Phase II Trial of RAD001 in Patients with Recurrent Low Grade Glioma

RAD001

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List of abbreviations

4E-BP1	4E-binding protein
ADR	Adverse Drug Reaction
AE	adverse event
ALT/SGPT	alanine aminotransferase/glutamic pyruvic transaminase/Serum glutamic-pyruvic transaminase
AST/SGOT	aspartate aminotransferase/glutamic oxaloacetic transaminase/Serum glutamic-oxaloacetic transaminase
ATC	Anatomical Therapeutic Chemical classification system
AUC	Area under the plasma-concentration time curve
BAC	Bronchoalveolar carcinoma
Cmax	Maximum plasma concentration
CR	Clinical research
CRF	Case report/Record form
CRO	Contract Research Organization
CT	Computer tomography
CTC	Common toxicity criteria
CV	Coefficient of Variation
CYP3A4	CytochromeP450 3A4 isoenzyme
DLT	Dose limiting toxicity
ECG	Electrocardiogram
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
eIF-4E	Eucariotic Initiation Factor 4E
EPR	Early progression rate
FDG-PET	Fluorine-18-2-fluoro-Deoxy-D-Glucose Positron Emission Tomography
FKBP-12	FK506-binding protein 12
GF	Growth factor
HDL	High-density lypoproteins

HER	Human Epidermal Receptor
HUVECS	human umbilical endothelial cells
IC50	Inhibitory concentration at 50%
IEC	Independent Ethics Committee
IGF1-R	Insulin-like Growth Factor 1 Receptor
IHC	immunohistochemistry
INN	International Non-proprietary Name
INR	International Normal Ratio
IRB	Institutional Review Board
LC-MS	liquid chromatography method with mass spectrometry
LDL	Low-density lypoproteins
LLOQ	Lower limit of quantification
MAPK	Mitogen Activated Protein Kinase
mRNA	messenger Ribonucleic acid
mTOR	mammalian Target of Rapamycin
NIH/NCI	National Institutes of Health/National Cancer Institute
nM	nano-molar
NSCLC	Non-small cell lung cancer
OS	overall survival
P-AKT	phosphor-AKT
PD	Pharmacodynamics
PET	Proton emission tomography
PFS	progression free survival
P-gp	P-glycoprotein
PI3K	Phosphoinositide 3-kinase
PK	Pharmacokinetics
PK/PD model	Pharmacokinetic/pharmacodynamic model
PT/PTT	prothrombin time
PTEN	Phosphatase and Tensin homolog deleted on chromosome 10
RBC	red blood cell count
REB	Research Ethics Board
RR	response rate
S6K1	S6 kinase 1
SAE	serious adverse event
SCLC	Small cell lung cancer
STAT3	Signal Transducer and Activator of Transcription 3
TK	Tyrosine kinase

TSC2	Tuberous Sclerosis Complex 2
TUNNEL	Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin Nick End Labeling
ULN	upper limit of normal
VEGF	Vascular Endothelial Growth Factor
WBC	total white blood cell count
WHO	World Health Organization

1 Introduction

1.1 RAD001 (everolimus)

RAD001 (everolimus) is a novel oral derivative of rapamycin.

RAD001 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. RAD001 has been in development for patients with various malignancies since 2002. RAD001 2.5mg, 5mg and 10mg tablets were approved under the trade name Afinitor® for patients with advanced renal cell carcinoma (RCC) after failure of treatment with Sutent® (sunitinib) or Nexavar® (sorafenib) in the US, EU and several other countries and is undergoing registration in other regions worldwide. Afinitor® was also recently approved for the treatment of patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis (TS) who require therapeutic intervention but are not candidates for curative surgical resection.

RAD001 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
- Indirectly by inhibiting angiogenesis leading to reduced tumor vascularity (via 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 proangiogenic factors as well as modulation of VEGFR signaling in endothelial cells.

1.1.1 mTOR pathway and mechanism of action

At cellular and molecular levels, RAD001 acts as a signal transduction inhibitor. RAD001 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 RAD001 (Reviewed in Boulay and Lane 2007).

mTOR is downstream of 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; through PI3K mutation/amplification; through AKT/PKB overexpression/overactivation; through 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.

The main known functions of mTOR include the following (Bjornsti and Houghton 2004; Boulay and Lane, 2007):

- 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) (Wullschleger et al. 2006).

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) (Bjornsti and Houghton 2004). 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 (Manning and Cantley 2007; Wang, et al. 2007).

mTORC2 (mTOR complex 2) is activated through a currently unknown mechanism, possibly by receptor tyrosine kinase (RTK) signaling (Manning and Cantley 2007). 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 (Wullschleger et al. 2006).

mTORC1-mediated signaling is subject to modulation by the macrocyclic lactone rapamycin and its derivatives, such as RAD001. 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 (Sabatini 2006)]. 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 (Bjornsti and Houghton 2004).

1.1.2 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 (Boulay and Lane, 2007). 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 (Francesc 1999, Yu 1999, Lane et al 2009) and have antiangiogenic activity in vivo (Guba 2002, Tsutsumi 2004, Mabuchi et al 2007, Lane et al 2009). Exactly which molecular determinants predict responsiveness of tumor cells to everolimus is still unclear. Currently, the activation status of the PI3K/AKT/mTOR/p70 S6K 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 Boulay and Lane, 2007, Mabuchi et al 2007). 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 (Galanis, et al 2005).

Everolimus is a highly specific inhibitor of mTOR, which is afforded by high-affinity binding to the protein FKBP-12 (IC_{50} 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 antiproliferative 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" (IC_{50} range 0.2 to 4125 nM) (O'Reilly and McSheehy, 2009). The median IC_{50} value of the 48 cell

lines was 0.5 nM. Similar findings have been observed for rapamycin (Huang and Houghton 2002). Everolimus was also shown to have activity in human pancreatic neuroendocrine cells, where induction of apoptosis was reported (Zitzmann, et al 2007), as well as in acute myeloid leukemia cells (Zeng, et al 2007), mantle cell lymphoma cells (Haritunians, et al 2007), adult T-cell leukemia cells (Ikezoe, et al 2007), diffuse large B cell lymphoma cells (Wanner, et al 2006), pancreatic tumor cells (Tuncyurek, et al 2007), ovarian cancer cells (Treeck, et al 2006, Mabuchi, et al 2007) and hepatocellular carcinoma cells (Sieghart, et al 2007).

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.

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Everolimus was effective and well tolerated against subcutanuous (s.c.) 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. Antitumor activity of everolimus has also been demonstrated in mouse models of ovarian (Mabuchi, et al 2007), breast (Lu, et al 2007, Torres-Arzayus, et al 2007) and gastrointestinal stromal tumors (Rossi, et al 2006).

Pre-clinical 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.

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.

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 women 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 [Investigator‟s Brochure].

1.1.3 Clinical experience

1.1.3.1 RAD001 Pharmacokinetics

Everolimus is rapidly absorbed with a median t_{max} of 1-2 hours. The steady-state $AUC_{0-\tau}$ 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. C_{max} 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 C_{max} 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 C_{max} by 54%. Light fat

meals reduced AUC by 32% and C_{max} 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 $AUC_{0-\tau}$ and pre-dose trough concentration at steady-state on the daily regimen. The mean elimination half-life of everolimus is approximately 30 hours.

Table 3-3 (Section 3.3.3) lists examples of clinically relevant CYP3A inhibitors and inducers.

Please refer to Section 3.3.3 for more information on the concomitant use of CYP3A4 inhibitors/inducers and other medications.

More information on RAD001 pharmacokinetics is provided in the [Investigator"s Brochure].

1.1.3.2 RAD001 Pharmacodynamic studies

Pharmacokinetic/pharmacodynamic modeling based on inhibition of the biomarker p70S6 kinase 1 [S6K1] 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], Lane et al 2003). In molecular changes in tumor were evaluated through serial biopsying before and during treatment. Biopsying on treatment took place in 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 pelF-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], Tabernero, 2008)

More information is provided in the [Investigator"s Brochure].

1.1.3.3 Clinical experience with RAD001

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. 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.

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. Please note that safety pharmacology and toxicology studies as well as some human pharmacology studies which have been conducted in support of the transplant indication, are described in the oncology IB due to the relevance of these data for the oncology indication. Malignancies that are currently being evaluated in Novartis sponsored studies include the following: metastatic renal cell carcinoma (mRCC), breast cancer, gastroenteropancreatic neuroendocrine tumors (GEP-NET), mantle cell lymphoma 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 Angiomyolypoma is also being evaluated. Colorectal cancer (CRC) is no longer being evaluated.

Everolimus 2.5mg, 5mg and 10mg tablets were approved under the trade name Afinitor® for patients with advanced renal cell carcinoma in the US, EU and several other countries and is undergoing registration in other regions worldwide. Recent approval was granted in the US for the treatment of patients with subependymal giant cell astocytoma (SEGA) associated with tuberous sclerosis (TS) who require therapeutic intervention but are not candidates for curative surgical resection.

