NCT# 02023905

PI3K/mTOR Pathway Activation Selected Phase II Study of Everolimus (RAD001) With and Without Temozolomide in the Treatment of Adult Patients with Supratentorial Low-Grade Glioma

Novartis Study Number CRAD001CUS225T

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Version number: 6.0
Release date: 20Feb2018

Referenced Investigator Brochure
Edition: Version #16, dated
08May2017

Protocol Signature Page

Protocol No.: **Version Date:**

1. I agree to follow this protocol version as approved by the UCSF Protocol Review Committee (PRC), Committee on Human Research (CHR), and Data Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with applicable CHR requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572), and with local regulatory requirements. In accordance with the FDA Modernization Act, I will ensure the registration of the trial on the www.clinicaltrials.gov website.
5. I agree to maintain adequate and accurate records in accordance with CHR policies, Federal, state and local laws and regulations.

UCSF Principal Investigator / Study Chair

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Participating Site(s): N/A

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List of abbreviations

4E-BP1	4E-binding protein
AE	Adverse event
ALT	Alanine aminotransferase/glutamic pyruvic transaminase/GPT
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase/glutamic oxaloacetic transaminase/GOT
AUC	Area under the curve
BAL	Bronchoalveolar lavage
BCG	Bacillus Calmette-Guérin
CDS	Core data sheet
CoA	Coenzyme A
CPK	Creatine phosphokinase
CRF	Case Report Form
CT	Computer tomography
CTCAE	Common terminology criteria for adverse events
CYP3A4	Cytochrome P450 3A4
DDI	Drug-drug interaction
DLCO	Diffusing capacity of the Lung for Carbon Monoxide
DNA	Deoxyribonucleic acid
DS&E	Drug Safety and Epidimology
EOT	End of treatment
EU	European Union
FDA	Food and drug administration
GERD	Gastroesophageal reflux disease
GBM	Glioblastoma
HbA1c	Hemoglobin A1c
HBcAb	Hepatitis B core antibody
HbsAb	Hepatitis B surface antibody
HbsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HMG	3-hydroxy-3-methyl-glutaryl
IB	Investigator brochure
INR	International Normalized Ratio
IRB	Institutional Review Board
IUD	Intrauterine device
IUS	Intrauterine system
IV	Intravenous
KPS	Karnofsky Performance Status score
LGG	Low-grade glioma
$\log_{10}$	Decadic logarithm (common logarithm)
MRI	Magnetic resonance imaging

mTOR	Mammalian target of rapamycin
NCI	National Cancer Institute
OS	Overall Survival
PCR	Polymerase chain reaction
PgP	P-glycoprotein
PFT	Pulmonary function tests
PFS	Progression-free Survival
PI3K	Phosphoinositide 3-kinase
PNET	Pancreatic neuroendocrine tumor
RCC	Renal cell carcinoma
RMP	Risk management plan
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SEGA	Subependymal giant cell astrocytoma
TS	Tuberous sclerosis
ULN	Upper limit of normal
US	United States
VEGF	Vascular endothelial growth factor
WOCBP	Women of child-bearing potential

Glossary of terms

Assessment	A procedure used to generate data required by the study
Baseline	For efficacy evaluations, the baseline assessment will be the last available assessment before or on the date of randomization. For safety evaluations (i.e. laboratory assessments and vital signs), the baseline assessment will be the last available assessment before or on the start date of study treatment. The value obtained at baseline assessments, referred to as "baseline value" will be used as reference for the patient.
Control drug	A study treatment used as a comparator to reduce assessment bias, preserve blinding of investigational drug, assess internal study validity, and/or evaluate comparative effects of the investigational drug
Dose level	The dose of drug given to the patient (total daily or weekly etc.)
Enrollment	Point/time of patient entry into the study; the point at which informed consent must be obtained (i.e. prior to starting any of the procedures described in the protocol)
Investigational drug	The study treatment whose properties are being tested in the study; this definition is consistent with US CFR 21 Section 312.3 and is synonymous with "investigational new drug."
Investigational treatment	Drug whose properties are being tested in the study as well as their associated placebo and active treatment controls (when applicable). This also includes approved drugs used outside of their indication/approved dosage, or that are tested in a fixed combination. Investigational treatment generally does not include other study treatments administered as concomitant background therapy required or allowed by the protocol when used in within approved indication/dosage
Medication number	A unique identifier on the label of each study treatment package which is linked to one of the treatment groups of a study
Other study treatment	Any drug administered to the patient as part of the required study procedures that was not included in the investigational treatment
Patient	A unique identifying number assigned to each patient/subject/healthy volunteer who enrolls in the study
Period	A subdivision of the study timeline; divides stages into smaller functional segments such as screening, baseline, titration, washout, etc.

Premature patient withdrawal	Point/time when the patient exits from the study prior to the planned completion of all study treatment administration and/or assessments; at this time all study treatment administration is discontinued and no further assessments are planned, unless the patient will be followed for progression and/or survival
Stop study participation	Point/time at which the patient came in for a final evaluation visit or when study treatment was discontinued whichever is later
Study treatment	Includes any drug or combination of drugs in any study arm administered to the patient (subject) as part of the required study procedures, including placebo and active drug run-ins. In specific examples, it is important to judge investigational treatment component relationship relative to a study treatment combination; study treatment in this case refers to the investigational and non-investigational treatments in combination.
Study treatment discontinuation	Point/time when patient permanently stops taking study treatment for any reason; may or may not also be the point/time of premature patient withdrawal
Supportive treatment	Refers to any treatment required by the exposure to a study treatment, e.g. premedication of vitamin supplementation and corticosteroid for pemetrexed disodium.
Variable	Identifier used in the data analysis; derived directly or indirectly from data collected using specified assessments at specified time points

1 Background

1.1 Overview of disease pathogenesis, epidemiology and current treatment

The incidence of gliomas in the United States (US) is about 18,000 per year, with a case fatality rate of about 10,000 per year. Gliomas account for up to 80% of primary malignant brain tumors and they account for significant mortality. Gliomas can be classified as either high-grade or low-grade. The low-grade gliomas (LGG) themselves are a diverse group of glial tumors with an annual incidence in the US of about 2500-3000 cases (Dolecek, et al. 2012). Histologic subtypes of LGG include diffuse astrocytomas (WHO grade II astrocytomas), oligodendrogliomas and mixed oligoastrocytomas (Ashby and Shapiro 2004). The majority of these tumors are of mixed histology.

Despite medical management with surgery and radiation, the median 10-year survival rates for LGG range from 17-49%. Survival is limited by recurrence and progression of LGGs to high-grade gliomas. Factors influencing survival in these patients include histologic subtype, age,

extent of surgical resection, and 1p/19q chromosomal status [reviewed in (Bourne and Schiff 2010)]. 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 LGGs in adults will be considered.

The management of patients with residual LGG following surgical resection is not standardized (Lang and Gilbert 2006). Options include surveillance scans, reserving treatment for 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 (Mittal, Szlaczky et al. 2008).

LGGs are diffuse in nature and the treatment fields for radiation therapy can be large. The timing of radiotherapy was investigated by the EORTC 22845 randomized trial that examined the long-term efficacy of early versus delayed radiotherapy for all comers with LGG in adults. The median overall survival (OS) in early versus delayed radiation showed no statistically significant difference (7.1 versus 7.9 years), with median PFS of 5.4 years versus 3.7 years (P = 0.003). One possible benefit of early radiation was better seizure control at 1 year post-radiotherapy (seizures present in 25% and 41% respectively) (van den Bent, Afra et al. 2005). 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 2006).

In an effort to delay the use of radiotherapy, several studies have evaluated the use of chemotherapy as initial treatment of LGG. Tumor regression using a combination of procarbazine, lomustine and vincristine in patients with an oligodendroglial component has been demonstrated (Buckner, Gesme et al. 2003; Stege, Kros et al. 2005). More recently, temozolomide (TMZ) has shown activity as treatment for LGG. Both the "standard" regimen of TMZ, 200 mg/m^2/day for 5 days of a 28-day cycle, and more protracted low-dose "metronomic" TMZ regimens have been evaluated and have shown activity. However, the patient populations studied have been heterogeneous in both the molecular subtypes of tumors included and the timing of TMZ administration. Most reported studies have treated patients at the time of tumor progression following a period of observation post-surgically, or have treated a mixture of patients with newly-diagnosed and progressive tumors (Kesari, 2009, Hoang-Xuan, 2004, Brada, 2003, Pace, 2003). As such, the role of TMZ in treatment of patients with newly diagnosed LGG remains under investigation. One recent retrospective study calculated progression-free survival (PFS) in patients with LGGs treated with TMZ after surgery but prior to progression, and the median PFS was approximately 35 months (Houillier, Wang et al. 2010). Additional treatment strategies are needed for this patient population. As our understanding of the signaling pathways important for tumor growth and invasion improves, novel targeted therapies are an appealing option to consider.

1.2 Introduction to investigational treatment(s) and other study treatment(s)

Everolimus is a novel derivative of rapamycin. It has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation. Everolimus is approved in Europe and other global markets (trade name: Certican®) for cardiac and renal transplantation, and in the United States (trade name: Zortress®) for the prevention of organ rejection of kidney transplantation.

Afinitor® was approved for adults with advanced renal cell carcinoma (RCC) after failure of treatment with sunitinib or sorafinib in 2009. In 2010, Afinitor® received United States (US) approval for patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC). Everolimus is also available as Votubia® in the European Union (EU) for patients with SEGA associated with TSC who require therapeutic intervention but are not candidates for curative surgical resection. Afinitor® was approved for "progressive pancreatic neuroendocrine tumor (PNET) in patients with unresectable, locally advanced, or metastatic disease" in 2011 in various countries, including European countries and the US. In 2012 Afinitor® received approval for the treatment of postmenopausal women with advanced hormone receptor-positive, HER2-negative breast cancer (advanced HR+ BC) in combination with exemestane, after failure of treatment with letrozole or anastrozole. Furthermore in 2012, Afinitor® received approval for the treatment of patients with TSC who have renal angiomyolipomas not requiring immediate surgery.

Approximately 25,645 cancer patients have been treated with everolimus as of 30-Sep-2012:

- 13,229 patients in Novartis-sponsored clinical trials
- 2,624 patients in the individual patient supply program
- 9,792 patients in investigator-sponsored studies.
- In addition, healthy volunteer subjects and non-oncology hepatically impaired subjects have participated in the clinical pharmacology studies as described in Section 5.2 (Investigator's Brochure Edition 11, Release date: 15-Nov-2012).

The following is a brief summary of the main characteristics of everolimus. More complete information can be obtained from the everolimus Investigator's Brochure (IB).

1.2.1 Overview of everolimus

Everolimus is a derivative of rapamycin that acts as a signal transduction inhibitor (Table 1-1, Figure 1-1). Everolimus selectively inhibits mTOR (mammalian target of rapamycin), specifically targeting the mTOR-raptor signal transduction complex. mTOR is a key serine-threonine kinase in the PI3K/AKT signaling cascade, which is known to be dysregulated in a wide spectrum of human cancers (Boulay and Lane 2007).

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

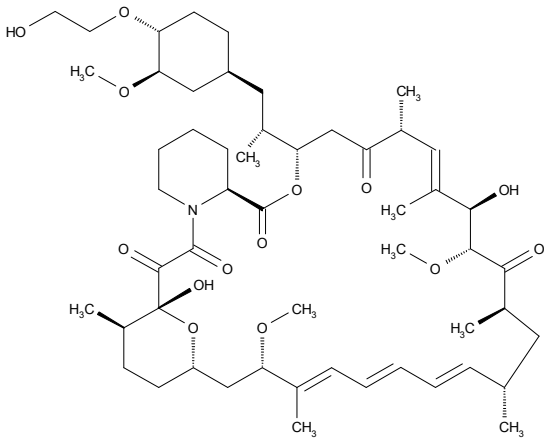
- directly on the tumor cells by inhibiting tumor cell growth and proliferation;

- indirectly by inhibiting angiogenesis leading to reduced tumor vascularity (via potent inhibition of tumor cell VEGF (vascular endothelial growth factor) production and VEGF-induced proliferation of endothelial cells).

Table 1-1 Everolimus - Drug substance

Chemical name	(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-{(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.$0^{4,9}$]hexatriaconta-16,24, 26,28-tetraene-2,3,10,14,20-pentaone
International non-proprietary name	Everolimus

Figure 1-1 Chemical structure of Everolimus



1.2.1.1 mTOR pathway and cancer

At the cellular and molecular level, everolimus acts as a signal transduction inhibitor. It selectively inhibits mTOR (mammalian target of rapamycin), a key protein kinase which regulates cell growth, proliferation and survival. The mTOR kinase is mainly activated via the phosphatidylinositol 3-kinase (PI3-Kinase) pathway through AKT/PKB and the tuberous sclerosis complex (TSC1/2). Mutations in these components or in PTEN, a negative regulator of PI3-kinase, may result in their dysregulation. Abnormal functioning of various components of the signaling pathways contributes to the pathophysiology of numerous human cancers. Various preclinical models have confirmed the role of this pathway in tumor development (Cohen et al 2005).

The main known functions of mTOR include the following (Bjornsti and Houghton 2004):

- mTOR functions as a sensor of mitogens, growth factors and energy and nutrient levels;
- Facilitating cell-cycle progression from G1-S phase in appropriate growth conditions;
- The PI3K/mTOR pathway itself is frequently dysregulated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors;

- PI3-kinase mutations have been reported in the primary tumor in 10-20% of human colorectal cancers (Frattini 2005, Velho 2005);
- The loss of PTEN protein, either through gene deletion or functional silencing (promoter hypermethylation), is reported in approximately 60% of primary human colorectal cancers (Goel et al 2004);
- The mTOR pathway is involved in the production of pro-angiogenic factors (i.e., VEGF) and inhibition of endothelial cell growth and proliferation;
- Through inactivating eukaryotic initiation factor 4E binding proteins and activating the 40S ribosomal S6 kinases (i.e., p70S6K1), mTOR regulates protein translation, including the HIF-1 proteins. Inhibition of mTOR is expected to lead to decreased expression of HIF-1.

