SUMMARY OF CHANGES

For Protocol Amendment #14 to: Protocol Title: I/II Trial of Temsirolimus and Perifosine for Recurrent or Progressive Malignant Gliomas

NCI Protocol #: 8249
Local Protocol #: 09-058

NCI Version Date: 04/01/2014
Protocol Date: 04/01/2014

#	*Section*	*Page(s)*	*Change*
1.	Header	All	The local IRB Protocol # has been updated to A(14) throughout the document.
2.	Face Page	1	The NCI version date has been updated to 04/01/2014.
3.	Face Page	2	Sumera Bukhari has replaced Brian Seko as the Responsible Data Manager for Memorial Sloan Kettering Cancer Center.
3.	Face Page	3	The "Version #15/Version Date 04/01/2014" was added to Protocol Type/Version #/ Version Date.
4.	Table of Contents	5-6	The table of contents was updated to reflect the body of the protocol.
5.	N/A	N/A	In response to NCI correspondence from Shanda Finnigan, MPH, RN, CCRC, dated January 10, 2014, references to the "Adverse Event Expedited Reporting System (AdEERS)" have been changed to "CTEP Adverse Event Reporting System (CTEP-AERS)" throughout the protocol.
6.	N/A	N/A	Formatting and editorial changes were made throughout the protocol document.

NCI Protocol #: 8249

Local Protocol #: 09-058

NCI Version Date: 04/01/2014

TITLE: Phase I/II Trial of Temsirolimus and Perifosine for Recurrent or Progressive Malignant Gliomas

Coordinating Center: Memorial Sloan Kettering Cancer Center

***Principal Investigator:**
Thomas Kaley, MD
Department of Neurology
Memorial Sloan Kettering Cancer Center
1275 York Avenue
New York, NY 10065
Tel 212-639-5122
Fax 646-422-0626
kaleyt@mskcc.org

Co-Investigators:
Elena Pentsova, MD (Neurology, Co-PI)
Lisa DeAngelis, MD (Neurology)
Craig Nolan, MD (Neurology: Suffolk)
Jerome Posner, MD (Neurology)
Igor Gavrilovic, MD (Neurology: Basking Ridge)
Antonio Omuro, MD (Neurology)
Ingo Mellinghoff, MD (Neurology)
Christian Grommes, MD (Neurology)
Philip Gutin, MD (Neurosurgery)
Viviane Tabar, MD (Neurosurgery)
Cameron Brennan, MD (Neurosurgery)
Eric Holland, MD (Neurosurgery)
Steven Larson, MD (Nuclear Medicine, Radiology)
Mario Lacouture, MD (Medicine, Dermatology)
Emmy Ludwig-Miller, MD (Medicine, Gastroenterology)
Sasan Karimi, MD (Neuro-radiology, Radiology)
Jason Huse, MD (Pathology)
Marc Rosenblum, MD (Pathology)

Memorial Sloan Kettering Cancer Center1275 York Avenue
New York, NY 10065

Consenting Professionals:

Thomas Kaley, MD (Neurology)
Elena Pentsova, MD (Neurology)
Lisa DeAngelis, MD (Neurology)
Craig Nolan, MD (Neurology)
Jerome Posner, MD (Neurology)
Igor Gavrilovic, MD (Neurology)
Antonio Omuro, MD (Neurology)
Ingo Mellinghoff, MD (Neurology)
Christian Grommes, MD (Neurology)

Statistician:

Katherine S Panageas, DrPH
Memorial Sloan Kettering Cancer Center
Epidemiology-Biostatistics
307 East 63rd Street
New York, NY 10021
Tel 646-735-8119
Fax 646-735-0010
panageak@mskcc.org

Responsible Research Nurse:

Mary Elizabeth Davis
Memorial Sloan Kettering Cancer Center
160 East 53rd Street 5th floor
New York, NY 10022
Tel 212-610-2662
davism@mskcc.org

Responsible Data Manager:

Sumera Bukhari
Memorial Sloan Kettering Cancer Ctr
633 3rd Avenue
New York, NY 10017
Tel 646-227-2269
bukharis@mskcc.org

Data Analysis Site:

Andrew B. Lassman, MD
Columbia University Medical Center
Department of Neurology
710 West 168th Street
New York, NY 10032
Tel 212-305-5051
Fax 212-305-6978
Abl7@columbia.edu

NCI Supplied Agent(s): **Temsirolimus (CCI-779) (NSC 683864) Perifosine (NSC# 639966)**

Protocol Type / Version # / Version Date:
Original/Version #4/Version Date 11/19/2009;
Version # 5/Version Date 1/8/2010;
Version #6/Version Date 3/24/2010;
Version #7/Version Date 5/5/2010;
Version #8/Version Date 11/30/2010;
Version #9/Version Date 3/4/2011;
Version #10/ Version Date 8/22/2011;
Version #11/Version Date 10/26/2011;
Version #12/Version Date 2/13/2012;
Version #13/Version Date 10/02/2013;
Version #14/Version Date 12/01/2013;
Version #15/Version Date 04/01/2014;

SCHEMA

Dose levels and treatment schema

Dose level	Temsirolimus (mg/week)	Perifosine load/maintenance (mg/day)
-6	5	150/50
-5	10	150/50
-4	15	150/50
-3	15	150/100
-2	15	300/100
-1	15	450/100
1 (starting)	15	600/100
2	25	600/100
3	50	600/100
4	75	600/100
5	115	600/100
6	170	600/100
7	170	900/100

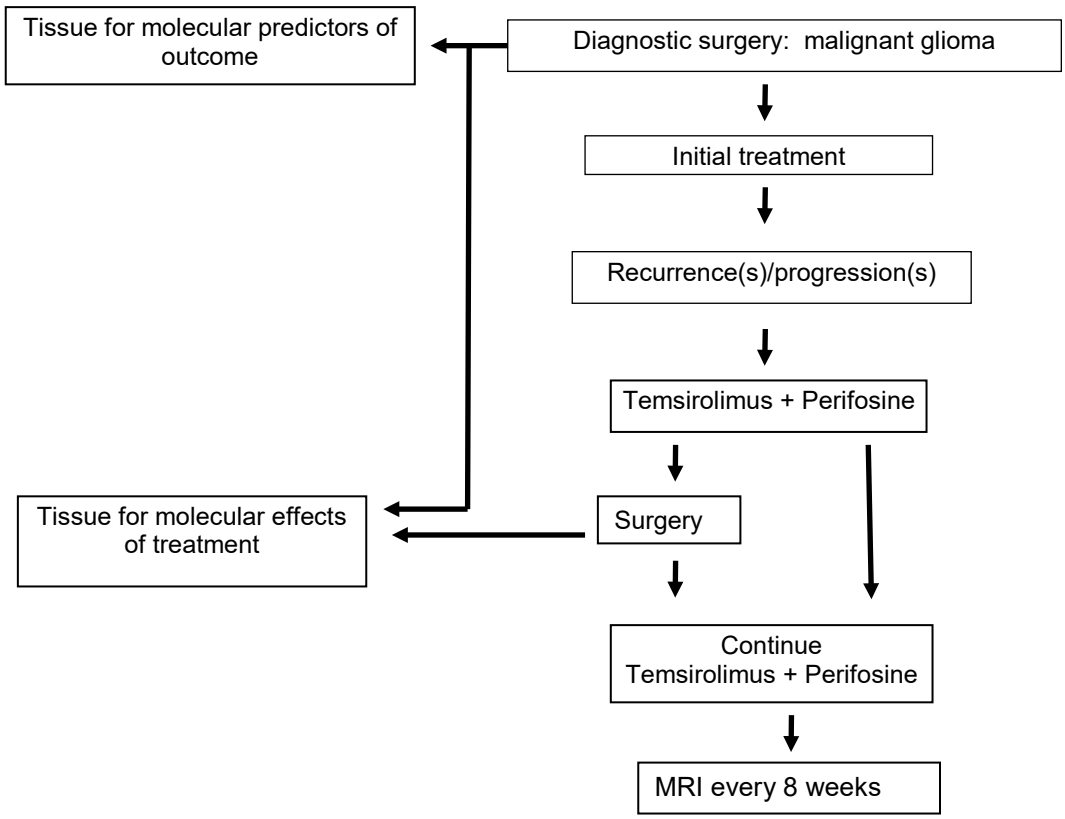

TABLE OF CONTENTS

1 OBJECTIVES

1.1 Primary:

1.1.1 Phase I: Define the maximum tolerated dose (MTD) of temsirolimus in combination with perifosine in patients with recurrent malignant gliomas who are not taking enzyme-inducing anti-epileptic drugs (EIAEDs).

1.1.2 Phase II: Determine the efficacy of temsirolimus in combination with perifosine in patients with recurrent/progressive GBMs not taking EIAEDs as measured by 6 month Progression-Free Survival (6mPFS) and radiographic response rates

1.2 Secondary (phase I and II):

1.2.1 Characterize the safety profile of perifosine and temsirolimus

1.2.2 Estimate median overall and progression-free survival.

1.2.3 Explore association of pre-treatment molecular phenotype with response to treatment.

1.2.4 Explore molecular effects during treatment including PI3K/AKT/mTOR/S6K and RAS/MEK/ERK signaling, proliferation, and apoptosis.

2 BACKGROUND

2.1 CTEP-Supplied Investigational Agents: temsirolimus and perifosine

Temsirolimus (CCI-779) (NSC 683864)

Temsirolimus (CCI-779, sirolimus 42-ester with 2,2-bis(hydroxymethyl) propionic-acid), an ester of the macrocyclic immunosuppressive agent sirolimus (rapamycin, Rapamune™), is a cytostatic cell cycle inhibitor with antitumor properties. The agent specifically inhibits the mammalian target of rapamycin (mTOR), a Ser/Thr kinase involved in the initiation of mRNA translation (reviewed in Dancey, 2002). Temsirolimus has been shown to inhibit the growth of a wide range of histologically diverse tumor cells, with the greatest sensitivity shown by cells derived from the central nervous system (CNS) cancers, leukemia (T-cell), breast cancer, prostate cancer, and melanoma [Investigator's Brochure 2008]. Temsirolimus is being developed as a cytostatic agent to delay the time to tumor recurrence or progression or to increase survival in patients with various malignancies. Key features of this agent include its good tolerability, unique mechanism of action, ability to arrest cells in the G_1 phase, and ability to induce apoptosis. Intermittent schedules of Temsirolimus administration have been evaluated in clinical studies because nonclinical data suggest such schedules minimize the agent's immunosuppressive effects while maintaining antitumor activity.

Mechanism of Action

The observed antitumor and immunosuppressive properties of rapamycin analogs are due to their ability to disrupt the mTOR-dependent signaling pathway (Sekulic *et al*., 2000). mTOR, a member of the phosphatidylinositide 3'-kinase (PI3K)-related family, is located predominantly in the nuclear fraction of both neoplastic and normal cells (Zhang *et al*., 2002). mTOR activation triggers resting cells to increase the translation of a subset of mRNAs whose proteins are required for cell cycle progression from G_1 to S phase. mTOR regulates essential signal transduction pathways and is involved in the coupling of growth stimuli with cell cycle progression. Experimental data indicate that mTOR acts downstream of the PI3K/Akt pathway and is phosphorylated in response to mitogenic signals (Sekulic *et al*., 2000). Early studies reported that mTOR was dedicated to initiating mRNA translation in response to favorable nutrient environments (Barbet *et al*., 1996). In fact, cells treated with rapamycin undergo changes that are strikingly similar to those observed during conditions of

starvation. These include mTOR inactivation, down regulation of translation, G_1 arrest, accumulation of glycogen stores and altered transcription patterns (Barbet *et al*., 1996). More recent studies have demonstrated that mTOR is involved in regulating many aspects of cell growth, including organization of the actin cytoskeleton, membrane traffic, protein degradation, protein kinase C (PKC) signaling, ribosome biogenesis, and transcription (reviewed in Schmelzle and Hall, 2000).

Temsirolimus reacts with the ubiquitous intracellular FK506-binding protein 12 (FKBP12), forming a Temsirolimus/FKBP12 complex that is a potent inhibitor of the highly conserved kinase mTOR (Armistead and Harding, 1993; Geoerger *et al*., 2001). Inhibition of mTOR leads to suppression of several downstream signaling effectors, including the ribosomal subunit $p70^{S6k}$ and the eukaryotic initiation factor 4 binding protein 1 (4E-BP1) (Brown *et al*., 1994). These two proteins play key roles in ribosomal biogenesis and cap-dependent translation, respectively (Brown and Schreiber 1996). The extent of phosphorylation of these two downstream proteins ($p70^{S6}$ kinase and 4E-BP1) may therefore serve as indicators of Temsirolimus biologic activity *in vivo*. Inhibition of the synthesis of ribosomal proteins and elongation factors, required to accelerate the process of cell division, are thought to contribute to the anti-proliferative effects of rapamycin analogs (Terada *et al*., 1994). While Temsirolimus inhibits the translation of only a subset of mRNAs, inhibition of mTOR can lead to a substantial decrease (~15%) in overall protein synthesis (Dudkin *et al*., 2001).

Tumors that rely on paracrine or autocrine stimulation of receptors that constitutively stimulate the PI3K/Akt/mTOR pathway or tumors with mutations that activate the PI3K/Akt signal transduction pathway may depend on rapamycin-sensitive pathways for growth and therefore may be particularly sensitive to rapamycin analogs. The tumor suppressor gene PTEN is known to play a major role in embryonic development, cell migration, and apoptosis (reviewed in Yamada and Araki, 2001). PTEN acts as a lipid phosphatase that regulates major signal transduction pathways and effectively terminates PI3K-mediated signaling (Podsypanina *et al*., 2001). PTEN mutation is associated with constitutive activation of the PI3K/Akt pathway, resulting in tumors that are generally resistant to apoptosis. PTEN status in tumor cells may therefore be an important predictor of sensitivity to rapamycin analogs (Sekulic *et al*., 2000). Preliminary evidence suggests that breast cancer cell lines containing PTEN mutations are sensitive to growth inhibition by rapamycin (Hidalgo and Rowinsky, 2000). Additional studies in PTEN-deficient human tumor cell lines and PTEN knockout mice have demonstrated sensitivity to growth inhibition by Temsirolimus. Temsirolimus produced remarkable sensitivity to G_1 arrest ($ID_{50} < 1nM$) in PTEN-deficient myeloma cell lines, while myeloma cells containing wild type PTEN were at least 1000-fold less sensitive to the agent (Shi *et al*., 2002). Also, studies of glioblastoma cell lines indicated that low PTEN protein expression was strongly linked with sensitivity to Temsirolimus-mediated growth arrest (Smith *et al*., 2002). Together, these studies indicate that the molecular identification of PTEN mutations or other mutations that lead to constitutive activation of the pathway within tumor cells might be predictive of sensitivity to Temsirolimus therapy (Neshat *et al*., 2001; Mills *et al*., 2001).

Summary of Clinical Studies

Temsirolimus safety, pharmacokinetics, and preliminary antitumor effects were evaluated in a phase 1 dose-escalation study with doses of 7.5-220 mg/m^2 given as a weekly intravenous (IV) infusion to 24 patients with advanced malignancies (Raymond *et al*., 2004). Although the maximum tolerated dose (MTD) was not reached, 220 mg/m^2 appeared to be the maximum acceptable dose, with thrombocytopenia being dose limiting with repeated dosing. No clinically relevant immunosuppressive effects were observed during treatment, although herpes simplex infections were observed in five patients. The most frequent drug-related adverse events were acneiform maculopapular rashes and mucositis/stomatitis (18 of 24 patients, 75%). Confirmed partial responses (PRs) were observed in 2 of 24 patients evaluated, one each with renal cell carcinoma (RCC) and breast cancer.

Data from *in vitro* studies of A498 human renal cell lines indicated that Temsirolimus had a median growth inhibitory concentration (IC_{50}) of 5 ng/mL [Investigator's Brochure (CCI-779) 2007]. Predicted modeling of IC_{50} (humans receiving doses as low as 10 mg) suggests that whole blood concentrations would be above the range of 1 ng/mL throughout the entire 1-week dose interval and above 5 ng/mL for the majority of this time period. It is expected that mTOR inhibition would be attained with a 25 mg dose.

Clinical pharmacokinetic data are available in patients with cancer receiving Temsirolimus both IV daily x 5 days every 2 weeks, once weekly schedules, and orally daily x 5 every 2 weeks. These data indicate that there is no appreciable drug accumulation between cycles and that distribution is extensive. With increasing dose, exposure (AUC) increases in a less than proportional fashion. The mean volume of distribution at steady state (V_{dss}) is large (57 L after 2 mg IV dose; 900 L following a 250 mg IV dose) and increases with dose. Exposure to the hydrolytic product sirolimus is substantial with mean values of approximately 1.5-2.3-fold greater than those seen with Temsirolimus following IV administration. Clearance (CL) of Temsirolimus from whole blood increases with increasing dose from approximately 5.2L/h after a 2 mg dose to 100L/h after a 250 mg dose. Intersubject variability in CL at a given dose was modest and ranged from 16-27%. The terminal half-life ($t_{1/2}$) following Temsirolimus doses of 25 to 250 mg is approximately 15 hours.

Pharmacokinetic results from the initial phase 1 study showed that the AUC increased proportionally with doses up to 150 mg, but doses higher than 300 mg yielded high AUCs and low CL in some patients (Raymond *et al*., 2004). The mean Vd_{ss} was large with mean values of 127-384 L, while the Temsirolimus mean terminal $t_{1/2}$ decreased from 22 hours (34 mg/m^2) to 13 hours (220 mg/m^2) as the dose increased. Similar pharmacokinetic data were reported for 16 patients following their initial dose of Temsirolimus of 25, 75, or 250 mg delivered as a weekly 30-minute IV (Atkins *et al*., 2004).

Phase II studies of single agent temsirolimus evaluating different doses of 25 mg, 75 mg, and/or 250 mg weekly IV have been undertaken in broad range of tumor histologies. The most promising activity has been seen in mantle cell lymphoma (Witzig *et al.,* 2005; Ansell et al., 2006), other B-cell lymphomas (Smith *et al.*, 2006) and endometrial carcinoma (Oza *et al.,* 2006) with objective tumor response rates of 25-40%. Moderate activity has been reported in breast (Chan *et al.,* 2005) and renal cell carcinoma (Atkins *et al.,* 2004). Minimal to modest single agent activity has been seen in SCLC (Pandya *et al.,* 2005), melanoma (Margolin *et al.,* 2005) and GBM (Chang *et al.,* 2004, Galanis *et al.,* 2006) and multiple myeloma (Farag *et al.,* 2006). In general, lower doses appear to be as active as higher doses with better tolerability.

Recently, a phase 3 trial of temsirolimus, temsirolimus with interferon versus interferon in poor prognosis patients with RCC has been reported (Hudes et al., 2006). Of the 626 patients, overall survival of patients treated with temsirolimus was significantly prolonged compared to those treated with interferon (median 10.9 months versus 7.3 months, HR 0.73, p 0.0069). The combination of interferon and temsirolimus did not confer greater benefit than interferon alone, possibly due to compromised dose delivery of the agent(s).

Safety issues

The safety profile of Temsirolimus has been similar across studies regardless of tumor type, schedule, or route of administration. The most frequently reported adverse events have been gastrointestinal (mucositis, diarrhea, nausea, anorexia, vomiting), dermatologic (maculopapular rash, acneiform rash, pruritus, nail disorder), hematologic (thrombocytopenia, anemia, leukopenia), and metabolic (hyperglycemia, hyperlipemia). Asthenia, headache, epistaxis, somnolence, and taste perversion have also been reported. Less common but related adverse events included mucositis, depression, and allergic reactions [Investigator's Brochure 2007].

Acute hypersensitivity reactions to Temsirolimus that begin shortly after the start of the intravenous (IV) infusion (usually, but not always, with the first infusion) and end after stopping the infusion have been reported. Signs and symptoms include flushing of the face and neck (sometimes also the extremities and trunk); descriptions of feeling hot, uncomfortable, and/or anxious; chest pain/tightness; shortness of breath; decrease in oxygen saturation and cyanosis; hypotension; lightheadedness; periorbital puffiness; descriptions of feeling like the head is swelling; nausea; back pain; numbness and tingling of hands/feet/face; and difficulty speaking. Because of these idiosyncratic reactions, subjects receiving IV Temsirolimus should be premedicated with diphenhydramine 25-50 mg IV (or a similar antihistamine) approximately 30 minutes before the start of the Temsirolimus infusion.

Similarly to organ-transplant patients receiving sirolimus, pneumonitis/pulmonary infiltrates and alveolitis have been reported among oncology patients receiving intravenous Temsirolimus. Some patients have been asymptomatic with pneumonitis detected on CT scan or chest x-ray, while others have had symptoms of dyspnea, cough and fever. These symptomatic patients have had Temsirolimus discontinued and improved after receiving corticosteroids and/or antibiotics. There have been a few instances of recurrence of the symptoms and signs of pneumonitis with Temsirolimus rechallenge. Patients with cough, dyspnea and fever should have Temsirolimus discontinued pending investigation and permanently discontinued if the diagnosis is confirmed and the reaction thought to be related to Temsirolimus.

Subjects may be immunosuppressed and should be carefully observed for the occurrence of infections, including opportunistic infections. Other serious adverse events seen in subjects treated on clinical studies of temsirolimus include abnormal wound healing, bowel perforation (including fatal outcome), renal failure (including fatal outcome) in subjects with advanced RCC and/or with pre-existing renal insufficiency, angioneurotic edema-type reactions have been observed in some subjects who received temsirolimus and ACE inhibitors concomitantly and cataracts have been observed in some subjects who received the combination of temsirolimus and interferon-a. Central nervous system (CNS) bleeding has been reported, primarily in patients with CNS tumors. Several of these patients were concurrently taking anticoagulants, so the relationship between Temsirolimus and the adverse event is not clear. Increased fibrinogen has been reported among oncology patients receiving Temsirolimus. The clinical significance of this event is not certain, although thromboembolic events have been reported. A list of Comprehensive Adverse Events and Potential Risks (CAEPR) in NCI-CTCAE terms is included in Section 7 of the protocol. Reference may also be made to the Investigators' Brochure.

Potential Drug Interactions
Temsirolimus and rapamycin are metabolized primarily by CYP3A4 in human liver microsomes. Since there are many agents that affect the metabolic activity of CYP3A4, there is a potential for drug-drug interactions to occur between Temsirolimus and co-administered drugs. Although formal drug-drug interaction studies have not been reported, current knowledge and available data suggest that Temsirolimus should be used with caution in combination with enzyme inducers or inhibitors. Changes in treatment regimens in which CYP3A4 inducers or inhibitors are being administered, or initiation of such regimens during Temsirolimus treatment, should be avoided if possible, but are not contraindicated. Temsirolimus can inhibit CYP2D6, suggesting a potential interaction with drugs that are substrates for CYP2D6.