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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 12,700 cancer patients have been treated with everolimus as of 30 SEP 2010:

- 5559 patients in Novartis-sponsored clinical trials
- 712 patients in the single patients use IND program for renal cell cancer
- 5474 in investigator-sponsored studies.
- In addition, healthy volunteer subjects have participated in the clinical pharmacology studies as described in Section 5.2.

As of 30 SEP 2010, there are a total of 11 Phase III trials ongoing in the indications mRCC (1), advanced NET (2), breast cancer (3), TSC (2), DLBCL (1), gastric cancer (1) and hepatocellular carcinoma (1). One additional Phase III trial in the TCS will be starting later in

2011. In addition, the Phase II SEGA in Tuberous Sclerosis study remains ongoing for follow-up.

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. Safety data from study [C2240] are described in detail in Section 6.5.

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 [Investigator‟s Brochure].

1.1.3.4 Gliomas

The incidence of primary brain tumors in the United States (US) is about 17,000 per year, with a case fatality of about 10,000 per year. Gliomas account for up to 80% of primary brain tumors and they account for significant mortality. Gliomas can be classified as either high-grade (or malignant), or low-grade. The low-grade gliomas (LGG) themselves are a diverse group of glial tumors with an annual incidence in the US is about 1800 cases (*CBTRUS: Statistical Report* 2002). Histologic subtypes of LGG include diffuse astrocytomas (WHO grade II astrocytomas), oligodendrogliomas and mixed oligoastrocytomas Ashby et al. 2004). The majority of these tumors are of mixed histology.

Despite medical management with surgery and irradiation the median 10-year survival rates for LGG range from 17-49%. Survival is limited by recurrence and progression of LGG to high-grade gliomas. Factors influencing survival in these patients include histologic subtype, age, extent of surgical resection, and presence of 1p 19q status (Pignatti et al 2002; Yeh et al. 2005; Smith et al. 2000. One particular subtype of LGG, the pilocytic astrocytomas, usually occurs in the cerebellum of children. These tumors are often completely resectable and are associated with a 10-year survival of >80%. For the purposes of this project only supratentorial non-pilocytic LGG in adults will be considered.

The management of patients with residual low grade glioma following surgical resection is not standardized (Lang et al. 2006). Options include surveillance scans and treatment at the time of tumor progression, versus the use of radiation therapy soon after the surgical procedure. Adjuvant chemotherapy following radiation is not considered a standard approach (Grier and Batchelor, 2003).

Low grade gliomas are diffuse in nature and the treatment fields for radiation therapy can be large. The potential complications of radiation therapy to the brain include seizures, cognitive decline, endocrinopathies, necrosis and vasculopathies. In addition, any of a number of secondary malignancies may arise after radiation treatment including meningiomas, gliomas and sarcomas. Given the potential for long term survival of patients with LGG and the morbidity associated with radiation therapy, alternative approaches in this setting are appealing and are being evaluated (Grier and Batchelor, 2003).

In an effort to reduce the use of radiotherapy, several studies have evaluated the use of chemotherapy as initial treatment of LGG. Tumor regressions using a combination of procarbazine, lomustine and vincristine in patients with an oligodendroglial component has been demonstrated (Buckner et al. 2003; Stege at al. 2005). More recently, temozolomide showed activity as treatment for recurrent LGG. However, the role of chemotherapy as up-front treatment of LGG continues to be evaluated (Grier and Batchelor, 2003; Hoang-Xaun et al. 2004; Brada et al. 2003).

The role of chemotherapy in adult patients with recurrent LGG has also not been extensively investigated (Quinn et al. 2003; Pace et al. 2003; Soffietti et al. 1998). Nitrosourea based chemotherapy and temozolomide have both been evaluated. In small studies, temozolomide

appears to have response rates ranging from 47-61% and 1 year progression free survival from 39-76% (Quinn et al. 2003; Pace et al. 2003). Although these numbers appear promising for the treatment of recurrent LGG with chemotherapy, other treatment strategies are needed for this patient population, especially in the setting of progression to malignant glioma. With a better understanding of the signaling pathways important for tumor growth and invasion, novel targeted therapies are being evaluated.

1.1.3.5 PTEN methylation and PKB/Akt phosphorylation in gliomas

Alterations in the tumor suppressor protein PTEN are common in gliomas and result in activation of the PI3K pathway, as evidenced by phosphorylation of PKB / Akt. Whereas mutation of PTEN is common in *de novo* malignant gliomas (Choe et al. 2003; Rasheed et al. 1997; Ermoian et al. 2002), methylation of the PTEN promoter is thought to be the underlying mechanism of PTEN alteration found in low-grade gliomas and secondary malignant gliomas (Wiencke et al. 2007).

In fact, methylation of the PTEN promoter occurs frequently in low-grade gliomas. Whereas there is no evidence of PTEN promoter methylation in normal brain and only 9% methylation in *de novo* glioblastiomas, low-grade tumors displayed methylation of PTEN promoter in 43-67% of low-grade glioma cases, and in 68-82% of secondary high grade glioma cases. The differences in PTEN promoter methylation frequencies for low-grade gliomas were highly statistically significant ($P<0.001$) (Wiencke et al 2007).

Not only is methylation of the PTEN promoter frequent and associated specifically with low-grade gliomas and secondary malignant gliomas, there is evidence that PTEN methylation also leads to functional activation of the PI3K pathway, which is thought to be important for tumor transformation and growth (Wiencke et al. 2007). Furthermore, a retrospective study of 43 LGG (grade II) tumor specimens from newly diagnosed patients show a trend towards decreased overall survival in low grade glioma patients with PTEN promoter methylation compared to those without methylation (McBride et al. 2007).

Because of the clinical significance of PTEN promoter methylation and its effects on the PI3K pathway, therapies that target tumors with PI3K activity may be of clinical benefit in low-grade gliomas. The mammalian target of rapamycin (mTOR) is downstream to the (PI3K) / PTEN-AKT survival pathway and is therefore an ideal target for low-grade glioma and secondary malignant glioma patients with PTEN promoter methylation (Choe et al. 2003; Xu et al. 2004; Neshat et al. 2001). In fact, there is evidence that tumors expressing activated PKB/Akt appear particularly sensitive to mTOR inhibition (Gera et al. 2004; Noh et al. 2004).

1.1.3.6 Clinical experience with mTOR inhibitors for gliomas

There is extensive clinical experience with mTOR inhibitors including the evaluation in the treatment of patients with malignant glioma (Reardon et al. 2006). Perhaps most exciting are recent studies in which patients with Tuberous Sclerosis Complex (TSC) and low-grade

gliomas were treated with an mTOR inhibitor (rapamycin most commonly), resulting in tumor regression in every treated patient. In one patient treatment was interrupted, resulting in tumor re-growth, followed by further regression when treatment with the mTOR inhibitor resumed (Franz et al. 2006). The success of this approach adds credence to our plan to treat low-grade gliomas with RAD001 because of the genetic underpinnings of low-grade gliomas in TSC patients. In brief, mutations in either hamartin or tuberin, the tuberous sclerosis gene products, result in elevated mTOR signaling. As a result, afflicted individuals develop hamartomatous growths in multiple organs, and 5-15% of TSC patients develop low-grade central nervous system neoplasms in the form of subependymal giant cell astrocytomas (SEGAs). Although SEGAs rarely respond to radiation or chemotherapy, striking tumor regressions were documented after treatment with mTOR inhibitor in these TSC patients.

1.4 Rationale

We have shown that methylation of the PTEN promoter occurs frequently in low-grade gliomas and secondary glioblastomas, and is associated with PI3K signaling and PKB/Akt phosphorylation (Wiencke et al. 2007). Given these findings, and the link between (mTOR) and the (PI3K) / PTEN-AKT survival pathway, we hope that RAD001 will also prove efficacious in the treatment of low-grade gliomas and secondary malignant gliomas as delineated in the study proposed herein.

In this study, molecular features, including PTEN status and PKB/Akt phosphorylation will be assessed prospectively in this trial to test the hypothesis that those tumors with PTEN methylation and consequent PKB/Akt phosphorylation will preferentially respond to mTOR inhibition.

1.5 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 (Just and Thelen 1988; Dean et al. 1990; Ginsberg et al. 1996). 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 (Earnest et al. 1988; Kleihues et al. 1993). 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.

2 Study objectives

Primary

To determine progression-free survival at 6 months associated with use of RAD001 in patients initially diagnosed with low-grade glioma who undergo biopsy or subtotal resection at the time of recurrence with pathologic evidence of recurrent low-grade glioma.

Secondary

1. To further delineate the safety profile of RAD001 in patients with recurrent LLG.

2. To assess overall survival (OS) in patients treated with RAD001.

3. To assess the Objective Response rate (ORR) in patients treated with RAD001.

4. To assess the correlation of phosphorylated PKB/Akt and PTEN expression with response, progression status by 6 months, and OS in patients treated with RAD001.