1.2.1.2 mTOR pathway and 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* high-grade gliomas (Rasheed, Stenzel et al. 1997; Ermoian, Furniss et al. 2002; Choe, Horvath et al. 2003), methylation of the *PTEN* promoter is thought to be the underlying mechanism of *PTEN* alteration found in LGGs and secondary high-grade gliomas (Wiencke, Zheng et al. 2007).

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

Not only is methylation of the *PTEN* promoter frequent and associated specifically with LGGs and secondary high-grade 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, Zheng et al. 2007). Furthermore, a retrospective study of 45 LGG (grade II) tumor specimens from newly diagnosed patients shows a negative correlation between activation of the PI3K/AKT/mTOR pathway and survival in LGG patients (McBride, Perez et al. 2010). These 45 LGG tumor specimens from newly diagnosed patients were analyzed for methylation of the putative 50-promoter region of *PTEN* using methylation specific PCR as well as phosphorylation of S6 (p-S6) and PRAS40 (p-PRAS40) and expression of PTEN protein using immunohistochemistry. There was a trend towards statistical significance, with *PTEN*-methylated patients having decreased survival ($P = 0.128$). Eight of 29 patients that expressed p-S6 died, whereas all 9 patients lacking p-S6 expression were alive at last follow-up. There was an inverse relationship between expression of p-S6 and survival ($P = 0.029$). There was a trend towards decreased survival in patients expressing p-PRAS40 ($P = 0.077$). Analyses of relationships between molecular markers demonstrated a statistically significant positive correlation between expression of p-S6 and p-PRAS40 ($P = 0.04$);

expression of p-S6 correlated positively with *PTEN* methylation (P = 0.04) and negatively with PTEN expression (P = 0.03). Survival of LGG patients negatively correlates with phosphorylation of S6 protein (McBride, Perez et al. 2010). Activation of the PI3K/AKT/mTOR pathway in approximately half of adult LGGs provides the pre-clinical rationale for the use of selective mTOR inhibitors in the treatment of LGG.

The mammalian target of rapamycin (mTOR) is downstream to the PI3K/PTEN-AKT survival pathway and is therefore an ideal target for LGG and secondary high-grade glioma patients with *PTEN* promoter methylation and activation of the PI3K/AKT/mTOR pathway. In fact, there is evidence that tumors expressing activated Akt appear particularly sensitive to mTOR inhibition (Gera, Mellinghoff et al. 2004; Noh, Mondesire et al. 2004).

1.2.1.3 Non-clinical experience with everolimus

Everolimus inhibits the proliferation of a range of human tumor cell lines *in vitro* including lines originating from lung, breast, prostate, colon, melanoma and glioblastoma. IC50s range from sub/low nM to µM. Everolimus also inhibits the proliferation of human umbilical vein endothelial cells (HUVECS) *in vitro*, with particular potency against VEGF-induced proliferation suggesting that everolimus may also act as an anti-angiogenic agent. The anti-angiogenic activity of everolimus was confirmed *in vivo*. Everolimus selectively inhibited VEGF-dependent angiogenic response at well tolerated doses. Mice with primary and metastatic tumors treated with everolimus showed a significant reduction in blood vessel density when compared to controls.

The potential of everolimus as an anti-cancer agent was shown in rodent models. Everolimus is orally bioavailable, residing longer in tumor tissue than in plasma in a subcutaneous mouse xenograft model, and demonstrating high tumor penetration in a rat pancreatic tumor model. The pharmacokinetic profile of everolimus indicates sufficient tumor penetration, above that needed to inhibit the proliferation of endothelial cells and tumor cell lines deemed sensitive to everolimus *in vitro*.

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

It is not clear which molecular determinants predict responsiveness of tumor cells to everolimus. Molecular analysis has revealed that relative sensitivity to Everolimus *in vitro* correlates with the degree of phosphorylation (activation) of the AKT/PKB protein kinase and the S6 ribosomal protein; in some cases (i.e., glioblastoma) there is also a correlation with PTEN status.

In vivo studies investigating the anti-tumor activity of everolimus in experimental animal tumor models showed that everolimus monotherapy typically reduced tumor cell growth rates rather than produced regressions. These effects occurred within the dose range of 2.5 mg to 10 mg/kg, orally once a day.

In preclinical models, the administration of everolimus is associated with reduction of protein phosphorylation in target proteins downstream of mTOR, notably phosphorylated S6 (p-S6) and p-4EBP1, and occasionally with an increase in phosphorylated AKT, a protein upstream of mTOR signaling pathway.

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

Further details can be found in the everolimus Investigator's Brochure.

1.2.1.4 Clinical experience with everolimus in gliomas

There is extensive clinical experience with mTOR inhibitors including the evaluation in the treatment of patients with malignant glioma (Galanis, et al. 2005, Cloughesy, et al. 2008). 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 everolimus 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 inhibitors in these TSC patients.

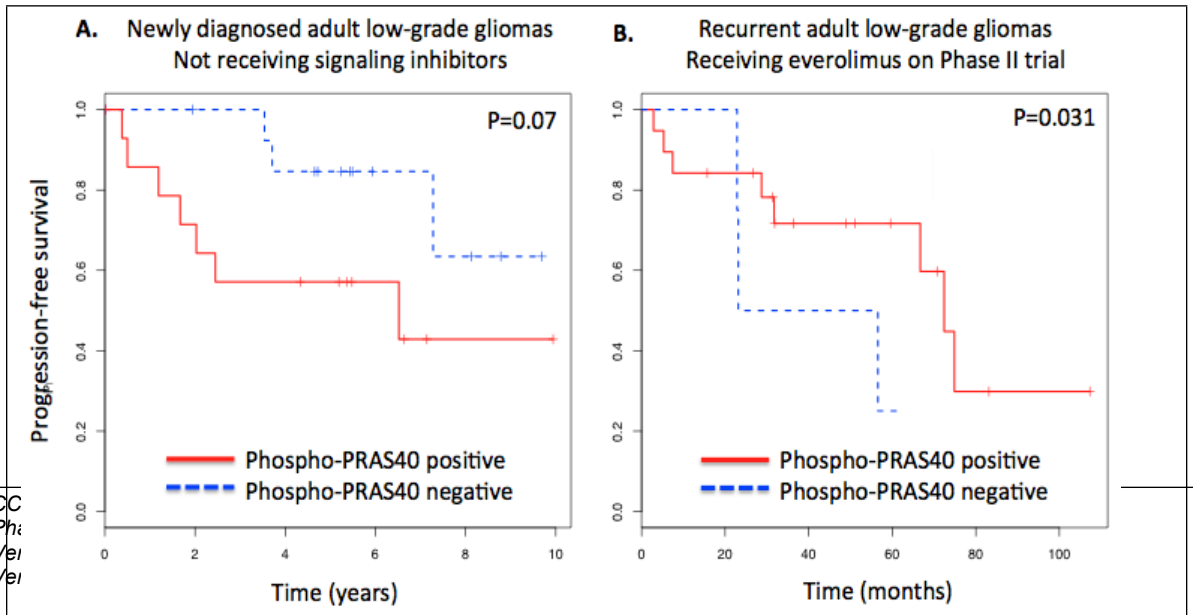


Fig. 1-2. Activation of mTOR in adult LGG correlates with poor outcome (A), but with response to mTOR inhibitor everolimus (B).

2 Rationale

2.1 Rationale for everolimus in adult low-grade gliomas (LGGs)

As explained above in Section 1.2.1.2, in adults with newly-diagnosed low-grade gliomas, we have previously shown that *PTEN* promoter methylation leads to functional activation of the PI3K pathway and that such pathway activation correlates with poor survival for these patients (Wiencke, Zheng et al. 2007; McBride, Perez et al. 2010). Furthermore, mTOR lies downstream in the PI3K/PTEN/AKT survival pathway and is therefore an ideal target for LGG and secondary high-grade glioma patients with *PTEN* promoter methylation and activation of the PI3K/AKT/mTOR pathway. In fact, there is evidence that tumors expressing activated AKT appear particularly sensitive to mTOR inhibition (Gera, Mellinghoff et al. 2004; Noh, Mondesire et al. 2004).

Thus, activation of the PI3K/AKT/mTOR pathway in approximately half of adult LGGs and association of this activation with glioma progression and poor clinical outcome, coupled with susceptibility of PI3K-activated tumors to mTOR inhibition, all establish a strong rationale for the treatment of newly-diagnosed LGGs with everolimus.

In addition to the above-stated pre-clinical rationale, promising results for the use of everolimus in adult LGGs have emerged from an ongoing phase II UCSF trial testing everolimus for adults with recurrent LGGs (Haas-Kogan, Phillips et al. 2013). In this study, 36 patients have enrolled, 12 continue on therapy, 15 had stable disease for >1 year, and 4 had stable disease for >2 years, most of them despite multiple prior recurrences. Staining for p-PRAS40 was associated with improved PFS (log-rank, p=0.03; Fig. 1-2B), which is in contradistinction to a cohort of newly diagnosed adult LGGs (not treated with signaling inhibitors) in which p-PRAS40 staining was associated with worse PFS (log-rank, p=0.07; Fig. 1-2A). These data represent an interm analysis of an ongoing Phase II study and therefore conclusions should be drawn with caution given their preliminary nature. In addition, it should be noted that although all patients receiving everolimus on this Phase II study had recurrent gliomas, molecular analyses were performed on available tissues that were from the primary tumor in some patients and from the recurrent tumor in others. In Figure 1, molecular analyses for three of the four p-PRAS40 negative tumors, were performed on primary tumor samples as the specimens for the recurrent tumors were too small for a large number of assays. Nonetheless, the apparent “reversal” of association of p-PRAS40 staining with PFS when everolimus is administered (Fig. 1-2) supports our central hypothesis that PI3K/mTOR pathway activation acts as a predictive marker of response to mTOR inhibition.

2.2 Rationale for combining everolimus and temozolomide in adult LGGs

There is a strong biological rationale for combining everolimus and TMZ for newly diagnosed patients with LGGs. Our collaborators at UCSF, as outlined below, have documented that TMZ treatment of LGGs in some cases appears to cause driver mutations in the PI3K/AKT/mTOR pathway that lead to malignant transformation. Therefore we believe that the addition of everolimus to TMZ will block emergence of PI3K/AKT/mTOR-activated tumor recurrence,

reduce the risk of TMZ-induced transformation of LGGs to higher-grade gliomas, and improve patient outcomes.

Alkylating agents such as TMZ are carcinogenic, and exposure to this class of systemic therapies is associated with a small but reproducible increased risk of developing treatment-related leukemias. In experimental systems, TMZ induces mutagenesis by methylating the O6 position of guanine, leading to mispairing with thymine. If not recognized and repaired by O6-methylguanine-DNA methyltransferase (MGMT) or by DNA mismatch repair (MMR), this results in a guanine to adenosine (G to A) transition mutation upon DNA replication. Previous analyses of recurrent GBMs have shown an association between TMZ or other alkylating agent treatment, MMR pathway mutations, and high rates of G to A mutagenesis. While MMR pathway mutations are associated with resistance to TMZ therapy, the clinical impact of TMZ-associated mutagenesis is unknown.

LGGs are slow-growing tumors that often undergo malignant transformation to an aggressive, high-grade glioma with a significantly worse prognosis. To investigate evolution of the mutational landscape over time, Dr. Costello at UCSF performed parallel sequencing of the exomes of the primary tumor, recurrent tumor, and matched normal control of 24 patients diagnosed with primary grade II astrocytomas. Six of eight patients treated with adjuvant TMZ therapy acquired more than 1,000 mutations, 95% of which were G to A, and in all six hyper-mutated cases, there was malignant progression to GBM. These TMZ-associated G to A mutations affected many genes linked directly to malignant phenotypes and resulted in both RB pathway deregulation and constitutive mTORC1 signaling in all six post-TMZ recurrent tumors relative to treatment naïve, patient matched primary tumors. While deregulation of these two pathways is associated with high-grade astrocytomas and has been implicated in malignant progression, the mechanisms driving these genetic changes are poorly understood. Therefore, our preliminary data suggests that TMZ therapy can drive the malignant progression of indolent grade II astrocytomas, in part through the induction of signature genetic alterations in these pathways. It is not yet clear why some but not all TMZ-exposed tumors undergo hypermutation. The somewhat predictable pattern of mutations however, suggests mTORC1 signaling is critical for malignant transformation, whether spontaneously occurring or induced by TMZ. Chronic inhibition of mTORC1 may therefore be one way to prevent or delay the initial outgrowth of malignantly transformed tumor sub-clones, and ultimately improve overall survival. Our preliminary data on the paired low-grade tumors and their high-grade recurrence support a model whereby genetic alterations that are sub-clonal can give rise to the recurrence. Thus, identification of such sub-clones at initial diagnosis is a critical step in identifying patients most likely to undergo transformation.

As malignantly transformed tumor sub-clones arise, either before or after surgical resection, initially they will be present in very few cells in the tumor, and may be undetectable by standard exome sequencing (mutation detection limit of approximately 1 in 100 cells). Thus, corresponding biomarkers of susceptibility to spontaneous or TMZ-associated malignant transformation may in fact be the alterations present in only rare cells of the initial tumor (varying degrees of sub-clonal). In a funded NIH study that will be complementary to what we propose in this clinical trial, exome sequencing of up to 2 samples per patient per surgery is

being used to define the frequency of TMZ induced hypermutation in a large patient cohort, including patients on this clinical trial.

