Type I error

rate for the two studies is set to α/2 = 0.025 while the Type II error probabilities are set at 0.2. For the $Path^{not\text{-}act}$ study everolimus will be of interest if the PFS6 is 70% or greater. Thus 18 $Path^{not\text{-}act}$ patients will be enrolled and everolimus will be considered preliminarily sufficiently active in this sub-population if 11 or more of the patients are PF at 6 months (the number of patients is denoted as $PF_1^{non\text{-}act}$ in Figure 1). For the $Path^{act}$ study everolimus will be of interest if the PFS6 is 80% or greater. Thus 7 patients will be enrolled and everolimus will be considered preliminarily sufficiently active in this sub-population if 5 or more of the patients are PF at 6 months (the number of patients is denoted as PF_1^{act} in Figure 1). In both studies, if the 'true' PFS6 rate is 50%, there is a 76% probability of ending the trial during the first stage. If less than 11 $Path^{not\text{-}act}$ patients **and** less than 5 $Path^{act}$ patients are PF at 6 months the trial will be closed to accrual and we will conclude that everolimus does not merit continued investigation in this disease due to lack of efficacy. Otherwise, recruitment will begin for the second stage.

Second Stage: Recruitment in the second stage is entirely dependent on the results of the first stage. If preliminary evidence in the first stage suggests efficacy of everolimus in $Path^{not\text{-}act}$ patients, enrollment in the second stage will be open to all patients regardless of PI3K/AKT/mTOR status (i.e. unselected patients). Thus 40 additional patients will be enrolled, regardless of PI3K/AKT/mTOR status, for a total of 65 patients.

- If 25 or more of the **total** number of $Path^{not\text{-}act}$ patients are PF at 6 months (denoted as $PF_T^{non\text{-}act}$ in Figure 1), everolimus will be considered sufficiently efficacious in the unselected population to warrant recommendation for continued investigation.

- If fewer than 25 of the total number $Path^{not\text{-}act}$ patients are PF at 6 months and 19 or more of the total number of $Path^{act}$ patients are PF at 6 months (denoted as PF_T^{act} in Figure 1), everolimus will be considered sufficiently efficacious in the $Path^{act}$ sub-population.

- If fewer than 25 $Path^{not\text{-}act}$ patients and fewer than 19 $Path^{act}$ patients are PF at 6 months ($PF_T^{non\text{-}act} < 25$ & $PF_T^{act} < 19$), everolimus will not be considered sufficiently efficacious.

With α = 0.0512, the power for concluding efficacy when PFS6 is independent of PI3K/AKT/mTOR status is > 95%. If the proportion of 40 patients with PI3K/AKT/mTOR activation is not approximately 50%, we will reassess the necessary calculations to avoid drawing incorrect conclusions.

On the other hand, if preliminary evidence in the first stage suggests efficacy of everolimus is limited to the $Path^{act}$ patients, enrollment for the second stage will be restricted to only $Path^{act}$ patients and no additional $Path^{not\text{-}act}$ patients will be accrued. An additional 20 $Path^{act}$ patients will be recruited for a total of 27 $Path^{act}$ patients. Everolimus will be considered sufficiently active in this sub-population to warrant recommendation for continued investigation if 19 or more of the 27 $Path^{act}$ patients are PF at 6 months. With α = 0.0512, the power for concluding efficacy in the $Path^{act}$ sub-population is 81%.

8.4.3 Secondary Analysis (or Analysis of Secondary Endpoints)

In this setting of 2-staged phase II trials, the secondary objectives are considered exploratory and will be reported descriptively based on appropriate statistical methods.

- Estimate PFS and OS distributions as well as objective response (CR+PR) rates associated with everolimus treatment in recurrent pediatric LGGs.

  The secondary endpoints for PFS and OS are survival times as defined in Section 7.1.1.3. Survival time distributions will be estimated using the Kaplan-Meier product limit

curve. Objective response rates and confidence intervals will be estimated based on the proportion of complete and partial responders.

- Explore associations between activation of the PI3K pathway as measured by expression of phospho-AKT, phospho-PRAS40, and phospho-4EBP1 and outcome as measured by the 6-month disease stabilization rates (a dichotomous variable; see section 7.1.2.2) as well as PFS for progressive or recurrent pediatric low-grade glioma patients with measureable disease treated with everolimus.