Exploratory

1. To determine progression-free survival at 6 months associated with use of RAD001 in patients initially diagnosed with low-grade glioma who have already received radiotherapy, and who undergo biopsy or subtotal resection at the time of recurrence with pathologic evidence of high-grade glioma.

2. Pending adequate funding, to assess the ability of metabolic and physiologic imaging parameters such as MR spectroscopy, perfusion-weighted imaging, and diffusion-weighted imaging to predict clinically-relevant endpoints such as time to progression and survival.

3 Investigational plan

3.1 Overall study design

A single-arm, one-stage phase II trial of RAD001 will be undertaken. Sixty patients will be enrolled. The target population will be patients with a diagnosis of low-grade glioma who experience a recurrence and who undergo a biopsy or subtotal resection at the time of recurrence with pathologic evidence of recurrent glioma. The purpose of this study is to accrue patients to evaluate a pharmacologic agent.

3.2 Study population

3.2.1 Patient population

It is anticipated that most low-grade glioma patients completing surgical resection for recurrence will be eligible for enrollment and will choose to participate. Based on current patient volume in the UCSF neurologic-oncology clinic, it is expected that a minimum of 3 patients per month will be enrolled. Therefore, accrual can be completed in a 20 month period. Patients will be recruited with no preference to gender. Minorities will actively be recruited to participate. In order to evaluate our primary objective, enrollment will continue until at least 40 low-grade glioma patients have enrolled.

3.2.2 Inclusion and exclusion criteria

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. Results of all baseline evaluations, which assure that all inclusion and exclusion criteria have been satisfied, must be reviewed by the Principal Investigator or his/her designee prior to enrollment of that patient. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

3.2.2.1 Baseline Characteristics Inclusion criteria

- Patients must have a Karnofsky performance status of ≥ 60
- Patients must have a life expectancy > 8 weeks
- Patients must be ≥ 18 years old
- All patients must sign an informed consent document indicating that they are aware of the investigational nature of this study
- Patients must sign an authorization for the release of their protected health information
- Patients must have an MRI scan performed within 14 days prior to initial protocol treatment
- Patients must be registered in the UCSF Neuro-Oncology database prior to treatment with study drug
- Adequate bone marrow function as shown by: ANC $\geq 1.5 \times 10^9$/L, Platelets $\geq 100 \times 10^9$/L, Hb >9 g/dL
- Adequate liver function as shown by:
  - serum bilirubin ≤ 1.5 x ULN
  - INR < 1.5 (Anticoagulation is allowed if target INR ≤ 1.5 on a stable dose of warfarin or on a stable dose of LMW heparin for > 2 weeks at the time of registration)
  - ALT and AST ≤ 2.5x ULN

- Adequate renal function: serum creatinine ≤ 1.5 x ULN
- Fasting serum cholesterol ≤300 mg/dL OR ≤7.75 mmol/L AND fasting triglycerides ≤ 2.5 x ULN. NOTE: In case one or both of these thresholds are exceeded, the patient can only be included after initiation of appropriate lipid lowering medication.

3.2.2.2 Histologic Inclusion Criteria

- Patients must have histologically proven intracranial low-grade glioma at initial diagnosis. Low-grade gliomas include: astrocytoma, oligodendroglioma and mixed oligoastrocytoma. Pilocytic astrocytomas are excluded
- Patients must have unequivocal evidence for tumor recurrence or progression by histology as determined by review of pathology by an attending neuro-pathologist at UCSF
- If most recent histology shows progression to high grade glioma, patients must have had prior radiotherapy in order to be eligible
- Paraffin-embedded sections of tissue acquired from surgery at the time of suspected recurrence must be available for analysis

3.2.2.3 Radiographic Inclusion Criteria

- Patients must have evidence for tumor recurrence or progression by MRI as determined by radiographic review of images by an attending neuro-oncologist or neuro-radiologist at UCSF

- If the steroid dose is increased between the date of the MRI and registration on the trial, a new baseline MRI is required. This MRI must be performed after ≥ 5 days on a stable or decreasing dose of steroids
- An MRI must be used throughout the period of protocol treatment for tumor measurement
- Patients must have evaluable disease

3.2.2.4 Prior Therapy Inclusion Criteria

- Patients may have had treatment (including radiotherapy) for any number of relapses prior to this recurrence
- Patients must be at least 4 weeks from the completion of any radiation therapy
- Patients must be less than 4 months from the surgical procedure for this recurrence
- Patients must have recovered from the toxic effects of prior therapy:
    1. 4 weeks from any investigational agent.
    2. 4 weeks from prior cytotoxic therapy (except 6 weeks from nitrosoureas, 3 weeks from procarbazine, 3 weeks from vincristine)

3. 3 weeks for non-cytotoxic or biologic agents e.g., interferon, tamoxifen, thalidomide, cis-retinoic acid, tarceva, etc. Note a 3-week washout is required for prior treatment with Bevacizumab

3.2.2.5 Exclusion criteria

- Patients, who have not recovered from the side effects of a major surgery or significant traumatic injury or patients that may require major surgery during the course of the study
- Patients receiving chronic, systemic treatment with corticosteroids or another immunosuppressive agent. Topical or inhaled corticosteroids, and treatment with low dose Decadron (≤ 3mg daily) are allowed.
- Other than surgery, patients may not have therapy for this recurrence (including radiation). Supportive care such as steroids or anti-epileptics does not constitute treatment of recurrence
- Patients must not have any significant medical illnesses that in the investigator"s opinion cannot be adequately controlled with appropriate therapy or would compromise the patient"s ability to tolerate this therapy.
- Patients with a history of any other cancer (except for adequately treated carcinoma of the cervix or basal or squamous cell carcinomas of the skin), unless in complete remission and off of all therapy for that disease for a minimum of 3 years are ineligible.
- 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.
- Uncontrolled brain or all leptomeningeal metastases, including patients who continue to require glucocorticoids for brain or leptomeningeal metastases
- Patients who have any severe and/or uncontrolled medical conditions or other conditions that could affect their participation in the study such as:
    - Symptomatic congestive heart failure of New York heart Association Class III or IV
    - unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction within 6 months of start of study drug, serious uncontrolled cardiac arrhythmia or any other clinically significant cardiac disease
    - severely impaired lung function
    - uncontrolled diabetes as defined by fasting serum glucose >1.5 x ULN (Note: Optimal glycemic control should be achieved before starting trial therapy.)
    - active (acute or chronic) or uncontrolled severe infections
    - liver disease such as cirrhosis or severe hepatic impairment (Child-Pugh class C).

- A Hepatitis B/C blood test must be done at screening for all patients. Patients who test positive for Hepatitis C antibodies and the Hepatitis B antigen are ineligible
- A known history of HIV seropositivity
- Impairment of gastrointestinal function or gastrointestinal disease that may significantly alter the absorption of RAD001 (e.g., ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome or small bowel resection)
- Impaired lung function: O_2 saturation 88% or less at rest on room air by Pulse Oximetry. If O_2 saturation is $\leq$ 88% at rest, further pulmonary function tests (PFTs) should be ordered to confirm normal pulmonary function and eligibility.
- Patients with an active, bleeding diathesis
- Female patients who are pregnant or breast feeding, or adults of reproductive potential who are not using effective birth control methods. Adequate contraception must be used throughout the trial and for 8 weeks after the last dose of study drug, by both sexes. (Women of childbearing potential must have a negative urine or serum pregnancy test within 7 days prior to administration of RAD001)
- Male patient whose sexual partner(s) are WOCBP who are not willing to use adequate contraception, during the study and for 8 weeks after the end of treatment
- Patients who have received prior treatment with an mTOR inhibitor (e.g., sirolimus, temsirolimus, everolimus).
- Patients with a known hypersensitivity to RAD001 (everolimus) or other rapamycins (e.g., sirolimus, temsirolimus) or to its excipients
- History of noncompliance to medical regimens
- Patients unwilling to or unable to comply with the protocol

3.2.3 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 RAD001 must be interrupted because of unacceptable toxicity, drug dosing will be interrupted or modified according to rules described in Table 3-1. Toxicity will be assessed using the NIH-NCI Common Terminology Criteria for Adverse Events, version 3.0 (CTCAEv3.0, (http://ctep.cancer.gov/forms/CTCAEv3.pdf)).