In summary, these data show the direct acquisition of functionally significant driver genetic lesions in the PI3K/AKT/mTOR pathway during malignant transformation and suggest they are induced by TMZ exposure. Thus, we hypothesize that combining TMZ with everolimus will block emergence of PI3K/AKT/mTOR-activated clones and improve efficacy, as well as reduce the risk of TMZ-induced transformation of LGGs to higher-grade gliomas.

Building on the preliminary results seen in the recently-completed study of everolimus in recurrent LGGs (Haas-Kogan, Phillips et al. 2013), the proposed study will select treatment for patients both by 1p/19q chromosomal status and by whether or not their LGG demonstrates activation of the PI3K/mTOR pathway, but will target patients who have received no treatment aside from surgery, rather than potentially heavily pre-treated recurrent patients. If 1p/19q co-deletion is present, patients will be treated with single-agent everolimus. If 1p/19q co-deletion is not present but PI3K/mTOR pathway activation is present, patients will be treated with single-agent everolimus. If 1p/19q co-deletion is not present and PI3K/mTOR pathway activation is also not present, patients will be treated with a combination of everolimus and TMZ. The combination of everolimus and TMZ has been tested and found to be tolerable in GBM patients (Mason, Macneil et al. 2012) as well as in patients with melanoma (Dronca, Allred et al. 2013), as described in Section 2.4.

2.3 Rationale for selection by 1p/19q deletion status

Combined 1p/19q deletion, typically involving the entire chromosome arms at both sites, is a molecular signature of oligendrogliomas, seen in 50%–80% of patients with this histology. 1p/19q co-deletion is also found in 30-50% of oligoastrocytomas and 0-15% of astrocytomas, with an overall frequency of approximately 30% in all adult LGGs [Reviewed in (Sanai, Chang et al. 2011)]. Comparative genomic hybridization, loss of heterozygosity, quantitative microsatellite analysis, and fluorescence *in situ* hybridization are all techniques used to identify tumors with 1p/19q co-deletion. The distinction between a prognostic indicator of clinical outcome regardless of therapy and a predictive measure of response to a particular therapy is very important and 1p/19q co-deletion appears to serve both roles. Its prognostic significance is reflected in longer progression-free survival and more indolent behavior in oligodendrogliomas with 1p/19q co-deletion. Its predictive value is reflected in studies that first suggested that 1p/19q co-deletion is a marker of response to PCV chemotherapy and then indicated that 1p/19q co-deleted LGGs also respond favorably to temozolomide [Reviewed in (Sanai, Chang et al. 2011)]. Houillier *et al.* reported that adult LGGs exhibited an objective response rate to TMZ of 66% if 1p/19q co-deletion was present but only 31% if 1p/19q co-deletion was absent (Houillier, Wang et al. 2010). Because of its role as a prognostic factor and predictive marker, paradigms for treatment of adult LGGs incorporate 1p/19q co-deletion status into the decision making process. Therefore, in our study of everolimus for newly diagnosed adult LGGs for whom treatment will be selected by activation of the PI3K/mTOR pathway, we have also chosen to select treatment based on patients' tumor 1p/19q co-deletion status. On this clinical trial, favorable patient with 1p/19q co-deletion will receive single agent everolimus.

The less favorable group that lacks 1p/19q co-deletion will be further selected by PI3K/mTOR pathway activation, with pathway activated tumors (reflected in positive phospho-PRAS40 staining) receiving single agent everolimus and pathway non-activated tumors (reflected in negative phospho-PRAS40 staining) receiving dual therapy with everolimus and temozolomide.

In contrast to oligodendrogliomas and oligoastrocytomas there is a high incidence of somatic mutations in the alpha thalassemia/mental retardation syndrome X-linked (ATRX) gene in diffuse astrocytomas with mutation in IDH1/IDH2 (Jio 2012; Kannan K 2012; Killela PJ 2013]. Based on our current understanding of diffuse glioma, in the vast majority of cases ATRX mutation is mutually exclusive with 1p/19q-coeletion and mutually exclusive with TERT promoter mutation, and several studies now demonstrate that ATRX mutation status can be used in combination with IDH1/IDH2 mutation status and 1p/19q-codeletion status to generate a molecular diagnostic algorithm that is superior to to histopathologic classification alone to identify patient subgroups with different outcomes (Wiestler B 2013; Reuss DE et al. 2014; Cancer Genome Atlas Research Network 2015; Eckel-Passow JE 2015). Thus, low-grade tumors with mutation in ATRX will lack 1p/19q-codeletion and low-grade tumors without ATRX mutation will have 1p/19q-codeletion.

2.4 Safety of combined everolimus and temozolomide

The combination of everolimus and temozolomide has been reported in a phase I study (Mason, Macneil, et al., 2012) combining the two drugs for treatment of GBM, either in the adjuvant setting immediately after radiation or in the setting of first recurrence after initial treatment, as well as in a phase II study in patients with metastatic, unresectable melanoma (Dronca, Allred, et al, 2013). In the phase I study, the recommended phase II dose was everolimus 10 mg/day with TMZ 150 mg/m2/d x 5 days out of 28. At this dose, myelosuppression was mild, with 2 patients each having grade 3 thrombocytopenia, lymphopenia, and granulocytopenia. Common adverse events otherwise included mild fatigue, flushing, pruritus, rash, mucositis, nausea and vomiting, headache, cough and mild pneumonitis. The combination of everolimus and TMZ at 200 mg/m2/d was attempted only in combination with an everolimus dose of 2.5 mg/day; this dose level had no DLTs, but 3 of 4 patients required a delay in restarting TMZ, and 2 of 3 patients were dose-reduced to TMZ 150 mg/m2/d, so no further attempts to treat patients at 200 mg/m2/d were made in that study. In the phase II study in melanoma patients, patients were treated with everolimus 10 mg/d 5 days/week, with TMZ dosed at 200 mg/m2/d x 5 days out of 28. 9 out of 48 patients required dose reduction due to myelotoxicity, but overall the combination was well-tolerated, with the most common other toxicities being fatigue, nausea and elevated liver enzymes.

Given this clinical experience with the combination of everolimus and TMZ, we will treat patients at 10 mg daily of everolimus and 150 mg/m2/d x 5 days out of 28 for the first cycle. If the first cycle is well-tolerated, the treating physician will have the option to increase TMZ to 200 mg/m2/d for the second cycle and beyond.

3 Objectives and endpoints

Objectives:

Primary objectives

Arm 1: To assess progression-free survival in patients with previously untreated ATRX lost and/or 1p/19q intact, PI3K/mTOR pathway-activated LGG treated with everolimus.

Arm 2: To assess progression-free survival in patients with previously untreated ATRX lost and/or 1p/19q intact, PI3K/mTOR pathway-non-activated LGG treated with everolimus and TMZ.

Arm 3: To assess progression-free survival in patients with previously untreated ATRX intact and/or 1p/19q co-deleted LGG treated with everolimus.

Secondary objectives

1) To assess overall and progression-free survival distributions (Arms 1, 2 & 3).

2) To assess the objective response rate to treatment (Arms 1, 2 & 3).

3) To further delineate the safety profile of the combination of everolimus and TMZ (Arm 2)

4) To assess whether treatment (Arms 1, 2 & 3) provides clinical benefit by reducing seizure frequency

Exploratory objectives

1) 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.

2) To assess for an association between the presence/absence of clonal or subclonal genetic mutations in the PI3K pathway and Median PFS, Objective Response Rate (ORR), *PTEN* methylation, and the immunohistochemical measurements of the PI3K pathway activation in patients treated with everolimus or TMZ and everolimus.

3) To longitudinally assess quality of life (QoL) in low-grade glioma patients over the course of treatment with everolimus (Arms 1 & 3).

Endpoints:

Primary Endpoint:

Arms 1 & 2: progression-free survival at 33 months (PFS-33)
Arm 3: progression-free survival at 38 months (PFS-38)

Secondary Endpoints (for all arms):

1) Median and distribution of overall survival (OS) and PFS

2) Objective Response Rate (ORR)

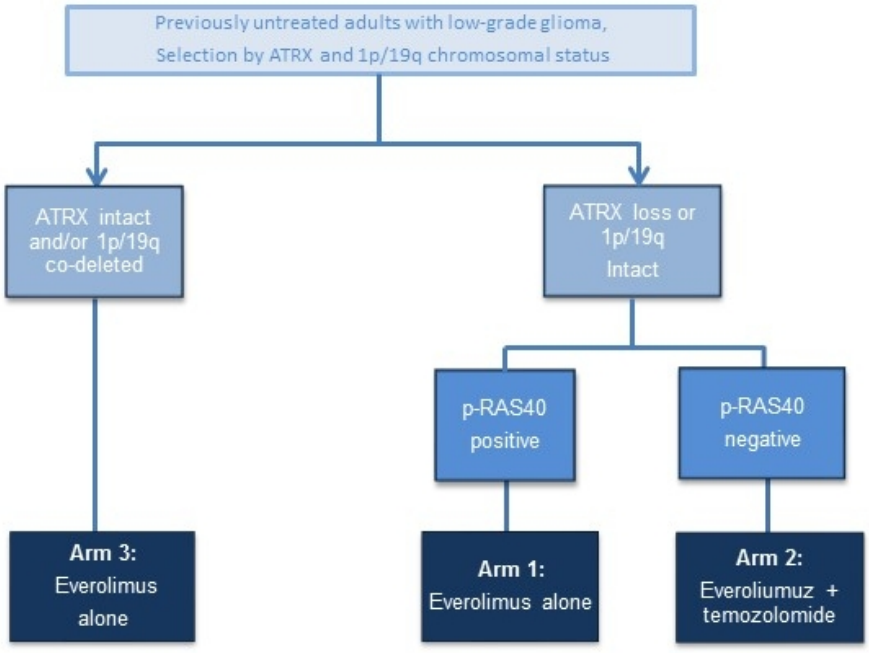
3) Number of adverse events related to combination treatment

4) Rate of Reduction in Seizures (as outlined in van den Bent, Wefel *et al.* 2011)

4 Study design

Treatment for patients with previously untreated LGG will be selected by whether or not their tumor has ATRX loss and/or 1p/19q chromosomal co-deletion. If the tumor is ATRX lost or 1p/19q intact, then patients will be further selected by whether or not their tumor demonstrates activation of the PI3K/mTOR pathway. If activation is present, patients will be treated in Arm 1 with single-agent everolimus at 10 mg daily continuously. If activation is not present, patients will be treated in Arm 2 with combined everolimus and TMZ. Everolimus will be given at 10 mg daily continuously, and TMZ will be dosed initially at 150 mg/m^2/day for 5 days out of a 28-day cycle. If ATRX is intact, 1p/19q co-deletion should be confirmed, or if only 1p/19q co-deletion is available and present, patients will be treated in Arm 3 with single-agent everolimus at 10 mg daily continuously. In all arms, treatment with everolimus will continue for up to 24 cycles, after which patients will be followed with interval MRIs until progression. In Arm 2, TMZ will be stopped after 12 cycles.

Figure 2-1: Study Schema



5 Population

The total number of patients enrolled in this study will be 105, with 35 patients in each of the three treatment arms.

5.1 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.

Inclusion criteria:

1. Age ≥ 18 years
2. KPS ≥ 60
3. Adequate bone marrow function as shown by: ANC ≥ 1.5 x 10^9/L, Platelets ≥ 100 x 10^9/L, Hb ≥ 9.0 g/dL;
4. Adequate liver function as shown by:
   a. Total serum bilirubin ≤ 2.0 mg/dL,

   b. ALT and AST ≤ 2.5x ULN,
   c. INR ≤ 2;
5. Adequate renal function: serum creatinine ≤1.5 x ULN;
6. Fasting serum cholesterol ≤ 300 mg/dL OR ≤ 7.75 mmol/L AND fasting triglycerides ≤ 2.5x 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 with confirmed reduction of lab values to within eligibility parameters;
7. Signed informed consent obtained prior to any screening procedures.
8. Patients must have histologically proven supratentorial low-grade glioma at initial diagnosis and at the time of any subsequent surgeries; pathology must have been reviewed by UCSF neuropathology. Eligible low-grade gliomas include: astrocytoma, oligodendroglioma and mixed oligoastrocytoma. Pilocytic astrocytomas are excluded.
9. Patient's tumor must have documentation of the presence of an IDH-1 and/or IDH-2 mutation of any type.
10. Results of ATRX and/or 1p/19q chromosomal status: if ATRX is lost, 1p/19q status is not required. If ATRX is intact, 1p/19q chromosomal status must be available to permit treatment selection.
11. Results of pRAS40 testing.
12. Patients must have evaluable disease. Patients must begin treatment within 120 days of most recent surgical procedure.