Perifosine (NSC 639966)
Alkylphospholipids represent a new class of lipid-related compounds that exhibit promising anticancer activity and a different spectrum of toxicity than conventional cytotoxic agents. Perifosine (1,1-dimethyl-4[[(octadecyloxy)hydroxyphosphinyl]oxy]-piperidinium inner salt, is a synthetic, substituted heterocyclic alkylphospholipid, structurally related to miltefosine (NSC 60558, D-18506). The anti-tumor activity of miltifosine was initially evaluated in the 1980's. However, the agent was unsuitable

for intravenous administration, as it caused hemolytic anemia, or oral administration as it caused gastrointestinal toxicity that prevented adequate dosing to reach effective drug levels. Further development of miltefosine was limited to topical applications, and the drug has recently been licensed in Europe as Miltex® for the treatment of patients with cutaneous metastases from breast cancer. Perifosine was identified as a potentially active and better tolerated analog of miltefosine. Its spectrum of activity across the NCI 60 cell line screen was very similar to miltefosine (Pearson correlation coefficient = 0.817). Both miltefosine and perifosine had very unique patterns of *in vitro* cell growth inhibition, unlike any "standard" chemotherapeutic agent. Perifosine has been shown to be more active and better tolerated than miltefosine in nonclinical models (Hilgard *et al*. 1997). Perifosine exhibited marked activity in animal and human tumor cell lines resistant to standard chemotherapeutic agents with relative sparing of normal cells, including macrophages and bone marrow cells.

While intracellular actions of alkylphospholipids have been studied in detail, their exact mechanism(s) of antitumor activity are unclear. Alkylphospholipids exert a broad spectrum of effects on cellular functions including proliferation, differentiation, invasion, and metastasis. Alkylphospholipids in general are absorbed directly into cell membranes where they accumulate (reviewed in Arthur and Bittman 1998). Although there is a widespread view that the plasma membrane is the primary site of action, alkylphospholipids may be widely distributed throughout the cytoplasm and possibly within the nucleus. Modulation of cell surface receptors and inhibition of lipid-mediated signal transduction pathways appear to play key roles in the observed anti-tumorigenic effects (Arthur *and* Bittman 1998; Ruiter *et al*. 1999; Maly *et al.* 1995; Verheij *et al.* 2002). Initial studies suggested that alkylphospholipids interfere with phosphoinositide metabolism, leading to inhibition of phospholipase C and protein kinase C (Maly *et al.* 1995). Treatment of head and neck squamous cell carcinoma (HNSCC) cell lines with perifosine led to loss of cyclin-dependent kinase (cdk) activity and subsequent cell cycle arrest in G_1-S and G_2-M phases. Perifosine-mediated inhibition of cell cycle events may be mediated in part through $p21^{WAF1}$ (Patel *et al*. 2002; Kondapaka *et al*. 2002) as a correlation was observed between inhibition of cdk activity and up regulation of $p21^{WAF1}$. Treatment with perifosine led to increased expression of $p21^{WAF1}$, resulting in inactivation of the cdk/cyclin complex and subsequent cell cycle arrest. Cells lacking p21 gene function ($p21^{WAF-/-}$) were insensitive to perifosine-mediated inhibitory effects. Perifosine induced disruption of signal transduction pathways and cell cycle progression are likely to have profound effects on tumor cell growth and function.

Nonclinical Anti-Tumor Efficacy

The activity of perifosine has been evaluated in numerous human and murine cell lines. In vitro, cell lines demonstrating the greatest sensitivity to perifosine included KB (larynx), LNCaP (prostate), MAI-PaCa-2 (pancreas), DLD-1 (colon), and SK-HEP-1 (liver) (IC_{50} 1.0 – 4.9 µg/mL) (Perifosine Investigator's Brochure 1998). In a soft agar tumor stem cell assay, the human KB (squamous) and murine L1210 (leukemia) cell lines were the most sensitive.(Perifosine Investigators Brochure, 1998) In the methylene blue exclusion assay, the five most sensitive lines include: KB (squamous mouth), LU 65A (lung), LNCaP (prostate), PC-1 (lung) and Hep-2 (larynx) with IC_{50} values of 0.8 – 3.4 µg/mL. In the SRB/metabolic capacity assay, KM12 (colon), PC3 (prostate), M14 (melanoma), HOP-92 (lung) and SF295 (CNS) cancer cell lines were the most sensitive with IC_{50} values of 0.2 – 3.1 µg/mL. The majority of cell lines (in all assays) were more sensitive to perifosine than to miltefosine. Compared with malignant cells, mouse bone marrow cells were shown to be resistant to perifosine in vitro.

The *in vivo* activity of perifosine has been evaluated via oral dosing in various schedules in several transplanted tumors, as well as in the dimethylbenzanthracene (DMBA) induced mammary tumor model of the rat. Two generally drug resistant tumors exhibiting sensitivity were the KB tumor (squamous cell) and AsPC-1 (human pancreatic carcinoma). In vivo, single oral doses of 511 mg/kg completely inhibited tumor growth, and higher doses (two doses of 203 or 300 mg/kg given 7 days apart) gave complete persisting remissions lasting >63 days in nude mice with subcutaneously

transplanted KB squamous cell tumors. Other xenografts in nude mice with good evidence of *in vivo* effect of orally administered perifosine include the Hep-2 laryngeal, R3327 rat prostate, SAS tongue, SC115 mouse breast and HCT-8 colon tumors treated on a daily x 21 day schedule. Perifosine was extremely active in the Sprague-Dawley rat model of dimethylbenzanthracene (DMBA) induced mammary tumors. Daily oral treatment using 21.5 mg/kg for 5 weeks led to complete regressions of tumors. Notably, complete regressions over the entire study period were also achieved by combining a high initial dose of 68.1 mg/kg followed by lower daily doses of 2.15 mg/kg over 20 days, whereas the loading dose alone caused only an arrest of tumor growth for 14 days. Even large, established DMBA induced mammary tumors (4-8 grams) in rats responded to daily doses of 14.7 to 68.1 mg/kg over 28 days, with a persisting effect of more than 20 days after cessation of treatment.

Nonclinical Pharmacology
Nonclinical studies of perifosine given as a single oral dose (10 mg/kg) demonstrated near complete absorption, with an absolute bioavailability of 81% and 95% in male and female rats (Perifosine Investigator's Brochure, 1998). For doses ranging from 1 to 50 mg/kg, maximum plasma concentration (C_{max}) values ranged from 0.34 to 10.5 μg/mL. Time to achieve C_{max} was reached at a median of 16 to 32 hours following administration of perifosine. The volume of distribution (V_d) was twice the physical body volume, and the terminal half-life ($t_{1/2}$) was 120.5 – 171.4 hours.

A study of [^{14}C] excretion (urine, feces) done in the rat after oral dose [^{14}C] perifosine at 10 and 50 mg/kg revealed the following proportions of renal and fecal excretion:

	Urine	Feces
Dose (mg/kg)		
10	<24 hr 1.0%, 360 hr 20.4 %	24 hr 24.6 %, 360 hr 42.9%
50	<24 hr 1.2%	<24 hr 22%, 48 hr 48.5%, 72 hr 52.6 %

In vitro binding of perifosine to human serum albumin or α_1-acid glycoprotein ranged from 92 to 98% (Perifosine Investigator's Brochure, 1998). No concentration dependence of protein binding was observed, suggesting a high binding capacity of the plasma proteins for perifosine.

Nonclinical Toxicology
In a subchronic toxicity study in rats, dose dependent toxicities were observed in the kidneys, gastrointestinal tract, skin, mammary glands, pituitary, hematopoietic tissue, spleen, ovaries, male genital tract, and eyes (Perifosine Investigator's Brochure, 1998). Effects on the hematopoietic tissue were characterized by an increase in cellularity for the bone marrow and an increased extramedullary hematopoiesis. Histopathologic changes seen in other tissues included evidence of chronic nephropathy, atrophy of hair follicles, atrophy of mammary glands, reduction of follicular development in the ovaries, degeneration of the germinative epithelium of the testes, atrophy/inactivation of prostate and seminal vesicals, secondary hypertrophy of the Leydig cells in the testes, and hypertrophy/hyperplasia of mucosal epithelium cells in stomach and small intestine. All toxicities, except those in the male genital tract and eyes, were reversible within 13 weeks. Most significant and not clearly reversible were ophthalmologic lesions, specifically, retinal degeneration and cataract formation. This toxicity was seen in rat but not in dog toxicity studies. Clinical chemistry revealed reversible increases in CK, BUN, SGOT, SGPT and reversible decrease of red blood cell parameters, total cholesterol, triglycerides, inorganic phosphorus and albumin and total protein.

In dogs, decreased food consumption, anorexia, diarrhea and vomiting were noted. Clinical chemistry

revealed reduced glucose levels in the group receiving the highest dose. All changes were reversible during a 6-week recovery period. Histopathologically, minimal to mild degeneration of proximal tubular cells of the kidneys were found in male animals.

Reproductive Toxicity
Perifosine impaired fertility and reproductive performance in male rats at doses of 2.37 and 8.25 mg/kg. Perifosine impaired the reproductive performance of female rats and possessed fetotoxic properties in doses of 3.16 and 21.5 mg/kg. A dose of 0.464 mg/kg is considered to be the no-observed-toxic-effect level on the female reproductive function and early embryonic development.

Mutagenicity/Carcinogenicity
In the assay systems employed (Salmonella typhimurium reverse mutation assay with and without S9-Mix, the V79 gene mutation test, and the micronucleus test *in vivo*) only a slightly increased number of mutants was found in the V79 gene mutation test without activation, a finding similar to that for miltefosine. Carcinogenicity studies of perifosine are not available; thus, a carcinogenic potential of perifosine cannot be excluded.

Clinical Results - Phase 1
Asta Medica sponsored three European phase 1 trials of perifosine evaluating weekly and daily dosing, as well as an enteric coated formulation. The study D-21266-3040 was a phase 1 study of escalating doses of perifosine given on a weekly schedule. Thirty-six patients were treated in sequential cohorts at dose levels of 100, 200, 350, 450, 600 and 800 mg/week. At 350 mg weekly, vomiting grade 3 occurred in the first two patients. These patients responded to anti-emetic prophylaxis with tropisetron, alizaprid, and dexamethasone, and all subsequent patients received anti-emetic prophylaxis. Among 7 patients treated at the maximum administered dose of 800 mg/wk, grade 2 nausea and vomiting of residual perifosine tablets and grade 4 diarrhea occurred. Preliminary data from analyzed plasma samples of patients receiving perifosine in the dose range of 100 to 600 mg indicated a dose-linearity of C_{max} and of the area under the concentration time curve (AUC) in this dose range. However, at 800 mg/week, the dose-AUC and C_{max} proportionality was reduced. C_{max} and $t_{1/2}$ values ranged from 8 – 25 hours and 96 – 225 hours, respectively (Perifosine Investigator's Brochure, 1998). After repeated dosing a slight accumulation of perifosine was seen, which is in accordance with the relatively prolonged terminal $t_{1/2}$ of the agent. Perifosine was not detectable in the patients' urine. One patient with chondrosarcoma treated at 350 mg/weekly achieved a partial response (Asta Medica update to the NCI, 2000).

In the phase 1 study D-21266-3079, 22 patients received perifosine at escalating doses of 50-350 mg daily x 21 days. Fatigue, nausea, vomiting and diarrhea were the most frequent toxicities experienced by patients on this study. The maximum tolerated dose on this study was 200 mg/day. Three of six patients treated at 250 mg/day and the one patient treated at 350 mg/day had intolerable fatigue and/or gastro-intestinal toxicity. (Asta Medica update to the NCI, 2000)

An enteric-coated formulation was evaluated on a weekly study in the trial D-21266-3087. A total of eight patients (4/dose level) were treated at 200 or 350 mg weekly. The enteric coating did not improve the tolerability of the oral administration of perifosine and reduced its bioavailability. (Asta Medica update to the NCI, 2000)

The Division of Cancer Treatment and Diagnosis, National Cancer Institute has sponsored two phase 1 trials of perifosine evaluating a loading dose followed by daily maintenance dose schedule. A loading dose/daily maintenance schedule was proposed due to improved efficacy in the DMBA-induced rat mammary carcinoma model with this schedule and the clinical PK data which showed a long terminal

$t_{1/2}$ of ~ 100 hours and a slow approach to steady state. A loading dose could potentially achieve active anti-tumor serum concentrations followed by tolerable daily maintenance dosing. The loading dose was fractionated and administered every 6 hours to avoid potential problems with bioavailability and possible C_{max} associated toxicity such as emesis. Similar to the results from the Asta Medica-sponsored studies, the most frequently observed toxicities in NCI sponsored phase 1 studies were gastrointestinal (nausea, vomiting, diarrhea) and fatigue. Chronic nausea among patients receiving perifosine appears to resolve approximately 48 hours following discontinuation of therapy. In contrast, fatigue may persist for weeks after therapy has ended.

The first trial examined a loading dose on day 1 followed by a maintenance dose schedule for 20 days in patients with solid tumors repeated every 28 days. In an attempt to minimize nausea, doses were administered with a meal or with maintenance doses at bedtime. In addition, prior to the loading dose, patients were given granisetron, metoclopramide, diphenhydramine, and dexamethasone as anti-emetic prophylaxis. As of June 2002, 30 patients had been treated on this study. Dose limiting nausea and vomiting occurred with loading doses of 1200-1500 mg. Persistent grade 2 nausea and vomiting despite anti-emetics, grade 3 arthralgias, arthritis, and fatigue occurred with a maintenance dose of 200 mg/day. Of note, while taking perifosine 200 mg/day two patients, one with history of gout and one with a history of hyperuricemia had episodes of gout. One patient without a prior history had an episode of pseudogout. The maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) were established as: Cycle 1: 900/150 mg loading/maintenance dose and for > Cycle 2: 300 mg day 1 loading dose followed by 150 mg days 2-21 in patients repeated every 28 days (Monga *et al*., 2002). Among 10 patients treated at the MTD/RP2D, no patients experienced grade 3-4 dose limiting events during the loading dose or maintenance dose periods in the first cycle. Grade 1-2 fatigue occurred in 2/10 patients and grade 2 gastrointestinal toxicity was intermittent and tolerable. No clinical responses have been observed; however, four patients with stable disease (duration 167-750 days) have been reported.

The second DCTD, NCI sponsored phase 1 trial is evaluating a loading dose followed by a continuous maintenance dose schedule in patients with advanced cancer. The loading dose is only given in course one, and maintenance dosing continues without a scheduled break. The cohorts of patients have received loading/maintenance doses ranging from 400/50 – 1200/150 mg. In this study, loading doses are split into 4-8 equal doses given at 6 hour intervals. As of June 2002, 41 patients have been enrolled on study. Reported severe toxicities for this trial include anorexia, fatigue, dehydration, nausea/vomiting, constipation, diarrhea, elevated liver function tests, and decreased hemoglobin. One partial response in a patient with uterine sarcoma has been reported. The MTD/recommended phase 2 dose for this schedule is 900 mg loading dose followed by 100 mg daily maintenance. Among 14 patients treated at the MTD, there were grade 3 events (fatigue, nausea and vomiting) during the first 28 days of treatment.

Pharmacokinetics
Pharmacokinetic analyses from both NCI sponsored studies showed rapid achievement of steady state plasma concentrations without evidence of drug accumulation for doses ranging from 300/50 – 900/150 (cycle 1) daily for the 21/28 days regimen and from 400/50 – 900/100 mg/day for the continuous treatment regimen. Plasma concentrations were within the range (i.e., 5-10 μg/mL) found to have biological activity in laboratory studies.

2.2 Other Agents: Not applicable (N/A)

2.3 Study Disease: Malignant Gliomas

Malignant gliomas are the most common type of brain tumor in adults. It is estimated that each year there are approximately 12,000 new cases in the United States. Despite optimal treatment with surgery, radiotherapy and chemotherapy with temozolomide, the prognosis remains poor and almost all patients

develop recurrent or progressive tumor. Patients with glioblastoma (GBM) have a median survival of approximately 14.6 months,(Stupp, Mason et al. 2005) while those with anaplastic astrocytomas (AA) have a median survival of 24 to 36 months. The median survival for patients with recurrent GBM is 6 months and for AA is 11 months. Once patients develop tumor progression, conventional chemotherapy is generally ineffective.

Approximately 70% of GBMs exhibit abnormally activated phosphotidylinositol 3-kinase (PI3K)/AKT signaling, usually as a result of loss or alteration of the tumor suppressor gene phosphatase and tensin homolog on chromosome ten *(PTEN)*.(reviewed in Lassman and Holland 2006) The PI3K/AKT pathway activates the mammalian target of rapamycin (mTOR) which activates p70S6 Kinase (S6K) and stimulates multiple oncogenic processes including translation of a number of key proteins required for cell-cycle progression. The presence of *PTEN* gene alterations and the subsequent activation of these downstream pathways have been associated with poor prognosis in AA, anaplastic oligodendroglioma (AO) and GBM. Targeting of the components of the PI3K/AKT/mTOR/S6K pathways can result in cell-cycle arrest, apoptosis, or reduced tumorigenicity.

Modeling brain tumors using the RCAS/tv-a system

One of the main objectives highlighted by the *Brain Tumor Progress Review Group (PRG)* was the need for animal models of brain tumors that accurately represent the histology and genetics of the disease in humans. The RCAS/tv-a system produces gliomas that fulfill this objective by achieving cell type-specific gene transfer to somatic cells post-natally. This system was developed by Dr. Eric Holland, now head of the Brain Tumor Center at Memorial Sloan Kettering Cancer Center. Through glial-specific gene transfer *in vivo*, this system permits determination of the role of single and multiple mutations on gliomagenesis in mice. Modeling with this system demonstrated that the combined activation of RAS/MAPK and PI3K/AKT signaling in GBMs is causal and not an epiphenomenon. Murine gliomas modeled with this system reproduce their human counterparts both histologically and radiographically with high fidelity (Figure 1).(Lassman and Holland 2006)

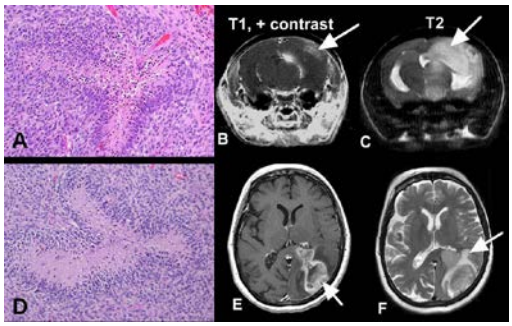

Figure 1: RCAS/tv-a modeled high grade gliomas (A,B,C) using forced PDGF overexpression accurately reflect their human counterparts (D,E,F) both histologically (A vs. D) and radiographically on contrast enhanced T1 (B vs. E) and T2 (C vs. F) weighed brain MRI.

2.4 Rationale for combined temsirolimus and perifosine in malignant gliomas

Temsirolimus

Temsirolimus crosses the blood-brain barrier and achieves therapeutic concentrations in brain tumor tissue.(Kuhn, Chang et al. 2007) It is also reasonably well-tolerated with toxicities usually mild including myelosuppression (especially thrombocytopenia), hyperlipidemia, stomatitis, skin rash and fatigue. However, temsirolimus has only modest antitumor activity as a single agent in recurrent high-grade gliomas. For example, the 6 month progression-free survival (6mPFS) rate for patients with recurrent GBM was approximately 2% in a North American Brain Tumor Consortium phase II trial, (Chang *et al*. 2005) and approximately 8% in a North Central Cancer Treatment Group phase II trial (Galanis *et al.* 2005). Possible explanations for the limited efficacy include the existence of mTOR independent activities of AKT uninhibited by temsirolimus, and the observation in other cell types that mTOR inhibition leads to upstream activation of AKT itself through a feedback loop.(Sun *et al.* 2005;

O'Reilly *et al.* 2006)

Perifosine

There has been extensive pre-clinical testing of perifosine in glia *in vitro* and in modeled gliomas *in vivo* in Dr. Eric Holland's laboratory at Memorial Sloan Kettering Cancer Center (MSKCC). For example, perifosine specifically has been shown to inhibit AKT signaling in transformed glia (Momota *et al.* 2005) as well as reduce proliferation of modeled gliomas in mice.(Momota *et al.* 2005; also reviewed in Lyustikman and Lassman 2006)

In addition, the effects of perifosine clearly extend beyond inhibition of the AKT signal transduction cascade. These effects, while not totally understood, are in fact advantageous and likely critically important to the anti-glioma activity of perifosine. For example, preclinical studies by Dr. Holland also demonstrated that perifosine potently inhibits MAPK/RAS signaling in glia.(Momota *et al.* 2005) Although this may be a cell type specific effect, this dual inhibition of AKT and MAPK/RAS signaling in glia is a major theoretical advantage over agents that inhibit only one but not both cascades, especially because these cascades are activated in ~70% and ~100% of GBMs,(Lassman and Holland 2006) respectively and forced activation of both cascades in mice is sufficient to induce GBMs.(Holland *et al*. 2000) Furthermore, other agents that inhibit AKT signaling can lead to MAPK/RAS activation through feedback loops (personal communication, Pier Paolo Pandolfi), and this potentially counterproductive effect is not seen with perifosine. Moreover, perifosine increases the penetrability of the blood brain barrier in our modeled gliomas *in vivo*, an effect not achieved by mTOR blockade. This may contribute to synergy with temsirolimus, allowing enhanced intratumoral penetration by temsirolimus over that seen in patients treated with temsirolimus monotherapy.

Based on these preclinical data, as well as the promising results of numerous phase I and II trials in patients with sarcomas and other solid tumors, in 2006 we conceived, wrote, and opened an investigator (Lassman) initiated phase II trial of perifosine monotherapy at MSKCC for patients with recurrent malignant gliomas. This was the first step in the translation of our extensive preclinical glioma data with perifosine into human glioma trials. Toxicity was minimal among 25 patients enrolled to date, with 1 episode each of grade 1 diarrhea, grade 1 nausea, grade 4 joint pain, and grade 3 difficulty walking as the only significant adverse events possibly, probably, or definitely related to perifosine. The known toxicities of perifosine include nausea, diarrhea, and exacerbation of gout; the joint pain was likely a gouty attack in a patient without a prior known gout diagnosis. Preliminary efficacy results, reported at the 2007 Society for Neuro-Oncology annual meeting, included 2 partial radiographic responses among 6 evaluable patients with recurrent anaplastic gliomas.. However, none of the first 12 patients with GBM reached 6 months of progression-free survival which was the primary efficacy endpoint; therefore, in accordance with the statistical design of the trial, accrual of patients with GBM was terminated because it was statistically unlikely (90% power) that the 6mPFS rate would reach or exceed 20%, the threshold to consider monotherapy with perifosine as promising for recurrent GBM.