Table 3.0 RAD001 dose level modification guidelines

Dose level	Dose and schedule
0 (starting dose)	10 mg daily
-1	5 mg daily
-2	5 mg every other day

Table 3-1 Criteria for dose-modification in case of suspected RAD001 toxicity and re-initiation of RAD001 treatment

Toxicity	Actions
Non-hematological toxicity	
Grade 2 (except pneumonitis – refer to Table 3-2)	**If the toxicity is tolerable to the patient, maintain the same dose. If the toxicity is intolerable to patient, interrupt RAD001 until recovery to grade ≤1. Then reintroduce RAD001 at same dose. If event returns to grade 2, then interrupt RAD001 until recovery to grade ≤1. Then reintroduce RAD001 at the lower dose level.**
Grade 3 (except hyperlipidemia*) (except pneumonitis – refer to Table 3-2)	**Interrupt RAD001 until recovery to grade ≤1. Then reintroduce RAD001 at the lower dose level. For pneumonitis consider the use of a short course of corticosteroids.**
Grade 4	**Discontinue RAD001.**
Hematological toxicity	
Grade 2 Thrombocytopenia (platelets <75, ≥ 50x10^9/L)	**Interrupt RAD001 until recovery to grade ≤1 (>75 x10^9/L). Then reintroduce RAD001 at initial dose. If thrombocytopenia again returns to grade 2, interrupt RAD001 until recovery to grade ≤1. Then reintroduce RAD001 at the lower dose level.**
Grade 3 Thrombocytopenia (platelets <50, ≥ 25 x10^9/L)	**Interrupt RAD001 until recovery to grade ≤1 (platelets ≥ 75 x10^9/L). Then resume RAD001 at one dose level lower. If grade 3 thrombocytopenia recurs, discontinue RAD001.**
Grade 4 Thrombocytopenia (platelets < 25 x10^9/L)	**Discontinue RAD001.**
Grade 3 Neutropenia (neutrophils <1, ≥0.5 x10^9/L)	**Interrupt RAD001 until recovery to grade ≤1 (neutrophils ≥ 1.5 x 10^9/L). Then resume RAD001 at the initial dose. If ANC again returns to Grade 3, hold RAD001 until the ANC ≥ 1.5 x 10^9/L. Then resume RAD001 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 RAD001 until recovery to grade ≤ 1 (neutrophils ≥ 1.5 x 10^9/L). Then resume RAD001 at the lower dose level. If grade 3 or grade 4 neutropenia occurs despite this dose reduction, discontinue RAD001.**
Grade 3 febrile neutropenia (not life-threatening)	**Interrupt RAD001 until resolution of fever and neutropenia to grade ≤ 1. Hold further RAD001 until the ANC ≥ 1,500/mm^3 and fever has resolved. Then resume RAD001 at the lower dose level. If febrile neutropenia recurs, discontinue RAD001.**
Grade 4 febrile neutropenia (life-threatening)	**Discontinue RAD001.**
Any hematological or non-hematological toxicity requiring interruption for ≥ 28 days.	**Discontinue RAD001**

*Grade 3 hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia) should be managed using medical therapies (see Sec. 3.2.5.2).

3.2.4 Monitoring of RAD001 suspected toxicities

Patients whose treatment is interrupted or permanently discontinued due to an adverse event or abnormal laboratory value suspected to be related to RAD001 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 > 28 days from the intended day of the next scheduled dose, then the patient must be discontinued from the study.

3.2.5 Known Undesirable Side Effects of RAD001

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 dyspnoea. The most common grade 3-4 adverse reactions (incidence ≥2%) were infections, stomatitis, fatigue, and pneumonitis.

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/flare of Hepatitis B (HBV) has been observed in patients with cancer receiving chemotherapy (Yeo 2004). 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 (Loomba 2008). A detailed assessment of Hepatitis B/C medical history must be done for all patients at screening, with testing performed prior to the first dose of everolimus.

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 are not allowed.

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 RAD001.

Table 3-1 provides general recommendations for the management of patients, with suspected drug toxicities while on treatment with RAD001 as single-agent therapy.

More detailed information regarding RAD001 reported suspected toxicities and individual cases is provided in the [Investigator"s Brochure].

3.2.5.1 Management of stomatitis/oral mucositis/mouth ulcers

Stomatitis/oral mucositis/mouth ulcers due to RAD001 should be treated using local supportive care.

When described, the disorder has been identified as inflammation or ulcers in the mouth. If exam reveals mouth ulcers, rather than a more general inflammation of the mouth, please classify the adverse event as „mouth ulcers". If inflammation is limited to the mouth without ulcers please use the term „stomatitis" rather than the less specific term „mucositis".

If the disorder is elsewhere than the mouth please describe the location as well as any specific procedures carried out for exploration (e.g. endoscopy).

Please use the Grading according to the NIH-NCI Common Terminology Criteria for Adverse Events, Version 3.0 (CTCAEv3.0; http://ctep.cancer.gov/forms/CTCAEv3.pdf).

Grade 1: minimal symptoms; normal diet

Grade 2: symptomatic but can eat and swallow modified diet

Grade 3: symptomatic and unable to adequately aliment or hydrate orally

Grade 4: symptoms associated with life-threatening consequences

Recommendations

1. To help avoid these effects patients should brush their teeth with a very soft toothbrush and if gums bleed patients should be instructed to brush their teeth with gauze instead, use an alcohol-free mouthwash up to 3 times a day, eat soft bland foods like puddings milkshakes and cream soups, avoid spicy, crunchy, acidic and very hot foods.
2. For mild toxicity (Grade 1), use conservative measures such as non-alcoholic mouth wash or salt water (0.9%) mouth wash several times a day until resolution.
3. 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% (e.g. Kenalog in Orabase®).

Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents. 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 RAD001 metabolism, therefore leading to higher RAD001 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.

3.2.5.2 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.

3.2.5.3 Management of Hepatitis B reactivation

If any patients experience reactivation of hepatitis B during treatment, the patient will be removed from study protocol and referred to the appropriate specialist for management of Hepatitis B.

3.2.5.4 Management of non-infectious pneumonitis

Non-infectious pneumonitis is a class effect of rapamycin derivatives. Cases of non-infectious pneumonitis (including interstitial lung disease) have also been described in patients taking everolimus (see Section 5 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 dyspnoea, 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.

Both asymptomatic radiological changes (grade 1) and symptomatic non-infectious pneumonitis (grade 2 = not interfering with activities of daily living or grade 3 = interfering with activities of daily living and oxygen indicated) have been noted in patients receiving RAD001 therapy. Non-infectious pneumonitis has been associated with RAD001 and other mTOR inhibitors (Atkins 2004). In order to monitor for asymptomatic (grade 1) pulmonary infiltrates, a chest X-ray is required if a CT scan of chest is not used for bi-monthly disease evaluations. Additional chest CT scans may be performed, when clinically necessary. If non-infectious pneumonitis develops, a consultation with a pulmonologist should be considered. If the patient develops grade 3 pneumonitis, treatment with RAD001 should be interrupted and the patient should be treated as medically indicated (short course corticosteroids, oxygen, etc).Management of non-infectious pneumonitis suspected to be associated with RAD001 and dose modifications instructions are provided in Table 3-2

Table 3-2 Management of non-infectious pneumonitis

Worst Grade Pneumonitis	Required Investigations	Management of Pneumonitis	RAD001 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 RAD001 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 RAD001 dose until recovery to ≤ Grade 1. RAD001 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.**

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

It will be documented 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. Reasons that a patient may discontinue participation in a clinical study are considered to constitute one of the following:

1. After administration of 24 cycles
2. adverse event(s) including re-activation of Hepatitis B
3. abnormal laboratory value(s)
4. abnormal test procedure result(s)

5. disease progression
6. protocol violation
7. subject withdrew consent
8. lost to follow-up
9. administrative problems
10. Medical or psychiatric illness which in the investigator's judgment renders the patient incapable of further therapy.
11. Death
12. Treatment delay due to toxicity greater than **28** days measured from the start of the preceding cycle.

3.3 Treatments

3.3.1 RAD001 Administration

The study drug RAD001 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. RAD001 will be administered orally as once daily dose of ***10 mg (two 5 mg tablets)*** continuously from study day 1 until progression of disease or unacceptable toxicity. Patients will be instructed to take RAD001 in the morning, at the same time each day.

RAD001 may be taken with or without food.

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.

RAD001 will be provided by Novartis. RAD001 is formulated as tablets for oral administration of 5 mg and 10mg strength. Tablets are blister-packed under aluminum foil in units of 10 tablets (16 units per box), which should be opened only at the time of administration as drug is both hygroscopic and light-sensitive.

3.3.2 Duration of Therapy

In the first year of therapy, patients will receive therapy continuously for approximately 2 four week cycles. At the end of every 2 cycles during the first year, patients will receive an MRI and return to clinic for clinical evaluation. The frequency of MRI‟s is based on clinical practice at the Neuro-oncology department at UCSF for patients on active therapy. Upon re-evaluation, patients who have not experienced excess toxicity or disease progression will continue on protocol therapy.

In the second year of therapy, patients will receive therapy continuously for approximately 3 four week cycles. At the end of every 3 cycles during the second year, patients will receive an MRI and return to clinic for clinical evaluation. Upon re-evaluation, patients who have not experienced excess toxicity or disease progression will continue on protocol therapy.

After administration of RAD001 for 2 years, patients will receive an MRI and return to clinic for clinical evaluation. At that time, they will be offered the opportunity to continue treatment if they have not experienced excess toxicity or disease progression. Patients will continue to be followed at three month intervals if after two years they continue to take RAD001.