  Association between activation of the PI3K pathway as measured by expression of phospho-AKT, phospho-PRAS40, and phospho-4EBP1 and 6-month response rate will be assessed via logistic regression. Estimates of the odds ratio and confidence intervals will be reported. Association between activation of the PI3K pathway as measured by expression of phospho-AKT, phospho-PRAS40, and phospho-4EBP1 and PFS will be assessed via a Cox proportional hazards model. Estimates of the hazard ratio and confidence intervals will be reported.

- Collect tissue from all enrolled patients and prospectively analyze key molecular features including activation of the PI3K, mTOR and MAPK pathways, aberrations in PTEN, p53, *PDGFRA* amplification, CDKN2A loss, and activating mutations in BRAF (KIAA1549-BRAF fusion and BRAFV600E missense BRAF mutation). In addition to the targeted approaches described above that examine previously reported alterations additional genomic profiling studies will be performed including targeted sequencing, RNA sequencing, and/or exome(plus) next generation sequencing if sufficient tissue is available. Such studies will include but are not limited to molecular analyses of DNA, RNA and protein in tumor biopsy specimens and blood samples. When possible and/or sufficient blood/tissue is provided, sample will be used to establish tumor cell cultures, cell lines and/or transplantation models. These studies will be carried out in collaboration with Dr. Adam Resnick, Children's Hospital of Philadelphia.

  Descriptive statistics will be reported for each molecular feature including means and variances for continuous variables and proportions for categorical variables.

- Explore MR quantitative measures of relative cerebral blood volume, permeability and apparent diffusion coefficient within the region of hyper-intensity on T2-weighted images as markers of disease response and/or progression in comparison to institutional evaluation of disease response and/or progression and quantitative measures of tumor response as determined by central review (based upon both area and volumetric measures).

  The relevant MR quantitative measures are defined in Section 7.1. We hypothesize that (a) high volumetric parameters, low nADC percentiles and high nCBV percentiles at the baseline exam are predictive of poor outcome and (b) that early changes in these parameters can predict tumor progression. To assess associations between the parameters at baseline and subsequent poor outcome (both PFS and OS), we will employ Cox proportional hazard models for each imaging parameter assessing whether it should be included as a continuous (as recorded or after an appropriate transformation) or a categorical (e.g. by dichotomizing or including as dummy variables) covariate. A smoother (e.g. LOESS) can be used to assess the appropriateness of the linearity assumption of the continuous covariate based on the martingale residuals. Estimates of the hazard ratio and confidence intervals will be reported. For b) the binary outcome denoting tumor progression (defined as categories 4—7 vs. categories 1—3 in

Evaluation of Response in Section 7.1.1.3), we will explore logistic regression models. Early changes in the imaging parameters will be included on a continuous scale unless a nonlinear association indicates an alternative high/low behavior in the risks. If the latter is true, a different scale or categorization will be employed. Estimates of the odds ratio and confidence intervals will be reported.

8.5 Evaluation of Safety

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the NCI CTCAE v4.0.

8.6 Study Results

The primary hypothesis of this study is to determine the efficacy of everolimus in LGG patients. This final analysis will occur after 65 unselected or 27 pathway-activated patients have been followed for at least 6 months following the start of treatment. It will include tabulation of all cases entered and those excluded from the analyses with the reasons for such given; the distribution of the important prognostic baseline variables; and observed results with respect to the primary and secondary endpoints.

9 Study Management

9.1 Pre-study Documentation

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

Before initiating this trial, the Investigator will have written and dated approval from the Institutional Review Board for the protocol, written informed consent form, subject recruitment materials, and any other written information to be provided to subjects before any protocol related procedures are performed on any subjects.

The clinical investigation will not begin until either FDA has determined that the study under the Investigational Drug Application (IND) is allowed to proceed or the Investigator has received a letter from FDA stating that the study is exempt from IND requirements.

The Investigator must comply with the applicable regulations in Title 21 of the Code of Federal Regulations (21 CFR §50, §54, and §312), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

9.2 Institutional Review Board Approval

The protocol, the proposed informed consent form, and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the UCSF Institutional Review Board (IRB). Prior to obtaining IRB approval, the protocol must be approved by the Helen Diller Family Comprehensive Cancer Center Site Committee and by the Protocol Review Committee (PRC). The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

9.3 Informed Consent

All participants must be provided a consent form describing the study with sufficient information for each participant to make an informed decision regarding their participation. Participants

must sign the IRB-approved informed consent and HIPAA form prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent/HIPAA document. The original signed copy of the consent and HIPAA document must be retained in the medical record or research file. All subsite consent forms must be reviewed and approved by PNOC OPS prior to submitting to the local IRB.