Although temsirolimus and perifosine have only limited activity as single agents, we explored the possibility that combinations of these agents could have synergistic therapeutic efficacy. While the phase II trial of perifosine monotherapy for recurrent gliomas has been ongoing, further pre-clinical evaluation of perifosine in combination with temsirolimus has been conducted. In modeled gliomas, perifosine reduces pAKT and reduces proliferation, but there is only minimal killing of tumor cells and only regional cell cycle arrest. In addition, despite the reduction in pAKT, the activity of the mTOR effector S6RP (assayed by pS6RP) is not decreased *in vivo* and may actually rise during perifosine treatment for reasons that remain unclear at this time. By contrast, temsirolimus reduces pS6RP levels and arrests proliferation, but also only causes minimal cell death. However, when the two agents are administered together, the combination induces widespread cell death, arrest of surviving cells, and inhibition of the entire AKT signal transduction cascade including both pAKT and pS6RP. Therefore, this extensive preclinical data suggests combining perifosine and temsirolimus has both superior anti-

tumor effects and superior inhibition of the AKT signal transduction cascade than either agent alone. For example, three-day treatment of perifosine (30mg/kg) in combination with temsirolimus (40mg/kg) leads to massive necrosis and cell cycle arrest almost in all treated mice (n=13). From the perspective of a clinical trial, with the exception of nausea/vomiting and diarrhea, the two agents have non-overlapping toxicities when used alone, and may therefore allow therapeutic doses of each drug together without substantial side effects.

The maximum tolerated dose (MTD) of combined temsirolimus and perifosine is required for a phase II study. Therefore, we will conduct a phase I/II study to both determine the MTD, safety, and efficacy of the combined drug therapy. We will also analyze pre-treatment archival tissue to identify molecular predictors of response and also assess tissue resected during therapy (through a surgical substudy as part of the phase II component) to determine molecular effects of the combination in human malignant gliomas in vivo.

Study Design and Rationale

Phase I Component

The phase I component of the study will define the maximum tolerated dose (MTD) of temsirolimus in combination with perifosine in patients with recurrent malignant gliomas not on EIAED. Temsirolimus is metabolized by cytochrome P450 3A4 which is induced by EIAED. As a result the therapeutic doses of temsirolimus, and to a lesser extent, perifosine, will be higher for patients on EIAED than for patients not taking these medications. Therefore, to reduce the study complexity and the number of patients required, eligibility will be restricted to patients not taking EIAED. If the combination of temsirolimus and perifosine shows efficacy, a separate phase I study to determine the MTD in patients on EIAED will be considered. Standard dose escalation in groups of three will be performed.

All patients will initially receive both drugs and none will receive placebo. There is no intra-patient dose escalation. The starting dose of perifosine will consist of a loading dose of 600 mg on day 1 followed by a fixed maintenance dose of 100 mg per day for days 2-28 of each 28 day cycle. Patients will receive an initial dose of temsirolimus of 15 mg intravenously once weekly. Depending on patient tolerance, subsequent temsirolimus doses will be escalated to a maximum of 170 mg intravenously weekly in subsequent cohorts with one final cohort comprised of an escalated perifosine dose. The maximum tolerated dose of temsirolimus will be based on the assessment of dose limiting toxicity DLT during the first 28 days of treatment only (cycle 1). If excessive toxicity is encountered with the combination of temsirolimus and perifosine, the doses will be reduced per section 6.

Phase II Component

The primary goal of the phase II component of the study is to determine the therapeutic efficacy of the combination of temsirolimus and perifosine in patients with recurrent malignant gliomas as measured by patients who obtain either a radiographic response or achieve 6-months of disease satibilization (6 month progression free survival). Patients will receive the dose of both agents determined from the phase I study. There is no intra-patient dose escalation.

An important limitation of prior studies of targeted molecular agents in GBMs is the lack of tumor tissue to determine whether the therapeutic agents under evaluation inhibited their putative molecular targets. We previously demonstrated such analyses are possible through a surgical substudy in which patients who undergo cytoreductive surgery for recurrent disease receive study drug pre-operatively and then restart the agents upon recovery from surgery.(Lassman *et al.* 2005) Therefore, in this study up to 10 patients enrolling into the phase II component will undergo resection of their tumor as part of the trial. These patients will receive temsirolimus and perifosine pre-operatively at the dose determined by the phase I. Tumor specimens resected in this manner will be analyzed for evidence of inhibition of PI3K/AKT/mTOR/S6K and RAS/MEK/ERK signaling by immunohistochemistry of

paraffin embedded tissue and Western blot of flash frozen tissue. PTEN, p-AKT, p-mTOR, total S6K, p-S6K, p-ERK, Ki-67, and apoptosis will be measured. These data will be compared to similar analysis performed on tumor tissue not exposed to temsirolimus and perifosine obtained from each patient's original or prior surgery (intra-patient comparison). After recovery from surgery, patients will resume treatment with temsirolimus and perifosine. Treatment duration will be measured in 4 week cycles.

Phase I and II

Patients will remain on treatment as long as there are no unacceptable toxicities until tumor progression. Responses will be assessed by MRI scans every 8 weeks. Fluorodeoxyglucose (FDG)-PET will be performed in phase II patients to evaluate the effects of the drugs on tumor metabolism. Paraffin tumor sections from the original surgical resection will be obtained from all patients. An attempt will be made to correlate the genotype patient's tumor specimens with response to treatment. In this way, molecular subsets of malignant gliomas that may be more or less likely to respond to treatment with temsirolimus and perifosine can be identified.

2.5 Correlative Studies Background

GBMs are molecularly heterogeneous. For example, the oncogenic mutations driving tumor growth differ between "primary" (or "de novo") GBMs which appear abruptly and "secondary" GBMs which arise from lower grade gliomas.(Kleihues and Ohgaki 1999) Similarly, tumors with *MGMT* promoter methylation appear to exhibit greater sensitivity to temozolomide and are associated with longer overall survival regardless of therapy in comparison to tumors with unmethylated promoters.(Hegi *et al.*, 2005) Finally, recent results also suggest a tripartite distribution of high grade gliomas (with prognostic significance) designated proneural, proliferative, and mesenchymal. (Phillips *et al.*, 2006)

This molecular heterogeneity is of potential importance in clinical trials. An experimental chemotherapy regimen may be declared ineffective during a trial involving GBMs of all molecular profiles despite responses in a small subset. For example, the EGFR inhibitors erlotinib and gefinitib as monotherapy are ineffective in recurrent GBMs when all tumors are analyzed as one cohort, associated with 6mPFS rates and response rates that are no better than historic controls (Lieberman *et al.*, 2003; Raizer *et al.*, 2004; Rich *et al.*, 2004; Vogelbaum *et al.*, 2004; Yung *et al.*, 2004) However, analysis of archival tissue in one study demonstrated that a specific molecular profile, present in only 10-15% of patients, strongly correlated with activity in the small subset of patients exhibiting radiographic responses.(Mellinghoff *et al*., 2005) Although others have questioned this conclusion, (Brandes *et al.*, 2008), the concept that treatment may be active in tumors with a specific molecular profile is important. Molecular profiling may allow identification of a subset of tumors sensitive to a particular therapy that would otherwise be declared inactive. Such identification may then allow individualization of therapy. In this trial, we also propose to molecularly profile archival tissue with the goal of determining the subset of patients more likely to respond.

Initial results from The Cancer Genome Atlas (TCGA) study of GBM (in which 200 tumors were analyzed for chromosomal copy number, gene expression, and large scale gene re-sequencing) confirmed the previously documented high incidence of *PDGFRA* amplification/mutation and *EGFR* amplification/mutation.(The Cancer Genome Atlas (TCGA) Research Network, submitted). This analysis also identified an unexpectedly high incidence of *NF1* mutation and gene deletion, found in >20% of primary GBMs. NF1 silencing, *PDGFRA* amplification/mutation, and *EGFR* amplification/mutation are mutually exclusive genetic lesions. Furthermore, unsupervised clustering of transcriptome data reveals 3 groups of GBM defined by their gene expression: these groups are strongly enriched for *EGFR*, *PDGFRA*, and *NF1* aberrations, respectively. If *NF1*, *PDGFRA*, and *EGFR* mutation are associated with distinct molecular subtypes of GBM, it is possible these subtypes may respond differently to various therapies.

Of note, the "PDGF" transcriptomal group in the TCGA data set is characterized by expression of markers previously associated with the "Proneural" class of GBMs, such as Delta Like 3 (DLL3), SOX4, NCAM1, and Doublecortin (Phillips *et al.*, 2006). These genes are expressed in tumors with *PDGFRA* amplification, but also in tumors without *PDGFR* mutations which co-cluster. (TCGA data). In addition, unpublished data from Memorial Sloan Kettering Cancer Center also demonstrates that PDGF ligand is overexpressed at the protein level in tumors that harbor no *PDGFR* amplification, accounting for approximately 20% of GBMs. (Cameron Brennan and Eric Holland, personal communication).

The PDGF group is of particular interest to this trial because the mouse model (Figure 1, reviewed in Lassman and Holland 2006) used to generate the GBMs for the pre-clinical data demonstrating synergistic activity of temsirolimus + perifosine are driven by forced PDGF overexpression in glia. Therefore, it may be important to identify human tumors exhibiting a PDGF-like profile, and such tumors may demonstrate enhanced sensitivity to temsirolimus + perifosine. Therefore, we plan to molecular profile archival tissue from all patients enrolled onto this trial as an exploratory endpoint.

To accomplish this, we will perform Fluorescent In-Situ Hybridization (FISH) for determination of *EGFR* amplification and *PDGFRA* amplification and *NF1* loss, immunohistochemistry for PDGF ligand overexpression at the protein level (which occurs in the absence of *PDGF* gene amplification), and immunohistochemistry for NF1 under-expression. We will also perform array based comparative genomic hybridization for other markers of the PDGF-like subclass including DLL3, SOX4, NCAM1, and Double Cortin. Classification of tumors will be made based on results of these analyses. The EGFR and NF-1 groups will be defined by amplification and loss/under-expression of *EGFR* and *NF1*/NF1, respectively. The PDGF group is more complex. Tumors will be classified as PDGF-like based on either: 1) *PDGFRA* amplification; or 2) PDGF ligand overexpression without *EGFR* amplification (Cameron Brennan and Eric Holland, personal communication). In addition, in patients with available frozen tissue, tumors with neither *PDGFRA* amplification nor PDGF ligand overexpression will be tested for "Proneural" class expression markers (Phillips *et al.*, 2006) that transcriptomally co-cluster with *PDGFRA* amplification (*e.g.*, DLL3, SOX4, NCAM1, and Doublecortin).

Finally, perifosine and temsirolimus both act on the PI3K/AKT/mTOR/S6K cascade, although they may also affect RAS/MEK/ERK signaling. In addition, temsirolimus causes proliferation arrest. In modeled mouse gliomas, combination therapy also leads to apoptotic death. Therefore, we will assess archival tissue for expression and activation status of these signaling molecules using commercially available antibodies by immunohistochemistry for PTEN, AKT, pAKT, mTOR, p-mTOR, S6K, pS6K, ERK, pERK, Ki67 and assess apoptosis by TUNEL. If flash frozen tissue is available for analysis, we will also perform Western Blot for PTEN, AKT, pAKT, S6K, pS6K, ERK, pERK, and proliferating cell nuclear antigen (PCNA). We will also extract DNA to sequence *PTEN*, *PI3K*, and other genes involved in AKT signal transduction to identify mutations that may confer drug sensitivity.

In addition, a subset of patients on the phase II study will receive dual drug therapy followed by surgical resection of tumor. This provides an opportunity to assess the molecular effects of therapy in humans in vivo. We will perform the above analysis for PI3K/AKT/mTOR/S6K and RAS/MEK/ERK signaling, proliferation, and apoptosis, using intrapatient comparison from pre-treatment archival tissue from an earlier resection as we have done in other studies.(Lassman *et al.*, 2005)

3 PATIENT SELECTION

3.1 General Eligibility Criteria

3.1.1 Patients must have unstained slides or tissue blocks available from at least one prior surgery. Frozen tissue is also requested if available.

3.1.2 Patients must have received prior radiotherapy and temozolomide. There is otherwise no limit on the number of prior recurrences/therapies.

3.1.3 At least 6 weeks (42 days) must have elapsed since completion of radiation therapy to initiation of study treatment.

3.1.4 At least 4 weeks (28 days) must have elapsed since most recent temozolomide and initiation of study treatment.

3.1.5 Patients must have recovered from the toxic effects of other prior direct inhibitors of VEGF/VEGFR: 4 weeks from prior therapy with agents such as bevacizumab (Avastin), aflibercept (VEGF-Trap), cedirinib (AZD2171), or XL-184 (BMS 907351); any questions regarding the definition of a direct anti-VEGF/VEGFR therapy must be discussed with the PI or co-PI. Patients must have recovered from the toxic effects of other prior therapy including: 4 weeks (28 days) from any investigational agent, two weeks (14 days) from vincristine, 6 weeks (42 days) from nitrosoureas, 3 weeks (21 days) from procarbazine administration, and 1 week (7 days) for non-cytotoxic agents, e.g., interferon, tamoxifen, thalidomide, cis-retinoic acid, etc. (radiosensitizer does not count), and 4 weeks (28 days) from any other prior cytotoxic therapy . Any questions related to the definition of non-cytotoxic agents should be directed to the co-PI.

3.1.6 Patients must have shown unequivocal evidence for tumor progression by MRI/CT on the baseline MRI/CT in comparison to a prior scan OR have recently undergone resection for recurrent/progressive disease per 3.1.17. The baseline brain MRI/CT must be performed 14 days or fewer prior to treatment. The same type of scan, i.e., MRI (or CT for patients who cannot undergo MRI) must be used throughout the period of protocol treatment for tumor measurement. Criteria in section 11 for progression on this study are not mandatory if the disease progression is obvious in the opinion of the investigator. Any questions should be addressed to the PI.

3.1.7 Patients must be on a stable or decreasing dose of corticosteroids for a minimum of 5 days before the baseline MRI/CT (and PET scans for patients on the phase II study) ***except*** patients undergoing surgery on the surgical substudy of phase II (section 5.1.1.7). If the corticosteroid dose is increased between the date of imaging and registration a new baseline MR/CT is required.

3.1.8 Age $\geq$18 years. Because no dosing or adverse event data are currently available on the use of temsirolimus in combination with perifosine in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric phase 1 combination trials.

3.1.9 Karnofsky Performance Status $\geq$60%

3.1.10 Life expectancy of greater than 8 weeks.

3.1.11 Patients must have normal organ and marrow function (WBC $\geq$ 2,000/µl, ANC $\geq$ 1,500/mm^3, platelet count of $\geq$ 100,000/mm^3, and hemoglobin $\geq$ 10 gm/dl), adequate liver

function (SGOT, SGPT and bilirubin < 2 times ULN), adequate renal function (creatinine < 1.5 mg/dL), calcium levels at or above the lower limit of normal, and phosphorus levels at or above the lower limit of normal before starting therapy. These tests must be performed within 14 days prior to registration. Eligibility level for hemoglobin may be reached by transfusion.

3.1.11.1 Platelet count of at least 100,000/mm3 on at least 2 consecutive blood draws, at least 1 week apart, with results stable/trending upward. Any question regarding the definition of stable/trending upward must be discussed with the PI.

3.1.12 Patients must have cholesterol level ≤ 350 mg/dl and triglycerides level ≤ 400 mg/dl because temsirolimus can induce hyperlipidemia.

3.1.13 Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation because the effects of temsirolimus and perifosine on the developing human fetus are unknown. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.14 Women of childbearing potential must have a negative B-HCG pregnancy test documented within 7 days prior to treatment.

3.1.15 Women must agree not to breast feed.

3.1.16 Patients must have the ability to understand and the willingness to sign a written informed consent document.

3.1.17 As a primary efficacy endpoint is 6mPFS rate, measurable disease is not required for eligibility in patients who recently underwent resection as long as progressive disease led to the surgery, and the histology of the most recent surgery documented recurrent/progressive/persistent malignant glioma.

3.1.17.1 If cytoreductive surgery is planned for tumor recurrence at the time of enrollment, such patients may be eligible for the surgical substudy (Phase II only), taking temsirolimus + perifosine pre-operatively and then re-initiating such therapy after recovering from the effects of surgery (section 5).

Phase I specific inclusion criteria:

3.1.18 Patients must have a EITHER

Histologically confirmed intracranial malignant glioma of the following types: Glioblastoma, Anaplastic astrocytoma (AA), Anaplastic oligodendroglioma (AO), Anaplastic oligo-astrocytoma (AOA) also called anaplastic mixed gliomas, Malignant glioma NOS (not otherwise specified). Patients will be eligible if the original histology was low-grade glioma and a subsequent histological diagnosis of a high grade (malignant) glioma is made.

OR

Histologically confirmed low grade (WHO grade II) gliomas (such as low grade astrocytoma, low grade oligodendroglioma, low grade oligo-astrocytoma (mixed gliomas),

or low grade glioma NOS) IF there is radiographic evidence by MRI or CT of malignant transformation but histologic confirmation of high grade (malignant) transformation would not be otherwise undertaken for routine clinical care. Inclusion of patients in this group will allow increased accrual rapidity by enrolling patients who are otherwise ineligible for almost all malignant glioma trials yet whom are treated presumptively for malignant glioma. The primary aim of the phase I study is not determination of efficacy. Therefore, inclusion of such patients will not affect efficacy analyses.

Phase II specific inclusion criteria:

3.1.19 Patients must have a histologically confirmed intracranial malignant glioma of the following types: Glioblastoma, Anaplastic astrocytoma (AA), Anaplastic oligodendroglioma (AO), Anaplastic oligo-astrocytoma (AOA) also called anaplastic mixed gliomas, Malignant glioma NOS (not otherwise specified). Patients will be eligible if the original histology was low-grade glioma and a subsequent histological diagnosis of a high grade (malignant) glioma is made.

Phase II patients enrolling on the surgical substudy to evaluate tissue correlates.

3.1.20 Patients eligible for the surgical subset have been identified as candidates for cytoreductive surgery by the treating physician and/or based on discussion in a multidisciplinary tumor board, with the input of other surgeons as well as that of the neuro-oncologists involved in the trial. For the patients in the preoperative component, a scan showing progression is required but stable corticosteroids are not required. Following surgery, a scan should be done less than 96 hours after surgery. If this is not performed, then a new baseline scan should be performed at least 4 weeks after surgery to avoid mis-interpretation of post-operative changes as enhancing disease. This scan will serve as the new baseline before restarting treatment post-operative, and it must be performed on a stable or decreasing dose of corticosteroids. (As above, regarding the baseline MRI or CT scan prior to registration, patients in the Phase II component who are NOT participating in the pre-operative component of the study should be on a steroid dose that has been stable for at least 5 days prior to the scan. If the corticosteroid dose is increased between the date of imaging and registration a new baseline MR/CT is required.)

3.1.21 Post-operatively, treatment with temsirolimus and perifosine must re-start no later than the 14th day after the scan. If the 96-hour scan is more than 14 days old before treatment is initiated, the scan needs to be repeated on a stable or decreasing steroid dose. Treatment must start no later than 56 days after surgery.

Phase II patients previously treated with becacizumab or other direct inhibitors of VEGF/VEGFR including Aflibercept (VEGF-Trap) and cedirinib and XL-184 (BMS 907351).

3.1.22 There is no limit on such therapy for patients accrued to phase I. For phase II, historical controls for this group of patients is poorly defined. Therefore, we will accrue up to 15 patients who received prior treatment with direct VEGF/VEGFR inhibitors in order to gain preliminary data for use as a comparison group in a follow up study. See section 13.2. All other inclusion/exclusion criteria also apply to this cohort. Any question about the definition of a direct VEGF/VEGFR inhibitor should be addressed to the PI or co-PI prior to registration.

3.2 Exclusion Criteria

3.2.1 Patients may not be receiving any other investigational agents.

3.2.2 Patients must not have a history of allergic reactions attributed to compounds of similar chemical

or biologic composition to temsirolimus or perifosine.

3.2.3 Patients must not be taking EIAED. If previously on an EIAED, the patient must be off of it for at least two weeks prior to treatment on study.

3.2.4 Patients must not have uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.5 Pregnant women are excluded from this study because temsirolimus and perifosine are agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with temsirolimus or perifosine, breastfeeding should be discontinued if the mother is treated with temsirolimus and perifosine.

3.2.6 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with temsirolimus and perifosine. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

Phase II specific exclusion criteria
3.2.7 Patients may not have received prior treatment with mTOR inhibitors such as temsirolimus, rapamycin (sirolimus), or RAD001 (everolimus). Any question regarding the definition of mTOR inhibiting therapy must be discussed with the PI. Such prior therapy is allowed for the phase I component.

3.2.8 Patients may not have previously received perifosine or other AKT targeting agents. Any question regarding the definition of AKT targeting therapy must be discussed with the PI. Such prior therapy is allowed for the phase I component.

3.2.9 Patients must not have received prior treatment with convection enhanced delivery, other catheter based intra-tumoral treatment, or carmustine (BCNU)/Gliadel wafers because of potential difficulty interpreting brain scans in such patients. Such prior therapy is allowed for the phase I component .

3.2.10 Patients with prior therapy that included stereotactic radiosurgery (including gamma-knife or cyber-knife) during therapy for newly diagnosed or recurrent disease, or re-irradiation of any type, must have confirmation of true progressive disease rather than radiation necrosis ***based upon surgical documentation of recurrent/progressive disease.*** Imaging with MRSpectroscopy, PET, or other techniques is not adequate to exclude radiation necrosis for this study. (Clarke *et al*., 2008) Such prior therapy is allowed for the phase I component and does not require surgical documentation of disease.

3.2.11 Patients with a history of any other cancer (except non-melanoma skin cancer or carcinoma in-situ of the cervix), unless in complete remission and off of all therapy for that disease for a minimum of 3 years are ineligible for the phase II study but are eligible for the phase I component.

3.3 Inclusion of Women and Minorities

This study was designed to include women and minorities, but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender. No exclusion to this study will be based on race.

4 REGISTRATION PROCEDURES

4.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Patient Selection.

Obtain informed consent by following procedures defined in section entitled Informed Consent Procedures.

During the registration process, registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm (ET) at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

4.2 Informed Consent Procedures

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information.

In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

5 TREATMENT PLAN

5.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for temsirolimus and perifosine are described in Section 7. Appropriate dose modifications for

temsirolimus and perifosine are described in Section 6.

5.1.1 CTEP IND Agents: temsirolimus and perifosine

5.1.1.1 Pre-medication for temsirolimus

Idiosyncratic hypersensitivity reactions have been seen, so patients receiving IV Temsirolimus should be premedicated with diphenhydramine 25 – 50 mg IV (or a similar antihistamine) approximately 30 minutes before the start of the Temsirolimus infusion. If the subject begins to develop a hypersensitivity reaction despite pretreatment with diphenhydramine, the infusion should be stopped for at least 30 – 60 minutes, depending upon the severity of the reaction. The infusion may be resumed by administering a histamine H_2-receptor antagonist approximately 30 minutes before restarting the Temsirolimus infusion. Famotidine 20 mg IV or ranitidine 50 mg IV are recommended rather than cimetidine because of the lack of likely metabolic/pharmacologic interactions with the former drugs. The rate of the Temsirolimus infusion may also be slowed from 30 minutes to over an hour. All subjects should be monitored while receiving the Temsirolimus infusion and emergency medical equipment and health care personnel must be readily available to respond to hypersensitivity reactions or other medical emergencies.