The study will be terminated for the patient if treatment is delayed due to toxicity greater than 28 days measured from the last dose of RAD001.

3.3.3 Concomitant therapy

All concomitant medications administered within 14 days of enrollment through treatment termination should be reported to the investigator and recorded in the Case Report Form (CRF). In particular, corticosteroid dosing will be recorded as use of corticosteroids may affect the appearance of T2 changes on MRI imaging. In addition, the definition of response is based on corticosteroid use (see section 3.42).

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

- Seville orange, star fruit, grapefruit and their juices affect P450 and PgP activity. These juices are potent CYP3A4-inhibitors. Concomitant use 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 RAD001 to 2.5 mg daily. Additional dose reductions to every other day may be required to manage toxicities. If the inhibitor is discontinued the RAD001 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.

Inducers of CYP3A4 and/or PgP

Avoid the use of strong CYP3A4 inducers. If patient requires co-administration of strong CYP3A4 inducers (i.e., phenytoin, carbamazepine, rifampin, rifabutin, Phenobarbital, St. John‟s wort)**,** an increase in the dose of everolimus from 10mg daily to 20mg daily, using 5mg increments. Enzyme induction usually occurs within 7-10 days, therefore everolimus dose should be increased by one increment 7 days after the start of the inducer therapy. If no safety concerns are seen within the next 7 days, the dose can be increased again one additional increment up to a maximum of twice the daily dose used prior to initiation of the strong CYP3A4 inducer. This dose adjustment of RAD001 is intended to achieve similar AUC to the range observed without inducers. However, there are no clinical data with this dose adjustment in patients receiving strong CYP3A4 inducers. If the strong inducer is discontinued the RAD001 dose should be returned to the dose used prior to initiation of the strong CYP3A4/PgP inducer.

- No chronic treatment with systemic steroids (at a dose equivalent of greater than 20 mg prednisone per day) 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 RAD001 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 coadministration of RAD001 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 3-3 (CYP3A4 inhibitors/inducers) and Table 3-6 (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.

Table 3-3 Examples of clinically relevant drug interaction: substrates, inducers and inhibitors of isoenzyme CYP3A.

Substrates (competitive inhibition)	
Antibiotics[1]: clarithromycin* erythromycin telithromycin* Anti-arrhythmics: quinidine Benzodiazepines: alprazolam diazepam midazolam triazolam Immune Modulators: cyclosporine tacrolimus (FK506) HIV Protease Inhibitors: indinavir* nelfinavir ritonavir* saquinavir* Prokinetic: cisapride Antihistamines: astemizole chlorpheniramine terfenadine	Calcium Channel Blockers: amlodipine diltiazem felodipine lercanidipine nifedipine nisoldipine nitrendipine verapamil HMG CoA Reductase Inhibitors[2]: cerivastatin lovastatin simvastatin Miscellaneous: Alfentanil, aprepitantaripirazole, 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
Inducers	
Barbiturates, Carbamazepine Glucocorticoids modafinil, oxcarbazepine, Phenobarbital Phenytoin*, pioglitazone, Rifabutin*	Rifampin* St John's wort Troglitazone efavirenz, nevirapine
Inhibitors	
Strong Inhibitors: indinavir, nelfinavir, ritonavir, clarithromycin, itraconazole, ketoconazole, nefazodone, saquinavir, telithromycin Posaconazole (Krishna et al, 2009) **Moderate inhibitors:** aprepitant, diltiazem, erythromycin, fluconazole, grapefruit juice, verapamil **Weak inhibitors:** Cimetidine, Seville orange (Malhotra et al, 2001) **Unclassified as per the Indiana University DDI listing:**	

Ciprofloxacin, delaviridine, troleandamycin, mibefradil, amiodarone, chloramphenicol, diethyldithiocarbamate, fluvoxamine, starfruit, gestodene, imatinib, mifepristone, norfloxacin, norfluoxetine, voriconazole*

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)

1.

Table 3-7 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

3.3.4 Treatment compliance

Records of study medication used, dosages administered, and intervals between visits will be recorded during the study. Drug accountability will be noted and patients will be asked to return all unused study medication.

3.4 Visit schedule and assessments

3.4.1 Visit schedule

3.4.1.1 Screening Assessments

Written informed consent must be obtained and documented in the medical record before starting protocol therapy. After receiving a subject‟s agreement to participate in the study and verifying that the subject eligibility criteria, the study site will begin further pretreatment evaluation as follows:

- Clinical Evaluation: A complete history and neurological examination (including clinical assessment of Karnofsky Performance Status, Neurological function, neurologic exam score, Vital signs, Physical, and Mental Status) is to be done within 14 days of initial protocol treatment.

- Radiographic Evaluation: An MRI scan must be performed within 14 days prior to initial treatment on a dose of steroids that has been stable or decreasing for 5 days or more. MR spectroscopy are optional.

- Vital Signs: Height, pulse, blood pressure, respiration rate, temperature and weight. Blood pressure, pulse and respiration rate should be measured on patients.

- Laboratory Evaluation: Blood tests may be performed at the UCSF GCRC, at the UCSF Neuro-oncology clinic, or at a local laboratory. Pre-study laboratory tests must be obtained with 14 days prior to initial protocol treatment. Pre-study laboratory tests shall include:

    - CBC, platelets, differential, PT (INR)
    - Sodium, potassium, chloride, bicarbonate, calcium, fasting glucose, albumin, total protein, BUN, serum creatinine
    - T. bilirubin, AST, ALT, alkaline phosphatase, uric acid, phosphorus,
    - Fasting serum lipid profile (total cholesterol,triglycerides, HDL and LDL
    - serum pregnancy test for women of childbearing potential (performed within 7 days of initial protocol treatment).

- Electrocardiogram (ECG): A standard 12 lead ECG is to be performed within 14 days of initial protocol treatment and significant findings must be recorded.

- Lung function: Pulse Oximetry and Chest X-ray must be performed within 14 days prior to initial protocol treatment

Hepatitis B/C testing: Patients will be tested for the following hepatitis serologic markers: HBsAg, HBs Ab, HBc Ab Total, HBc IgM Ab and HCV antibodies.

3.4.1.2. Assessments During Treatment

- Clinical Evaluation: Clinical assessments will be required every 2 months for the first 12 months, then every 3 months for the next 12 months. After 4 weeks of treatment, a toxicity check will be performed with a local MD or telephone. Whenever possible, all assessments at each scheduled time-point will be completed in a single clinic visit. Performance of a full clinical evaluation should take between 1-1.5 hours. Clinical assessments performed at required visits will include the following:

  Karnofsky performance status (Appendix 17.2)

  Neurological function / Neurologic exam (see below)

  Physical Examination

  Pulse Oximetry measurements

  Neuro Exam: Neurologic status should be recorded at each patient visit according to the scale below (compared to baseline, defined as neurologic exam at time of screening).

  +2 Definitely better
  +1 Possibly better
  0 Unchanged
  -1 Possibly worse
  -2 Definitely worse

  Vital Signs: pulse, blood pressure, respiration rate, temperature and weight. Blood pressure, pulse and respiration rate should be measured on patients

- Radiographic Evaluation: An MRI will be required every 2 months for 12 months and every 3 months for the next 12 months. MR spectroscopy scans at these time points are optional. A research MRI/MRS scan maybe performed during Cycle 1 week 3. Required assessments will include measurement of bi-

directional diameters for patients who continue to have measurable disease. Uni-dimensional maximal diameter will also be recorded. MRI will be evaluated by Central Radiology review by both a neuro-oncology attending.

- Chest X-ray: A chest x-ray is required every 2 months for 12 months and every 3 months for the next 12 months.

  Course Response: This will be recorded every time a patient has an imaging study.

+3	=	Disappearance of tumor (CR)
+2	=	Definitely better (PR)
+1	=	Possibly better
0	=	Unchanged
-1	=	Possibly worse
-2	=	Definitely worse (PD)
-3	=	Development of a new lesion (PD)

- Laboratory Evaluation: Laboratory tests except serum pregnancy tests shall be performed every 2 weeks during the first 2 months of treatment, and then monthly thereafter:

  - CBC, platelets, differential
  - Sodium, potassium, chloride, bicarbonate, calcium, fasting glucose, albumin, total protein, BUN, serum creatinine
  - T. Bilirubin, AST, ALT, alkaline phosphatase, uric acid, phosphorus
  - Fasting serum lipid profile (total cholesterol, triglycerides, HDL and LDL)
  - Serum pregnancy test: for women of childbearing potential every 2 months for 12 months and then every 3 months while on protocol therapy and 12 weeks after discontinuing protocol therapy.
  - Note the coadministration of RAD001 and oral anticoagulants is possible but should be subject to verification of coagulation (INR) once steady state is reached (after one week‟s treatment).

- Hepatitis B testing: Patients who test positive for hepatitis B antibodies without receiving prior vaccination will be monitored for HBsAg every 4 weeks.