Exclusion criteria:

1. Patients may not have had any prior tumor treatment except for surgery, and must have adequately recovered from surgery.
2. Known intolerance or hypersensitivity to everolimus or other rapamycin analogs (e.g. sirolimus, temsirolimus) or to temozolomide;
3. Known impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of oral everolimus or temozolomide;
4. Uncontrolled diabetes mellitus as defined by HbA1c > 8.0% despite adequate therapy. Patients with a known history of impaired fasting glucose or diabetes mellitus (DM) may be included, however blood glucose and antidiabetic treatment must be monitored closely throughout the trial and adjusted as necessary;
5. Patients who have any severe and/or uncontrolled medical conditions such as:
   a. unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction ≤6 months prior to start of everolimus, serious uncontrolled cardiac arrhythmia, or any other clinically significant cardiac disease
   b. symptomatic congestive heart failure of New York heart Association Class III or IV
   c. active (acute or chronic) or uncontrolled severe infection, liver disease such as cirrhosis, decompensated liver disease, and chronic hepatitis (i.e. quantifiable HBV-DNA and/or positive HbsAg, quantifiable HCV-RNA),
   d. known severely impaired lung function (spirometry and DLCO 50% or less of normal and O_2 saturation 88% or less at rest on room air),

e. active, bleeding diathesis;

6. Chronic treatment with corticosteroids or other immunosuppressive agents. Topical or inhaled corticosteroids are allowed, and treatment with low dose Decadron (≤ 3mg daily) is allowed;
7. Known history of HIV seropositivity;
8. Positive serological test results for hepatitis B (see Section 5.2, Table 5)
9. Positive serological test result for hepatitis C (see Section 5.2)
10. Patients who have received live attenuated vaccines within 1 week of start of treatment and during the study. Patient should also avoid close contact with others who have received live attenuated vaccines. Examples of live attenuated vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines;
11. Patients who have a history of another primary malignancy, with the exceptions of: non-melanoma skin cancer, and carcinoma in situ of the cervix, uterus, or breast, unless the patient has been disease free for ≥ 3 years;
12. Patients with a history of non-compliance to medical regimens or who are considered potentially unreliable or will not be able to complete the entire study;
13. Patients who are currently part of or have participated in any clinical investigation with an investigational therapeutic drug within 1 month prior to dosing;
14. Pregnant or nursing (lactating) women;
15. Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant, who are not willing to use adequate methods of contraception during the study and for 8 weeks after the end of treatment.

    Women are considered post-menopausal and not of child-bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks prior to randomization. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child-bearing potential.
16. Male patients whose sexual partner(s) are WOCBP who are not willing to use adequate contraception, during the study and for 8 weeks after the end of treatment

5.2 Screening for hepatitis B and C

Prior to start of everolimus, patients should be tested for hepatitis B serologic markers, that is, HBsAg, HBs Ab, and HBc Ab. Patients should also be tested for hepatitis C using HCVAb.

Table 5-1 Action to be taken based on screening hepatitis B results

Test	Result	Result	Result	Result
HBsAg	+	-	-	-
HBsAb	+ or -	+ and no prior HBV vaccination	+ or -	- or + with prior HBV vaccination
HBcAb	+ or -	+ or -	+	-
Recommendation	Patient is not eligible for study			Patient is eligible for study

6 Treatment

6.1 Study treatment

6.1.1 Everolimus

Everolimus is supplied by Novartis. See Section 1.2.1 for additional drug information. Everolimus is formulated as tablets for oral administration, of 2.5mg, 5mg, and 10mg strength. Tablets are blister-packed under aluminum foil, which should be opened only at the time of administration as drug is both hygroscopic and light-sensitive.

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 everolimus will be described on the medication label.

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

6.1.2 Temozolomide

Temozolomide for this trial will be obtained from commercial suppliers. Formulation and packaging may vary depending on the commercial suppliers; there will be no study-specific labeling unless required by local regulations.

Please refer to the package insert for information regarding the preparation and dispensing of temozolomide (see Appendix 4).

6.1.3 Treatment assignment

Patient treatment will be selected by whether or not their tumor tissue demonstrates 1p/19q chromosomal co-deletion and by PI3K/mTOR pathway activation (see Section 7.3.2). As per Section 4, patients whose tumors are ATRX lost and/or 1p/19q intact and that demonstrate pathway activation will be treated on Arm 1 with single-agent everolimus. Patients whose tumors are ATRX lost and/or 1p/19q intact but do not demonstrate pathway activation will be treated on Arm 2 with the combination of everolimus and temozolomide. Patients whose tumors are 1p/19q co-deleted will be treated on Arm 3 with single-agent everolimus regardless of mTOR pathway status. Patients whose tumors are ATRX intact must be tested for 1p/19q

co-deletion and, if co-deletion is confirmed they will be treated on Arm 3 with single-agent everolimus regardless of mTOR pathway status.

6.1.4 Everolimus dosing regimen

The investigator should promote compliance by instructing the patient to take the study drug exactly as prescribed and by stating that compliance is necessary for the patient's safety and the validity of the study. The patient should be instructed to contact the investigator if he/she is unable for any reason to take the study drug as prescribed.

Everolimus should be administered orally once daily at the same time every day, either consistently with or consistently without food. The initial dose will be 10 mg daily. The tablets should be swallowed whole with a glass of water and should not be chewed or crushed. For patients unable to swallow tablets, the tablet(s) should be dispersed completely in a glass of water (containing approximately 30 mL) by gently stirring, immediately prior to drinking. The glass should be rinsed with the same volume of water and the rinse completely swallowed to ensure the entire dose is administered.

If vomiting occurs, no attempt should be made to replace the vomited dose. Patients should be instructed that if they miss a dose on one day, they must not take any extra dose the next day, but instead to immediately contact the study center as soon as possible to ask for advice.

6.1.5 Temozolomide dosing regimen

Temozolomide will be orally self-administered at approximately the same time each day on days 1 through 5 of each 28 day dosing cycle. A temozolomide dose of 150 mg/m^2 per day will be given for Cycle 1 of TMZ. This dose may increase to 200 mg/m^2 body surface area per day in subsequent TMZ cycles per the treating physician's discretion. Any change in weight of more than 10% will require re-calculation of the administered temozolomide dose; otherwise, dosing may be based on the baseline weight. The total dose of TMZ shall be rounded to the nearest 5 mg. Dosing should occur according to instructions in the product label and per standard clinical practice.

6.2 Dose modifications

6.2.1 Dose modification and dose delay

For patients who do not tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to allow the patient to continue the study treatment. Details of study treatment dose levels are provided in Table 6-1.

Table 6-1a Everolimus dose levels

Dose level	Dose and schedule
0 (starting dose)	10 mg daily
-1	5 mg daily
-2	2.5 mg daily

Table 6-1b Temozolomide dose levels

Dose level	Dose and schedule
+1 (dose increased at cycle 2 at treating MD's discretion)	200 mg/m^2/day for days 1-5 of each 28-day cycle
0 (starting dose)	150 mg/m^2/day for days 1-5 of each 28-day cycle
-1	100 mg/m^2/day for days 1-5 of each 28-day cycle
-2	75 mg/m^2/day for days 1-5 of each 28-day cycle

If a patient has already decreased 2 dose levels of a specific drug, no further dose reduction is permitted. Patients who need an additional dose reduction will be required to discontinue the drug in question.

In the combination treatment arm, hematologic toxicity will initially be presumed due to temozolomide. Nausea, emesis and constipation during or within a few days after temozolomide dosing will be presumed due to temozolomide. Acneiform rash will be presumed due to everolimus; all other rash will initially be presumed due to temozolomide. Elevation of liver enzymes, hyperglycemia, hyperlipidemia, mucositis, diarrhea, and noninfectious pneumonitis will initially be presumed due to everolimus.

Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.

6.2.2 Management of specific everolimus toxicities

Table 6-2 and Table 6-3 list the dosing guidelines for everolimus-related non-hematologic and hematologic toxicities.

Table 6-2 Dosing guidelines for Everolimus-related non-hematologic toxicities

Toxicity	Action
Non-Infectious Pneumonitis	Please refer to Table 6-6.
AST or ALT elevation Grade 1 (> ULN - 3.0 x ULN) Grade 2 (> 3.0 - 5.0 x ULN)	Maintain current dose level
AST or ALT elevation Grade 3 (> 5.0 - 20.0 ULN)*	Interrupt everolimus administration until resolution to ≤ grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤ 7 days, everolimus should be re-started at the dose level prior to interruption. If resolution takes > 7 days, or if event recurs within 28 days, hold everolimus until recovery to ≤ grade 1 or baseline grade / value and reintroduce everolimus at one dose level lower, if available.
AST or ALT elevation Grade 4 (> 20 x ULN)* Recurrence of grade 4 after dose reduction or toxicity requiring Everolimus interruption for > 28 days	Interrupt everolimus administration until resolution to ≤ grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤ 7 days, everolimus should be re-started at one dose level lower. If resolution takes > 7 days, discontinue everolimus. Discontinue everolimus.
Intolerable grade 2 mucositis, or grade 3 AE, except hyperglycemia or hypertriglyceridemia or hypercholesterolemia (see Section 6.2.2.5)	Interrupt everolimus administration until resolution to ≤ grade 1 or baseline grade / value. If resolution occurs within ≤ 7 days, everolimus should be re-started at the dose level prior to interruption. If resolution takes > 7 days, or if event recurs within 28 days, hold everolimus until recovery to ≤ grade 1 or baseline grade / value and reintroduce everolimus at one dose level lower, if available. Patients will be withdrawn from the study if they fail to recover to ≤ grade 1 or baseline grade / value within 28 days.
Any other grade 4	Hold everolimus until recovery to grade ≤ 1 or baseline value Reintroduce everolimus at one dose level lower, if available.
Grade 3 or 4 clinical liver failure (asterixis or encephalopathy/coma)	Discontinue everolimus
Recurrence of intolerable grade 2 mucositis or grade 3 event after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of everolimus is 2.5 mg daily. Below this level, everolimus must be discontinued.
Recurrence of grade 4 after dose reduction	Discontinue everolimus
Any non-hematologic toxicity requiring Everolimus interruption for > 28 days	Discontinue everolimus

Table 6-3 Dosing guidelines for everolimus-related hematologic toxicities

Toxicity	Action
Grade 2 thrombocytopenia (platelets <75, ≥ 50x109/L)	No action

Grade 3 thrombocytopenia (platelets <50, ≥ 25 x109/L)	Interrupt everolimus until resolution to grade ≤1 If resolution occurs ≤ 7 days, reintroduce everolimus at the dose level prior to interruption. If resolution occurs > 7 days and ≤ 28 days, reintroduce everolimus at one dose level lower, if available.
Grade 4 thrombocytopenia (platelets < 25 x109/L)	Interrupt everolimus until recovery to grade ≤ 1. Then reintroduce everolimus at one dose level lower, if available.
Grade 3 neutropenia or anemia (neutrophil <1, ≥0.5 x109/L)	Interrupt everolimus until resolution to grade ≤1 or baseline value If AE resolution occurs ≤ 7 days, reintroduce everolimus at the same dose level. If AE resolution occurs > 7 days and ≤28 days, reintroduce everolimus at one dose level lower, if available.
Grade 4 neutropenia or anemia	Interrupt everolimus until recovery to grade ≤ 1 or baseline value. Reintroduce everolimus at one dose level lower, if available.*
Febrile neutropenia	Interrupt everolimus until resolution to grade ≤ 1 (or baseline value) and no fever. Reintroduce everolimus at one dose level lower, if available.*
Recurrence of grade 3 toxicity after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of everolimus is 5 mg every other day (2.5 mg daily). Below this level, everolimus must be discontinued.
***Recurrence of grade 4 toxicity (including febrile neutropenia) after dose reduction**	**Discontinue everolimus**
***Any hematologic toxicity requiring Everolimus interruption for > 28 days**	**Discontinue everolimus**

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

Adverse events most frequently observed with everolimus are stomatitis, rash, diarrhea, fatigue, infections, asthenia, nausea, peripheral edema, decreased appetite, headache,dysgeusia, epistaxis, mucosal inflammation, pneumonitis, weight decreased, vomiting, pruritus, cough, dyspnea, dry skin, nail disorder, and pyrexia. Overall, the most frequently observed laboratory abnormalities include decreased hematology parameters including hemoglobin, lymphocytes, platelets, and neutrophils (or collectively as pancytopenia).; increased clinical chemistry parameters including cholesterol, triglycerides, glucose, aspartate transaminases,creatinine, alanine transaminases, and bilirubin; and decreased clinical chemistry parameters including phosphate and potassium. The majority of these AEs have been of mild to moderate severity (NCI CTC grade 1-2).

6.2.2.1 Management of infections

Everolimus has immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoal infections, including infections with opportunistic pathogens. Localized and systemic infections, including pneumonia, other bacterial infections, invasive fungal infections,

such as aspergillosis or candidiasis and viral infections including reactivation of hepatitis B virus, have been described in patients taking everolimus. Some of these infections have been severe (e.g. leading to respiratory or hepatic failure) and occasionally have had a fatal outcome.

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

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

6.2.2.2 Management of skin toxicity

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

6.2.2.3 Management of stomatitis / oral mucositis / mouth ulcers

Table 6-4: Management of stomatitis/oral mucositis/mouth ulcers

Adverse Drug Reaction	Severity	Afinitor Dose Adjustment and Management Recommendations
Stomatitis	Grade 1	No dose adjustment required. Manage with non-alcoholic or salt water (0.9%) mouth wash severaltimes a day.
	Grade 2	Temporary dose interruption until recovery to grade ≤1. Re-initiate everolimusat same dose. If stomatitis recurs at grade 2, interrupt dose until recovery to grade ≤1. Re-initiate everolimus at lower dose. Manage with topical analgesic mouth treatments (e.g. benzocaine,butyl aminobenzoate, tetracaine hydrocholoride, methol or phenol)with or without topical corticosteroids (i.e. triamcinolone oral paste)*.
	Grade 3	Temporary dose interruption until recovery to grade ≤1. Re-initiate everolimus at lower dose. Manage with topical analgesic mouth treatments (i.e. benzocaine,butyl aminobenzoate, tetracaine hydrocholoride, methol or phenol) with or without topical corticosteroids (i.e. triamcinolone oral paste)*
	Grade 4	Discontinue everolimus and treat with appropriate medical therapy.

* Avoid using agents containing hydrogen peroxide, iodine, and thyme derivatives in management of stomatitis as they may worsen mouth ulcers.