Anti-emetic prophylaxis. For the 1st dose of temsirolimus, the prophylaxis for the loading dose of perifosine (below) will also be used as prophylaxis for temsirolimus. Starting with the 2nd dose of temsirolimus, nausea/vomiting induced by study agents should be treated initially with a serotonin type-3 (5-HT(3)) antagonist. If this is inadequate, phenothiazine (prochlorperazine 10 mg q8h PO prn or promethazine 12.5-25 mg IV q6h prn should be added until acute nausea is controlled. Should this prove inadequate acutely, a corticosteroid may be added (*e.g.*, dexamethasone 4 mg q6h prn) but note that corticosteroid use can confound response interpretation. Alternative or additional antiemetics may be prescribed at the discretion of the investigator as needed.

5.1.1.2 Pre-medication for perifosine

Anti-emetic prophylaxis for loading dose -- The loading dose will be administered in the adult day hospital (or equivalent outpatient chemotherapy unit) in divided doses with anti-emetic prophylaxis, IV if needed. A divided dose that occurs in the evening can be taken by the patient at home at the discretion of the investigator.

Administer together 0.5-1 hour before intake of the first loading dose:

- granisetron (1 mg PO, or 10 :g/kg IV) or equivalent $5HT_3$ antagonist
- metoclopramide (20 mg PO or 1-2 mg/kg IV)
- diphenhydramine (25 mg, IV or PO)
- dexamethasone (8 mg, IV or PO) – Note any brain MRI scan within 5 days of the loading dose is therefore not evaluable for best radiographic response because of the potential confounding effects of dexamethasone on contrast enhancement.

Three hours after intake of the first divided dose, and one hour before the next divided dose is given:

- metoclopramide (20-40 mg, IV or PO)
- diphenhydramine (25 mg, IV or PO)

This may be repeated every 3-4 hours if needed during the loading dose period. Alternative or additional antiemetics may be prescribed at the discretion of the investigator as needed.

Diphenhydramine may be held for sedation.

Anti-emetic prophylaxis for maintenance dose -- The daily perifosine maintenance dose will be continued at home without routine anti-emetic prophylaxis, but anti-emetics can be used on an as needed basis. Maintenance doses of the study medication will be administered together with food at bedtime to minimize nausea not controlled by anti-

emetics. Nausea/vomiting requiring anti-emetic treatment during the maintenance doses may be treated with the following regimen: granisetron 1 mg PO or 10 :g/kg IV or equivalent $5HT_3$ antagonist, followed, if ineffective, by metoclopramide (20 mg PO or 1-2 mg/kg IV plus diphenhydramine (25 mg IV or PO). After acute nausea has resolved, consideration should be given to initiation of prophylactic antiemetic therapy for subsequent doses. If nausea recurs despite reasonable medical intervention (as outlined above), dose reduction will be needed as described in Section 6. Alternative or additional antiemetics may be prescribed at the discretion of the investigator as needed.

Hyperuricemia/gout - Prior to beginning perifosine, all patients should be questioned about a history of hyperuricemia and/or gout. Patients with a known history of hyperuricemia and/or gout should receive prophylactic treatment with allopurinol 300 mg orally daily. If severe gout does occur or uric acid levels increase, the dose of allopurinol should be increased and/or other medications used at physician discretion.

5.1.1.3 Route of administration for temsirolimus

Temsirolimus is administered intravenously. Temsirolimus is incompatible with polyvinyl chloride (PVC) equipment or devices that are plasticized with di- (2-ethylhexyl) pthalate (DEHP). Administer Temsirolimus over approximately 30 minutes as an IV infusion via an automatic dispensing pump (e.g. IMED, Harvard, Travenol) using non-polyvinyl chloride (PVC) tubing with the appropriate filter. List of filters for tubing with preattached filters that are permissible are found the in the pharmaceutical information section. It is recommended but not mandatory that CCI-779 (temsirolimus) be administered through either a "long line" venous access devise such as a PICC line, a mediport, or a central venous catheter.

5.1.1.4 Route of administration for perifosine

Perifosine will be taken orally. All doses are administered with food. Pills should remain intact and should not be crushed *except* to administer via g-tube.

5.1.1.5 Phase I treatment schedule

Temsirolimus: The starting dose for temsirolimus in the phase I component of the study is 15 mg IV weekly (table 1).

Perifosine: The starting dose for perifosine in the phase I component of the study is a loading dose of 600 mg orally on day 1 and then a maintenance dose of 100 mg orally daily thereafter on days 2-28. The loading dose is only administered during cycle 1 unless treatment is interrupted for >7 days for which patients will receive a repeat loading dose upon restarting perifosine (table 1).

The loading dose should be divided into doses of 150 mg each administered every 4 hours until the complete load has been administered. The loading dose is given only on cycle 1 day 1. The first 3 divided doses of the load should be administered under supervision in the adult day hospital (or equivalent outpatient chemotherapy unti) where patients will have access to intravenous antiemetics if needed. Patients may receive the 4^{th}-6^{th} doses of the load at home if they have tolerated the first 3 divided doses at the discretion of the investigator. On cycle 1 days 2-28, patients take a maintenance dose every night with food and not on an empty stomach.

Cycles $\geq$2 consist of daily maintenance doses taken every night on days 1-28 without a loading dose. However, if treatment is interrupted for >7 days for any reason, patients will undergo a repeat loading dose of perifosine upon re-starting therapy.

See section 5.2 for discussion of dose escalations and maximum tolerated dose.

Table 1: The following temsirolimus and perifosine dose levels will be used to determine dosing for specific cohorts in the Phase I component.

Dose level	Temsirolimus (mg/week)	Perifosine load/maintenance (mg/day)
-6	5	150/50
-5	10	150/50
-4	15	150/50
-3	15	150/100
-2	15	300/100
-1	15	450/100
1 (starting)	15	600/100
2	25	600/100
3	50	600/100
4	75	600/100
5	115	600/100
6	170	600/100
7	170	900/100

Treatment with both agents will occur simultaneously in the following manner: after anti-emetic prophylaxis, patients will receive the first divided dose of the perifosine loading dose. Patients will be observed for 30 minutes to ensure there has been adequate anti-emetic prophylaxis, and then patients will receive temsirolimus at their assigned dose administered over 30 minutes IV. The remaining divided doses of the perifosine loading dose will then be administered. Patients will then return weekly for infusion of temsirolimus over 30 minutes IV. Dosing will be continuous although for the purposes of evaluation, a cycle will be defined as 4 weeks (28 days). It is expected that temsirolimus administration will occur on the same day of the week throughout the treatment period. However, it may be given up to 3 days early or late due to holiday or other scheduling reasons.

Prior to beginning cycle 3, patients undergo clinical and radiographic tumor restaging. As long as the tumor is stable or smaller in size and the patient is clinically stable or improved, they will go on 2 more 4 week cycles of therapy with temsirolimus and perifosine. Treatment will continue indefinitely as long as there are no unacceptable toxicities (DLTs) and no clinical or radiographic progression.

5.1.1.6 Phase II treatment schedule for patients ***NOT*** participating in the surgical substudy

Temsirolimus: The starting dose for temsirolimus will be determined from the phase I component. There is no intra-patient dose escalation.

Perifosine: The starting dose (load and maintenance) for perifosine will be determined from the phase I component. The loading dose is only administered during cycle 1 unless treatment is interrupted for >7 days for which patients will receive a repeat loading dose upon restarting perifosine (table). There is no intra-patient dose escalation.

Treatment with both agents will occur simultaneously in the following manner: after anti-emetic prophylaxis, patients will receive the first divided dose of the perifosine loading dose. Patients will be observed for 30 minutes to ensure there has been adequate anti-emetic prophylaxis, and then patients will receive temsirolimus administered over 30 minutes IV. The remaining divided doses of the perifosine loading dose will then be administered. Patients will then return weekly for infusion of temsirolimus over 30 minutes IV. Dosing will

be continuous although for the purposes of evaluation, a cycle will be defined as 4 weeks (28 days). It is expected that temsirolimus administration will occur on the same day of the week throughout the treatment period. However, it may be given up to 3 days early or late due to holiday or other scheduling reasons.

Prior to beginning cycle 3, patients undergo clinical and radiographic tumor restaging. As long as the tumor is stable or smaller in size and the patient is clinically stable or improved, they will go on 2 more 4 week cycles of therapy with temsirolimus and perifosine. Treatment will continue indefinitely as long as there are no unacceptable toxicities (DLTs) and no clinical or radiographic progression.

5.1.1.7 Phase II treatment schedule for patients who ***ARE*** participating in the surgical substudy

Patients who choose to participate in this component of the study must have a contrast enhanced brain MRI performed within 14 days of taking pre-operative temsirolimus and perifosine. Patients unable to undergo MRI will have CT scans. A brain PET scan must also be performed within the 14 days before taking pre-operative temsirolimus and perifosine, and again (research PET) after completing the full loading dose of perifosine and 1 dose of temsirolimus but before surgery to correlate metabolic effects with tissue analysis. A repeat PET is again requested but is not mandatory for patients with residual measurable disease post-operatively before restarting study drugs (research PET).

Before surgery

The loading dose of perifosine and the dose of temsirolimus will be administered on the same day. This may be up to 1-4 days pre-operatively

Temsirolimus: Patients will receive one treatment of temsirolimus intravenously prior to surgery analogous to phase II patients NOT on the surgical substudy (section 5.1.1.6). The dose will be determined by the phase I component of the study. This dose of temsirolimus may be administered up to the 4th pre-operative day. This dosing allows for some flexibility in scheduling surgery.

Perifosine: Patients will receive a loading dose of perifosine prior to surgery analogous to phase II patients NOT on the surgical substudy (section 5.1.1.6), Patients will receive a loading dose of perifosine the same day they receive pre-operative temsirolimus. They will receive a maintenance dose nightly starting the next day until the night before surgery. If the loading dose is administered on the pre-operative day, then they will not receive a maintenance dose pre-operatively. The dose will be determined by the phase I component of the study.

Both agents should be administered as in section 5.1.1.6. **Both agents should be started up to 4 days before surgery. If clinical or other scheduling reasons alter the plan or timing for surgery, the PI must be notified and best physician practice applied to care for the patient.**

Day of surgery

Tissue freezer vials should be labeled with the patient's protocol study Identifier number(s). Additionally, a thermos with liquid nitrogen (preferably), or if not possible, an ice bucket with dry ice should be prepared. All attempts will be made to obtain specimens immediately adjacent to the areas of resection taken for "permanent sections" in order to optimize the likelihood that the tumor seen on permanent sections is representative of that taken for analysis. Once the pathologist has made a diagnosis of "tumor" on frozen section at the time of surgical resection, the tumor tissue should be divided into 50 – 100mg pieces.

At the time of surgical resection, the following specimens will be obtained:

- Fresh tumor tissue frozen in liquid nitrogen for evaluation of activation status of PI3K/AKT/mTOR/S6K, RAS/MEK/ERK, proliferation, and gene profiling.

- Paraffin block or slides for evaluation of activation status of PI3K/AKT/mTOR/S6K, RAS/MEK/ERK, proliferation, apoptosis, and gene profiling.

It is important that the exact times of collection of these specimens be recorded.

Pre and post-operative evaluations will consist of standard clinical practice. Patients should have weekly CBC and differential in the postoperative period. Once patients have recovered from the effects of surgery, they will start the phase II component of the study. Patients are not required to have residual disease to continue onto the phase II component.

Following surgery

A post-operative MRI/CT scan should be done no later than 96 hours from surgery on a steroid dose that is stable or decreasing. Treatment with temsirolimus and perifosine post-operatively should start no later than 14 days after the scan. If the 96-hour scan is more than 14 days before treatment is initiated, the scan needs to be repeated on a stable or decreasing steroid dose. The post-operative scan should be assessed for the presence of residual measurable disease. A repeat PET is again requested but is not mandatory for patients with residual measurable disease post-operatively before restarting study drugs.

Treatment may be instituted starting 7 days postoperatively if patients have recovered from effects of surgery and demonstrated wound healing. Treatment with temsirolimus and perifosine post-operatively should start no later than 56 days after surgery.

Day 1 of treatment following surgery will represent the first day of treatment for determination of the primary endpoint of 6 month progression free survival. The dose of temsirolimus and perifosine will be determined by the phase I component of the study. Patients will undergo a loading dose of perifosine when re-initiating treatment post-operatively. Patients will receive treatment in 4 week cycles in the same manner as patients not on the surgical substudy. Drug treatment will be started analogously to patients in the phase II component NOT on the surgical substudy.

In the unlikely event that the analysis of the tissue specimen demonstrates necrosis or lack of active disease, the PI should be notified and the patient disenrolled from the trial as they are not eligible without recurrent/progressive disease. Such patients may be replaced.

5.1.2 Other Agents: N/A

5.1.3 Other Procedures

5.1.3.1 Pre-treatment evaluations

A complete history, physical, and neurological examination (to include documentation of the patients Karnofsky Performance Status per Appendix A, as well as neuro-imaging confirming tumor progression shall be performed on all patients. The baseline scan should be performed within 14 days of treatment initiation. Brain PET (to evaluate tumor metabolism) should be performed as well for patients on phase II.

Prestudy laboratory tests shall include CBC, differential, platelets, PT, PTT, INR, carbon dioxide, BUN, creatinine, glucose, potassium, sodium, calcium, phosphorus, chloride, total protein, albumin, alkaline phosphatase, total bilirubin, SGOT (AST), SGPT (ALT), uric acid, cholesterol, triglycerides. Antiepileptic drug levels may be drawn if they are used clinically to monitor patient's AED dose. These pr-study laboratory tests must be obtained within 14 days of treatment initiation. Women of childbearing potential must obtain a serum pregnancy test within 7 days prior to registration.

Unstained paraffin tissue from surgical samples: Every reasonable effort will be made to obtain a

representative paraffin tissue block (at least 5 mm x 5 mm) or at least 15 unstained paraffin slides (preferably 30) in all patients from original surgery and/or definitive surgery and/or the surgery closest to initiation of this clinical trial in patients on phase I. Prior tissue is mandatory for patients on phase II. Paraffin slides will be used for DNA isolation and immunohistochemistry to evaluate molecular pathways described in section 2.5 and section 9. Array comparative genomic hybridization (array CGH) will also be performed on these specimens. We will attempt to correlate tumor genotype with response to treatment.

5.1.3.2 Evaluation during study: Phase I requirements

Following the 1st treatment, CBC, differential, and platelets, and serum chemistries (BUN, creatinine, glucose, calcium, alkaline phosphatase, phosphorus, total bilirubin, SGOT (AST), SGPT (ALT), fasting cholesterol and triglycerides will be performed every week for the first 4 weeks and then every 2 weeks thereafter. All labs are permitted to be obtained up to 3 days prior to the weekly Temsirolimus IV infusion. AED levels (if clinically appropriate) will be measured every 2 weeks for the first 4 weeks and then every 4 weeks. β-HCG will be measured prior to each 4-week cycle. For patients on warfarin, a PT/INR will be checked every 1-2 weeks, or sooner if clinically indicated. Uric acid will be re-checked as clinically indicated.

A head CT (wthout contrast) or MRI with gradient echo must also be performed during week 2 of treatment to evaluate for subclinical hemorrhage observed in mice treated with the combination of temsirolimus and perifosine**.**

A brain MRI/CT will be done prior to every other cycle (up to 2 weeks prior). An MRI/CT scan done at 6 months from initiation of therapy will be performed while the patient is still in the study to ensure assessment of the primary endpoint.

A complete physical and neurologic exam (to include documentation of the patients Karnofsky Performance Status) will be performed up to 3 days prior to every cycle.

Phase I patients will be evaluated for adverse events at least weekly during their first cycle of therapy. These adverse events will be reported to the PI.

Adverse events for subsequent cycles will be reported prior to the beginning of each cycle. In addition all serious adverse events will be reported to the NCI and the PI.

All relevant information regarding drug doses, concomitant medications, and doses, measurable lesions with measurements, tumor response, laboratory examinations, and treatment-related toxicities shall be documented in the patient's medical record and flow sheets.

All patients will be followed for overall survival, when possible.

- Patients who discontinue treatment due to progression will be followed for survival every 3 months.
- Patients who come off therapy for reasons other than progression should be followed until progression or institution of new anti-tumor therapy. They should then be followed for survival.

5.1.3.3 Evaluation during study: Phase II requirements

Following the 1st treatment, CBC, differential, and platelets, and serum chemistries (BUN, creatinine, glucose, calcium, alkaline phosphatase, phosphorus, total bilirubin, SGOT (AST), SGPT (ALT), fasting cholesterol and triglycerides will be performed every week for the first 4 weeks and then every

2 weeks thereafter. All labs are permitted to be obtained up to 3 days prior to the weekly Temsirolimus IV infusion. Patients with normal bloodwork may have these laboratory tests performed every 4 weeks after the first 16 weeks. AED levels (if clinically appropriate) will be measured every 2 weeks for the first 4 weeks and then every 4 weeks. β-HCG will be measured prior to each 4-week cycle. For patients on warfarin, a PT/INR will be checked every 1-2 weeks, or sooner if clinically indicated. Uric acid will be re-checked as clinically indicated.

A head CT (without contrast) or MRI with gradient echo must also be performed during week 2 of treatment to evaluate for subclinical hemorrhage observed in mice treated with the combination of temsirolimus and perifosine. Note that the CT of the head performed with the PET scan (i.e., in patients undergoing a PET/CT) is NOT sufficient.

A brain MRI/CT will be done prior to every other cycle (up to 2 weeks prior). A brain MRI/CT done at 6 months from initiation of therapy will be performed while the patient is still in the study to ensure assessment of the primary endpoint.
A brain PET scan for research purposes will be perfomed after 1-2 weeks of treatment. A follow up PET will then be performed after every 4th cycle (approximately 16 weeks). The PET scans must be performed at MSKCC in order to allow easier comparison and interpretation.

A complete neurologic exam (to include documentation of the patients Karnofsky Performance Status) will be performed up to 3 days prior to every other cycle.

Phase II patients will be evaluated for adverse events at the end of each cycle. In addition all serious adverse events will be reported to the NCI and the PI.

All relevant information regarding drug doses, concomitant medications, and doses, measurable lesions with measurements, tumor response, laboratory examinations, and treatment-related toxicities shall be documented in the patient's medical record and flow sheets.

All patients will be followed for overall survival, when possible.

- Patients who discontinue treatment due to progression will be followed for survival every 3 months.
- Patients who come off therapy for reasons other than progression should be followed until progression or institution of new anti-tumor therapy. They should then be followed for survival.

Patients enrolled in surgical substudy must have a brain MRI/CT scan obtained within 96 hours post-operatively. Treatment with temsirolimus and perifosine post-operatively should start no less than 7 days and no later than 56 days after surgery. If post-operative scan (performed within 96-hour after surgery) is more than 14 days old when treatment is re-initiated post-operatively, the scan needs to be repeated.

5.2 Definition of Dose Limiting Toxicity

5.2.1 Definition of dose limiting toxicities (DLT):

Toxicities will be graded according to the CTCAE version 4.0. If multiple toxicities are seen, the presence of DLT should be based on the most severe toxicity experienced. DLT will be defined as any of the following events occurring during treatment with temsirolimus and perifosine and attributable (possible, probable, or definite) to either study drug or the combined therapy:

- Any grade 3 thrombocytopenia, grade 4 anemia, and/or grade 4 neutropenia.

- Any unacceptable grade 2 non-hematologic toxicity, despite maximal medical treatment, that results in dose interruption of greater than 7 days within the first 28 days,.*
- Any non-hematologic grade 3 or 4 toxicity, excluding alopecia, despite maximal medical treatment.*
- Failure to recover from toxicities to be eligible for re-treatment with temsirolimus and perifosine within 2 weeks of the last dose of temsirolimus and perifosine.

*Non-hematologic toxicities diarrhea and mucositis will only be considered a DLT if they remain grade 3 or unacceptable grade 2, despite maximal medical therapy.

*Hypercholesterolemia and hypertriglyceridemia and other Metabolism and Nutrition Disorders (such as hypophosphatemia and hypocalcemia) will only be considered a DLT if they remain grade 3 or unacceptable grade 2, despite maximal medical therapy
.

5.2.2 Maximum Tolerated Dose (MTD)

The MTD will be based on the assessment of DLT during the first 28 days of treatment only (cycle 1). Escalations are planned in groups of three patients, with an additional three patients to be added at the first indication of Dose Limiting Toxicity (DLT). (Please see next two paragraphs). **There is NO intra-patient dose escalation.** The MTD of temsirolimus in combination with perifosine will be defined as the dose at which fewer then one-third of patients experience a DLT. Therefore, the MTD is the dose level at which 0/3 or 1/6 (or up to 2/9, etc.) patients experience DLT with the next higher dose having at least 2/3 or 2/6 patients encountering DLT. Up to three patients may be enrolled simultaneously at each dose level. These patients should be observed for DLT for at least 4 weeks from the first day of treatment before new patients are enrolled at the next highest dose level (Table 2). Patients completing 28 days of therapy or patients removed for toxicity earlier than 28 days are evaluable. Patients removed from study within 28 days for reasons other than toxicity may be replaced.

Table 2: Dose levels used to determine dosing for specific cohorts in the Phase I component

Dose level	Temsirolimus (mg/week)	Perifosine load/maintenance (mg/day)
-6	5	150/50
-5	10	150/50
-4	15	150/50
-3	15	150/100
-2	15	300/100
-1	15	450/100
1 (starting)	15	600/100
2	25	600/100
3	50	600/100
4	75	600/100
5	115	600/100
6	170	600/100
7	170	900/100

The following dose escalation rules will be used:

- Three patients are studied at the first dose level
- If none of these three patients experience DLT, then the dose is escalated to the next higher level in the three subsequent patients.
- If one of the three patients experience DLT at the current dose, then three more patients are accrued at the same dose. If none of these three additional patients suffer DLT, then the dose is escalated in subsequent

patients. If one or more of these three additional patients experiences DLT, the MTD has been exceeded and three more patients are treated at the next lower dose (if only three patients were previously treated at that prior dose).

- If two or more patients encounter DLT, then the MTD has been exceeded and three more patients are treated at the next lower dose level (if only three patients were previously treated at that prior dose).
- At least 6 patients will be treated at the MTD.
- All escalations will occur in separate cohorts. There is no intra-patient dose escalation of either drug.