- Medication Evaluation: 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 at 1 week, and then every 2 months for the first 12 months, then every 3 months until the patient discontinues therapy.

3.4.1.3 Follow-Up Assessments

- Protocol Completion Assessment:
  - After administration of RAD001 for two years, patients will be offered the opportunity to continue treatment. There is no limit to the course of treatment an individual patient may receive. However, the patient will continue to be evaluated at 3 month intervals while on RAD001 and a decision to discontinue treatment after 2 years of therapy will be made on clinical grounds.
  - All patients must be observed for safety for 30 days following the last dose of RAD001 prior to withdrawal from study. This defines study completion.
  - Physical examination and comprehensive neurologic examination and grading, vital signs including weight, Karnofsky Performance status, all laboratory values, and MRI of the brain must be repeated within 2 weeks of the last day of study only if these have not been evaluated within 14 days, inclusive, prior to the date of withdrawal from study.

- Long-term Follow-up:
  - Patients will be followed according to routine clinical practice after removal from protocol therapy or until death, whichever occurs first.
  - Patients removed from protocol therapy for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.
  - All patients will be followed for PFS and OS while on protocol therapy and while still receiving subsequent routine clinical care. Patients off protocol therapy will continue to be followed for PFS and OS only.

3.4.1.4 Summary of Assessments

	Initial Screen	**1-month Assessment**	**Assessments every 8 weeks (+3/-7days unless otherwise noted) for year 1 of therapy**	**Assessments every 12 weeks (+3/-7days unless otherwise noted) after first year of therapy**	**30 days after completing protocol therapy**
Procedure:					
Clinical Evaluation	X		X	X	

Vital Signs	X		X	X	
Neurologic Exam	X		X	X	
Toxicity Check	X	X	X	X	X
Chest X-Ray	X		X Q 8 weeks (+/-7days)	X Q 12 weeks (+/-7days)	
Pulse Oximetry	X		X	X	
ECG	X				
Laboratory/ Diagnostic Tests:					
Serum pregnancy for women of child-bearing potential	X		X Q 8 weeks (+/- 3 days)	X Q 12 weeks (+/- 3 days)	
CBC with diff and platelts	Laboratory evaluation at baseline, Q 2 weeks (+/- 3 days) for the first two months, then Q 4 weeks (+/- 3 days) while on therapy.				
Chemistries					
Liver function tests					
Fasting cholesterol / triglycerides					
MRI of the brain	X		X	X	
Hepatitis B/C testing HBsAg, HBs Ab, HBc Ab Total, HBc IgM Ab and HCV antibodies	X	Patients who test positive for HBV antibodies without prior vaccination will be tested for HbsAg every 4 weeks (+/- 7 days) for re-activation			
Translational Research:					
Archival tumor tissue	X				
Study Drug:					
RAD001 starting dose:		10mg			

3.4.2 Efficacy assessments

3.4.2.1 Response Definitions

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 gioma. 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.

One of the secondary endpoints will be response rate, based on the best response achieved (see below for definition of best response). In order to ensure comparability of data from this trial with data from earlier trials, this trial will retain the traditional evaluation definition as the primary way to define response. Response will be determined by the bi-dimensional diameters. However, RECIST criteria will be collected and used for secondary evaluation.

Definitions of Response

Measurable Disease: Bidimensionally measurable lesions with clearly defined margins by MRI scan. A measurable lesion must be 1 mm or larger in longest diameter.

Evaluable Disease: Unidimensionally measurable lesions, masses with margins not clearly defined.

Objective Status, To Be Recorded at Each Evaluation: If there are too many lesions to measure at each evaluation, choose the largest two to be followed before a patient is entered on study. The remaining lesions will be considered evaluable for the purpose of objective status determination. Unless progression is observed, objective status can only be determined when ALL measurable and evaluable sites and lesions are assessed.

Complete Response (CR): Complete disappearance of all measurable and evaluable disease. No new lesions. No evidence of non-evaluable disease. All measurable, evaluable and non-evaluable lesions and sites must be assessed using the same techniques as baseline. Patients must be on minimal or no steroids.

Partial Response (PR): Greater than or equal to 50% decrease under baseline in the sum of products of perpendicular diameters of all measurable lesions. No progression of evaluable disease. No new lesions. All measurable and evaluable lesions and sites must be assessed using the same techniques as baseline. Responders must be on the same or decreasing doses of dexamethasone.

Partial Response, Non-Measurable (PRNM): Not applicable.

Stable / No Response (NR) / No Change (NC): Does not qualify for CR, PR, or progression. The designation of Stable/No Response requires a minimum of 8 weeks duration. All measurable and evaluable sites must be assessed using the same techniques as baseline.

Progression: 25% increase in the sum of products of all measurable lesions over smallest sum observed (over baseline if no decrease) using the same techniques as baseline, OR clear worsening of any evaluable disease, OR appearance of any new lesion/site, OR failure to return for evaluation due to death or deteriorating condition (unless clearly unrelated to this cancer).

Unknown: Progression has not been documented and one or more measurable or evaluable sites have not been assessed.

Best Response: This will be calculated from the sequence of objective statuses.

For patients with all disease sites assessed every evaluation period, the best response will be defined as the best objective status as measured according to the previously noted definitions. Best response is unknown if all objective status determinations before progression are unknown.

Neurological Exam: Although not used for determining response, it is useful to evaluate improvement in the neurologic exam that should coincide with objective measurement of tumor size.

+2 Definitely better
+1 Possibly better
0 Unchanged
-1 Possibly worse
-2 Definitely worse
(compared to baseline)

Time to Death: From date of registration to date of death due to any cause.

3.4.2.2. Pathology Review

Pathology must be reviewed in the UCSF pathology department.

Obtaining tissue samples:

- The UCSF Neurosurgery Tissue Bank has a general consent that patients sign to authorize release of their samples from previous surgeries at outside institutions.
- Slides and tissue blocks, along with a path report, are sent to UCSF -- c/o one of the NeuroOnc study coordinators or directly to the Neurosurgery tissue bank manager (currently Cynthia Cowdrey)

Preparation of tissue samples:

- Once received, samples are logged into the Pathology computer system and assigned an SF#.
- If H&E slides are not provided, the tissue bank manager (currently Cynthia Cowdrey) will prepare unstained slides from paraffin blocks.
- The unstained slides will be staining at the UCSF Core lab.

Review of tissue samples:

- Pathology Materials Required for Review:
  - A copy of the Pathology Reports and the Operative Reports.
  - A Protocol Specific Pathology Submission Form.
- A neuropathologist () will review all H&E slides and will determine if the histology corresponds to the report and if it is adequate / appropriate for the study.
- 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.

Immunohistochemical assays:

All antigens to be assayed in this protocol are cytoplasmic or membranous and will be scored using a semi-quantitative scale as described herein:

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.

The designated neuropathologists will identify areas 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 in a similar fashion to positive control cells. Based on this interpretation, the results are categorized into four groups:

0- negative staining = no staining in any of the tumor cells
1- focal positive staining = staining similar to positive control in less than one quarter of the tumor cells as determined by review of the entire slide and visual estimate.
2- Intermediate positive staining = staining similar to positive control in greater than one quarter but less than three quarters of tumor cells as determined by the review of the entire slide and visual estimate.
3- Diffuse positive staining = staining similar to positive control in greater than three quarters of the tumor cells as determined by the review of the entire slide and visual estimate.

Visual estimation may be aided by dividing the interpretable areas of the tissue on the slide into four quadrants using a tissue marker. It is beneficial to provide a visual scale in the form of a figure for the initial publications that will utilize the grading system. The results of this scoring can be reported as either a number representing each category, or descriptively using the terms negative, focal, intermediate, and diffuse.

Table 1: Summary of Assays to evaluate Phosphorylated Akt pathway

Molecular feature	**Assay (reagents)**
Phosphorylated PKB/Akt (phospho-PKB/Akt) expression	IHC: Polyclonal antibody Ser473; Cell Signaling Technology, Inc., Beverly, MA
PTEN promoter expression	IHC
Ribosomal S6 phosphorylation	IHC: phospho-specific antibody.
PRAS40	IHC

3.4.2.3 Radiology review

All objective responses must be reviewed by a neuro-oncologist at UCSF. Images will be viewed on the WebPACs 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 in section 9.1. Discrepancies between measurements will be adjudicated by another neuro-oncologist at UCSF.

3.4.3 Safety assessments

Safety assessments will consist of monitoring and recording all adverse events and serious adverse events, the regular monitoring of hematology, blood chemistry and regular measurement of vital signs and the performance of physical examinations.

These assessments should be performed within ±2 days of the scheduled day of assessment except for adverse events that will be evaluated continuously through the study. Safety and tolerability will be assessed according to the NIH/NCI CTC http://ctep.cancer.gov/forms/CTCAEv3.pdf.