Patients with a clinical history of stomatitis/mucositis/mouth ulcers and those with gastrointestinal morbidity associated with mouth/dental infections, irritation of esophageal

mucosa e.g. gastroesophageal reflux disease (GERD) and pre-existing stomatitis/mucositis must be monitored even more closely. Patients should be instructed to report the first onset of buccal mucosa irritation/reddening to their study physician immediately.

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

1. For mild toxicity (grade 1), no dose adjustment required. Manage with non-alcoholic mouth wash or salt water (0.9%) mouth wash several times a day until resolution.
2. For more severe toxicity (grade 2 in which case patients have pain but are able to maintain adequate oral alimentation, or grade 3 in which case patients cannot maintain adequate oral alimentation), the suggested treatments are topical analgesic mouth treatments (i.e., local anesthetics such as, benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or phenol) with or without topical corticosteroids, such as triamcinolone oral paste 0.1% (Kenalog in Orabase®).
3. Agents containing alcohol, hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. These agents should be avoided.
4. Antifungal agents should be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (ketoconazole, fluconazole, itraconazole, etc.) should be avoided in all patients due to their strong inhibition of everolimus metabolism, therefore leading to higher everolimus exposures. Therefore, topical antifungal agents are preferred if an infection is diagnosed.

6.2.2.4 Management of diarrhea

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

6.2.2.5 Management of hyperlipidemia and hyperglycemia

Table 6-5: Management of hyperlipidemia and hyperglycemia

Adverse Drug Reaction	Severity	Everolimus Dose Adjustment and Management Recommendations
Metabolic events (e.g. hyperglycemia, dyslipidemia)	Grade 1	No dose adjustment required. Initiate appropriate medical therapy and monitor.
	Grade 2	No dose adjustment required. Manage with appropriate medical therapy and monitor.
	Grade 3	Interrupt everolimus administration until resolution to ≤ grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). Re-initiate everolimus at lower dose. Manage with appropriate medical therapy and monitor.
	Grade 4	Discontinue everolimus and treat with appropriate medical therapy.

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

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

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

6.2.2.6 Management of non-infectious pneumonitis

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

- A diagnosis of non-infectious pneumonitis should be considered in patients presenting with non-specific respiratory signs and symptoms such as hypoxia, pleural effusion, cough or dyspnea, and in whom infectious, neoplastic and other non-medicinal causes have been excluded by means of appropriate investigations. Patients should be advised to report promptly any new or worsening respiratory symptoms.

- Patients who develop radiological changes suggestive of non-infectious pneumonitis and have few or no symptoms may continue everolimus therapy without dose alteration. If symptoms are moderate (grade 2), consideration should be given to interruption of therapy until symptoms improve. The use of corticosteroids may be indicated. Everolimus may be reintroduced at a daily dose approximately 50% lower than the dose previously administered.
- For cases where symptoms of non-infectious pneumonitis are severe (grade 3 or 4), everolimus therapy should be discontinued and the use of corticosteroids may be indicated until clinical symptoms resolve. For cases of grade 3 non-infectious pneumonitis, Therapy therapy with everolimus may be re-initiated at a daily dose approximately 50% lower than the dose previously administered depending on individual clinical circumstances

If non-infectious pneumonitis develops, the guidelines in Table 6-6 should be followed. Consultation with a pulmonologist is recommended for any case of pneumonitis that develops during the study.

Table 6-6 Management of non-infectious pneumonitis

Worst grade pneumonitis	Suggested investigations	Management of pneumonitis	Everolimus dose adjustment
Grade 1	CT scans with lung windows.	No specific therapy is required	No dose adjustment required. Initiate appropriate monitoring.
Grade 2	CT scan with lung windows. Consider pulmonary function testing includes: spirometry, DLCO, and room air O_2 saturation at rest. Consider a bronchoscopy with biopsy and/or BAL. Monitoring at each visit until return to ≤ grade 1. Return to initial monitoring frequency if no recurrence.	Symptomatic only. Consider corticosteroids and/or other supportive therapy if symptoms are troublesome.	Rule out infection and consider interruption of everolimus until symptoms improve to Grade ≤ 1. Re-initiate everolimus at one dose level lower. Discontinue everolimus if failure to recover within ≤ 28 days.
Grade 3	CT scan with lung windows and pulmonary function testing includes: spirometry, DLCO, and room air O2 saturation at rest. Monitoring at each visit until return to ≤ grade 1. Return to initial monitoring frequency if no recurrence. Bronchoscopy with biopsy and/or BAL is recommended.	Consider corticosteroids if infective origin is ruled out. Taper as medically indicated.	Rule out infection and interrupt everolimus until symptoms improve to Grade ≤ 1. Consider re-initiating everolimus at one dose level lower (approximately 50% lower than the dose previously administered depending on individual clinical circumstances) Discontinue everolimus if failure to recover within ≤ 28 days.
Grade 4	CT scan with lung windows and required pulmonary function testing, if possible, includes: spirometry, DLCO, and room air O2 saturation at rest. Monitoring at each visit until return to ≤ grade 1. Return to initial monitoring frequency if no recurrence. Bronchoscopy with biopsy and/or BAL is recommended if possible.	Consider corticosteroids if infective origin is ruled out. Taper as medically indicated.	Rule out infection and discontinue everolimus.

For patients receiving both everolimus and temozolomide: if everolimus is stopped due to toxicity, patients may continue to be treated with temozolomide at the discretion of the treating investigator.

6.2.3 Management of specific temozolomide toxicities

Temozolomide will be administered every 4 weeks, unless the patient has not recovered from treatment-related adverse events associated with a prior course. Recovery has occurred once all of the following conditions have been met:

- ANC ≥1500/μL;
- Platelet count ≥100,000/μL;
- All drug-associated non-hematological toxicities have recovered to ≤ grade 2 or to baseline levels and the investigator does not feel that any of these toxicities present any hazard to patient safety or care.

If recovery has not occurred by Day 28, the subsequent course of treatment will be delayed until these criteria have been met. The dose of temozolomide in subsequent courses will be individually titrated (see Table 6-7 below). Growth factors cannot be used to induce elevations in neutrophil count for the purposes of administration of temozolomide on the scheduled dosing or to allow treatment with temozolomide at a higher dose.

Hematological Criteria: If temozolomide cannot be administered on the scheduled day of dosing, the CBC will be repeated weekly for up to and including 4 weeks until the ANC is ≥1500/μL and platelet count is ≥100,000/μL. If these hematological criteria are met, chemotherapy may be administered according to the dose adjustments outlined in Table 6-5 below. If the ANC remains <1500/μL or platelet count is <100,000/μL at 4 weeks, the decision to restart must be reviewed with the study chair or their designated alternate before proceeding. No more than two dose reductions will be allowed.

The dose of temozolomide administered for subsequent cycles will be determined according to the nadir ANC and nadir platelet count of the immediately previous cycle for temozolomide (see Table 6-7).

Table 6-7 Temozolomide Dose-Adjustment Criteria for HematologicToxicity

Nadir Toxicity Level	Nadir ANC/μL	Nadir Platelets/μL	Temodar Modification
1	1500–1999	75–99, 999	Dose unchanged from previous
2	1000–1499	50–74, 999	Dose unchanged from previous
3	500–999	25–49, 999	Decrease dose to next lower dose level
4	<500	<25,000	Decrease dose to next lower dose level

Note: Dose levels (daily dose): 200 mg/m^2/day, 150 mg/m^2/day, 100 mg/m^2/day.

Non-Hematological Criteria: Subsequent courses will start (as long as the treatment is beneficial) after complete resolution of toxicities to grade 2 or less. A minimum 2-week rest period will be required if there is grade 3 or greater non-hematological toxicity, and dosages for the subsequent course will be one dose level below the dose that produced toxicity of grade 3 or greater, except doses will not be reduced for grade 3 nausea/emesis unless it is grade 3 despite optimal medical management.

If the patient experiences grade 3 or greater hematological or non-hematological toxicity after 2 dose reductions of temozolomide, he or she will discontinue temozolomide for the remainder of the study. Patients may continue to be treated with everolimus.

6.3 Concomitant medications

Patients must be instructed not to take any medications (over-the-counter or other products) during the protocol treatment period without prior consultation with the investigator. The investigator should instruct the patient to notify the study site about any new medications he/she takes after the start of study drug. All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) taken within 14 days of starting study treatment through the 30-day safety follow up visit should be reported on the 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 7.2.3).

6.3.1 Permitted concomitant therapy

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

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: preclinical and clinical data have shown everolimus to have CYP3A4 inhibitory activity rather than induction activity, so induction of metabolism of contraceptive hormones by everolimus is unlikely. Consequently, administration of everolimus should not reduce the efficacy of oral contraceptives.

Cytochrome P450 and P-glycoprotein inhibitors/inducers/substrates

Co-administration with strong inhibitors of CYP3A4 or PgP should be avoided; and may cause increased everolimus concentrations. For a current table of Substrates, Inhibitors and Inducers please access the following website:

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm

Everolimus is metabolized by CYP3A4 in the liver and to some extent in the intestinal wall.

Therefore, the following are recommended:

- Co-administration with strong inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole, ritonavir) or P-glycoprotein (PgP) inhibitor should be avoided.
- Co-administration with moderate CYP3A4 inhibitors (e.g., erythromycin, fluconazole) or PgP inhibitors should be used with caution. If a patient requires co-administration of moderate CYP3A4 inhibitors or PgP inhibitors, reduce the dose of everolimus . Additional dose reductions may be required to manage toxicities. If the inhibitor is discontinued, the everolimus dose should be returned to the dose used prior to initiation of the moderate CYP3A4/PgP inhibitor after a washout period of 2 to 3 days.
- Grapefruit or citrus juices affect P450 and PgP activity. Concomitant use should be avoided.
- Co-administration with strong inducers of CYP3A4 should be avoided. If a 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 up to twice the currently used daily dose should be considered, 5 mg 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 everolimus 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 everolimus dose should be returned to the dose used prior to initiation of the strong CYP3A4/PgP inducer.

Please refer to Table 6-6 listing relevant inducers and inhibitors of CYP3A and Table 6-7 for a list of relevant substrates, inducers, and inhibitors of PgP.

Table 6-8 Clinically relevant drug interactions: inducers, and inhibitors of isoenzyme CYP3A

Inducers
carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, phenytoin, pioglitazone, rifabutin, rifampin, St. John's wort, troglitazone, efavirenz, nevirapine, topiramate, avasimibe, bosentan, etravirine, nafcillin, ritonavir, talviraline (not available in US market), tipranavir, amprenavir, aprepitant, armodafinil (R-modafinil), dexamethasone, nevirapine, prednisone, pleconaril (not available in US market), rufinamide
Inhibitors
Strong inhibitors: clarithromycin, conivaptan, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, troleandamycin, voriconazole, tipranavir, elvitegravir, Posaconazole (Krishna et al 2009) **Moderate inhibitors:** aprepitant, atazanavir, casopitant, cimetidine, ciprofloxacin, darunavir, diltiazem, erythromycin, fluconazole, grapefruit juice (citrus parasidi fruit juice), imatinib, tofisopam, verapamil, amprenavir, fosamprenavir, dronedarone

Table 6-9 Clinically relevant drug interactions: substrates, inducers, inhibitors of PgP and PgP/CYP3A dual inhibitors

Substrates
digoxin, fexofenadine, indinavir, vincristine, colchicine, topotecan, paclitaxel
Inducers
rifampin, St John's wort
PgP Inhibitors and PgP/CYP3A Dual Inhibitors
amiodarone, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, elacridar, erythromycin, felodipine, fexofenadine, ginkgo (ginkgo biloba), indinavir, itraconazole, , lopinavir, mibefradil, milk thistle (silybum marianum), nifedipine, nitrendipine, quercetin, quinidine, ranolazine, ritonavir, saquinavir, Schisandra chinensis, St John's wort (hypericum perforatum), talinolol, telmisartan, tipranavir, valspodar, verapamil
Reference: Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated Oct. 2, 2011, 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.

Vaccinations

Immunosuppressants may affect the response to vaccination and vaccination during treatment with everolimus may therefore be less effective. The use of live vaccines should be avoided during treatment with everolimus. Examples of live vaccines are: intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid vaccines.

6.3.2 Prohibited concomitant therapy

None.

7 Visit schedule and assessments

7.1 Study flow and visit schedule

7.1.1 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 assessment of Karnofsky Performance Status [Appendix 1], vital signs [height, weight, blood pressure, pulse, respiration rate, temperature and pulse oximetry], physical and neurologic examination) is to be done within 14 days of initial protocol treatment.

- Radiographic Evaluation: An MRI scan must be performed within 28 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.

- Laboratory Evaluation: Blood tests may be performed at the UCSF clinical laboratory, or at a local laboratory. Pre-study laboratory tests must be obtained with 14 days prior to initial protocol treatment except as noted below. 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, 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).
    - Hepatitis B/C testing: Patients will be tested for the following hepatitis serologic markers: HBsAg, HBs Ab, HBc Ab Total, and HCV antibodies (performed within 28 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.

- Tumor tissue testing for treatment selection, as per Section 7.3.2

- Research blood sample (if needed): If blood has not already been collected for a patient in the BTRC Tissue Bank, then one tube of blood will be drawn to serve as a comparison for correlative tissue studies described in Appendix 2, Section C.

- Quality of Life Surveys (optional): 2 surveys will be completed during the screening period at UCSF. The surveys will ask patients about his/her feelings, beliefs and behaviors before beginning treatment.

7.1.2 Assessments During Treatment

- A cycle will be 28 days in length regardless of treatment delays; in particular, for patients also receiving temozolomide, if there is a delay in re-initiating temozolomide, Day 1 of that cycle will not shift with the temozolomide.

- At the end of cycle 1 (Day 28, +/- 7 days), a toxicity check will be performed via telephone call.