9.4 Changes in the Protocol

Once the protocol has been approved by the UCSF IRB, any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the Study Chair and approved by PNOC, the PRC and the IRB prior to implementation.

If it becomes necessary to alter the protocol to eliminate an immediate hazard to patients, an amendment may be implemented prior to IRB approval. In this circumstance, however, PNOC must then notify the IRB in writing within five (5) working days after implementation. PNOC OPS will be responsible for updating any participating sites. Once released by PNOC OPS, all major protocol amendments must be submitted to the local IRB within 30 business days by each participating institution. Minor amendments may be submitted at the time of continuing review.

9.5 Handling and Documentation of Clinical Supplies

Each participating site will maintain complete records showing the receipt, dispensation, return, or other disposition of all investigational drugs. The date, quantity and batch or code number of the drug, and the identification of patients to whom study drug has been dispensed by patient number and initials will be included. The sponsor-investigator will maintain written records of any disposition of the study drug.

The Principal Investigator shall not make the investigational drug available to any individuals other than to qualified study patients. Furthermore, the Principal Investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

9.6 Case Report Forms (CRFs)

Each participating site will complete study specific Case Report Forms (CRFs) for safety monitoring and data analysis. Each site will enter the study data into OnCore® via standardized CRFs in accordance with the CTMS study calendar, using a secure access account. The participating site's Clinical Research Coordinator (CRC) will complete the CRFs; the Investigator will review and approve the completed CRFs – this process must be completed within the timelines specified in Appendix 7.. Study data from the participating site will be reported and reviewed in aggregate with data from patients enrolled at the coordinating center. All source documentation and CTMS data will be available for review/monitoring as needed.

The information collected on CRFs shall be identical to that appearing in original source documents. For participating sites, source documents will be maintained per institutional guidelines. All source documentation should be kept in separate research folders for each patient.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto CRFs. The PI will approve all completed CRFs to attest that the information contained on the CRFs is true and accurate.

The Principal Investigator at each participating institution will be responsible for ensuring the accurate capture of study data. At study completion, when the CRFs have been declared to be complete and accurate, the database will be locked. Any changes to the data entered into the

CRFs after that time can only be made by joint written agreement among the Study Chair, the Trial Statistician, and the PNOC Project Leader.

9.7 Oversight and Monitoring Plan

This is a multicenter trial. The UCSF Helen Diller Family Comprehensive Cancer Center Data Safety Monitoring Committee (DSMC) will be the main monitoring entity for this study. The UCSF DSMC will work together with participating member institution DSMCs to monitor the study in accordance with the available NCI approved Data Safety and Monitoring Plans (DSMPs). For member institutions that do not follow an NCI approved DSMP, the UCSF DSMC will be considered the institutional DSMC. The DSMC will routinely review all adverse events and suspected adverse reactions considered "serious". The UCSF DSMC will audit study-related activities to ensure that the study is conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). Significant results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable. Please see Appendix 5 PNOC Data Safety and Monitoring Plan for more information.

9.8 Multicenter Communication

The PNOC operations office provides administration, data management, and organizational support for the participating sites in the conduct of the clinical trial. The PNOC Operations Office will coordinate, at minimum, quarterly conference calls with the PNOC member institutions to discuss risk assessment. The following items will be discussed, as appropriate:

- Enrollment information
- Adverse events (i.e. new adverse events and updates on unresolved adverse events and new safety information)
- Protocol violations
- Other issues affecting the conduct of the study

9.9 Record Keeping and Record Retention

The Principal Investigator for each PNOC institution is required to maintain adequate records of the disposition of the drug, including dates, quantity, and use by subjects, as well as written records of the disposition of the drug when the study ends per institutional guidelines.

The site Principal Investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

Study documentation includes all CRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

In accordance with FDA regulations, the investigator shall retain records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified.

9.10 Coordinating Center Documentation of Distribution

It is the responsibility of the PNOC operations office to maintain adequate files documenting the distribution of study documents as well as their receipt (when possible). The HDFCCC recommends that the PNOC operations office maintain a correspondence file and log for each segment of distribution (e.g., FDA, drug manufacturer, participating sites, etc.).

Correspondence file: should contain copies (paper or electronic) of all protocol versions, cover letters, amendment outlines (summary of changes), etc., along with distribution documentation and (when available) documentation of receipt.

Correspondence log: should be a brief list of all documents distributed including the date sent, recipient(s), and (if available) a tracking number and date received.

At a minimum, the PNOC operations office must keep documentation of when and to whom the protocol, its updates and safety information are distributed.