3.4.3.1 Adverse events

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

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug even if the event is not considered to be related to study drug. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

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

1. the severity grade (mild, moderate, severe) or (grade 1-4)
2. its relationship to the study drug(s) (suspected/not suspected)
3. its duration (start and end dates or if continuing at final exam)
4. action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization)
5. whether it constitutes a serious adverse event (SAE)

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

Information about common side effects already known about the investigational drug can be found in the [Investigators‟ Brochure]. This information should be included in the patient informed consent and should be discussed with the patient during the study as needed.

3.4.3.2 Serious adverse events

Information about all serious adverse events will be collected and recorded. To ensure patient safety each serious adverse event must also be reported to Novartis within 24 hours of learning of its occurrence. A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)
- elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
- treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
- social reasons and respite care in the absence of any deterioration in the patient‟s general condition

- is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

3.4.4 Novartis instructions for rapid notification of serious adverse events

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

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

All events must be reported, by FAX (888-299-4565), 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.

3.4.5 Data Safety Monitoring Plan

3.4.5.1 Oversight and Monitoring Plan

The UCSF-CCC Data Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UCSF-CCC institutional clinical studies. A summary of DSMC activities for this study includes:

- Review of subject data in each cohort
- Quarterly review for progress and safety
- Review of all serious adverse events
- Minimum of a yearly audit

3.4.5.2 Monitoring and Reporting Guidelines

Investigators will conduct continuous review of data and patient safety at monthly study group or site committee meetings where the results of each patient‟s treatment are discussed and the discussion is documented in the minutes. The discussion will include the number of patients, significant toxicities as described in the protocol, doses adjustments, and observed responses. Quarterly summaries will be submitted to the DSMC for review. All grade 3-5

AE‟s and SAE‟s will be entered in the CCC Oncore database.

3.4.5.3 Review and Oversight Requirements

3.4.5.4 Adverse Event Monitoring

Adverse Events (AEs) will be recorded on the Oncore database, all grade 3-5 expected and unexpected AEs will be recorded and updated at each visit.

Serious Adverse Event Reporting

Serious Adverse Event reporting will be in accordance with the UCSF- Committee on Human Research Regulations and Code of Federal Regulation Title 21 Volume 5 Part 312.32.

UCSF CHR website for guidance in reporting serious adverse events
http://www.research.ucsf.edu/chr/Guide/Adverse_Events_Guidelines.pdf

FDA website for guidance in reporting serious adverse events
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.32

MedWatch forms and information:
http://www.fda.gov/medwatch/getforms.htm

Serious Adverse events will be reported on the MedWatch form. A copy of the MedWatch report and CHR forms must be sent to CCC- DSMC at Box 1297. The date the SAE was sent to all required reporting agencies will be documented in Oncore, and hard copies of the report will be maintained in the regulatory files.

If the SAE is death and determined to be possibly, probably or definitely related to the investigational drug or any research related procedure the event must be reported to the DSMC Chair, or his designee with in 24 business hours. The reporting procedure is by personal communication via phone or in person with written documentation of the 1:1 communication via e-mail with a copy of the e-mail to DSMC Administrator and DSMC Coordinator.

If any of the above action occurs in multiple-institutional clinical trial coordinated by the UCSF-CCC, the Study Coordinator will insure that all participating sites are notified.

3.4.6 Review of Adverse Event Rates

If the study has an increase of unexpected or expected Adverse Events grade 3 or 4 above the rate reported in the Investigational Brochure or package insert, the increase rate of AEs will be reported to the DSMC at the time of Identification. The Chair and PI will discuss the finding and proceed with a written course of action. Each quarterly report will indicate if the AE incidence is within the scope of the investigational brochure or package insert. If at any time the Investigator stops enrollment or stops the study due to safety issues the DSMC Chair and Administrator must be notified within 24 business hours via e-mail. The DSMC must receive a formal letter within 10 business days and the CHR must be notified.

If any of the above action occurs in multiple-institutional clinical trial coordinated by the UCSF-CCC, the Study Coordinator will insure that all participating sites are notified.

3.4.7 Study Progress – Quarterly Review

Principal Investigators are required to submit quarterly study progress reports to determine whether accrual projections are being met, to summarize grade 3 and 4 toxicities (expected and unexpected) and SAE reports. This report will also indicate if the rate of all grade 3-5 AE"s are above the AE rates documented in the Investigational Brochure or package insert. In addition, a progress report on recruitment and subjects known responses to the investigational therapy must be submitted. At this time also send the committee all external DSMB reports and formal audit reports.

These quarterly reports are reviewed at Data Safety Monitoring Committee meetings. These reports are required: February 1, May 1, August 1, and October 1. Failure to submit such reports may result in trial suspension. Send reports to: DSMC Box 1297.

Data Safety Monitoring Committee Contacts:
DSMC Chair: Alan Venook, MD
Phone (415) 353-2745
Email venook@cc.ucsf.edu
Box 1705

DSMC Administrator: Diane Davies, RN
Phone (415) 353-9510
Email ddavies@cc.ucsf.edu
Box 1297

In addition all SAEs must be reported to:
Include the Pharmaceutical Company contact for SAE reporting in this section.

3.4.7.1 Pregnancies

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

3.4.7.2 Laboratory evaluations

- *please see section 3.4.1 for schedule for initial assessment and during follow up*

Hematology

Hematology must include hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential. PT (INR) evaluation will be included for baseline evaluations.

Blood chemistry

Blood chemistry must include sodium, potassium, chloride, bicarbonate, calcium, glucose, creatinine, blood urea nitrogen, albumin, total protein, SGOT (AST), SGPT (ALT), total bilirubin, alkaline phosphatase, uric acid, phosphorus, serum lipid profile (triglycerides, total cholesterol, HDL and LDL).

Because accurate serum glucose and lipid measurements are required, patients should be fasting at the time of the blood sampling.

Hepatitis B Virus testing

Patients will be tested for the following hepatitis B serologic markers prior to enrolling on the trial: HBsAg, HBs Ab, and HBc Ab Total, and HBc IgM Ab.

HbsAg monitoring should be performed every 4 weeks if a patient tests positive for the hepatitis B antibodies without prior vaccination

Hepatitis C Virus testing

Patients with be tested for HCV Ab prior to enrolling on the trial.

3.4.7.3 Vital signs

Vital sign assessment consists of height (first visit), pulse, blood pressure, respiration rate, temperature and weight. Blood pressure, pulse and respiration rate should be measured on patients

3.4.7.4 Physical examination

Physical examination will be performed which must comprise a total body examination (general appearance, skin, neck, including thyroid, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities and basic nervous system).

Please see 3.4.1 for detailed neurological assessment.

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

3.4.7.5 ECG

A standard 12 lead ECG is to be performed during screening and significant findings must be recorded.

3.4.7.6 Performance status

Performance status will be assessed.

- *Karnofsky score as outlined in 3.4.1.*

3.4.7.7 Special tests

- *MRI as outlined in 3.4.1; neurological evaluatiuon as outlined in 3.4.1*

3.4.8 Drug levels and pharmacokinetic assessments

- ***Drug levels will not be monitored***

4 Protocol amendments, or changes in study conduct

Any change or addition (excluding administrative) to this protocol requires a written protocol amendment that must be reviewed by Novartis and the investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study require additional approval by the IRB at each study center. A copy of the written approval of the IRB must be provided to Novartis. Examples of amendments requiring such approval are:

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

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by Novartis in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the

investigator and is implemented for safety reasons Novartis must be notified and the IRB at the center must be informed immediately. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB approval include:

1. changes in the staff used to monitor trials
2. minor changes in the packaging or labeling of study drug.

5 Data management

5.1 Data collection

- Investigators must record the information required by the protocol.

6 Statistical methods

6.1 Statistical methods

6.1.1. **Stopping Rules**

If the discontinuation rate due to toxicity is 20% or greater and the lower bound for the one-tailed 95% confidence interval is > 10%, this strategy would not be considered feasible. At the time we observe any toxicity that requires permanent discontinuation of drug, we will estimate, with confidence intervals, the rate of toxicity. If there is a greater than 20% rate for discontinuation of drug during therapy the study will stop and we will assess the study design. Because the confidence interval may be calculated multiple times, the actual confidence level will be overstated. This increases the likelihood of rejecting the therapy due to toxicity. However, this is felt to be an acceptable risk in order to prevent continuation of a therapy that may have a toxicity rate that precludes its routine use.

If there is any treatment related mortality event, the study accrual will be suspended until all data pertaining to the event is reviewed by the Study Chair and co-investigators.

6.1.2. **Endpoint Definitions and Sample Size**

The following definitions will be used for statistical analysis:

- Response: Response is based on the best response. The best response is defined as the best objective status as measured by the sum of products of perpendicular diameters of all measurable lesions.
- Progression-free survival: Number of days from the day the patient was enrolled to the day the patient experiences an event of disease progression or recurrence or to the date of death if disease progression or recurrence is not reached. All events of disease progression or recurrence will be included, regardless of whether the event

occurred while the patient was still taking drug or had previously discontinued study drug. All events of death will be included for patients who had not experienced disease progression or recurrence. If the patient does not have an event of disease progression or recurrence nor as the patient died, the patient"s data will be censored at the date of last contact with the patient.