- Clinical Evaluation: Clinical assessments will be required every 2 cycles for the first 12 cycles, then every 3 cycles for the next 12 cycles. Whenever possible, all assessments at each scheduled time-point will be completed in a single clinic visit. Clinical assessments performed at required visits will include the following:

    - Karnofsky performance status (Appendix 1)

    - Neurologic Examination (see below)

    - Physical Examination

    - Pulse Oximetry measurements

    - Vital Signs: pulse, blood pressure, respiration rate, temperature and weight.

    - A Neuro Exam Score should be recorded at each patient visit according to the following scale (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

- Radiographic Evaluation: An MRI will be required every 2 cycles for 12 cycles, and every 3 months for the subsequent 12 cycles. MR spectroscopy scans are optional, and will be done at the following timepoints: baseline, at the end of cycles 2 and 6, and at the time of tumor progression.

    - Imaging Score: 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:

    - **Arms 1 and 3** (everolimus alone):
        - CBC, platelets, differential (Day 28 (+/- 7 days) of each cycle)

- **Arm 2** (everolimus and temozolomide)
  - CBC, platelets, differential (Days 21 (+/- 3 days) and 28 (+/- 3 days) after the first dose of temozolomide while on temozolomide and everolimus, then Day 28 (+/- 7 days) when on everolimus alone.)

- **All Arms:**
  - Sodium, potassium, chloride, bicarbonate, calcium, fasting glucose, albumin, total protein, BUN, serum creatinine (Day 28 (+/- 7 days) of each cycle)
  - T. Bilirubin, AST, ALT, alkaline phosphatase, phosphorus (Day 28 (+/- 7 days) of each cycle)
  - Fasting serum lipid profile (total cholesterol, triglycerides, HDL and LDL) (Day 28 (+/- 7 days) of each even cycle for first 12 cycles, then Day 28 (+/- 7 days) of every 3rd cycle thereafter)
  - Serum pregnancy test: for women of childbearing potential (Day 28 (+/- 7 days) of each cycle).
  - Note the coadministration of everolimus and oral anticoagulants is possible but should be subject to verification of coagulation (INR) once steady state is reached (after one week's treatment).

*The intent is for these tests to be checked approximately every 28 days for all patients, regardless of treatment delays due to myelosuppression.

- Documentation: 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 every 2 cycles for the first 12 months, then every 3 cycles until the patient discontinues therapy.

- Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.

- Quality of Life Surveys (optional): 2 surveys will be completed at the end of every 6 cycles while on treatment, (if possible) prior to tumor assessment discussion with the physician.

7.1.3 Follow-up Assessments

- Protocol Completion Assessment:

- After administration of everolimus for two years, patients will have completed study treatment.

- All patients must be observed for safety for 30 days following the last dose of everolimus prior to withdrawal from study. This defines completion of active study participation.

- 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 4 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, if patients are able to return to do so. If patients are unable to return for an in-person visit, then they will be contacted by phone to assess toxicity at approximately 4 weeks after that last day of study.

- Quality of Life Surveys (optional): 2 surveys will be completed 1 year post-completion of treatment or at the time of tumor progression if sooner than 1 year post-completion of treatment.

- Long-term Follow-up:

  - After completion of study treatment, patients whose tumors have not progressed will be followed with interval MRIs. These should be done every 4 months (+/- 2 weeks) for 1 year, then every 6 months (+/- 4 weeks) for an additional 2 years, and thereafter as per the discretion of the managing physician.

  - 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.

  - Patients off protocol therapy will continue to be followed for PFS and OS only for the purposes of this protocol. If patients are not being seen regularly in clinic, then phone calls for PFS and OS will be made every 6 months.

Table 7-1 Visit evaluation schedule (note: 1 cycle = 28 days)

Procedures:	Initial Screen	1-month Assessment (by phone)	Assessments every 2 cycles (+/-7 days unless otherwise noted) for cycles 1-12 of therapy	Assessments every 3 cycles (+/-7 days unless otherwise noted) for cycles 13-24 of therapy	28 days after completing protocol therapy (+/- 7 days)†	Long Term Follow-Up
Clinical Evaluation: ±						
Medical History	X					
Vital Signs and KPS¥	X		X	X	X	
Physical Exam, Neurologic Exam	X		X	X	X	
Toxicity Check	X£	X	X	X	X	
Pulse Oximetry	X		X	X	X	
ECG	X					
QoL questionnaires	X	X§				
Laboratory/ Diagnostic Tests: ±						
Serum pregnancy for women of child-bearing potential	X	Day 28 (+/- 7 days) while on therapy			X	
CBC with diff and platelets*	X	Arms 1 and 3: Day 28 (+/- 7 days) while on therapy Arm 2: Day 21 (+/- 3 days) and day 28 (+/- 3 days) from 1st day of temozolomide treatment while on dual therapy with both drugs, then Day 28 (+/- 7 days) while on everolimus alone			X	

Chemistries & liver function tests*	X	Day 28 (+/- 7 days) while on therapy			X	
Fasting cholesterol / triglycerides*	X	Day 28 (+/- 7 days) while on therapy			X	
MRI of the brain**	X		X	X		X±
Hepatitis B/C testing*	X					
Tissue testing for treatment selection***	X					
Translational Research:						
Archival tumor tissue	X					
Research blood draw****	X					
Survival						X±

*See sections 7.1.1 and 7.1.2 for lists of specific tests to be ordered at screening and during treatment, respectively

**With optional MRS at baseline, end of cycles 2 and 6, and at the time of tumor progression

*** See section 7.3.2 for specific tests to be done

****If sample not already available in UCSF Tissue Bank

†If patients are unable to return for an in-person visit, a phone toxicity check will be acceptable

¥Vital signs include temp, BP, heart rate, respiratory rate, and weight. Height will be done at screening only.

£At screening, "toxicity check" refers to assessment of baseline conditions

±Please refer to protocol text (Section 7.1) for time windows for all assessments. Phone contact for survival is also acceptable as per protocol text.

§Surveys will be filled out at the end of every 6 cycles while on treatment and then at 1 year post-completion of treatment or at the time of tumor progression if sooner than 1 year post-completion of treatment

7.2 Imaging Assessment of Efficacy

Standard anatomic MRI in conjunction with clinical evaluation such as neurologic status and corticosteroid use, remains the key determinant of response to therapy and the evaluation of tumor recurrence for low grade glioma. Increase in contrast enhancement, worsening cerebral edema, and increased 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.

The majority of previously untreated LGG are non-enhancing on the post-Gadolinium T1-weighted images. This means that the primary analysis is based upon the interpretation of regions with hyperintensity on T2-weighted images, which are unable to distinguish edema from invasive tumor. After treatment, some lesions become enhancing, but that can be due either to treatment effect or to the presence of recurrent, progressive tumor. This complicates the interpretation of anatomic images and has motivated the search for alternative imaging modalities.

The recent review of response criteria for LGG performed by the RANO working group made the clear recommendation that alternative imaging approaches should be considered in evaluating such lesions (van den Bent, Wefel et al. 2011). Our previous research has made a concerted effort to determine which non-invasive metabolic and physiological imaging parameters are associated with residual disease and to identify markers that predict malignant transformation by using image guided tissue samples from patients with recurrent LGG (Bian, Khayal et al. 2009). This analysis has confirmed the relationship between in vivo tissue markers of increased blood volume, restricted diffusion and increased metabolite levels with histological evidence of tumor progression.

Of particular interest for the proposed study are the results from applying physiological and metabolic imaging methods to monitor response to therapy in the ongoing phased II clinical trial of everolimus for patients with recurrence. These are applying MR spectroscopic imaging (MRSI), Dynamic Contrast Enhanced (DCE) or Dynamic Susceptibility Contrast (DSC) imaging and diffusion-weighted imaging (DWI) in conjunction with standard anatomic MRI (Bian, Khayal et al. 2009). Early analysis of these data has revealed that the capillary density and vascular permeability measures estimated from DCE-MRI at 4 and 6 months after the start of everolimus decreased significantly in patients with stable disease but not in those with progressive disease. This suggests that decreased angiogenesis may represent an early marker of response to everolimus.

While metabolic imaging has not yet been widely evaluated for evaluation of response to temozolomide in LGG, we have shown that baseline and early changes in metabolites assessed using H-1 MRSI are predictive of outcome for patients with high-grade glioma who are being

treated with therapies that include temozolomide (Nelson 2011). We have also shown in pre-clinical models using C-13 metabolic imaging that early changes in the lactate/pyruvate ratio are associated with response to treatment with temozolomide (Park, Bok et al. 2011). This suggests that the combination of metabolic and physiological imaging will be valuable for this study.

7.2.1 Imaging Protocol

Data will be acquired using 3T clinical or research scanners and multi-channel phased array head coils in order to provide the highest possible sensitivity. These scanners are kept at similar software and hardware revisions and are connected by the PACS network. The following is the advanced imaging protocol currently being used for patients with glioma.

a) Calibration/Localization images: These include a 3-D localizing scan to define the graphical prescription and low resolution (64x64 matrix with 26cm FOV) multi-slice gradient echo calibration images obtained with minimal contrast and with the data from different elements of the multi-channel head coil reconstructed separately. The individual images provide estimates of the coil sensitivities for use in reconstructing parallel imaging reconstructions of subsequent anatomical and spectroscopic imaging data to accelerate acquisition times. (Tacq < 2min)

b) T2 FLAIR images: 3-D sagittally acquired images with typical parameters of TR/TE/TI = 6000/134/2200ms, with 1mm isotropic voxels (256x256 matrix and 26cm FOV) and 3-fold parallel imaging acceleration, reformatted to axial and coronal planes for analysis and clinical interpretation. (Tacq = 8 min)

c) Pre-contrast 3D T1 IR-SPGR images: 3-D axial T1- weighted, inversion recovery prepared, spoiled gradient echo images with typical parameters TR/TE/TI = 8/2/400ms, flip angle 15^{o}, 2-fold parallel imaging acceleration, and 1mm in plane resolution (256x256 matrix, 24cm FOV) with 1.5mm slice thickness. (Tacq = 4 min)

d) Diffusion-weighted Imaging (DWI): Diffusion weighted spin echo single shot echo planar images with b = 1000 s/mm^2, 6 gradient directions, 4 NEX, TR/TE = 7000/76ms, 128x128 matrix with 22cm FOV, 2-fold parallel imaging acceleration to reduce distortion, and 3-4mm slice thickness. (Tacq = 4 min)

e) Lactate-edited 3-D MRSI: Water suppressed, lactate-edited ^{1}H MRSI with automated PRESS volume and out of voxel saturation bands prescription, and 2-D phase encoding and flyback EPSI encoding to cover the lesion, surrounding tissue, and as much of the contralateral normal-appearing brain as possible. Typical parameters are TR/TE=1000-1100/144, matrix size 16x16x16 and nominal spatial resolution 1cc (10min)

f) Perfusion-weighted Imaging (PWI): Dynamic gradient-echo, single-shot echo-planar images acquired during the bolus of a Gadolinium contrast agent, 0.1mm/Kg, injection speed 5ml/s. Typical acquisition parameters are TR/TE/Flip = 1500ms/35ms/35^{o}, 80 time points, 22 slices,

4mm slice thickness, 26cm FOV, 128x128 matrix, and 2-fold parallel imaging acceleration factor to reduce distortion (Tacq = 2min).

g) Post-contrast anatomic images: These will include 3D T1 IR-SPGR images as described above and T1-weighted spin echo images as a clinical reference. (Tacq = 10 min)

In addition to the scans described above that are used for routine follow-up, MR examinations acquired immediately prior to surgery include T2-weighted FSE anatomical images and DTI images prior to gadolinium injection with parameters as follows:

h) T2-weighted FSE: 3-D axial images with typical parameters of TR/TE/ETL = 4300/102/33, with 24cm FOV, 256x256 matrix, 2-fold parallel imaging acceleration, and 1.5mm slice thickness. (Tacq = 9 min)

i) Diffusion Tensor Imaging (DTI): Diffusion-weighted single-shot echo planar spin-echo pulse sequence will be employed with 55 diffusion-gradient directions, with isotropic 2.2 mm voxel resolution, FOV 280x280 mm, 128x128 matrix, 2.2-mm interleaved slices with no gaps, at b=2000 s/mm^2. (Tacq = 7 min)

7.2.2 Imaging Time Points

Standard anatomic MR imaging will be performed as outlined in Table 7. The full imaging examination described above will optionally be performed at baseline, at the end of cycles 2 and 6, and at the time of suspected progression. The anatomic imaging components are standard of care, with the other parts being research add-ons. After the end of treatment and prior to progression, patients will be followed with anatomic imaging alone. The goal will be to determine whether the parameters estimated from advanced imaging are early predictors of response to therapy.