9.11 Regulatory Documentation

Prior to implementing the protocol at each PNOC institution, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be first approved by the UCSF Institutional Review Board (IRB) and by the PNOC operations office. Prior to implementing this protocol at the participating sites, approval for the UCSF IRB approved protocol must be obtained from the participating site's IRB.

Appendix 6 lists the documents which must be provided to PNOC Operations Office before the participating site can be initiated and begin enrolling participants.

Upon receipt of the required documents, PNOC operations office will formally contact the site and grant permission to proceed with enrollment.

10 References

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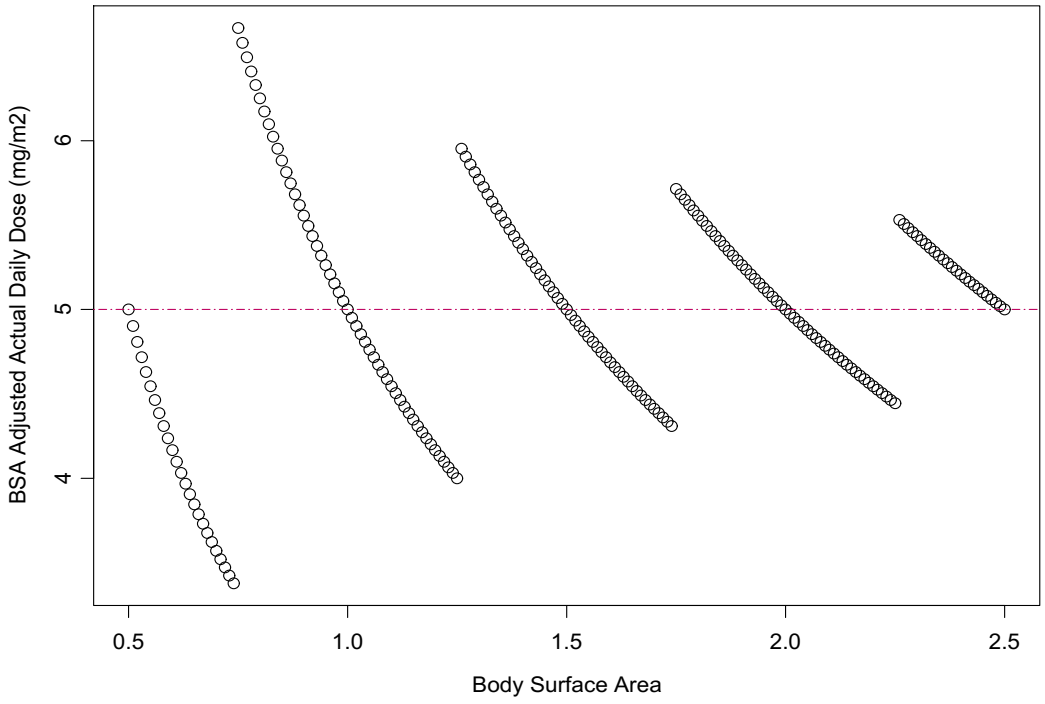
11 Appendices

Appendix 1 BSA Adjusted Daily Dose for RAD001

Target dose: 5 mg/m2 daily

Pill Sizes: 2.5 mg, 5 mg, 10 mg

The plot below illustrates what the BSA adjusted doses will look like if the objective is to get as close to the 5mg/m2 dose as possible.