- Overall survival: Number of days from the day the patient was enrolled until the date of death. If the patient is lost to follow-up, the patient"s data will be censored at the date of last contact with the patient.

For this single-arm phase II trial, the primary endpoint will be 6-month progression-free survival (PFS-6). Patients will be analyzed based on intention to treat. The primary determination of treatment success will be based on the results for those patients who are shown to still be low-grade. The intent is to perform the primary analysis at the time the 6 month PFS is known for all patients.

Precise documentation of prognosis among recurrent low grade glioma patients treated with targeted therapy is limited in literature. A recent study based on the experience of the North American Brain Tumor Consortium (NABTC) was conducted to assess whether progression status at 6 months predicts survival from that time among recurrent high-grade glioma adult patients (Lamborn, Yung et al. 2008). In that study, it was reported that the estimated PFS-6 among recurrent grade III glioma patients who were not treated with temozolomide, a chemotherapy which is part of the standard of care for newly diagnosed gliomas, is approximately 17%. Since we expect patients with recurrent low grade gliomas to have better prognosis than grade III patients, we consider the lower threshold for the probability of 6-month PFS to be 17%. The calculation of sample size is based on a binary endpoint with the goal to increase 6-month PFS rate to 40%. This translates into a hazard ratio of 0.52 (experimental/historical). 40 patients will provide over 90% power using a one-sided test with alpha of 0.05.

6.1.3. **Accrual Objectives**

The study plan is to accrue 60 patients. It is anticipated that most recurrent low-grade glioma patients completing surgical resection will be eligible for enrollment and will choose to participate. Based on current patient volume in the UCSF neurologic-oncology clinic, it is expected that a minimum of 3 patients per month will be enrolled.

6.1.3. Estimated Duration of Study

With enrollment of 3 patients per month, accrual can be completed in a 20 month period. With up to an additional 6 months of treatment to assess best response for the last accrued patient, the approximate duration of study is 26 months.

Based on review of UCSF historical data, we expect approximately one-third of recurrent low-grade glioma patients to present with high-grade gliomas. Therefore, we should be able to complete enrollment of 40 low-grade glioma patients and 20

secondary malignant glioma patients at approximately the same time. In order to evaluate our primary objective, enrollment will continue until at least 40 low-grade glioma patients have enrolled.

6.1.4 Analyses Time-points and planned analysis

Primary end-point – 6-month PFS:

Analyses will be performed after all enrolled patients have completed 6 months on study drug, or whenever the progression status of all patients has been established, whichever comes first. The primary analysis will be based on Kaplan-Meier method. Kaplan-Meier estimates and the associated 95% CIs will be calculated for the 6-month PFS separately for low-grade glioma patients and secondary malignant glioma patients. The Cox proportional hazards model will also be used to allow for adjustment of the prognostic factors including age, KPS, extent of resection, and number of prior chemotherapy regimens.

Secondary end-point – overall survival and objective response rate:
The method described above for the analysis of PFS-6 will be applied to the analysis of OS. Response is based on the best response, defined as the best objective status as measured by the sum of products of perpendicular diameters of all measurable lesions. The point estimate and the associated 2-sided 95% CI for the response rate will be calculated separately for low grade and secondary malignant tumors.

Secondary end-point – biologic correlates:

The endpoints of overall survival,objective response rate and progression status at 6 months will be evaluated for impact of phosphorlyated PKB/Akt status. We anticipate that the response rate may be more dependent on phosphorylated PKB/Akt status than on current tumor grade. We also expect approximately 50% of the low-grade tumors to be phosphorylated PKB/Akt positive and 75% of the higher-grade tumors to be positive. This will result in approximately 20 phosphorylated PKB/Akt positive low-grade cases and 15 phosphorylated PKB/Akt positive high-grade cases for secondary analyses. A consideration of phosphorylated PKB/Akt status as a predictor of response will use the Cochran Mantel-Haenzel test stratified by grade. If the number of responses is sufficient, logistic regression will be used to evaluate the potential impact of phosphorylated PKB/Akt status on the likelihood of response adjusting for grade. Estimates of the odds ratio and its associated 95% confidence interval will be calculated to determine if future studies of RAD001 should require prior assessment of phosphorylated PKB/Akt status. The same methods will be used to determine if phosphorylated PKB/Akt status predicts progression status at 6 months stratified by grade. The impact of phosphorylated PKB/Akt status on OS will be assessed using the proportional hazards models adjusting for grade. Estimates of the hazard ratio and its associated 95% confidence interval will be calculated. We will repeat these analyses on patients based on PTEN promoter methylation status.

Exploratory analysis – radiographic responses:

We will determine if the pretreatment imaging and changes from pre-treatment to first post-treatment scan correlate with the clinical endpoints of time to tumor progression and survival. A scoring system will be developed for predicting outcome based on the initial, pretreatment scan. Since all of these patients will have surgery, it will be possible to prospectively determine if the imaging score can predict grade as assessed by the neuropathologist. However, since most patients do not have surgery at time of progression, and time to progression varies even among those with the same tumor grade, we want to determine how useful this score will be beyond the current histology information. We plan to test the usefulness of this score in predicting PFS and survival using Cox regression. If the number of patients with responses is sufficient we will also assess the ability of the imaging score to predict response using logistic regression. The models will include, in addition to the imaging score, age, KPS, extent of resection, time since diagnosis, and initial diagnostic histology scored as oligodendroglioma only vs. those with an astrocytoma component. Because PKB/Akt phosphorylation status is predicted to impact outcome for this particular therapy, this will also be included in the model. The inclusion of these additional variables is to adjust for known or potential confounding risk factors. The primary hypothesis test will focus on the ability of the prediction score to add information beyond what would have been known based on the clinical data.

The patients will initially be categorized as predicted upgraded (Yes/No) consistent with the primary question posed. For supplementary analyses the prediction score will be included as a continuous variable, or we may create a score – based, for example, on the quartiles of the values observed in the previously studied cases – to determine if a further refinement in prediction of outcome can be achieved. A further analysis will include the known current histologic grade to determine if the score provides information even adjusted for this variable. Based on preliminary data we expect 1/3 of low-grade patients to be upgraded to high-grade glioma on recurrence. If we make the assumption that the predicted distribution will be the same, then we will be comparing 20 predicted high grade vs. 40 predicted low grade patients for the overall assessment. Because these patients are being treated at time of progression, we anticipate that we will know the progression time for most of them at the time of the analysis. Assume that the analysis is done when 50 of the 60 event times are known and alpha=0.05 one-tailed. There would be over 80% power to detect the difference if the hazard ratio was 2.2.

The analysis of the change from initial to first post treatment scan will be performed in a similar manner except that there will be the additional covariate of baseline prediction score, since we will want to know if the change from baseline adds information beyond what was available at the initial scan. The primary endpoints will be

PFS and survival. Fewer patients will be available for this assessment since some will progress at the time of their first post treatment scan. The power for testing hypotheses within this clinical trial is limited by the number of patients enrolled on the trial. We plan to estimate hazard ratios together with confidence intervals to determine if the MR parameters continue to look promising in this treatment group.

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8 Procedures and instructions

8.1.1 Publication of results

Any formal presentation or publication of data from this trial may be published after review and comment by Novartis and prior to any outside submission. Novartis must receive copies of any intended communication in advance of publication (at least fifteen working days for presentational materials and abstracts and thirty working days for manuscripts). These requirements acknowledge Novartis' responsibility to provide peer input regarding the scientific content and conclusions of such publications or presentations. Principal Investigation/Institution shall have the final authority to determine the scope and content of its publications, provided such authority shall be exercised with reasonable regard for the interests of Novartis and, in accord with the trial contract and shall not permit disclosure of Novartis confidential or proprietary information.

8.1.2 Disclosure and confidentiality

The investigator agrees to keep all information provided by Novartis in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC/REB. Study documents provided by Novartis (investigators' brochures and other material) will be stored appropriately to ensure their confidentiality. The information provided by Novartis to the investigator may not be disclosed to others without direct written authorization from Novartis, except to the extent necessary to obtain informed consent from patients who wish to participate in the trial.

8.1.3 Discontinuation of study

Novartis reserves the right to discontinue any study under the conditions specified in the clinical trial agreement.

8.2 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and the principles of Good Clinical Practice, as described in Novartis standard operating procedures and:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki and amendments, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

8.2.1 Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC/REB). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC/REB must be given to Novartis before study initiation. Any amendments to the protocol, other than administrative ones, must be reviewed by Novartis approved by this committee.

8.2.2 Informed consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB/IEC/REB approval.

8.2.3 Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki. Copies of the Declaration of Helsinki and amendments will be provided upon request or can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.