7.2.3 Imaging Response Criteria

Imaging assessment will be done by a UCSF neuro-oncologist according to the RANO criteria for low-grade gliomas (van den Bent, et al, Lancet Oncology 2011):

> **Complete response:** Complete response requires all the following criteria compared with the baseline scan:
>
> (1) Complete disappearance of the lesion on T2 or FLAIR imaging (if enhancement had been present, it must have resolved completely);
>
> (2) no new lesions, no new T2 or FLAIR abnormalities apart from those consistent with radiation eff ects, and no new or increased enhancement;
>
> (3) patients must be off corticosteroids or only on physiological replacement doses;
>
> (4) patients should be stable or improved clinically

Partial response: Partial response requires all of the following criteria compared with the baseline scan:

(1) greater than or equal to 50% decrease in the product of perpendicular diameters of the lesion on T2 or FLAIR imaging sustained for at least 4 weeks compared with baseline;

(2) no new lesions, no new T2 or FLAIR abnormalities apart from those consistent with radiation eff ects, and no new or increased enhancement; and

(3) patients should be on a corticosteroid dose that should not be greater than the dose at time of baseline scan, and should be stable or improved clinically

Minor response: Minor response requires the following criteria compared with baseline:

(1) a decrease of the area of non-enhancing lesion on T2 or FLAIR MR imaging between 25% and 50% compared with baseline;

(2) no new lesions, no new T2 or FLAIR abnormalities apart from those consistent with radiation eff ect, and no new or increased enhancement; and

(3) patients should be on a corticosteroid dose that should not be greater than the dose at time of baseline scan, and should be stable or improved clinically

Stable disease: Stable disease is present if the changes do not qualify for complete, partial, or minor response, or progression and requires:

(1) stable area of non-enhancing abnormalities on T2 or FLAIR imaging;

(2) no new lesions, no new T2 or FLAIR abnormalities apart from those consistent

with radiation eff ect, and no new or increased enhancement; and

(3) patients should be on a corticosteroid dose that should not be greater than the dose at time of baseline scan, and should be stable or improved clinically

Progression: Progression is defined by any of the following:

(1) development of new lesions or increase of enhancement (radiological evidence of malignant transformation);

(2) a 25% increase of the T2 or FLAIR non-enhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy, not attributable to radiation eff ect or to comorbid events;

(3) definite clinical deterioration not attributable to other causes apart from the tumour, or decrease in corticosteroid dose; or

(4) failure to return for evaluation because of death or deteriorating condition, unless caused by documented non-related disorders

7.3 Pathology and Molecular Assessments

Pathology must be reviewed in the UCSF pathology department.

7.3.1 Obtaining Tissues 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.

- Unstained slides and/or paraffin tissue blocks, along with a pathology report, are sent to UCSF -- c/o one of the NeuroOnc study coordinators or directly to the Neurosurgery tissue bank manager (currently Anny Shai)

  Preparation of tissue samples:

- Once received, samples are logged into the Tissue Bank computer system and assigned an SF#.

- If paraffin blocks are provided unstained slides will be prepared from these.

- If H&E slides are not provided they will be prepared from unstained slides.

  All staining and immunohistochemistry will be performed at the UCSF Brain Tumor Research Center Biomarkers Laboratory in the Neurosurgery Tissue Bank or in the UCSF Department of Pathology

  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 provided tissue is adequate / appropriate for the study and the best block for additional assays will be selected.

- In general, one to two representative H&E stained slides will be reviewed.

7.3.2 Treatment Selection Factors

Patient treatment will be selected by 1p/19q chromosomal status as determined by fluorescence in-situ hybridization (FISH) and/or ATRX mutation as determined by immunohistochemistry. Briefly, deletion of 1p and 19q will be determined based on the determination of the ratio of test probe to reference probe. A deletion is defined as a ratio of test probe to reference probe <0.8 and >15% of the cells showing a reference:target ratio of at least 2:1. Patients whose tumor is 1p/19q intact will be further selected by tumor PI3K/mTOR pathway activation ($Path^{act}$) as determined by immunohistochemistry for phospho-PRAS40. Pathway activation will be defined as a score of greater than or equal to 1 based on an assessment of tumor cells immunopositive for phospho-PRAS40 as follows: 0, no significant positive staining in tumor cells; 1, 1-33% of tumor cells positive; 2, 33-66% positive; 3, greater than 66% tumors cells positive. A similar scoring system was used previously to assess pathway activation in low grade glioma [McBride et al. 2010]. Based on this study which identified 18 of 35 patients with p-PRAS40-positive tumors, we anticipate approximately 50% of newly diagnosed low-grade gliomas will be $Path^{act}$ regardless of 1p/19q status.

The ATRX mutation typically results in a truncated protein and abrogated protein expression [Heaphy CM 2012; Liu XY 2012]. Thus, immunohistochemistry for ATRX can be performed and loss of immunostaining can be used as a surrogate for detecting ATRX mutations. For the evaluation of ATRX immunohistochemistry, only nuclear staining is assessed. A tumor is considered to be ATRX mutant when there is loss of ATRX in all tumor cell nuclei while nuclear positivity is present in non-neoplastic cells, including blood vessels, microglia, reactive astrocytes, and entrapped neurons. This assay will be performed clinically in a CLIA-certified laboratory, interpreted by a board-certified neuropathologist, and reported in the clinical chart.

7.3.3 Molecular and Biologic Correlative Studies

7.3.3.1A Immunohistochemical Assays

See Appendix 2, Section A for details.

7.3.3.1B Molecular Analyses

See Appendix 2, Section B for details.

7.3.3.2 Genomic Analyses: Custom capture Approach

See Appendix 2, Section C for details.

7.4 Quality of Life Assessment

Very little data is available prospectively assessing quality of life in patients with low-grade glioma, and it is increasingly appreciated that this is an important aspect of caring for patients with brain tumors. As such, patients on Arms 1 and 3 (those receiving everolimus as monotherapy) will be asked to participate in a quality of life component of the study, filling out questionnaires at enrollment and at the end of every 6 cycles during treatment (at the time of the MRI and clinic visit), then at 1 year post-completion of treatment, again associated with the corresponding MRI and clinic visit, or at the time of tumor progression if that occurs sooner than 1 year post-completion of treatment. Participation in this portion of the study will be voluntary. The survey instruments used will be the FACT-Br and the MDASI-BT, both of which are validated in patients with brain tumors (Weitzner, Meyers, et al. 1995; Liu, Solheim, et al. 2009; Armstrong, Gning, et al. 2009). Whenever possible, patients should fill out the questionnaires at each visit *prior to* meeting with the physician to discuss MRI results.

8 Safety monitoring and reporting

The study will use the CTCAE v4.0 for reporting of non-hematologic adverse events and modified criteria for hematologic adverse events. See Section 6.2 for details.

8.1 Definitions of Adverse Events

8.1.1 Adverse Event

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

8.1.2 Adverse reaction

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

8.1.2.1 Suspected

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

8.1.2.2 Unexpected

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

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

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

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

8.1.2.3 Serious

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

- Death
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Congenital anomaly/birth defect

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

8.1.2.4 Life-threatening

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

8.2 Recording of an Adverse Event

All grade 3 and above adverse events will be entered into OnCore®, whether or not the event is believed to be associated with use of the study drug. Data about these events and their severity will be recorded using the NCI CTCAE v4.0.

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

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

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

Grade 0 No AE (or within normal limits)

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

Grade 2 Moderate; minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Death related to AE

8.3 Follow-up of Adverse Events

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

8.4 Adverse Events Monitoring

All adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®, as noted above.

The Investigator will assess all adverse events and determine reportability requirements to the UCSF Data and Safety Monitoring Committee (DSMC) and UCSF's Institutional Review Board, the Committee on Human Research (CHR); and, when the study is conducted under an Investigational New Drug Application (IND), to the Food and Drug Administration (FDA) if it meets the FDA reporting criteria..

All adverse events entered into OnCore® will be reviewed by the Helen Diller Family Comprehensive Cancer Center Site Committee on a weekly basis. The Site Committee will review and discuss at each weekly meeting the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

All grade(s) 3-5 adverse events entered into OnCore® will be reviewed on a monthly basis at the Site Committee meetings. The Site Committee will review and discuss the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

In addition, all suspected adverse reactions considered "serious" entered into OnCore®, will be reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at DSMC meetings, which take place every six weeks.

8.5 Expedited Reporting

Reporting to the Data and Safety Monitoring Committee

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the Investigator or his/her designee must notify the DSMC Chair (or qualified alternate) within 1 business day of knowledge of the event. The contact may be by phone or e-mail.

Reporting to UCSF Committee on Human Research (Institutional Review Board)

The Principal Investigator must report events meeting the UCSF CHR definition of "Unanticipated Problem" (UP) within 10 business days of his/her awareness of the event.

Reporting to Novartis

Reporting of SAEs: The principal investigator has the obligation to report all serious adverse events to the Novartis Pharmaceuticals Drug Safety and Epidemiology Department (DS&E).

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

- after the patient has provided informed consent and until atleast 30 days after the patient has stopped study treatment/participation
- after protocol-specified procedures begin (e.g., placebo run-in, washout period, double-blind treatment, etc.) and 30 days after the patient has stopped study treatment
- after the start of any period in which the study protocol interferes with the standard medical treatment given to a patient (e.g., treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication) and until 30 days after the patient has stopped study treatment

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

Any SAEs experienced after this 30 day period should only be reported to Novartis if the investigator suspects a causal relationship to the study drug. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. A SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. The end date of the first event must be provided.

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

Follow-up information is sent to the same fax number as the original SAE Report Form was sent, using a new fax cover sheet, stating that this is a follow-up to the previously reported SAE, and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and

how it was treated, whether the blind was broken or not (if applicable), and whether the patient continued or withdrew from study participation.

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

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

Reporting of Pregnancy: Preclinical data regarding reproductive toxicity is described in the most recent Investigator Brochure. The potential reproductive risk for humans is unknown. Women of childbearing potential should be advised to use highly effective contraception methods while they are receiving everolimus and up to 8 weeks after treatment has been stopped.

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. The newborn will be followed for at least 12 months.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the oncology Novartis Drug Safety and Epidemiology Department (DS&E). Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study treatment and any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

9 Statistical methods and evaluation of results

9.1 Statistical methods

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 assessed by the RANO criteria.
- 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 has 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.

9.2 Treatment Selection Factors

Patient treatment will be selected by tumor ATRX and/or 1p/19q chromosomal status and by tumor PI3K/mTOR pathway activation ($Path^{act}$) as determined by immunohistochemistry for phospho-PRAS40 (see Section 7.3.2 for details).

9.3 Determination of Sample Size and Accrual Rate

Documentation of prognosis among LGG patients treated with TMZ is limited in the published literature. A recent study was conducted to assess whether IDH1 or IDH2 mutations predict longer survival in LGG patients (Houillier, Wang et al. 2010). In this study, it was reported that the median PFS among LGG patients whose tumors were 1p-19q co-deleted and IDH mutated was 37.9 months while it was 32.9 months for the patients whose tumors were 1p-19q intact and IDH mutated.

Arm 1: As we expect patients with LGGs that are IDH-mutated with ATRX loss and/or 1p/19q chromosomes intact, and with PI3K/mTOR pathway activated ($Path^{act}$) treated with everolimus to have better prognosis than if treated with TMZ alone, we consider the lower threshold for the probability of 33-month PFS to be 50%. If the true PFS-33 proportion is 70%, with 35 patients we will have 86% confidence in the PFS-33 estimate with a one-tailed 90% confidence interval.

Arm 2: Similarly, we expect patients with LGGs that are IDH-mutated with ATRX loss and/or 1p/19q chromosomes intact, and with PI3K/mTOR pathway non-activated ($Path^{non\text{-}act}$) treated with the combination of everolimus and TMZ to have better prognosis than if treated solely with TMZ, we again consider the lower threshold for the probability of 33-month PFS to be 50%. If the true PFS-33 proportion is 70%, with 35 patients we will have 86% confidence in the PFS-33 estimate with a one-tailed 90% confidence interval.

Arm 3: We expect patients with ATRX intact and/or 1p-19q co-deleted (regardless of PI3K/mTOR pathway status) LGGs treated with everolimus to have better prognosis than if treated with TMZ alone. Therefore, we consider the lower threshold for the probability of 38-month PFS to be 50%. If the true PFS-38 proportion is 70%, with 35 patients we will have 86% confidence in the PFS-38 estimate with a one-tailed 90% confidence interval.

9.4 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. Six months after the 20th patient is enrolled and six months after the 40th patient is enrolled, we will assess the frequency of toxicity requiring permanent discontinuation of the drug. If there is a greater than 20% rate for discontinuation of drug due to toxicity during therapy at either time point, the study accrual will be suspended 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.

9.5 Analysis Plans

For the first two arms, the primary endpoint will be 33-month progression-free survival (PFS-33) and for the third arm it will be 38-month PFS (PFS-38). Patients will be analyzed based on intention to treat. The primary analyses will be performed at the time the 33- (38-) month PFS is known for all patients.

9.5.1 Analysis Population

The study plan is to accrue 105 patients. It is anticipated that many low-grade glioma patients completing initial 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 2 patients per month will be enrolled.

With enrollment of 2 patients per month, accrual can be completed in a 53 month period. With 38 months from the time of enrollment of the last accrued patient in order to assess PFS-38, the approximate duration of study is expected to be 91 months.

9.5.2 Analysis of Primary Endpoints

33-(38-)month PFS: Analyses will be performed after all enrolled patients have completed 33 (38) months on study, 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 33-(38-)month PFS separately for the three arms. The Cox proportional hazards model will also be used to allow for adjustment of the prognostic factors including age, KPS, and extent of resection.

9.5.3 Analysis of Secondary Endpoints

Overall survival and objective response and seizure rate: The method described above for the analysis of PFS-33 and PFS-38 will be applied to the analysis of OS. Response is based on the best response, defined as the best objective status as assessed by the RANO criteria. The point estimate and the associated 2-sided 95% CI for both the response rate and the seizure rate will be calculated separately for the three arms. To assess the reduction in seizure rate we will compare the seizure rate on study to that experienced one month prior to enrolling in the study. We will follow the RANO LGG guideline (van den Bent, Wefel, et al 2011) which calls a 50% or more reduction number of monthly seizures an 'improvement'; a 50% or more increase a 'worsening'; and anything less than 50% in either direction a 'stable seizure rate'.