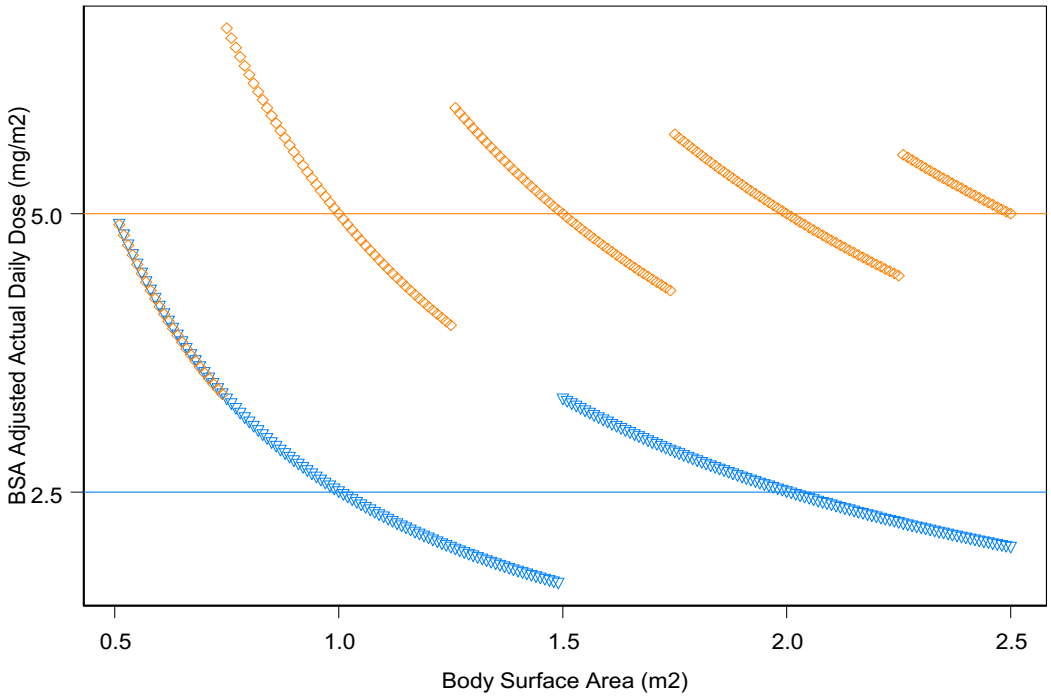
The table below contains the same information as the plot above where each row of the table corresponds to one of the arcs on the plot. The table provides instructions for the optimal dosing strategy for various BSA ranges and the associated mg/m2 doses. For example based on this dosing strategy patients within the 1.26-1.74 m2 BSA range should be given 7.5mg and the resulting BSA adjusted doses for this group will range from 4.31-5.95 mg/m2.

Dosing table for 5mg/m2 daily

BSA Range (m2)	Mg Dose	Mg/m2 Dose Range
0.5-0.74	2.5	3.38-5.00 mg/m^2

0.75-1.25	5	4.00-6.67 mg/m^2
1.26-1.74	7.5	4.31-5.95 mg/m^2
1.75-2.25	10	4.44-5.71 mg/m^2
2.26-2.50	12.5	5.00-5.53mg/m^2

The plot below shows the overlaps for 2.5mg/m2 daily vs. 5mg/m2 based on the minimum pill size of 2.5mg. The orange dots are the BSA adjusted doses for 5mg/m2/day and the blue ones are for 2.5mg/m2/day. The overlap on the far left is the region where dose reduction is not possible on the daily schedule. These are the patients who are already receiving 2.5mg/day total dose (BSA range 0.5-0.74 in the table above) and hence cannot be dose reduced further unless they go to the every other day schedule. The dosing table for the 2.5mg/m2/day dose level is also below.



Dosing table for 2.5mg/m2 daily

Note that patients must have BSA≥0.75m2 in order to be dosed at the 2.5mg/m2/day.

BSA Range (m2)	Mg Dose	Mg/m2 Dose Range

0.75-1.49	2.5	1.68-3.33 mg/m^2
1.50-2.50	5.0	2.0-3.33 mg/m^2

Dosing table for 2.5mg/m^2 every other day

Please use the same dosing table as above for 2.5 mg/m^2 daily but dose will be administered only every other day. No dosing will be possible using 2.5 mg/m^2 every other day if less than 0.75 m^2.

Appendix 2 Performance Status Criteria

Karnofsky		**Lansky**	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
80	*Normal activity with effort; some signs or symptoms of disease.*	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

Appendix 3 PNOC001 Archival Tissue Confirmation Form

This form may be submitted to confirm tissue eligibility at the time of registration.

Patient Name
institutional MRN:
Date of Surgery/Biopsy:
Date of Consent:
Institution Name:
Institution Address:
Contact Person:
Contact Phone #:

Please indicate what type of tissue will be submitted:

☐ Formalin fixed, paraffin embedded (FFPE) tumor material from any prior surgery (pathologist should choose block(s) that is best representative of the tumor.)

- Paraffin block should be in a container labeled with the PNOC patient ID and institutional accession number

OR

☐ Unstained slides tumor material from any prior surgery

- Please provide at least 15 unstained slides (5 um thick) labeled with the PNOC patient ID and institutional accession number.

If available, sites should also collect and submit fresh frozen tissue (see appendix 8).

I certify that the above-mentioned material is available for the stated participant and that tissue will be provided as requested.