Table 3: Escalation rules

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
≥2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. However, a cohort may be expanded to more than 6 patients if the PI and CTEP medical monitor agree more information is needed to clarify whether a DLT has occurred or the MTD has been reached.
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

5.3 General Concomitant Medication and Supportive Care Guidelines

5.3.1 Nausea/vomiting - see 5.1.1

5.3.2 Hyperuricemia/gout – see 5.1.1

5.3.3 Hypersensitivity reactions – see 5.1.1

5.3.4 Mucositis - Mucositis is common with CCI-779 (temsirolimus). This can be troublesome in the initial few weeks but usually subsides with time. Early treatment with mouthwash is recommended (e.g. Magic mouth wash (Maalox, viscous lidocaine and benadryl in 1:1:1 mixture) or kaopectate/ diphenhydramine/ lidocaine solution in 1:1:1 mixture). Antifungal agents should be used if oral candidiasis is present. Consider GI consultation.

5.3.5 Hyperlipidemia - It is recommended that patients with hyperlipidemia be treated with pravastatin (which dose not affect cytochrome metabolism), extended release niacin, or gemfibrozil (Lopid),

such as Lopid 600 mg BID.

5.3.6 Diarrhea should be managed with loperamide: 4 mg at first onset, then 2 mg every 2-4 hours until diarrhea is controlled (maximum = 16 mg loperamide/day). Additional antidiarrheal measures may be used at the discretion of the treating physician as warranted by the patient's condition. Consider GI consultation.

5.3.7 Routine supportive measures for cancer patients such as erythropoietin, analgesics, blood transfusions, antibiotics, bisphosphonates, and hematopoietic colony stimulating factors for treatment of cytopenias are permitted.

5.3.8 CY450 metabolism - In vitro studies with Temsirolimus in human liver microsomes indicate that CYP3A4 may be the primarily enzyme responsible for the agent's metabolism, suggesting that compounds known to modulate CYP3A4 activity may therefore affect the metabolism of Temsirolimus. Inhibitors of CYP3A4 may decrease the metabolism and increase Temsirolimus levels, while inducers of CYP3A4 may increase the metabolism and decrease Temsirolimus levels. Temsirolimus or its metabolites may also interact with the CYP450 enzyme system by inhibiting CYP2D6. Because there is a potential for interaction of temsirolimus and potentially perifosine with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes. **Every effort should be made to avoid cytochrome P450 inducing medications. Please see Appendix B and Appendix C.**

5.3.9 Therapeutic anticoagulation - Patients on therapeutic anticoagulation should have PT/INR or PTT (whichever is appropriate) monitored closely during therapy (*e.g.*, weekly for the first month and weekly for a minimum of 2 weeks following discontinuation of the agent). Therapy should be held if the coagulation parameters are higher than the intended therapeutic range.

5.3.10 Corticosteroids should be used in the smallest dose to control symptoms of cerebral edema and mass effect, and discontinued if possible.

5.3.11 Anti-seizure medications should be used as indicated. **IT IS STRONGLY RECOMMENDED THAT EVERY EFFORT SHOULD BE MADE TO MAINTAIN THE PATIENT ON A NON-EIAED RATHER THAN EIAED IF ANTI-SEIZURE PROPHYLAXIS IS REQUIRED. (APPENDIX B).**

5.3.11.1 Patients who were previously on a non-EIAED and need to change anticonvulsants, should be started on another non-EIAED if at all possible. No delays in treatment would be required.

5.3.11.2 Patients who were previously on a either no AED or a non- EIAED and started on an EIAED should immediately be started on another non-EIAED and the EIAED discontinued/tapered off as quickly as possible. The patient may continue the current treatment dose while a non-EIAED is re-started.

5.3.11.3 Patients who were previously on a non-EIAED and need to permanently change anticonvulsant, but who cannot change to another non-EIAED **MUST BE DISCUSSED WITH THE STUDY PI OR CO-PI**. These patients will be taken off-study unless it is felt that they have benefited from the therapy following assessment by the investigator, PI, and CTEP medical monitor. In some cases, this may be associated with an increase in the dose(s) used.

5.3.12 Other Concomitant Medications - Therapies considered necessary for the well being of the patient may be given at the discretion of the investigator. Other concomitant medications should be avoided except for analgesics, chronic treatments for concomitant medical conditions, or agents required for life-threatening medical problems. All concomitant medications must be recorded.

5.3.13 Surgery - If neurosurgical or other surgical management is required for reasons not due to tumor progression, these procedures must be documented, including the indications for surgery, the surgical operative note and pathology report.

5.3.14 Other Anticancer or Experimental Therapies - No other anticancer therapy (including chemotherapy, radiation, hormonal treatment or immunotherapy) of any kind is permitted during the study period. No other drug under investigation may be used concomitantly with the study drug. If patients are subsequently found to have been taking such therapy before or during initiation of study treatment, the patient can be replaced and will not be included in toxicity or efficacy analyses following discussion by the investigator, PI, and CTEP medical monitor.

5.4 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment will continue indefinitely or until one of the following criteria applies:

- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

Prior to cycle 3, patients undergo clinical and radiographic tumor restaging. As long as the tumor is stable or smaller in size and the patient is clinically stable or improved, they will go on to another 2 four week cycles of therapy with temsirolimus and perifosine. Treatment will continue indefinitely as long as there are no unacceptable toxicities (DLTs) and no tumor progression.

5.5 Duration of Follow Up

Patients will be followed every 3 months for survival after removal from study. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. They should then be followed for survival every 3 months.

5.6 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6 DOSING DELAYS/DOSE MODIFICATIONS

6.1 Dose Modifications

Patients with stable or responding disease may be retreated at the same dose or at a reduced dose level, depending upon the adverse events observed in the current cycle and any adverse events present on the first day of the next cycle. If multiple toxicities are seen, the dose administered in a subsequent cycle should be based on the most

severe toxicity experienced in the current cycle. Dose modifications or delays should be made based upon whether toxicities occur within a 4-week treatment cycle or at the expected start of the next treatment cycle.

Patients who experience dose-limiting toxicity should have laboratory testing at least weekly until the toxicity has resolved.

6.1.1 Within Treatment Cycle:

If a patient experiences DLT (as defined in Section 5.2) during the current treatment cycle, the treatment will be immediately suspended for a minimum of 1 week. If the toxicity resolves to less than or equal to grade 1 (or to within 1 grade of starting values for pre-existing laboratory abnormalities) within 2 weeks of the last dose of both temsirolimus and perifosine (grade 2 for diarrhea and hyperlipidemia), the patient will be retreated at one or two dose levels lower (see Section 6.1.2 - 6.1.5). Patients experiencing DLT at the initial starting dose of temsirolimus of 15 mg i.v. weekly will have the dose reduced according to Table 4. No dose reduction below 5 mg i.v. weekly is allowed**.** Otherwise, there is no limit on the number of dose level reductions**.** If temsirolimus-related toxicity persists at dose level -2 (5 mg i.v. weekly), the patient should be taken off study.

Doses that are reduced for temsirolimus or perifosine related toxicity will not be re-escalated, even if there is minimal or no toxicity with the reduced dose. Patients whose dose has been reduced for adverse events that are subsequently not felt to be related to temsirolimus or perifosine may have the dose re-escalated after completion of one cycle with toxicities less than or equal to grade 1. If any patient has further toxicities that would require additional reductions, or if treatment is held for more than 2 consecutive weeks because of ongoing toxicity, the investigator and the CTEP medical monitor will assess if the patient should remain in the study (if the patient has benefited from treatment).

Table 4: Temsirolimus Dose Modifications Table:

Dose Modifications	
Dose Level	**Dose of Temsirolimus (mg/week intravenously)**
Level -2	5 mg
Level -1	10 mg
Starting dose (Level 1)	15 mg
Level 2	25 mg
Level 3	50 mg
Level 4	75 mg
Level 5	115 mg
Level 6	170 mg

Table 5: Perifosine Dose Modifications Table for Maintenance Dose

Dose Modifications	
Dose Level	**Dose of perifosine**
Level -2	50 mg every other day

Level -1	50 mg per day
Starting dose (Level 1)	100 mg per day

6.1.2 Hematologic Toxicity

No dose adjustment or reduction of perifosine will be made for hematologic toxicity.

Dose Modifications of CCI-779 (Temsirolimus) Based on Weekly ANC and Platelet Counts:

ANC (/mL)		**Platelets (/mL)**	**% of Planned temsirolimus**
≥ 1000	and	≥ 75,000	100%
750-999	or	50,000 to < 75,000	hold*
< 750	or	< 50,000	hold**

* Upon recovery to ANC ≥ 1,000/mL and platelets to ≥ 100,000/mL, one lower dose level of temsirolimus will be administered (See Table 4). Patients already at the lowest dose level (5 mg i.v. weekly) will be removed from study.

** Upon recovery to ANC ≥ 1,000/mL and platelets to ≥ 100,000/mL, two lower dose levels will be administered. The exception will be patients at dose level -1 (10 mg i.v. weekly) who will be dose-reduced by only one level to 5 mg IV weekly (See Table 4). Patients already at the lowest dose level (5 mg i.v. weekly) will be removed from study.

6.1.3 Non-hematologic toxicity

Dose Modifications Based on perifosine and temsirolimus-related Non-Hematologic Toxicities and dose adjustments for each drug shown in tables 4, 5, and 6:

NCI Grade		**% of Planned perifosine and temsirolimus dose**
0-2+	100%+	
3*		hold**
4		hold***

\+ For symptomatic Grade 2 toxicity, the dose may be held until recovery to CTC Grade 0-1, then continue at the same dose or reduce by one level of temsirolimus and/or perifosine at the investigator's discretion. Grade 2 diarrhea does not require temporary discontinuation of treatment as this toxicity may improve despite continued treatment. For grade 2 diarrhea that is unacceptable to the patient for symptomatic reasons, hold perifosine temporarily held until resolution ≤ grade 1 and subsequently re-started at the same dose. If symptomatic grade 2 diarrhea recurs after re-instituting treatment at the same daily dose and require temporary discontinuation, treatment of both agents should be held until resolution to ≤ grade 1 and re-instituted at a reduced doses of both agents (see Table 4, 5, and 6).

\+ In addition, areas of intratumoral micro-hemorrhage were noted on necroscopy of mice harboring modeled GBMs following combined treatment of perifosine and temsirolimus. Significance of this in humans is unclear, and it may represent an early response to therapy. However, for patient safety, a non-contrast head CT or MRI with gradient echo imaging is required during the 2nd week of therapy to evaluate for such microbleeds. CTCAE grade 1 (asymptomatic, radiographic findings only) do not require treatment modification. CTCAE grade 2 (medical intervention indicated) may require corticosteroids as treatment for associated edema or other intervention at the discretion of the investigator, but treatment can continue at the discretion of the investigator.

* Except nausea/vomiting (unless patients are on maximal antiemetic therapy) or hypercholesterolemia and hypertriglyceridemia and other Metabolism and Nutrition Disorders (such as hypophosphatemia and hypocalcemia) unless remaining grade 3 or unacceptable grade 2, despite maximal medical therapy.

** Hold (no minimum time unless a DLT) until recovery to CTC Grade 0-1 (or to within 1 grade of starting values for pre-existing laboratory abnormalities), and then resume per table 6. For diarrhea, and hyperlipidemia, hold until recovery to CTC grade 2, unless the patient cannot tolerate symptoms in which case the dose may be held until recovery to CTC Grade 0-1. Antilipid therapy should be initiated for hyperlipidemia such as with pravastatin (which dose not affect cytochrome metabolism), extended release niacin, or gemfibrozil (Lopid) (see 5.3.5 and 6.1.5). Note that Subjects with toxicities that are manageable with supportive therapy do not require dose reductions unless they remain grade 3 or unacceptable grade 2 despite maximal medical therapy.

*** Hold (no minimum time unless a DLT) until recovery to CTC Grade 0-1 (or to within 1 grade of starting values for pre-existing laboratory abnormalities), and then resume per table 6. The exception will be patients at dose level -1 of temsirolimus (10 mg i.v. weekly) who will be dose-reduced by only one level to 5 mg i.v. weekly. For diarrhea and hyperlipidemia, hold until recovery to CTC grade 2, unless the patient cannot tolerate symptoms in which case the dose the dose may be held until recovery to CTC Grade 0-1. Antilipid therapy should be initiated for hyperlipidemia such as with pravastatin (which dose not affect cytochrome metabolism), extended release niacin, or gemfibrozil (Lopid) (see 5.3.5 and 6.1.5).

An important exception to this scheme are patients with symptoms suggestive of interstitial pneumonitis. Because pneumonitis is an anticipated toxicity for temsirolimus, patients with signs/symptoms of pneumonitis should have treatment held and be promptly evaluated. Those patients subsequently diagnosed with pneumonitis thought to be possibly related to study agent(s) should have treatment discontinued and be removed from study.

6.1.3.1 Perifosine related toxicity

Dose Modifications for Toxicity During the Loading Dose: Nausea and vomiting are the only toxicities likely to occur during the loading dose that may require dose modification. In patients who have emesis and are unable to retain perifosine, every attempt should be made to obtain control of nausea and vomiting using additional antiemetic medication. The dose of perifosine may be repeated if emesis occurs within 30 minutes of taking the tablet(s) OR all the tablets are seen in the emesis.

Dose Modifications for Toxicity during Maintenance Dosing:
For Grade 1 toxicity, treatment with perifosine will not be interrupted.

For Grade 2 nausea, vomiting, or diarrhea:
(a) For nausea, vomiting and diarrhea, maintain dosing with symptomatic treatment. The dose of perifosine may be repeated if emesis occurs within 30 minutes of taking the tablet(s) OR all the tablets are seen in the emesis.
(b) For persistent nausea, vomiting or diarrhea despite symptomatic treatment that remains unacceptable to the patient, hold both agents and then reduce dose of each by one dose level. If patients are receiving 50 mg/day of perifosine maintenance at the time of occurrence/recurrence of toxicity, change the schedule of administration to 50 mg every other day (q 2 days, dose level -2) Reductions below this dose/schedule will not be allowed.

For grade 3 joint pain (gout):
Hold perifosine and start allopurinol and/or other agents at the discretion of the investigator. Reevaluate the patient at least weekly (more frequently is allowed) until toxicity improves to $\leq$ Grade 1 or grade 2 that is acceptable to the patient. Then reduce the maintenance dose of perifosine by one dose level. If patients are receiving 50 mg/day at the time of occurrence/recurrence of toxicity, change the schedule of administration to 50 mg every other day (q 2 days, dose level -2). Reductions below this dose/schedule will not be

allowed.

For Unresolved Toxicity

If a patient has unresolved toxicity (which have not returned to ≤ grade 1 or grade 2 that is acceptable to the patient OR to within 1 grade of starting values for pre-existing laboratory abnormalities) greater than 14 days from the last dose of both temsirolimus and perifosine, the patient should discontinue all treatment and be taken off study. However, if it is the treating physician's opinion that the patient may benefit from continued treatment, the patient may continue on study with a dose reduction. Dose reduction below 50 mg PO q 2 days are not allowed unless it is the opinion of the treating physician that the patient may benefit from continued treatment. The decision to proceed with treatment must be made in consultation with the PI and the CTEP Senior Investigator/Medical Monitor.

Table 6: Summary of Dose Modification for perifosine and temsirolimus		
Toxicity	**Perifosine**	**Temsirolimus**
Hematologic	Continue drug; no dose reduction	Hold drug and reduce dose per Section 6.1.2 and dose levels in table 4
Rash	Continue drug; no dose reduction	Hold temsirolimus and reduce dose per Section 6.1.3 and dose levels in table 4. Consider Dermatology consultation.
Diarrhea, nausea, vomiting	Hold perifosine first. Resume at same dose after improvement per section 6.1.3 and Table 5. If no improvement, hold and reduce dose per section 6.1.3 and dose levels in Table 5. Consider GI consultation	If no improvement after holding perifosine, hold temsirolimus and reduce dose per section 6.1.3 and dose levels in table 4. Consider GI consultation.
Stomatitis	Continue drug; no dose reduction	Hold temsirolimus and reduce dose per Section 6.1.3 and dose levels in table 4. Consider GI consultation.
Pulmonary	Continue drug; no dose reduction	Hold drug per Section 6.1.3
Hepatic	Continue drug; no dose reduction	Hold drug and reduce dose per Section 6.1.3 and dose levels in table 4
Hyperlipidemia	Continue drug; no dose reduction	Hold drug and reduce dose per Section 6.1.3 and dose levels in table 4. Initiate antilipid therapy
Joint pain/gout attack	Hold perifosine. Start allopurinol 300 mg per day or other regimen at discretion of investigator. If persists, hold perifosine. Reduce dose per section 6.1.3.1 and dose levels in Table 5. Continue allopurinol at same or inceased dose with other agents at the discretion of the investigator to treat gout.	Continue drug; no dose reduction

All other toxicities	Continue drug; no dose reduction	Hold drug and reduce dose per Section 6.1.3 and dose levels in table 4 except Subjects with toxicities that are manageable with supportive therapy do not require dose reductions unless they remain grade 3 or unacceptable grade 2 despite maximal medical therapy

6.1.4 At the Start of the Next Treatment Cycle:

Additional cycles of therapy may be administered provided that the patient meets the following criteria on Day 1 of each cycle (labs may be drawn up to 3 days before the start of the cycle):

- ANC $\geq$ 1,000/mcL
- Platelets $\geq$ 100,000/mcL
- Non-hematologic toxicity recovered to $\leq$ grade 1 (or tolerable grade 2) EXCEPT
    - Non-hematologic toxicities such as rash, diarrhea and mucositis will only be considered a DLT if they remain grade 3 or unacceptable grade 2, despite maximal medical therapy.
    - *Hypercholesterolemia and hypertriglyceridemia and other Metabolism and Nutrition Disorders (such as hypophosphatemia and hypocalcemia) will only be considered a DLT if it remains grade 3 or unacceptable grade 2, despite maximal medical therapy.
- No evidence of progressive disease

In the event of toxicity, the doses of temsirolimus will be adjusted according to the guidelines shown in the Dose Delays/Dose Modifications table7 below. If an adverse event is not covered in the table, doses may be reduced or held at the discretion of the investigator for the subject's safety. Dose adjustments for hematological toxicity are based on the blood counts obtained in preparation for the day of treatment.

Patients requiring dose reductions should not have the dose re-escalated with subsequent treatments.

Subjects with toxicities that are manageable with supportive therapy do not require dose reductions unless they remain grade 3 or unacceptable grade 2 despite maximal medical therapy: hyperlipidemia may be treated with statins, such as pravastatin (which does not affect cytochrome metabolism), extended release niacin, or gemfibrozil; nausea/vomiting may be treated with antiemetics; and diarrhea may be treated with loperamide rather than by dose reduction

Subjects will be withdrawn from the study if they fail to recover to CTC Grade 0-1 or tolerable grade 2 (or within 1 grade of starting values for pre-existing laboratory abnormalities) from a treatment-related toxicity (EXCEPT toxicities that are manageable with supportive therapy as above) within 14 days from the last dose of both temsirolimus and perifosine OR they experience agent related adverse events requiring dose modification despite two previous dose reductions (i.e. would require a 3rd dose reduction) unless the investigator, PI, and CTEP monitor agree that the subject should remain in the study because of evidence that the patient is/may continue deriving benefit from continuing study treatment. The appropriate reduced dose will be determined after discussion between the principal investigator and CTEP monitor.

Table 7

Adverse Event	Agent	Treatment Modifications
Blood/Bone Marrow		
Grade 3: Neutrophils ANC 500-1000 Platelets 50,000-75,000	**Temsirolimus**	• Delay Temsirolimus until recovery to ≤ grade 1. • Retreat at a one dose level reduction • If recovery requires > 14 days from the last dose of both temsirolimus and perifosine, discontinue all study treatment.
Grade 4: Neutrophils ANC <500 Platelets <25,000		• Delay Temsirolimus until recovery to ≤ grade 1. • Retreat at two dose levels reduction • If recovery requires > 14 days from the last dose of both temsirolimus and perifosine, discontinue all study treatment.
Pulmonary/Upper Respiratory		
Pneumonitis (cough, dyspnea, fever)	**Temsirolimus**	Discontinue Temsirolimus pending investigation. If diagnosis is confirmed and events are considered at least possibly due to Temsirolimus, the agent is removed from the study.
All other non-hematologic adverse events except nausea, vomiting, and diarrhea		
Grade 0-2	**Temsirolimus**	Grade 2 toxicities that are persistent and intolerable (i.e. stomatitis) can result in dose delays and dose reductions to the next lower dose level
Grade 3-4		Hold dose. • Re-evaluate until AE resolved to ≤ 1 or tolerable grade 2. • Re-treat at a one dose level reduction • If recovery requires > 14 days from the last dose of both temsirolimus and perifosine, discontinue all study treatment. • Patients with grade 4 AEs related to agent may be taken off study at investigator's discretion.

6.1.5 For treatment or dose modification related questions, please contact the PI at 212-639-5122 or kaleyt@mskcc.org.

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited (via CTEP-AERS) **in addition** to routine reporting.

7.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system.

In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (ASAEL) contains events that are considered '**expected**' for expedited reporting purposes only. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification.