9.5.4 Analysis of Exploratory Endpoints

Metabolic and physiologic imaging parameters: We will determine if metabolic and physiologic imaging parameters correlate with the clinical endpoints of time to progression and survival. We will assess the usefulness of these parameters in predicting PFS and OS 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. In addition to the imaging parameters, the models will include: age, KPS, extent of resection, time since diagnosis, Arm 1, 2 vs. 3, and initial diagnostic histology scored as oligodendroglioma only vs. those with an astrocytoma component. The inclusion of these additional variables is to adjust for known or potential confounding risk factors.

Clonal or subclonal genetic mutations: We will assess the association between the presence/absence of clonal or subclonal genetic mutations in the PI3K pathway and median PFS, Objective Response Rate (ORR), *PTEN* methylation, and the immunohistochemical measurements of the PI3K pathway activation in patients together and by arm. Tests for association will be used as appropriate including chi-square for proportions and contingency tables and t-tests for continuous valued variables.

Quality of Life Parameters: Data from the end of 12 cycles of therapy, the end of 24 cycles of therapy, and one year post-therapy will be compared to baseline data (at study enrollment) to see if there are consistent temporal trends between patients over time in total scores as well as in specific functional domains. Paired T-Tests will be employed to make these comparisons. In addition, variability analyses using all available timepoints will be undertaken to evaluate trends within single patients over time, for total scores as well as specific functional domains.

9.6 Evaluation of Safety

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the NCI CTCAE v4.0. Safety and tolerability will be assessed by incidence, severity, and changes from baseline of all relevant parameters including AEs, laboratory values, and vital signs.

AEs will be coded using the latest available version of the Medical Dictionary for Regulatory Activities (MedDRA). AEs will be summarized for each treatment arm by the number and percentage of patients who experienced the event, according to system organ class (SOC) and preferred term (PT). Additional summaries will also be provided by severity grade and relationship to study drug, and for SAEs and events resulting in the permanent discontinuation of therapy. A subject reporting multiple cases of the same AE will be counted once within each SOC and similarly counted once within each PT, and AEs will be graded by worst severity grade. Unless specified otherwise, the denominator for these calculations will be based on the number of patients in each treatment arm who receive at least one dose of study drug, irrespective of the total number of doses administered.

Vital sign results (systolic and diastolic blood pressure, pulse, respiration, and body temperature) will be summarized descriptively for each scheduled and unscheduled protocol time point. Changes will be calculated relative to the assessments at baseline.

The changes in hematology, chemistry, and other laboratory values will be summarized descriptively for each scheduled and unscheduled protocol assessment time point. Changes will be calculated relative to the values collected at baseline. Data listings of all laboratory data collected during the study will be presented. Laboratory values outside normal limits will be identified in data listings and will include flags for high and low values.

10 Study Management

10.1 Pre-study Documentation

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

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

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

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

regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

10.2 Institutional Review Board Approval

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

10.3 Informed Consent

All participants must be provided a consent form describing the study with sufficient information for each participant to make an informed decision regarding their participation. Participants must sign the CHR-approved informed consent form prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

10.4 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:

- increases in drug dose or duration of exposure of subjects,
- significant changes in the study design (e.g. addition or deletion of a control group),
- increases in the number of invasive procedures,
- 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.

10.5 Handling and Documentation of Clinical Supplies

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

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

10.6 Case Report Forms (CRFs)

The Principal Investigator and/or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into OnCore® via standardized CRFs in accordance with the CTMS study calendar, using single data entry with a secure access account. The Clinical Research Coordinator (CRC) will complete the CRFs as soon as possible upon completion of the study visit; the Investigator will review and approve the completed CRFs.

The information collected on CRFs shall be identical to that appearing in original source documents. Source documents will be found in the patient's medical records maintained by UCSF personnel. All source documentation should be kept in separate research folders for each patient.

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

All source documentation and CTMS data will be available for review/monitoring by the UCSF DSMC and regulatory agencies.

The Principal Investigator will be responsible for ensuring the accurate capture of study data. At study completion, when the CRFs have been declared to be complete and accurate, the database will be locked. Any changes to the data entered into the CRFs after that time can only be made by joint written agreement among the Study Chair, the Trial Statistician, and the Protocol Project Manager.

10.7 Oversight and Monitoring Plan

The UCSF Helen Diller Family Comprehensive Cancer Center DSMC will be the monitoring entity for this study. The UCSF DSMC will monitor the study in accordance with the NCI-approved Data and Safety Monitoring Plan (DSMP). The DSMC will routinely review all adverse events and suspected adverse reactions considered "serious". The DSMC will audit

study-related activities to ensure that the study is conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). Significant results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable. See Appendix 3, Data and Safety Monitoring Plan for a Phase 2 or 3 Institutional Study, for additional information.

11 Protection of Human Subjects

11.1 Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the CHR mechanism and the process of informed consent. The CHR reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The CHR also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

11.2 Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign the HIPAA form and informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document. The use and disclosure of protected health information will be limited to the individuals described in the informed consent document.

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13 Appendices

Appendix 1: Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self, unable to carry on normal activity or to do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled, requires special care and assistance
30	Severely disabled, hospitalization indicated Death not imminent
20	Very sick, hospitalization indicated Death not imminent
10	Moribund, fatal processes progressing rapidly
0	Dead

Appendix 2: Molecular Assays

Section A: Immunohistochemical Assays

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

The designated neuro-pathologists 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 of the tumor cells

1- Focal positive staining = staining similar to positive control in less than or equal to 33% of the tumor cells;

2- Intermediate positive staining = staining similar to positive control in positive staining in greater than 33% but less than or equal to 66% of the tumor cells;

3- Diffuse positive staining = staining similar to positive control in greater than 66% of the tumor cells.

Visual estimation may be aided by dividing the interpretable areas of the tissue on the slide into four quadrants using a tissue marker. 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.

All tumor tissues will be analyzed to assess both 1p/19q chromosomal status and activation of PI3K/Akt/mTOR signaling. 1p/19q chromosomal status will be performed and interpreted in a laboratory which is CAP accredited and CLIA certified to perform high complexity testing. The 1p/19q Deletion by FISH assay is designed to identify chromosomal deletions involving the 1p36 and 19q13 regions. It utilizes two Vysis(TM) dual-color DNA probe sets. Probe set one includes the loci-specific identifier (LSI) 1p36 that hybridizes to the 1p target on chromosome 1, producing an orange signal while LSI 1q25 serves as a reference probe for 1q, producing a green signal. Likewise, probe set two includes LSI 19q13 that hybridizes to the 19q target on chromosome 19, producing an orange signal, and a 19p reference probe LSI 19p13 that produces a green signal. Formalin-fixed, paraffin-embedded tissue on two glass slides is de-paraffinized and then treated with pepsin to digest tissue proteins and allow for probes to reach target DNA. The DNA is then heat denatured and subsequently allowed to hybridize with the two probe sets, one per slide. After hybridization, the slides are washed to remove any of the excess unbound probes and the nuclei are counterstained with DAPI (4,6 diamidino-2-phenylidole). Enumeration of 1p, 1q, 19p and 19q signals is conducted by

microscopic examination of cell nuclei using a flourescence microscope equipped with appropriate excitation and emission filters. An adjacent H&E stained section of the case is reviewed to evaluate tumor and normal tissue morphology and to identify the appropriate area of FISH analysis.

In addition, all tumors will be molecularly profiled based on known alterations in adult LGGs. First, tumors will be divided into one of two categories, PI3K/AKT/mTOR activated ($Path^{act}$) or non-activated ($Path^{non\text{-}act}$), based upon the results of immunohistochemistry (IHC) for p-PRAS40 as described below. Second, all tumor samples will be further characterized by IHC, tumor-DNA sequencing, and FISH, for additional molecular aberrations, as described below.

PI3K/AKT/mTOR activated tumors ($Path^{act}$) will be those with a score of 1 or higher for phosphorylated PRAS40. We will analyze expression and phosphorylation of other proteins in the pathway by IHC with antibodies listed in the Table above and score them as described in detail above. Additional immunohistochemical assays will include IHC to determine p53 expression, Ki67 expression as determined by MIB-1, IDH1R132H, and ATRX.

Section B: Molecular Analyses

To further characterize the tumor samples we will perform FISH on FFPE samples to determine *EGFR* amplification, *PDGFRA* amplification, and *CDKN2A* loss. To examine methylation of the *PTEN* promoter, we will use methylation-specific primers previously used to demonstrate methylation of the *PTEN* promoter in non-small-cell-lung cancer as well as adult and pediatric gliomas (McBride, Perez et al. 2010; Mueller, Phillips et al. 2012). 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.

Summary of Immunohistochemical and Molecular Assays:

Molecular feature	**Assay (reagents)**
PTEN expression IHC	Cell Signaling #9559 (Rabbit)
Phosphorylated S6 235/236	IHC: Cell Signaling #2211 (Rabbit)
Phosphorylated S6 240/244	IHC: Cell Signaling #2215 (Rabbit)
Phosphorylated PRAS40 (pT246)	IHC: Cell Signaling #2997 (Rabbit)
Phosphorylated 4EBP1	IHC: Cell Signaling #2855 (Rabbit)
Phosphorylated Erk1/2	IHC: Zymed #18-2389 (Rabbit)
IDH1R132H	IHC: Dianova (DIA H09)
MIB-1 (Ki67)	IHC: Ventana #790-4286

P53	IHC: Dako M7001
ATRX	IHC: Sigma-Aldrich #HPA001906
P53 sequencing, when possible	Sanger sequencing
Sequence cases negative for IDH1 and IDH2 mutations, when IHC negative and sufficient tissue	Sanger sequencing
1p19q deletions	FISH
EGFR	FISH
PDGFRA	FISH
CDKN2A	FISH
PTEN promoter methylation	Methylation-specific primers as described

Section C: Genomic Analyses: Custom capture Approach

In this clinical trial of everolimus, we propose a radically more in-depth analysis of the pre-treatment tumors using custom capture and sequencing of approximately 300 selected cancer genes including all genes known genes in the PI3K/mTOR pathway, rather than all 20,000 genes captured in exome sequencing. Custom capture has a 10- to 100-fold increased sensitivity for detection of cells harboring mutations in the selected genes relative to exome sequencing. Furthermore, with the custom capture approach, 10 independent exome libraries will be multiplexed with barcodes so all 10 can be deeply sequenced using a single sequencing lane. Thus, with just 3 lanes of sequencing, 30 independent pieces of tumor from a single patient can be assayed.

In a funded NIH study that will be complementary to what we propose in this clinical trial, exome sequencing of up to 2 samples per patient per surgery is being used to define the frequency of TMZ induced hypermutation in a large patient cohort, including patients on this clinical trial. Leveraging the personnel and infrastructure supported by the NIH R01 project at these same surgeries, we will collect up to 5 spatially distinct pieces of tumor at each resection, and macrodissect them each into 6 individual pieces (30 tumor pieces total per patient). This standardization of tissue collection for all newly diagnosed LGG patients at UCSF will ensure that the patients that enroll in this clinical trial (enrollment is post-operative) will have the necessary tissue for in-depth analysis of clonal and subclonal drivers, with emphasis on known components of the PI3K pathway. Not all patients on this trial will have their surgeries at UCSF. For the few cases that are non-UCSF surgeries, we will have fewer tumor pieces to sequence, but they will be sequenced to greater depth.

Because *PTEN* methylation and/or PI3K pathway activation were associated with shorter survival, we will test for an association between the presence/absence of clonal or subclonal genetic mutations in the PI3K pathway and clinical features including Median PFS, Objective Response Rate (ORR), and the immunohistochemical measurements of the PI3K pathway. We

will test for potential additive or synergistic effects of *PTEN* methylation and genetic mutation in PI3K pathway activation.

Appendix 3: Data and Safety Monitoring Plan for Phase 2 or 3 Inst. Study

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and subject safety for all HDFCCC institutional clinical studies. A summary of DSMC activities for this study include:

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

Monitoring and Reporting Guidelines

Investigators will conduct continuous review of data and subject safety and discuss each subject's treatment at monthly Site Committee meetings. These discussions are documented in the Site Committee meeting minutes. The discussion will include the number of subjects, significant toxicities in accordance with the protocol, and observed responses.

All institutional Phase 2 or 3 studies are designated with a moderate risk assessment. The data is monitored twice per year with twenty percent of the subjects monitored (or at least three subjects if the calculated value is less than three).

Adverse Event Review and Monitoring

All grade(s) 3-5 adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®, UCSF's Clinical Trial Management System.

All grade(s) 3-5 adverse events entered into OnCore® will be reviewed on a monthly basis at the Site Committee meetings. The Site Committee will review and discuss the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

In addition, all suspected adverse reactions considered "serious" entered into OnCore®, will be reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at DSMC meetings, which take place every six weeks.

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the Investigator or his/her designee must notify the DSMC Chair within **1 business day** of knowledge of this event. The contact may be by phone or e-mail.

Increase in Adverse Event Rates

If an increase in the frequency of Grade 3 or 4 adverse events (above the rate reported in the Investigator Brochure or package insert) is noted in the study, a report should be submitted to the DSMC at the time the increased rate is identified. The report will indicate if the incidence of adverse events observed in the study is above the range stated in the Investigator Brochure or package insert.

If at any time the Investigator stops enrollment or stops the study due to safety issues, the DSMC Chair and DSMC Manager must be notified within 1 business day via e-mail. The DSMC must receive a formal letter within 10 business days and the CHR must be notified.

Data and Safety Monitoring Committee Contacts:

DSMC Chair:	[redacted]	DSMC Monitors
Phone:	[redacted]	[redacted]
Email:	[redacted]	UCSF Helen Diller Family Comprehensive Cancer Center
Address:	[redacted] UCSF San Francisco, CA 94115	San Francisco, CA 94115

Appendix 4: Temozolomide Package Insert

http://www.merck.com/product/usa/pi_circulars/t/temodar_capsules/temodar_pharm.pdf