_____ _____

Neuropathologist Date

Appendix 4 Imaging Guidelines

Pre-Study Imaging Qualification

The most critical aspect of the advanced imaging being performed in this study is to match quantitative exam protocols prior to the initial treatment and at follow-up studies, so that direct comparisons of parameter values can be made. Clearly each site must also be satisfied that the anatomic imaging sequences being used at these times satisfy clinical criteria for evaluating their patients. Hence, while there should be an attempt to make the protocols as similar as possible between institutions, it may not be feasible for them to be identical, and so any comparisons that are being made will focus on changes within the patient rather than differences between individuals. The images generated should be anonymized and sent to UCSF, either electronically or by CD so that they can be evaluated and confirmation provided that the protocol satisfies the requirements of the study. Sites should batch ship all images pertaining to each patient at the time the patient comes off treatment.

Guidelines for Imaging Protocols

Serial exams should be performed on the same 3T MR system using the commercial 8-channel or other multi-channel head coil. The sequences may either be performed in a pure axial orientation or lined up with the AC-PC line, as is the default in many institutions.

Outline of Protocol:

1. 3-plane localizer
2. T1-weighted pre-Gadolinium images: used as a reference for comparing with the post-Gadolinium images and to identify any sign of hemorrhage.
3. T2-weighted images: used in conjunction with the FLAIR images to define the spatial extent of the T2 lesion.
4. FLAIR images: required for defining treatment response using the RANO criteria.
5. Diffusion weighted images: the entire brain should be covered with at least 6 different gradient directions at b=1000 and with one acquisition having b=0. The slice thickness and spatial resolution should be chosen to allow calculation of maps of apparent diffusion coefficient and fractional anisotropy.
6. Echo planar gradient echo dynamic susceptibility contrast (DSC) images: A series of images should be acquired during the injection of a bolus of 0.1mmol/kg of Gadolinium contrast agent that is delivered at a rate of 3-5ml/s using a power injector and with a 15-20ml flush of normal saline delivered at the same rate. Slice thickness (3-5mm) and location should be chosen to cover as much of the T2 lesion as possible. The injector delay should be set at 15-30s to allow a good definition of baseline intensities from the pre-bolus images.
7. Post-Gadolinium T1-weighted volumetric images: this high-resolution image is used to define the spatial extent of the enhancing volume and for registration between examinations.
8. Post-Gadolinium T1-weighted images: these should match the pre-Gadolinium images are used to define the extent of the enhancing lesion.

Sequences 5, 6 and 7 represent the advanced imaging components that will be used for quantitative analysis of treatment effects. The other anatomic sequences are the ones commonly used for pre-surgery exams in a clinical setting. They will are used for clinical evaluation of the patient and to perform volumetric analysis of the anatomic lesions. The dose and timing of Gadolinium should be kept consistent to facilitate clinical interpretation. Please note that the radiologist at each site should interpret the anatomic images for clinical purposes and send them to UCSF for quantitative analysis.

De-Identification and Labeling

De-Identification of Digital Images

It is the responsibility of the sites to de-identify images according to HIPAA, Institutional Review Board (IRB) guidelines, GCPs and local regulatory requirements.

- Do not remove the date of the exam and the technical information (eg, slice location, kVP, echo time, etc).
- Do not modify time or date information before or during the de-identification process.

Labeling of Digital Images

Use the patient ID, the date (ddmmyy) and scan number (01 or 02 for the two advanced imaging exams) to label the data as follows: PatientID_date_xx

Checklist for Media Submission

- Completed Exam Data Sheet
- De-identified DICOM images

Sending Digital Images via FedEx:

- Sites should batch ship all images pertaining to each patient at the time the patient comes off treatment.
- All MRIs should be de-identified prior to shipment.
- All shipping materials should be provided by the site
- All shipments require signature for delivery
- Include checklist items and address packages to:

Attn: PNOC001
PNOC Operations Office
UCSF - Mission Bay
[redacted]

DATA ANALYSIS PERFORMED AT UCSF

The anatomic images are used to manually define the contrast enhancing lesion (CEL) and the T2 lesion (T2L). The T1 weighted pre-contrast image is used to define a brain mask so that intensity values can be normalized. The diffusion images are processed to generate maps of apparent diffusion coefficient (ADC) and fractional anisotropy (FA). The perfusion data are processed to calculate maps of relative cerebral blood volume (rCBV), peak height (PH) and percentage recovery (RECOV). Both sets of images are registered to the anatomic images and histogram analysis of the map intensities within the CEL and T2L performed. Where relevant the estimated parameters are normalized by the median intensity in normal appearing white matter and used to define metrics for assessing treatment effects as follows:

i) Volumes of the CEL and T2L

ii) Volume within the T2l where nADC < 1.5

iii) Volume within the T2L where nCBV >3

iv) 10^{th} and 25^{th} percentile of nADC in the T2L

v) 75^{th} and 90^{th} percentile of nCBV in the T2L.