7.1.1 Comprehensive Adverse Events and Potential Risks list (CAEPR) For Temsirolimus (CCI-779, NSC 683864)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1927 patients.* Below is the CAEPR for temsirolimus (CCI-779, Torisel).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.4, July 2, 2013[1]

Adverse Events with Possible Relationship to Temsirolimus (CCI-779, Torisel) (CTCAE 4.0 Term) [n= 1927]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
	Febrile neutropenia		***Febrile neutropenia (Gr 3)***
ENDOCRINE DISORDERS			
	Endocrine disorders - Other (decreased testosterone)		***Endocrine disorders - Other (decreased testosterone) (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal distension		***Abdominal distension (Gr 2)***
	Abdominal pain		***Abdominal pain (Gr 3)***
	Anal mucositis[2]		***Anal mucositis[2] (Gr 2)***
	Constipation		***Constipation (Gr 3)***
Diarrhea			***Diarrhea (Gr 3)***
		Gastrointestinal fistula[3]	
		Gastrointestingal perforation[4]	***Gastrointestinal perforation[4] (Gr 2)***

Mucositis oral[2]			***Mucositis oral[2] (Gr 3)***
Nausea			***Nausea (Gr 3)***
	Rectal mucositis[2]		***Rectal mucositis[2] (Gr 2)***
	Small intestinal mucositis[2]		***Small intestinal mucositis[2] (Gr 2)***
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills (Gr 2)***
	Edema face		***Edema face (Gr 2)***
	Edema limbs		***Edema limbs (Gr 3)***
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
	Flu like symptoms		***Flu like symptoms (Gr 2)***
	Non-cardiac chest pain		***Non-cardiac chest pain (Gr 2)***
	Pain		
IMMUNE SYSTEM DISORDERS			
	Allergic reaction[5]		***Allergic reaction[5] (Gr 2)***
INFECTIONS AND INFESTATIONS[6]			
	Infection[7]		***Infection[7] (Gr 3)***
INJURY, POISONING AND PROCEDURAL COMPLICATIONS			
	Wound dehiscence[8]		***Wound dehiscence[8] (Gr 2)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 3)***
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr 2)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 3)***
Cholesterol high[9]			***Cholesterol high[9] (Gr 4)***
	Creatinine increased		***Creatinine increased (Gr 3)***
	Fibrinogen decreased		***Fibrinogen decreased (Gr 2)***
	GGT increased		***GGT increased (Gr 2)***
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
	Neutrophil count decreased[10]		***Neutrophil count decreased[10] (Gr 4)***
Platelet count decreased[10]			***Platelet count decreased[10] (Gr 4)***
	Weight loss		***Weight loss (Gr 3)***
	White blood cell decreased		***White blood cell decreased (Gr 4)***
METABOLISM AND NUTRITION DISORDERS			
	Acidosis		***Acidosis (Gr 2)***
Anorexia			***Anorexia (Gr 3)***
	Glucose intolerance[11]		***Glucose intolerance[11] (Gr 2)***
	Hyperglycemia[11]		***Hyperglycemia[11] (Gr 3)***
	Hypertriglyceridemia[9]		***Hypertriglyceridemia[9] (Gr 4)***
	Hypocalcemia		***Hypocalcemia (Gr 3)***
	Hypokalemia		***Hypokalemia (Gr 4)***
	Hypophosphatemia		***Hypophosphatemia (Gr 3)***
	Metabolism and nutrition disorders - Other (hyperlipidemia)[9]		***Metabolism and nutrition disorders - Other (hyperlipidemia)[9] (Gr 4)***

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		***Arthralgia (Gr 2)***
	Back pain		***Back pain (Gr 2)***
	Myalgia		***Myalgia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
	Depressed level of consciousness		***Depressed level of consciousness (Gr 2)***
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 3)***
PSYCHIATRIC DISORDERS			
	Depression		***Depression (Gr 2)***
	Insomnia		***Insomnia (Gr 2)***
	Libido decreased		***Libido decreased (Gr 2)***
RENAL AND URINARY DISORDERS			
		Acute kidney injury[12]	
REPRODUCTIVE SYSTEM AND BREAST DISORDERS			
	Erectile dysfunction		***Erectile dysfunction (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 3)***
	Epistaxis		***Epistaxis (Gr 2)***
	Laryngeal mucositis[2]		***Laryngeal mucositis[2] (Gr 2)***
	Pharyngeal mucositis[2]		***Pharyngeal mucositis[2] (Gr 2)***
	Pleural effusion		***Pleural effusion (Gr 3)***
	Pneumonitis[13]		***Pneumonitis[13] (Gr 3)***
	Sinus disorder		***Sinus disorder (Gr 2)***
	Tracheal mucositis[2]		***Tracheal mucositis[2] (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Dry skin		***Dry skin (Gr 2)***
	Pruritus		***Pruritus (Gr 2)***
	Rash acneiform		***Rash acneiform (Gr 2)***
Rash maculo-papular			***Rash maculo-papular (Gr 3)***
	Skin and subcutaneous tissue disorders – Other (nail disorder/nail changes)[15]		***Skin and subcutaneous tissue disorders – Other (nail disorder/nail changes)[15] (Gr 2)***
	Urticaria		***Urticaria (Gr 2)***
VASCULAR DISORDERS			
	Hypertension		***Hypertension (Gr 3)***
	Hypotension		***Hypotension (Gr 3)***
		Thromboembolic event	***Thromboembolic event (Gr 4)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Mucositis/stomatitis: Gingivitis, mucositis/stomatitis, ulcers in mouth and throat, pharyngitis, and dysphagia have been reported in subjects receiving temsirolimus.

[3]Gastrointestinal fistula includes Anal fistula, Colonic fistula, Duodenal fistula, Esophageal fistula, Enterovesical fistula, Gastric fistula, Gastrointestinal fistula, Ileal fistula, Jejunal fistula, Oral cavity fistula, Pancreatic fistula, Rectal fistula, and Salivary gland fistula under the GASTROINTESTINAL DISORDERS SOC.

[4]Gastrointestinal perforation includes Colonic perforation, Duodenal perforation, Esophageal perforation, Gastric perforation, Ileal perforation, Jejunal perforation, Rectal perforation, and Small intestinal perforation under the GASTROINTESTINAL DISORDERS SOC. GI perforation (including fatal outcome) has been observed in subjects who received temsirolimus.

[5]Hypersensitivity /infusion reactions (including some life threatening and rare fatal reactions), including and not limited to flushing, chest pain, dyspnea, hypotension, apnea, loss of consciousness, hypersensitivity, and anaphylaxis, have been associated with the administration of temsirolimus. These reactions can occur very early in the first infusion, but may also occur with subsequent infusions. Patients should be monitored early during infusion and appropriate supportive care should be available. Temsirolimus infusion should be interrupted in all patients with severe infusion reactions and appropriate medical care administered. A risk-benefit assessment should be done prior to the continuation of temsirolimus therapy in patients with severe life-threatening reactions.

[6]Infections: Bacterial and viral infections including opportunistic infections have been reported in subjects. Infections may originate in a variety of organ systems/body regions and may be associated with normal or grade 3-4 neutropenia. Bacterial and viral infections have included cellulitis, herpes zoster, herpes simplex, bronchitis, abscess, pharyngitis, urinary tract infection (including dysuria hematuria, cystitis, and urinary frequency), rhinitis folliculitis, pneumonia, and upper respiratory tract infection.

[7]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[8]Wound Dehiscence: The use of temsirolimus has been associated with abnormal wound healing. Therefore, caution should be exercised with the use of temsirolimus in the perisurgical period.

[9]Cholesterol High: The use of temsirolimus in subjects has been associated with increases in serum levels of triglycerides and cholesterol. This may require initiation of or increase in the dose of lipid-lowering agents.

[10]Thrombocytopenia and Neutropenia: Grades 3 and 4 thrombocytopenia and/or neutropenia have been observed at higher frequency in subjects with mantle cell lymphoma (MCL).

[11]Hyperglycemia/Glucose Intolerance: The use of temsirolimus in subjects was associated with increases in serum glucose level. This may result in the need for an increase in the dose of, or initiation of, insulin and/or oral hypoglycemic agent therapy.

[12]Acute Kidney Injury: Renal failure (including fatal outcome) has been observed in subjects receiving temsirolimus for advanced RCC and/or with pre-existing renal insufficiency.

[13]Interstitial Lung Disease: There have been cases of nonspecific interstitial pneumonitis, including rare fatal reports. Some subjects were asymptomatic with pneumonitis detected on computed tomography scan or chest radiograph. Others presented with symptoms such as dyspnea, cough, and fever. Some subjects required discontinuation of temsirolimus or treatment with corticosteroids and/or antibiotics, while some subjects continued treatment without additional intervention.

[14]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage,

Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[15]Nail Disorder/Nail Changes includes Nail discoloration, Nail loss, and Nail ridging under the SKIN AND SUBCUTANEOUS TISSUE DISORDERS SOC.

Also reported on temsirolimus (CCI-779, Torisel) trials but with the relationship to temsirolimus (CCI-779, Torisel) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (coagulopathy); Hemolysis; Leukocytosis
CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Cardiac arrest; Chest pain - cardiac; Heart failure; Left ventricular systolic dysfunction; Myocardial infarction; Pericardial effusion; Right ventricular dysfunction; Sinus tachycardia; Supraventricular tachycardia; Ventricular fibrillation; Ventricular tachycardia
EAR AND LABYRINTH DISORDERS - Vertigo
ENDOCRINE DISORDERS - Endocrine disorders - Other (Cushing's syndrome); Endocrine disorders - Other (diabetes mellitus)
EYE DISORDERS - Blurred vision; Cataract; Conjunctivitis; Dry eye; Eye disorders - Other (diplopia); Eye pain; Flashing lights; Photophobia; Retinopathy
GASTROINTESTINAL DISORDERS - Anal pain; Anal ulcer; Ascites; Bloating; Colitis; Colonic obstruction; Colonic ulcer; Dry mouth; Duodenal ulcer; Dyspepsia; Dysphagia; Enterocolitis; Esophageal pain; Esophageal ulcer; Esophagitis; Flatulence; Gastritis; Gastrointestinal disorders - Other (anal fissure); Gastrointestinal disorders - Other (gastroenteritis); Gastrointestinal disorders - Other (mouth ulceration); Gastrointestinal hemorrhage[14], Hemorrhoids; Ileus; Oral pain; Pancreatitis; Periodontal disease; Proctitis; Rectal pain; Small intestinal obstruction; Stomach pain; Typhlitis
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema trunk; Facial pain; Gait disturbance; Injection site reaction; Localized edema; Malaise; Multi-organ failure; Sudden death NOS
HEPATOBILIARY DISORDERS - Hepatic failure
IMMUNE SYSTEM DISORDERS - Anaphylaxis
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Bruising; Fracture; Postoperative hemorrhage; Vascular access complication; Wound complication
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Blood bilirubin increased; CD4 lymphocytes decreased; INR increased (potential interaction with Coumadin); Investigations - Other (BUN increased); Investigations - Other (lactic dehydrogenase increased); Lipase increased; Lymphocyte count increased; Serum amylase increased; Weight gain
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hyperuricemia; Hypoalbuminemia; Hypoglycemia; Hypomagnesemia; Hyponatremia; Metabolism and nutrition disorders - Other (albuminuria) ; Metabolism and nutrition disorders - Other (blood urea increased);
Metabolism and nutrition disorders - Other (hypoproteinemia)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthritis; Avascular necrosis; Bone pain; Chest wall pain; Generalized muscle weakness; Joint effusion; Muscle weakness lower limb; Musculoskeletal and connective tissue disorder - Other (muscle cramps); Neck pain; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Leukemia secondary to oncology chemotherapy; Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (carcinoma of the lung); Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (lymphoma); Treatment related secondary malignancy
NERVOUS SYSTEM DISORDERS - Ataxia; Cognitive disturbance; Dizziness; Dysesthesia; Hydrocephalus; Intracranial hemorrhage; Lethargy; Neuralgia; Paresthesia; Peripheral motor neuropathy;

Peripheral sensory neuropathy; Reversible posterior leukoencephalopathy syndrome; Seizure; Somnolence; Spasticity; Stroke; Syncope
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Mania; Psychiatric disorders - Other (bipolar disorder); Psychosis
RENAL AND URINARY DISORDERS - Bladder spasm; Cystitis noninfective; Hematuria; Hemoglobinuria; Proteinuria; Renal hemorrhage; Urinary frequency; Urinary retention; Urinary tract pain; Urinary urgency
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Female genital tract fistula; Hematosalpinx; Irregular menstruation; Menorrhagia; Ovarian hemorrhage; Prostatic hemorrhage; Spermatic cord hemorrhage; Testicular disorder; Testicular hemorrhage; Testicular pain; Uterine hemorrhage; Vaginal discharge; Vaginal dryness; Vaginal fistula; Vaginal hemorrhage; Vaginal inflammation
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Allergic rhinitis; Bronchopulmonary hemorrhage; Bronchospasm; Hiccups; Hypoxia; Nasal congestion; Pharyngolaryngeal pain; Pleuritic pain; Productive cough; Pulmonary edema; Pulmonary fibrosis; Pulmonary hypertension; Respiratory failure; Voice alteration
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Erythema multiforme; Hyperhidrosis; Pain of skin; Palmar-plantar erythrodysesthesia syndrome; Photosensitivity; Skin and subcutaneous tissue disorders - Other (angioneurotic edema); Skin ulceration; Stevens-Johnson syndrome
VASCULAR DISORDERS - Flushing; Phlebitis; Superficial thrombophlebitis; Visceral arterial ischemia

Note: Intracerebral Bleeding: Subjects with central nervous system (CNS) tumors (primary CNS tumors or metastases) and/or receiving anticoagulation therapy may be at an increased risk of intracerebral bleeding (including fatal outcomes) while receiving therapy with temsirolimus (CCI-779, Torisel).

Note: Temsirolimus (CCI-779, Torisel) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.1.2 Comprehensive Adverse Events and Potential Risks list (CAEPR) for Perifosine (NSC 639966)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (SPEER) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 1586 patients*. Below is the CAEPR for Perifosine.

> **NOTE**: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Adverse Events with Possible Relationship to Perifosine (CTCAE 4.0 Term) [n= 1586]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr. 2)***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr. 2)***
	Constipation		***Constipation (Gr. 2)***
Diarrhea			***Diarrhea (Gr. 2)***
	Flatulence		***Flatulence (Gr. 2)***
Nausea			***Nausea (Gr. 2)***
Vomiting			***Vomiting (Gr. 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr. 2)***
INFECTIONS AND INFESTATIONS			
	Infection[2]		***Infection[2] (Gr. 2)***
INVESTIGATIONS			
	Alkaline phosphatase increased		***Alkaline phosphatase increased (Gr. 2)***
	Creatinine increased		***Creatinine increased (Gr. 2)***
	GGT increased		***GGT increased (Gr. 2)***
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr. 2)***
	Weight loss		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr. 2)***
	Dehydration		***Dehydration (Gr. 2)***
	Hyperglycemia		***Hyperglycemia (Gr. 2)***
	Hypoalbuminemia		***Hypoalbuminemia (Gr. 2)***
	Hypocalcemia		
	Hypokalemia		
	Hyponatremia		***Hyponatremia (Gr. 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Back pain		***Back pain (Gr. 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Dyspnea		***Dyspnea (Gr. 2)***
	Hiccups		***Hiccups (Gr. 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@*CTEP*.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Also reported on Perifosine trials but with the relationship to Perifosine still undetermined:

CARDIAC DISORDERS - Atrial fibrillation; Cardiac arrest; Cardiac disorders - Other (cardiac valve disorder); Conduction disorder; Myocardial infarction; Palpitations; Pericardial effusion; Sinus bradycardia; Sinus tachycardia
EAR AND LABYRINTH DISORDERS - Ear pain; Middle ear inflammation; Tinnitus
EYE DISORDERS - Blurred vision; Cataract; Conjunctivitis; Dry eye; Eye pain; Flashing lights; Keratitis; Photophobia; Watering eyes
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Colitis; Colonic obstruction; Dry mouth; Dyspepsia; Dysphagia; Esophageal pain; Esophageal perforation; Esophageal stenosis; Esophagitis; Gastric hemorrhage; Gastritis; Gastrointestinal pain; Gingival pain; Hemorrhoids; Ileus; Lower gastrointestinal hemorrhage; Mucositis oral; Oral hemorrhage; Oral pain; Rectal hemorrhage; Rectal pain; Small intestinal obstruction; Stomach pain; Toothache; Upper gastrointestinal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema limbs; Fever; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (visceral edema); Localized edema; Non-cardiac chest pain; Pain
HEPATOBILIARY DISORDERS - Hepatic failure; Hepatic pain
IMMUNE SYSTEM DISORDERS - Allergic reaction
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Arterial injury; Bruising; Fracture
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alanine aminotransferase increased; Aspartate aminotransferase increased; Blood bilirubin increased; CPK increased; Carbon monoxide diffusing capacity decreased; Cholesterol high; INR increased; Investigations - Other (granulocytopenia); Lipase increased; Neutrophil count decreased; Platelet count decreased; Serum amylase increased; Weight gain; White blood cell decreased
METABOLISM AND NUTRITION DISORDERS - Acidosis; Glucose intolerance; Hypercalcemia; Hyperkalemia; Hypernatremia; Hyperuricemia; Hypoglycemia; Hypomagnesemia; Hypophosphatemia; Metabolism and nutrition disorders - Other (blood bicarbonate decreased)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Arthritis; Bone pain; Buttock pain; Chest wall pain; Generalized muscle weakness; Joint range of motion decreased; Muscle weakness left-sided; Muscle weakness lower limb; Muscle weakness upper limb; Musculoskeletal and connective tissue disorder - Other (upper extremity dysfunction); Myalgia; Myositis; Neck pain; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (incl cysts and polyps) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (massive tumor hemorrhage); Tumor pain
NERVOUS SYSTEM DISORDERS - Acoustic nerve disorder NOS; Ataxia; Cognitive disturbance; Depressed level of consciousness; Dizziness; Dysarthria; Dysgeusia; Extrapyramidal disorder; Facial nerve disorder; Headache; Intracranial hemorrhage; Memory impairment; Neuralgia; Peripheral sensory neuropathy; Pyramidal tract syndrome; Seizure; Syncope; Tremor; Trigeminal nerve disorder; Vagus nerve disorder
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression; Insomnia; Libido decreased; Personality change; Psychosis
RENAL AND URINARY DISORDERS - Acute kidney injury; Cystitis noninfective; Proteinuria; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract obstruction; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Breast pain; Pelvic pain; Scrotal pain; Vaginal discharge; Vaginal hemorrhage

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Atelectasis; Bronchial obstruction; Bronchospasm; Cough; Epistaxis; Hypoxia; Nasal congestion; Pharyngolaryngeal pain; Pleural effusion; Pleuritic pain; Pneumonitis; Pneumothorax; Voice alteration
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Dry skin; Hyperhidrosis; Pain of skin; Photosensitivity; Pruritus; Purpura; Rash acneiform; Rash maculo-papular; Skin atrophy; Skin hyperpigmentation; Skin hypopigmentation; Skin ulceration
VASCULAR DISORDERS - Flushing; Hot flashes; Hypertension; Hypotension; Superficial thrombophlebitis; Thromboembolic event

Note: Perifosine in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR (Section 7.1.1).

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP home page (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP home page (http://ctep.cancer.gov).

In the rare event when Internet connectivity is disrupted, a 24-hour notification is to be made to NCI by telephone at 301-897-7497 or 301-897-7402 for CIP studies. An electronic report MUST be submitted immediately upon re-establishment of internet connection. **Please note that all paper AdEERS forms have been removed from the CTEP website and will NO LONGER be accepted.**

7.4 Expedited Reporting Guidelines for Phase I – CTEP-AERS Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent on Phase 1 Trials

Phase 1 Trials								
	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]
				Unexpected		Expected		
	Unexpected and Expected	Unex-pected	Expected	with Hospitali-zation	without Hospitali-zation	with Hospitali-Zation	without Hospitali-zation	Unexpected and Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:
- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 4 unexpected events
- Grade 5 expected events and unexpected events

[2] Although an CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

December 15, 2004

7.4.1 <u>Expedited Reporting Guidelines for phase II</u>– CTEP-AERS Reporting Requirements for Adverse Events that occur within 30 Days[1] of the Last Dose of the Investigational Agent on Phase 2 and 3 Trials

Phase 2 and 3 Trials									
	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]	Grades 4 & 5[2]
				Unexpected		Expected			
	Unexpected and Expected	Unex-pected	Expected	with Hospitali-zation	without Hospitali-zation	with Hospitali-zation	without Hospitali-zation	Unex-pected	Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:
- Grade 4 and Grade 5 unexpected events

CTEP-AERS 10 calendar day report:
- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although an CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

December 15, 2004

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

7.3.4 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, certain AEs/grades are exceptions to the Expedited Reporting Guidelines and do not require expedited reporting (*i.e.*, CTEP-AERS). The following AEs must be reported through the routine reporting mechanism (Section 7.4):

AEs assessed by the investigator as possibly, probably, or definitely due to Temsirolimus:

CTCAE Category	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Comments
Blood/Bone Marrow	**↓ Hemoglobin, leukocytes (total WBC), lymphopenia, ↓ neutrophils/granulocytes (ANC/AGC), ↓ platelets**	1-4	No	These AEs do not require expedited reporting unless patient is hospitalized for management
Gastro-intestinal	**Diarrhea, nausea, vomiting, stomatitis**	1-3	Yes	Hospitalization for grade 3 AEs does not require expedited reporting
Metabolic/ Laboratory	**Hypercholesterolemia, hyperglycemia, hypertriglyceridemia**	1-4	No	These AEs do not require expedited reporting unless patient is hospitalized for management

AEs assessed by the investigator as possibly, probably, or definitely due to perifosine of grade 1-4 unless the patient is hospitalized for management

Category	Adverse Event
Constitutional Symptoms	Fatigue (lethargy, malaise, asthenia)
Gastrointestinal	Anorexia
Gastrointestinal	Dehydration
Gastrointestinal	Diarrhea
Gastrointestinal	Flatulence
Gastrointestinal	Nausea
Gastrointestinal	Vomiting
Hepatic	Alkaline phosphatase
Hepatic	GGT (Gamma-Glutamyl transpeptidase)
Pulmonary	Hiccoughs (hiccups, singultus)
Renal/Genitourinary	Creatinine

7.4 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must also be reported in routine study data submissions.**

Note: as in section 12.1: This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be found on the CTEP web site (http://ctep.cancer.gov).

Note: **All** adverse events that have occurred on the study, including those reported through CTEP-AERS, must be reported via CDUS.

7.5 Secondary AML/MDS

Investigators are required to report cases of secondary AML/MDS occurring on or following treatment on NCI-sponsored chemotherapy protocols using CTEP-AERS. Refer to http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm for additional information about secondary AML/MDS reporting.

7.6 Serious Adverse Event (SAE) Reporting at MSKCC

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from the CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)

- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following information:
  - An explanation of how the AE was handled
  - A description of the subject's condition
  - Indication if the subject remains on the study
  - If an amendment will need to be made to the protocol and/or consent form

The PI's signature and the date it was signed are required on the completed report.

8 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7.1.

8.1 CTEP-Supplied Investigational Agents: temsirolimus and perifosine

Temsirolimus (Torisel®) (CCI-779) (NSC#683864)

8.1.1 **Chemical Name:** Sirolimus 42-ester with 2,2-bis (hydroxymethyl)-propionic acid

8.1.2 **Other Names:** CCI-779, Torisel®, Rapamycin analog, WAY-130779

8.1.3 **Classification:** Cell cycle inhibitor

8.1.4 **Molecular Formula:** $C_{56}H_{87}NO_{16}$ **MW:** 1030.30 daltons

8.1.5 **Mode of Action:** Temsirolimus [an ester of the immunosuppressive compound sirolimus, (rapamycin, Rapamune®)] blocks cell cycle progression from the G1 to the S phase by binding to the intracellular cytoplasmic protein, FK506 binding protein (FKBP)12. This complex inhibits activity of the enzyme mTOR (mammalian target of rapamycin), inhibiting translation of several key proteins that regulate progression through the G1 phase in response to growth factors. Sirolimus, temsirolimus's major metabolite, also binds to FKBP12.

8.1.6 **How Supplied:** TORISEL (temsirolimus) is supplied as a commercially labeled kit consisting of the following:

8.1.6.1 TORISEL (temsirolimus) injection (25 mg/mL). The TORISEL vial includes an overfill of 0.2 mL. Inert ingredients in the drug vial include dehydrated alcohol, d,l-alpha-tocopherol, propylene glycol, and anhydrous citric acid.

8.1.6.2 DILUENT for TORISEL. The DILUENT vial includes a deliverable volume of 1.8 mL. The diluent vial contains polysorbate 80 NF, polyethylene glycol 400 NF, and absolute alcohol USP.

8.1.7 **Preparation:** These mixing instructions apply to commercial TORISEL only. The investigationally labeled product is mixed differently.