We hypothesize that a) high volumetric parameters, low nADC percentiles and high nCBV percentiles at the baseline exam are predictive of poor outcome and b) that early changes in these parameters can predict tumor progression, based upon the RANO criteria.

Appendix 5 PNOC Data Safety and Monitoring

PNOC Data Safety and Monitoring Plan for a Phase 2 Study

It is the responsibility of each PNOC member institution to follow the National Cancer Institute (NCI) approved Data Safety and Monitoring Plan (DSMP) for their site, and to be internally monitored by a Data Safety Monitoring Committee/Board (DSMC/DSMB). In addition to the guidelines laid out in this document, each PNOC member institution must comply with the policies and standards put forward by their own institutional DSMC/DSMB. For member institutions that do not follow an NCI-approved DSMP, the UCSF DSMC will be considered the "institutional DSMC" mentioned in this document. Such institutions will be electronically monitored and visited annually by the UCSF DSMC.

The institutional DSMC/DSMB activities for this study will include:

- Review of subject data
- Review of suspected adverse reactions considered "serious" (SAEs)
- Monitoring every six months (depending on patient accrual)
- Minimum of a yearly regulatory audit

Monitoring and reporting guidelines

All institutional Phase 2 therapeutic studies are designated with a moderate risk assessment. The data is monitored every six months by the institutional DSMC/DSMB, with twenty percent of the subjects monitored (or at least three subjects if the calculated value is less than three).

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) DSMC is responsible for monitoring data quality and patient safety for all HDFCCC institutional clinical studies. In the case of all PNOC protocols, the UCSF DSMC will work together with non-UCSF PNOC member institution DSMC/DSMBs in order to ensure DSMP compliance. Each non-UCSF DSMC/DSMB will be responsible for providing regular monitoring reports to PNOC and the UCSF DSMC. These reports will be used by the UCSF DSMC to assess data quality, patient safety, and protocol compliance as well as to make decisions about dose escalations, where applicable.

PNOC and the UCSF DSMC reserve the right to conduct on-site monitoring at any non-UCSF member institution if DSMP requirements are not being met. If the need to perform a monitoring visit at a non-UCSF member institution arises, source documents will be provided by the member institution prior to the visit in order for the UCSF DSMC to monitor protocol compliance, patient safety, and to verify data entry.

The PNOC Operations Office provides administration, data management, and organizational support for the PNOC member institutions in the conduct of any PNOC clinical trial. The PNOC Operations Office will summarize and communicate adverse events, safety data, and other study matters to the PNOC member institutions on a quarterly basis.

The Study Chair is responsible for the overall conduct of any PNOC trial and for monitoring its safety and progress at all participating sites (as outlined in the PNOC Study Chair and Co-Chair Responsibilities SOP). The Study Chair will conduct continuous review of data and subject safety and discuss each subject's treatment with the PNOC Operations Office. The discussions are documented in the PNOC Operations Office meeting minutes.

Multicenter communication

The PNOC Operations Office will coordinate, at minimum, quarterly conference calls with the PNOC member institutions to discuss risk assessment. The following items will be discussed, as appropriate:

- Enrollment information
- Adverse Events (e.g. new AEs, unresolved AEs, and new safety information)
- Protocol violations
- Other study conduct issues

Adverse event review and monitoring

PNOC uses the web-based OnCore® Clinical Trials Management System for all patient registrations and data entry. The OnCore® System will also track patient level protocol compliance and safety information.

For Phase II studies, all Grade 3-5 AEs will be entered into OnCore®, regardless of relationship.

All Grade 3-5 Adverse Events entered into OnCore® will be reviewed on a monthly basis by the PNOC Operations Office. The PNOC Operations Office will discuss the toxicity, grade, and relationship to study intervention for all AEs in question.

All Adverse Events must be entered into OnCore® within **10 business days** of becoming aware of the event. Member institutions will submit this information to PNOC via the Adverse Event Form within OnCore®.

In addition, all adverse reactions considered “serious” (also called Serious Adverse Events, or SAEs), regardless of relationship, must be entered in OnCore® and reported to the PNOC Operations Office within **1 business day**. SAEs will be reviewed and monitored by the UCSF DSMC on an ongoing basis, and will be discussed at the UCSF DSMC meeting, which take place every six (6) weeks.