8.1.7.1 Protect from excessive room light and sunlight during preparation.

8.1.7.2 Follow this two step dilution process (TORISEL should only be diluted with the supplied diluent):

Step 1
Inject 1.8 mL of DILUENT for temsirolimus into the vial of temsirolimus concentrate for injection (25 mg/mL). A total volume of 3 mL (10 mg/mL) will be obtained. Mix well by gentle inversion of the vial. DO NOT SHAKE. Allow sufficient time for air bubbles to subside. The solution should be clear to slightly turbid, colorless to light-yellow in color and free from visual particulates.

Step 2
Withdraw the required amount of temsirolimus from the 10 mg/mL concentrate/diluent mixture prepated in Step 1. For doses less than 10 mg, filter the concentrate/diluent mixture using a syringe filter unit before measuring required volume. Further dilute with 0.9% sodium chloride injection in glass or polyolefin containers to a final concentration between 0.04 mg/mL and 1 mg/mL. Mix by inversion of the bag and avoid excessive shaking. Inspect for visual particulates and discoloration prior to administration.

Route of administration: Intravenous with an appropriate in-line filter (i.e. 0.2 to 5 micron) for all temsirolimus doses equal to or greater than 10 mg. To avoid drug loss, prepare doses by less than 10 mg by filtering the concentrate/diluent mixture as noted previously between steps 1 and 2 using a syringe filter unit.

8.1.8 **Storage:** Refrigerate intact TORISEL kit at 2º-8ºC and protect from light.

8.1.9 **Stability:** The 10 mg/mL drug solution/diluent mixture is stable for 24 hours at room temperature.

Administer within 6 hours of the final dilution in 0.9% NaCl. Store at room temperature (20º-25ºC) and protect from light.

8.1.10 **Route of Administration:** Intravenous with an appropriate in-line filter (i.e. 0.2 to 5 micron) for all temsirolimus doses equal to or greater than 10 mg. Do not use an inline filter for temsirolimus doses less than 10 mg. Protect from light during administration.

8.1.11 **Incompatibilities:** Avoid contact of the diluted product with polyvinyl chloride (PVC) equipment or devices that are plasticized with di- (2-ethylhexyl)pthalate (DEHP) to prevent DEHP leaching. Store diluted temsirolimus solutions in bottles (glass) or plastic bags (polyolefin or polypropylene).

Temsirolimus is compatible with most infusion sets that are acceptable with paclitaxel.

Infusion sets which have been qualified for use with temsirolimus include the following:

- Baxter vented paclitaxel set
- Baxter unvented paclitaxel set
- Abbott #11947 tubing set
- Alaris #72953 tubing set

Other non-PVC tubings can be used with the following in-line filters:

- IV 6200 Disposable I.V. Filter 0.2 micron by EPS®, Inc
- IV 6120 Disposable I.V. Filter 1.2 micron by EPS®, Inc
- LV 5000 Large Volume 5 micron Conical Filter by B.Braun

- Baxter Paclitaxel IV 0.2 micron filter set (2C7555)
- Codan 5 micron monofilter
- Alaris extension filter set #20350E
- *Other polyethersulfone filters may be used.*

8.1.12 **Potential Drug Interactions:**
Temsirolimus is a CYP3A4 substrate. Avoid concomitant treatment of temsirolimus with potent CYP3A4 inhibitors and agents that have CYP3A4 induction potential.

The combination of temsirolimus and sunitinib resulted in dose limiting toxicity at low doses of both agents. Avoid concomitant sunitinib during temsirolimus treatment.

Temsirolimus and warfarin may interact to increase INR. Monitor warfarin patient's PT/INR after starting and stopping temsirolimus.

The combination of temsirolimus and ACE inhibitors resulted in angioedema-type reactions (including delayed reactions occurring up to 2 months after initiation of therapy).

8.1.13 **Patient Care Implications:** For hypersensitivity prophylaxis, give diphenhydramine 25-50 mg I.V. (or comparable antihistamine) approximately 30 minutes before starting temsirolimus infusion. Infuse over 30 minutes. If a patient develops a hypersensitivity reaction despite diphenhydramine pretreatment, stop the infusion and wait 30 to 60 minutes (depending upon the reaction severity). At the physician's discretion, it may be possible to resume treatment by administering an H2 blocker approximately 30 minutes before restarting the infusion. The manufacturer recommends famotidine 20 mg IV, rather than cimetidine, because it lacks reported drug interactions. If famotidine is unavailable, administer ranitidine 50 mg IV. Re-attempt infusion at a slower rate, possibly over one hour.

Vaccinations: Avoid the use of live vaccines during temsirolimus treatment.

8.1.14 **Agent Ordering:** CCI-779 (temsirolimus, Torisel®) may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

CCI-779 (temsirolimus, Torisel®) may be requested electronically to PMB. Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account https://eapps-ctep.nci.nih.gov/iam/ and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.15 **Agent Accountability**
Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD

using the NCI Drug Accountability Record Form (DARF). (See the CTEP home page at http://ctep.cancer.gov for the Procedures for Drug Accountability and Storage and to obtain a copy of the DARF and Clinical Drug Request form.)

Perifosine (NSC # 639966)

8.1.16 **Chemical name:** 1,1-dimethyl-4-[[(octadecyloxy)hydroxyphosphinyl]oxy]-piperidinium inner salt

8.1.17 **Classification:** Alkylphospholipid

8.1.18 **Other names:** Octadecylphosphopiperidine (OPP), D-21266

8.1.19 **Molecular Formula:** $C_{25}H_{52}NO_4P$ **MW:** 461.66 g/mol

8.1.20 **Approximate Solubility:**

- Water: 300 mg/mL
- Methanol: >800 mg/mL
    - 0.1 m HCL: 280 mg/mL
    - 0.1 m NaOH: 320 mg/mL
    - 1-Octanol: 200mg/mL

8.1.21 **Mode of Action:** Perifosine accumulates in cellular membranes and interferes with signal transduction pathway(s)

8.1.22 **How Supplied:** Perifosine is supplied as a film-coated tablet containing 50 mg of active ingredient, in bottles of 30 tablets.

8.1.23 **One film-**coated tablet contains:

- Perifosine 50 mg
- Other ingredients (tablet core):
    - Corn Starch 112mg
    - Microcrystalline Cellulose (Avicel PH 101) 40mg
    - Dibasic Calcium PhosphateColloidal 40mg
    - Silicon Dioxide (Aerosil V 200) 5mg
    - Magnesium Stearate 5 mg
- **Other Ingredients (film coating):** hydroxypropyl methycellulose (Pharmacoat 603), polyethylene glycol 6000, titanium dioxide, simethicone (Silbione antimousse 70454)

8.1.24 **Storage:** Store closed containers at room temperature (20-25° C). Perifosine is hygroscopic, so containers must be kept closed and dry.

8.1.25 *Perifosine tablets must be dispensed in the original container.*

8.1.26 **Stability:** Stability testing program ongoing.

8.1.27 **Route of Administration:** Oral with meal(s).

8.1.28 **Potential Drug Interactions:** None reported. However, perifosine is extensively bound to human serum proteins including albumin and alpha-acid glycoprotein.

8.1.29 **Availability**: Perifosine is an investigational agent supplied to investigators by the Division of Cancer

Treatment and Diagnosis (DCTD), NCI.

Perifosine is provided to the NCI under a Cooperative Research and Development Agreement (CRADA) between Zentaris AG (formerly Asta Medica) and the DCTD, NCI (see Section 12.3).

8.1.30 **Agent Ordering:** NCI-supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Agent may be requested electronically to PMB. Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account https://eapps-ctep.nci.nih.gov/iam/ and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

8.1.31 **Agent Accountability**

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the CTEP home page at http://ctep.cancer.gov for the Procedures for Drug Accountability and Storage and to obtain a copy of the DARF and Clinical Drug Request form.)

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form. See the CTEP web site for Policy and Guidelines for Accountability and Storage of Investigational Drugs (http://ctep.cancer.gov/requisition/storage.html).

8.2 Other Investigational Agent(s): N/A

8.3 Commercial Agent(s): N/A

9 CORRELATIVE/SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 All patients – molecular profiling of archival tissue

9.1.1.1 *Collection of Specimen(s):*

Pre-treatment tissue will be collected in all patients. If available, this will be from the surgery that occurred most recently in relationship to study registration. However, specimens from other prior surgery(s) may also be collected if available. For patients who develop tumor progression while

on study and undergo re-operation, tissue resected following disease progression will be used to determine molecular correlates of treatment failure.

A representative paraffin tissue block (at least 5 mm x 5 mm) or at least 15 unstained slides (preferably 30) will be requested from the Department of Pathology at Memorial Sloan Kettering Cancer Center (MSKCC) for patients who underwent a prior surgery at MSKCC. For patients who underwent surgery elsewhere, the block (or 30 unstained slides if the block is unavailable) will be requested and sent as 5.1.3.1 and in 9.1.1.3.

Patients undergoing tumor resection at MSKCC routinely have tissue flash frozen in liquid nitrogen immediately after removal and stored at -70 degrees Celsius. When available, such tissue will also be procured from the brain tumor bank of the MSKCC Brain Tumor Center.

Optional: One tube of blood will also be collected before starting treatment and research for future research purposes.

9.1.1.2 *Handling of Specimens(s):*
Paraffin blocks will be cut by the Department of Pathology into unstained slides using standard procedures for routine clinical testing by immunohistochemistry and FISH. Unstained slides will also be handled according to routine procedures at room temperate.

9.1.1.3 *Shipping of Specimen(s):*
For patients who underwent surgery at a center other than MSKCC, a representative paraffin tissue block (at least 5 mm x 5 mm) or at least 15 unstained slides (preferably 30) will be requested and shipped to the PI, Thomas Kaley, MD by overnight courier at room temperature.

9.1.1.4 *Site Performing Correlative Studies:*
FISH analysis will be performed in the FISH laboratory in the Pathology Department at Memorial Sloan Kettering Cancer Center and other assays in the laboratories of Cameron Brennan, MD and Eric Holland, MD, PhD at Memorial Sloan Kettering Cancer Center or their designee(s).

The following studies will be performed:

- FISH for *EGFR* amplification, *PDGFRA* amplification, and *NF1* loss and others
- Immunohistochemistry for NF1 under-expression, PDGF ligand, PTEN, AKT, pAKT, mTOR, p-mTOR, S6K, pS6K, ERK, pERK, and Ki67 and others
- Western blot of homogenized flash frozen tissue (when available) for NF1, PDGF (ligands), PTEN, AKT, pAKT, S6K, pS6K, ERK, pERK, and proliferating cell nuclear antigen (PCNA) and others if immunohistochemistry fails for these markers.
- TUNEL

If sufficient tissue is available (paraffinized and/or frozen), we will also perform

- RNA extraction for RTPCR analysis of DLL3, SOX4, NCAM1, and Doublecortin overexpression and others
- DNA extraction for mutational analysis of *PTEN*, *AKT*, *PI3K*, *mTOR*, *EGFR*, *NF1*, and others

9.1.2 Molecular effects during treatment

The evaluation of the in vivo biological activity of these agents will be based on a within patient comparison of PI3K/AKT/mTOR/S6K signaling and cell proliferation in pre-treatment archived tissue to the tissue acquired during treatment as part of the phase II surgical substudy. The therapy will be considered molecularly effective if the phosphorylation of PI3K/AKT/mTOR/S6K is decreased by at least one level based on the pathology grading for those markers which are positive in the pretreatment tissue for

that patient. (See discussion of the assays to be used in the laboratory correlates section.) Given the small number of patients who will undergo surgery, these studies will also be considered exploratory.

9.1.2.1 *Collection of Specimen(s)*:
see section 5.1.3.2

9.1.2.2 *Handling of Specimens(s)*:
see section 5.1.3.2

9.1.2.3 *Shipping of Specimen(s)*:
Not applicable (all resections on the surgical substudy will occur at Memorial Sloan Kettering Cancer Center)

9.1.2.4 *Site Performing Correlative Study:*
All assays will be performed in the laboratories of Cameron Brennan, MD and Eric Holland, MD, PhD at Memorial Sloan Kettering Cancer Center or their designee(s).

As in 9.1.1, assays for effects on AKT and RAS signaling, proliferation, and apoptosis will be performed including: immunohistochemistry for PTEN, AKT, pAKT, mTOR, p-mTOR, S6K, pS6K, ERK, pERK, Ki67; TUNEL; Western Blot for PTEN, AKT, pAKT, S6K, pS6K, ERK, pERK, and proliferating cell nuclear antigen (PCNA).

9.2 Special Studies: N/A

10 STUDY CALENDAR

	Pre-Study	Wk 1[i]	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12 , etc	Off Study
Temsirolimus and perifosine[a]		X	X	X	X	X	X	X	X	X	X	X	X	
Informed consent	X													
Demographics	X													
Medical history	X					X				X				X
Concurrent meds	X	X----------											----------X	
Physical/Neuro exam	X					X				X				X
Vital signs	X	X				X				X				X
Height	X													
Weight	X	X				X				X				X
Performance status	X	X				X				X				X
CBC w/diff, plts[b]	X		X	X	X	X		X		X		X		X
PT, PTT, INR[c]	X		X[c]	X[c]	X[c]	X[c]								
Serum chemistry[d,e]	X		X	X	X	X		X		X		X		X

Serum lipids[f]	X		X	X	X	X		X		X		X		X
Adverse event evaluation		X ----------X												X
Tumor measurements	X	Tumor measurements are repeated every 8 weeks (May be performed up to 2 weeks early)												X
Radiologic evaluation[g]	X	Radiologic measurements should be performed every 8 weeks (May be performed up to 2 weeks early)												X
B-HCG	X[h]					X				X				

a: Temsirolimus administered weekly, Perifosine administered daily.
b: Following the 1st treatment: Weekly for first 4 weeks and then every 2 weeks. Phase II patients with stable bloodwork after 16 weeks can have these laboratory tests monthly.
c: Following the 1st treatment: For patients on warfarin, every 1-2 weeks, or sooner, if clincically indicated; otherwise, at the discretion of the treating physician.
d. Following the 1st treatment: Alkaline phosphatase, total bilirubin, carbon dioxide, BUN, calcium, chloride, creatinine, glucose, phosphorus, sodium, potassium, total protein, SGOT[AST], SGPT[ALT] weekly for 4 weeks then every 2 weeks. Phase II patients with stable bloodwork after 16 weeks can have these laboratory tests monthly. Uric acid should be checked at baseline and then during treatment as clinically indicated.
e. Patients with diarrhea should have more frequent monitoring of electrolytes at the discretion of the investigator.
f. Following the 1st treatment: Fasting triglycerides and cholesterol weekly for first 4 weeks then every 2 weeks. Phase II patients with stable bloodwork after 16 weeks can have these laboratory tests monthly.
g. MRI or CT at baseline and every 8 weeks (may be performed up to 2 weeks early). **A**n FDG-PET scan should be performed after 1-2 weeks of therapy and every 4th cycle (16 weeks). **A NON-CONTRAST HEAD CT OR AN MRI WITH GRADIENT ECHO SEQUENCE MUST ALSO BE PERFORMED DURING WEEK 2 TO EVALUATE FOR HEMORRHAGE (see 5.1.3).**
h: Serum pregnancy test (women of childbearing potential).
i. Note: all tests and evaluations performed for baseline can also serve as those required for week 1, as long as they are within the starting timeframes of treatment initiation.

NOTE: All laboratory tests, assessments, and treatments are permitted a window of +/- 3 days to accommodate patient scheduling, weekends, and/or holidays

11 MEASUREMENT OF EFFECT

11.1 Antitumor Effect

The primary endpoint of this study is a hybrid of: 6-month progression free-survival (with the start date defined as the first treatment dose in the phase II component for patients not on the surgical substudy, and from the first post-operative treatment dose for patients enrolled on the phase II surgical substudy) and radiographic response (among patients with measurable disease at the start of treatment). Patients enrolled to the phase I component who are treated at the MTD and otherwise qualify for the phase II study will be included in all phase II efficacy analyses.

As the primary efficacy analysis includes prolonged disease stabilization as a success (6mPFS), patients without measurable disease are eligible whether they enroll post-operatively following a gross-total resection or whether they undergo a gross total resection as part of the phase II surgical substudy. However, for patients with measurable disease either at the start of treatment (at study entry or post-operatively on the surgical substudy), radiographic responses will be measured.

For the purposes of this study, patients should be re-evaluated for response approximately every 8 weeks. The same technique (CT or MRI) must be used for intrapatient comparisons throughout the study.

11.2 Definitions

Evaluable for toxicity: All patients who receive any study treatment will be evaluable for toxicity from the time of their first treatment with temsirolimus and/or perifosine.

Evaluable for 6-month progression-free survival: All patients who receive any study treatment on the phase II study and those on the phase I treated at the MTD who otherwise qualify for the phase II will be evaluable for 6mPFS except those that are removed from the study before the end of cycle 1 for reasons other than clinical progression (such as toxicity). Patients who suffer clinical progression without radiographic confirmation of progression will be considered to have progressive disease in determination of 6mPFS. Clinical progression requires the absence of other causes of decline, such as anticonvulsant toxicity or overly rapid corticosteroid taper, and that steroids be increased to a prior level before scoring as progression.

Evaluable for radiographic response (CR or PR): Only those patients who have measurable disease (defined below) present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated radiographically will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. Patients with measurable disease at study entry who suffer clinical progression without radiographic confirmation of progression will be considered to have progressive disease in determination of radiographic response rate. Clinical progression requires the absence of other causes of decline, such as anticonvulsant toxicity or overly rapid corticosteroid taper, and that steroids be increased to a prior level before scoring as progression.

Disease will be defined as either measurable or non-measurable:
Measurable Disease: Bi-dimensionally measurable lesions with clearly defined margins by CT or MRI scan of at least 1 cm by 1 cm. For patients on the surgical substudy, the baseline study used to determine existence of measurable disease will be either the post-operative MRI or a later MRI used as the new baseline before re-starting therapy post-operatively. Note that patients who undergo gross total resection, either on the phase II surgical substudy or immediately pre-enrollment not as part of the protocol, have non-measurable disease. The surgical cavity without a measurable mass of tumors should not be defined as measurable.

Objective Status, To Be Recorded at Each Evaluation: If there are too many measurable lesions to measure at each evaluation, choose the largest two to be followed before a patient is entered on study.

Progression-Free Survival: Duration of time from the date of the start of treatment to the date of progression or death from any cause.

Overall survival: Duration of time from the date of start of treatment to date of death from any cause.

Start of treatment: Date of the first treatment dose for all patients except those on the surgical substudy of phase II for whom it is defined as the date of the first post-operative drug administration.

11.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers or electronically using imaging software. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 2 weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

11.4 Response Criteria

Response and progression will be evaluated in this study using standard criteria for patients with malignant gliomas.(Macdonald, Cascino et al. 1990) A major difference from RECIST and other criteria for measuring response in solid tumors is the requirement that patients be on a stable or decreasing dose of corticosteroids when evaluating for response because of the potentially confounding impact of corticosteroids on contrast enhancement during brain tumor imaging by CT(Cairncross, Macdonald et al. 1988) or MRI(Watling, Lee et al. 1994) scans. The tumor size will be measured in

millimeters and is the largest cross-sectional area using perpendicular measurements of contrast enhancing abnormality.

If there are too many measurable lesions to measure at each evaluation, choose the largest two to be followed before a patient is entered on study. The remaining lesions will not be considered measurable for the purpose of objective status determination. Unless progression is observed, objective status can only be determined when ALL measurable and sites are assessed.

Complete Response (CR): Complete disappearance of all measurable disease and all non-measurable disease using the same technique (MRI or CT) as baseline. Patients must be on no steroids. Confirmed on follow up MRI/CT at least 1 month later.

Partial Response (PR): Greater than or equal to 50% decrease **under baseline** in the sum of products of perpendicular diameters of all measurable lesions. No progression of non-measurable disease. No new lesions. All measurable and non-measurable lesions and sites must be assessed using the same techniques as baseline. The steroid dose at the time of the scan evaluation should be no greater than the maximum dose used in the first 8 weeks from initiation of therapy. Confirmed on follow up MRI/CT at least 1 month later.

Stable/No Response: Does not qualify for CR, PR, or progression. All measurable and non-measurable sites must be assessed using the same techniques as baseline. The steroid dose at the time of the scan evaluation should be no greater than the maximum dose used in the first 8 weeks from initiation of therapy.

Progression: 25% increase in the sum of products of all measurable lesions over **smallest sum observed during treatment** (which may be smaller than baseline) using the same techniques as baseline, OR clear worsening of any non-measurable disease, OR appearance of any new lesion/site, OR clear clinical worsening or failure to return for evaluation due to death or deteriorating condition (unless clearly unrelated to this cancer).

Unknown: Progression has not been documented and one or more measurable or non-measurable site(s) have not been assessed.

Best Overall Response: For patients with all disease sites assessed every evaluation period, the best response will be defined as the best objective status as measured according to Section 11.4. If the response does not persist on an MRI at least 1 month later, the response will still be recorded based on the prior scan, but will be designated as a non-sustained response. If the response is sustained, e.g. still present on the subsequent MRI, it will be recorded as a sustained response, lasting until the time of tumor progression. Best response is unknown if the patient did not have measurable disease either at study entry or post-operatively for patients on the surgical substudy of phase II or if all objective status determinations before progression are unknown.

12 DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

Method: This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31, and October 31. Instructions for submitting data using the CDUS can be

found on the CTEP web site (http://ctep.cancer.gov). **Note**: **All** adverse events that have occurred on the study, including those reported through CTEP-AERS, must be reported via CDUS.

Responsibility for Data Submission: N/A (single institution study)

12.2 CTEP Multicenter Guidelines: N/A

12.3 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industry) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements , the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data."):

    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.

    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used, and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Email: ncicteppubs@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13 STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

Phase I:
Up to 42 patients will be enrolled in the phase I portion of this study (6 patient X 7 cohorts= 42 total patients). A standard phase I design will be used to determine the MTD of combined perifosine and temsirolimus. To determine the MTD, a dose escalation scheme with patients entering in cohorts of 3 at each dose level will be used. The MTD will be defined as the dose at which fewer than one-third of patients experience a DLT.

The dose of temsirolimus will be escalated in groups of 3 patients, with 3 additional patients enrolled at the first sign of a DLT. There will be no intra-patient dose escalation. If 0 of 3 patients experience a DLT at a dose level, then 3 patients will be enrolled at the next dose level. If 1 of 3 patients experiences a DLT at a dose level, then 3 additional patients will be enrolled at the same dose level. If 1 of 6 patients experiences a DLT at that dose level, then 3 additional patients will be enrolled at the next dose level. However, if 2 or more of 6 patients (or 3 or more of 9 patients, etc.) at a dose level experiences a DLT, then the MTD is defined as the previous dose level. Six patients will be treated at a dose level being considered as the MTD. A cohort may be expanded to more than 6 patients if the PI and CTEP medical monitor agree more information is needed to clarify whether a DLT has occurred or the MTD has been reached.