If a death occurs during the treatment phase of the study, or within 30 days after the last administration of the study drug(s), and is determined to be related either to the investigational drug or to any research related procedure, the Study Chair and the PNOC Operations Office must be notified by the member institution within **1 business day.** The Study Chair or the PNOC Operations Office must then notify the UCSF DSMC Chair, or qualified alternate, within 1 business day of this notification. The contact may be by phone or e-mail.

Increase in adverse event rates

If an increase in the frequency of Grade 3, 4, or 5 Adverse Events (above the rate reported in the Investigator Brochure or package insert), the Study Chair or the PNOC Operations Office is responsible for notifying the UCSF DSMC at the time the increased rate is identified.

If at any time the Study Chair or the PNOC Operations Office halts enrollment or ends the study due to safety issues, the UCSF DSMC Chair and Manager must be notified within **1 business day** via e-mail. The UCSF DSMC must receive a formal letter within **10 business days,** and the UCSF IRB must be notified.

UCSF data and safety monitoring committee contacts:

UCSF DSMC Chair

[redacted]
UCSF-[redacted]
San Francisco, CA 94143

UCSF DSMC Manager

[redacted]
UCSF-[redacted]
San Francisco, CA 94143

UCSF DSMC Monitors

UCSF[redacted]
San Francisco, CA 94143

Appendix 6 PNOC Institutions Required Regulatory Documents

Prior to opening a study at any member institution, the following regulatory documents must be submitted to the PNOC Operations Office:

- Participating Site IRB approval(s) for the protocol, appendices, informed consent form and HIPAA authorization
- Participating Site IRB approved consent form
- Participating Site IRB membership list
- Participating Site IRB's Federal Wide Assurance number and OHRP Registration number
- Copy of the 1572 (Note: 1572 form is not required for FDA-exempt trials)
- Curriculum vitae and medical license for each investigator and consenting professional
- Documentation of Human Subject Research Certification training for investigators and key staff members at the Participating Site
- Participating site laboratory certifications and normals
- Signed copy of the completed delegation of authority log (found in PNOC Documents > Forms)
- Signed copy of the protocol signature page
- Signed copy of the final contract
- Confirmation of a "Site Initiation Visit" (SIV) teleconference

Upon receipt of the required documents, the PNOC Operations Office will formally contact the site and grant permission to proceed with enrollment. All documents can uploaded directly to SharePoint by navigating to your site's page and clicking "Add Documents".

Each PNOC site is responsible for ensuring all regulatory documents in SharePoint are up to date. Sites will upload new or revised documents as applicable to reflect any changes, including changes in staff and approved/expired documents.

Appendix 7 Required Data and Time Table for Submission

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 days of registration
Baseline Assessment Forms	Within 14 days of registration
Treatment Forms	Within 10 days of the last day of the cycle
Adverse Event Report Forms	Within 10 days of the last day of the cycle
Serious Adverse Event Reporting	Please refer to section 7.6 Expedited Reporting
Response Assessment Forms	Within 10 days of the completion of the cycle required for response evaluation
Off Treatment/Off Study Forms	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 14 days of the protocol defined follow up visit date

Appendix 8 PNOC Specimen Collection and Shipping

Research Tissue (required)

Depending on the stage of the protocol, PI3K activation status needs to be known prior to trial enrollment. A pre-screening process is now integrated into PNOC001 trial enrollment. To establish activation status for the PI3K a minimum of 2 unstained slides needs to be provided. Once the patient is deemed to be eligible additional tumor material needs to be submitted to UCSF. Tumor samples (at least 15 unstained slides or a tissue block) from a prior surgery will be used to test for molecular analyses. A completed Archival Tissue Confirmation Form (appendix 3) or an email confirmation may be submitted at the time of registration to confirm tissue availability. The tissue itself must be submitted within 90 days of registration. It is the responsibility of each participating institution to request and submit this tissue.

For details and shipping instruction refer to the PNOC Member's SharePoint website under the documents section.

Fresh Frozen Tissue (if available)

If fresh frozen tissue is available we strongly encourage sending such frozen tissue with the paraffin embedded tissue. The study will provide adequate shipping boxes. For details and shipping instruction refer to the PNOC Member's SharePoint website under the documents section.

Research Blood (required)

In addition to tumor tissue, blood samples from subjects will be obtained prospectively within 90 days of registration for genomic profiling studies. At least one (1) blood sample (approximately 8.5 ml) from the subject (research related, obtained for the purpose of this study).

After the eligibility of a patient is confirmed and the patient is enrolled in study, the enrolling site will be provided with blood collection tubes for subsequent DNA isolation. For details and shipping instruction refer to the PNOC Member's SharePoint website under the documents section.