If the first dose level exceeds the MTD, then the dose will be de-escalated to level -1. If one-third or more of patients experience DLT at level -1, then the level will be reduced to level -2, etc. Using this

scheme, the probability of escalating to the next higher dose level, based on the true rate of DLT at the current dose, is:

	True Toxicity at a Given Dose					
	10%	20%	30%	40%	50%	60%
Probability of Escalating	.91	.71	.49	.31	.17	.08

Therefore, if the true underlying proportion of DLTs is 50% at a given dose level, then there is a 17% chance of escalating to the next dose. Baseline characteristics will be summarized across all enrolled patients.

Patients completing 28 days of therapy or patients removed for toxicity earlier than 28 days are evaluable. Patients removed from study within 28 days for reasons other than toxicity may be replaced.

An accounting will be done of all patients registered in the study. The number of patients who died or withdrew before treatment began will be specified. Patients who did not meet all eligibility criteria will be described. Patients who were evaluated separately due to first-cycle non-drug-related death or cessation of first-cycle therapy prior to DLT will be characterized. Treatment administration will be described for all cycles. Doses administered, dose modifications or delays, and duration of therapy will be evaluated. Safety variables will be summarized by descriptive statistics. Adverse events that occur will be reported for each dose level and described in terms of incidence and severity. Laboratory data will be presented by dose level at each observation time. Values outside of normal limits will be identified and their frequency calculated. Parameters will be described based on the CTCAE version 4.0 severity grading. Distribution by CTC severity grade (when applicable) and clinical relevance will be given.

Phase II:

The primary clinical objective of this phase of the study will be to determine if temsirolimus and perifosine in combination can significantly delay progression and/or induce tumor responses in patients with recurrent malignant gliomas. The trial cohort will include both GBM and Anaplastic Glioma (AG) patients. The primary endpoint is a hybrid endpoint of progression-free survival (PFS) at 6 months and radiographic response. The calculation of PFS for patients will be determined from the first day of study drug administration except for those in the surgical substudy for whom it will be calculated from the first day of study drug administration following surgery.

The historical values for comparison are from a database of 225 GBM patients enrolled in 8 previous phase II studies (in which none of the treatments were considered particularly effective).(Wong, Hess et al. 1999) In this database, the 6 month PFS was 15% for GBM. The 6 month PFS for patients treated with BCNU for recurrence/progression was 17.5%.(Brandes, Tosoni et al. 2004) Therefore, we would expect 6 month PFS to be less than 20% in molecular unselected recurrent GBMs.

However, these ineffective 6mPFS rates of 15%-20% were derived from trials that accrued patients without regard to molecular profiling, and without accepting radiographic responses as evidence of efficacy. The modeled GBMs in mice used in the pre-clinical testing of combined therapy with temsirolimus and perifosine are driven by forced PDGF ligand expression. Therefore, this therapy may be more effective in human tumors also exhibiting a PDGF-like signaling profile than in other tumors. Molecular profiling work from The Cancer Genome Atlas and at Memorial Sloan Kettering demonstrates 3 main profiles of GBMs: PDGF, EGFR, and NF1. The accrual for this study will include a mixture of enriched patients that present with the PDGF-like molecular profile and nonenriched patients that do not have this profile. It is expected that the accrual ratio of nonenriched to

enriched patients will be 3:1 because tumors of other molecular profiles are approximately 3 times as common as those with the PDGF-like profile. We will profile tumors in all patients (phase I and II) and may observe a higher level of efficacy among patients with tumors exhibiting the enriched profile.

For the phase II study, a two-stage design will be used to assess treatment activity for this mixture patient population. For the purpose of this study, a patient will be defined as a success if he/she remains alive and progression free for six months from the start of treatment or attains a radiographic response. We will set the insufficient success rates at ≤0.25 for the enriched patient population and ≤0.15 for the non-enriched patient population. Sufficient success rates for the enriched and nonenriched populations are set at ≥0.45 and ≥0.35, respectively. In the first stage, 20 patients will be enrolled in the study. If ≤4 successes (either response or 6mPFS) are observed, then the trial will be stopped. If ≥5 successes are observed, then 22 additional patients will be accrued, for a total of 42 patients.

At the end of the second stage, if ≤9 patients with GBM reach 6 months of progression-free survival, then, we will conclude that the treatment is not sufficiently active in recurrent/progressive GBMs. The design has power 0.90 for a population success proportion of 0.45/0.35 in the enriched/nonenriched populations, using a 0.10 size test for success rates of 0.25/0.15 in the enriched/nonenriched populations.

In addition to the assessment of the population success rate in this population, the crude incidence rates for the probability of remaining alive and progression free for six months, patient response, and toxicity will be computed along with the attendant 95% confidence intervals. Kaplan-Meier estimates of the progression-free and overall survival distributions will be computed as a function of time in the whole group and stratified by molecular profile.

Although it is estimated that 25% of patients will be enriched patients, we do not stratify accrual based on this factor. If the treatment regimen appears promising given the observed mixture of enriched/nonenriched patients then additional patients may be accrued in order to obtain 10 enriched patients. Outcomes (e.g., response, progression rate, toxicity) will be summarized descriptively for these patients.

Anaplastic gliomas

Up to 10 patients with anaplastic gliomas will be accrued to the phase II study. Endpoints such as response, median overall survival, and median progression-free survival will be computed in order to gain preliminary data for these other malignant glioma subtypes. However, the historical controls for patients with anaplastic glioma are less well defined than for GBM; therefore, only patients with GBM will be included in analyses of efficacy. Archival tissue will be profiled to explore whether profiling influences response in such patients.

Prior treatment with bevacizumab or other direct inhibitors of VEGF/VEGFR including Aflibercept (VEGF-Trap) and cedirinib and XL-184 (BMS 907351).

There is no limit on such therapy for the phase I study for which determination of MTD is the primary endpoint. For the phase II study, however, the historic control data available for efficacy goals after treatment with bevacizumab or other similar therapy is extremely limited. For example, the only such data that exists is from two retrospective analyses, currently published only in abstract form: one of 44 patients at Dana Farber Cancer Center (Quant *et al*., 2008) and the other of 14 patients at Memorial Sloan Kettering Cancer Center (Lassman *et al*., 2008). The historic control data sets typically used for recurrent GBM trials (Wong *et al*., 1999; Lamporn, et al., 2008) did not include patients treated with bevacizumab or similar drugs. Therefore, it is difficult to reasonably estimate efficacy goals for this population. In addition, discontinuation of such agents can lead to rapid clinical deterioration and rebound contrast enhancement that can confound MRI interpretation.

However, it is possible that during the course of this study, the use of bevacizumab or other direct VEGF/VEGFR inhibitors will become routine for treatment of gliomas. Therefore, to explore efficacy of the experimental regimen in this protocol following treatment with bevacizumab or other VEGF/VEGFR directed agents, we will accrue up to 15 patients who received prior treatment with direct VEGF/VEGFR inhibitors in order to gain preliminary data for use as a comparison group in a follow up study. Although there are not a sufficient number of patients in each group to formally test treatment efficacy, the clinical outcomes for these patients will be summarized at the conclusion of the study. Endpoints such as response, median overall survival, and median progression-free survival will be computed,. However, only patients with GBM who are naïve to prior treatment with direct VEGF/VEGFR inhibitors will be included in analyses of efficacy. Archival tissue will be profiled to explore whether profiling influences response in such patients.

Surgical substudy

In order to obtain specimens for study of molecular effects of drug therapy in tumor tissue, we plan to accrue up to 10 subjects to a preoperative treatment arm. Although patients on the surgical substudy will have resections of tumor at the initiation of treatment, the natural history of recurrent GBM strongly supports the concept that nearly all patients will have tumor progression by 6 months if no further treatment or ineffective treatment is provided post-operatively. For example, a review of patients with recurrent GBM treated for first relapse at the MD Anderson Cancer Center also supports the concept that gross total resection does not impact on 6-month PFS rate. During the time from of 2001-2003, 220 patients were treated at MD Anderson for first relapse or progression of GBM. There was no difference in 6-month PFS between those patients who had a gross total resection at relapse compared with all other patients. (Mark Gilbert, personal communication). Hence, we plan to combine data from patients on and not on the surgical substudy in efficacy analyses. The calculation of PFS for patients enrolled in the pre-operative component of the study will be determined from the first day of study drug administration following surgery.

Molecular profiling of pre-treatment tissue

The analysis of the biological correlate data has the overall goal of providing an increased understanding of the nature of the response to temsirolimus and perifosine, and how it may be influenced by genetic characteristics of the tumor. The amount of data available for the various measures is uncertain. Assays utilizing historical paraffin tissues should be relatively complete, although the information may be limited by the impact of intervening treatment. The nature of the analyses and the strength of the conclusions from these laboratory studies depend not only on the amount of data available, but also on the nature of patient response to therapy. If a substantive response to treatment is found, there will be more opportunity to detect biological correlates. If the response is not favorable, the data may be used to understand better the reason for the failure. Thus it is difficult to pre-specify the nature of the analyses and all should be considered exploratory. However, as in section 2.5, archival tissue will undergo molecular analysis for PI3K/AKT/mTOR/S6K and RAS/MEK/ERK signaling; proliferation; apoptosis; and categorized as PDGF, EGFR, or NF1-like if possible. As above, we may observe more activity among tumors with PDGF than other profiles, and efficacy goals are higher for this group.

Slides analyzed by immunohistochemistry will be scored for immunostaining on a 4 point scale (0-3) analogous to that developed by others. (Choe *et al.* 2003) Band intensity on Western blot will be similarly scored on a 4 point scale as we have done previously (Lassman *et al.* 2005). Gene amplification by FISH will be defined as the presence of >4 gene copies to avoid categorizing tumors with polysomies as containing genes that are amplified For example, polysomy for chromosome 7 is a relatively common finding in GBMs, leading to the presence of 4 copies, but does not correlate with *EGFR* amplification. Finally, tumor DNA will be extracted from pre-treatment tissue and AKT, PI3K, PTEN, RAS, and ERK will be sequenced in all responders to identify mutations that may increase

perifosine sensitivity. Such sequencing is crucial because it is an emerging paradigm that mutations in the genes encoding the targets of small molecule inhibitors can sensitize those targets to their inhibitors including mTOR inhibitors such as temsirolimus in myeloma (Shi *et al*., 2002) and GBM (Smith *et al*., 2002) as MSKCC investigators have demonstrated in both non-small cell lung cancer (Pao *et al*., 2004) and glioblastoma (Mellinghoff *et al.* 2005; Cloughesy *et al.* 2007)

We hypothesize that patients who respond to combination therapy of temsirolimus and perifosine or with at least 6 months of progression free-survival will have pre-treatment tumor tissue exhibiting high levels of AKT and RAS/ERK signaling because we hypothesize that these targets must be active for drug action, and because these signal transduction cascades are activated in the mouse GBM model system used in the ascertainment of the preclinical data. As above, we hypothesize that tumors exhibiting a PDGF-like molecular profile may also exhibit greater sensitivity to the study treatment because the mouse GBM model used is a PDGF-ligand driven tumor.

Molecular effects during treatment

The evaluation of the in vivo biological activity of these agents will be based on a within patient comparison of PI3K/AKT/mTOR/S6K signaling and cell proliferation in pre-treatment archived tissue to the tissue acquired during treatment as part of the phase II surgical substudy. The therapy will be considered molecularly effective if the phosphorylation of PI3K/AKT/mTOR/S6K is decreased by at least level by immunohistochemistry (and Western blot when sufficient frozen tissue is available for analysis) based on the pathology grading for those markers which are positive in the pretreatment tissue for that patient. (See discussion of the assays to be used in the laboratory correlates section.) Effects on proliferation will be assessed by counting mitoses on standard H&E staining, and by Ki-67 immunostaining to estimate the proliferation index (% of tumors staining per HPF). Effects on apoptosis (TUNEL) will also be analyzed in paraffin embedded tissue. Tissue data will be compared to data from our perifosine monotherapy trial (analysis underway) to determine molecular correlates of perifosine alone versus perifosine + temsirolimus. Given the small number of patients (maximum 10) who will undergo surgery, these studies will also be considered exploratory.

13.2 Sample Size/Accrual Rate

It is anticipated that the many dose levels of phase I will not be needed, that phase I will likely include patients with AG, and that patients treated on Phase I at the MTD who are otherwise eligible for Phase II will count toward Phase II accrual goals and be included in phase II efficacy analyses. Therefore, although maximum enrollment is up to 92 patients (42 in phase I, 42 with GBM in phase II, 10 with AG if not already accrued to phase I and II), we anticipate the number of accrued patients will be far less.

For phase II, we anticipate accrual of 3 patients per month. Therefore, it is anticipated that the first 20 on phase II stage I patients will be accrued in approximately 9 months. In order to increase accrual rapidity, enrollment of patients to stage 2 will open and continue while patients on stage 1 are followed for response and PFS, and we will accept non-sustained radiographic responses as evidence of activity in stage 1 to fully accrue stage 2 (above). However, if accrual to stage 1 is halted for lack of efficacy, then enrollment to stage 2 will terminate, and any patients still on trial with any histology may continue until progression or death at the discretion of the investigator, PI, and CTEP medical monitor.

Patients removed for toxicity are evaluable. Phase I patients removed from study within 28 days forreasons other than toxicity may be replaced.

Accrual Targets								
Ethnic Category	**Sex/Gender**							
	Females			**Males**			**Total**	
Hispanic or Latino	4		+	4		=	8	
Not Hispanic or Latino	12		+	22		=	34	
Ethnic Category: Total of all subjects	16	(A1)	+	26	(B1)	=	42	(C1)
Racial Category								
American Indian or Alaskan Native	0		+	0		=		
Asian	4		+	4		=		
Black or African American	4		+	4		=		
Native Hawaiian or other Pacific Islander	0		+	0		=		
White	8		+	18		=		
Racial Category: Total of all subjects	16	(A2)	+	26	(B2)	=	42	(C2)
	(A1 = A2)			(B1 = B2)			(C1 = C2)	

Accrual Rate: 3 pts/month **Total Expected Accrual:** 3 Min 92 Max

13.3 Stratification Factors

The following cohorts will be accrued:

Study Phase: Phase I or Phase II

Prior direct VEGF/VEGFR inhibitor: Yes or No

histologically documented malignant glioma: Yes or No

Histology at Registration:

1) Glioblastoma (GBM), with the additional designation of:

- GBM
- Gliosarcoma (GS)
- GBM with oligodendroglioma features
- Giant cell GBM

2) Anaplastic Glioma (AG) with the additional designation of:

- Anaplastic Astrocytoma (AA)
- Anaplastic Oligodendroglioma (AO)
- Anaplastic Oligo-astrocytoma (AOA)
- High grade glioma NOS (Difficult to subclassify)

Surgical substudy (phase II):	Yes or No
Measurable disease at registration for non-surgical patients, post-operatively for surgical substudy patients:	Yes or No
Molecular profile of baseline tissue:	Enriched (PDGF) or non-enriched (other) with the additional designation for non-enriched of: • EGFR • NF1 • Undetermined/Other Note this will be assessed in all patients but will not be determined prior to registration and treatment initiation.

13.4 Analysis of Secondary Endpoints

Radiographic response rate will be reported according to the definitions above (Section 11.4). This will be reported using all eligible patients who receive at least 1 dose of protocol therapy with temsirolimus and/or perifosine, and another section will detail the response rate for evaluable patients only (those with measurable disease at study entry or post-operatively among those enrolled on the phase II surgical substudy). A response rate analysis on this subset will include an explanation of which patients were excluded from the analysis and for which reasons. Calculations of 95% confidence limits will be given where applicable. Median overall and progression-free survival will be calculated and include all eligible patients who received at least 1 dose of protocol therapy with temsirolimus and/or perifosine. Biologic correlative goals include molecular analysis of pre-treatment tissue to identify both molecular predictors of response (using Fishers exact test) and molecular effects during treatment (using Wilcoxon rank sum test) as described in section 2.5 and section 9.

13.5 Reporting and Exclusions

13.5.1 **Evaluation of toxicity.** All patients will be evaluable for toxicity from the time of their first treatment with temsirolimus or perifosine.

13.5.2 **Evaluation of efficacy.** All patients who receive any treatment with temsirolimus or perifosine will be assessed for the primary efficacy endpoint of 6 month progression-free survival, even if there are major protocol treatment deviations or if they are ineligible.

13.5.3 **Response rate:** All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible.

Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the “unknown” status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria and who have measurable disease except those who received no study medication will be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug

administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

14 REFERENCES

Armistead, D., and M.W. Harding. (1993). Immunophilins and immunosuppressive drug action. *Ann Rep Med Chem*. 28:207.

Arthur, G. and R. Bittman. (1998). The inhibition of cell signaling pathways by antitumor ether lipids. *Biochim Biophys Acta.* 1390:85-102.

Ansell, S.M., Geyer, S.M., Kurtin, P.J., *et al*. (2006) Anti-tumor activity of mTOR inhibitor temsirolimus for relapsed mantle cell lymphoma: A phase II trial in the North Central Cancer Treatment Group. *J Clin Oncol 2006 ASCO Annual Meeting Proceedings Part 1* 24(18S (June Suppl)):Abstract 2732.

Atkins, M.B., M. Hidalgo, W.M. Stadler, *et al*. (2004). A randomized phase 2 study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J Clin Oncol.* 22:909-918.

Barbet, N.C., U. Schneider, S.B. Helliwell, *et al.* (1996). TOR controls translation initiation and early G_1 progression in yeast. *Mol Biol Cell*. 7(1):25-42.

Brandes, A. A., A. Tosoni, *et al*. (2004). How effective is BCNU in recurrent glioblastoma in the modern era? A phase II trial. *Neurology* 63(7): 1281-4.

Brandes, A. A., E. Franceschi, *et al*. (2008). Epidermal growth factor receptor inhibitors in neuro-oncology: hopes and disappointments. *Clin Cancer Res* 14(4): 957-60.

Brown, E.J., M.W. Albers, T.B. Shin, *et al*. (1994). A mammalian protein targeted by G_1-arresting rapamycin-receptor complex. *Nature*. 369:756-758.

Brown, E.J., and S.L. Schreiber. (1996). A signaling pathway to translational control. *Cell*. 86:517-520.

Chan, S., Scheulen, M.E., Johnston, S., *et al.* (2005) Phase II study of temsirolimus (CCI-779), a novel inhibitor of mTOR, in heavily pretreated patients with locally advanced or metastatic breast cancer. *J Clin Oncol* 23(23):5314-22.

Cairncross, J. G., D. R. Macdonald, *et al.* (1988). Steroid-induced CT changes in patients with recurrent malignant glioma. *Neurology* 38(5): 724-6.

Chang, S.M., J. Kuhn, P. Wen, *et al*. (2004). Phase I/pharmacokinetic study of CCI-779 in patients with recurrent malignant glioma on enzyme-inducing antiepileptic drugs. *Invest New Drugs*. 22:427-435.

Chang, S. M., P. Wen, *et al.* (2005). Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme. *Invest New Drugs* 23(4): 357-61.

Choe, G., S. Horvath, *et al.* (2003). Analysis of the phosphatidylinositol 3'-kinase signaling pathway in glioblastoma patients in vivo. *Cancer Res* 63(11): 2742-6

Clarke, J. L., L. E. Abrey, *et al*. (2008). Pseudoprogression (PsPr) after concurrent radiotherapy (RT) and temozolomide (TMZ) for newly diagnosed glioblastoma multiforme (GBM) [abstract 2025]. J Clin Oncol **26**(May 20 suppl): abstr 2025.

Cloughesy, T. F., K. Yoshimoto, *et al*. (2008). Antitumor activity of rapamycin in a Phase I trial for patients with recurrent PTEN-deficient glioblastoma. *PLoS Med* 5(1): e8.

Dancey, J.E. (2002). Clinical development of mammalian target of rapamycin inhibitors. *Hematol Oncol Clin North Am*. 16:1101-1114.

Farag, S.S., Zhang, S., Miller, M., *et al.* (2006) Phase II trial of temsirolimus (CCI-779) in patients with relapsed or refractory multiple myeloma (MM): Preliminary results. J *Clin Oncol 2006 ASCO Annual Meeting Proceedings Part 1*. 24:Abstract 7616.

Dudkin, L., M.B. Dilling, P.J. Cheshire, *et al*. (2001). Biochemical correlates of mTOR inhibition by the rapamycin ester CCI-779 and tumor growth inhibition. *Clin Cancer Res.* 7:1758-1764.

Hilgard, P., T. Klenner, J. Stekar, *et al*. (1997). D-21266, a new heterocyclic alkylphospholipid with antitumor activity. *Eur J Cancer.* 33:442-446.

Hudes, G., Carducci, M., Tomczak, P., *et al.* (2006) A phase 3, randomized, 3-arm study of temsirolimus (TEMSR) or interferon-alpha (IFN) or the combination of TEMSR + IFN in the treatment of first-line, poor-risk patients with advanced renal cell carcinoma (adv RCC). *J Clin Oncol 2006 ASCO Annual Meeting Proceedings Part I.* 24(8S (June 20 Supplement)):Abstract LBA4.

Galanis, E. Buckner, J.C., Maurer, M.J., *et al.* (2005) Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol* 23(23):5294-304.

Geoerger, B., K. Kerr, C-B. Tang, *et al*. (2001). Antitumor activity of the rapamycin analogue CCI-779 in human primitive neuroectodermal tumor/medulloblastoma models as single agent and in combination chemotherapy. *Cancer Res*. 61:1527-1532.

Hegi, M. E., A. C. Diserens, *et al.* (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 352(10): 997-1003.

Hidalgo, M., and E.K. Rowinsky. (2000). The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. *Oncogene*. 19:6680-6686.

Hidalgo, M., M.B. Atkins, W.M. Stadler, *et al*. (2003). A randomized double-blind phase 2 study of intravenous (IV) CCI-779 administered weekly to patients with advanced renal cell carcinoma (RCC): Prognostic factor analysis. *Proc Am Soc Clin Oncol*. Abstract 804.

Holland, E. C., J. Celestino, *et al.* (2000). Combined activation of Ras and Akt in neural progenitors induces glioblastoma formation in mice. *Nat Genet* 25(1): 55-7.

Investigator's Brochure (Temsirolimus, CCI-779) 2007. Wyeth Research.

Kleihues, P. and H. Ohgaki (1999). Primary and secondary glioblastomas: from concept to clinical diagnosis. *Neuro-oncol* 1(1): 44-51.

Kondapaka, S., S. Singh, G. Dasmahapatra, *et al*. (2002). Perifosine – a novel alkyllysophospholipid, modulates the Akt signaling pathway. *Proc Am Assoc Cancer Res*. Abstract 2977.

Kuhn, J. G., S. M. Chang, *et al.* (2007). Pharmacokinetic and tumor distribution characteristics of temsirolimus in patients with recurrent malignant glioma. *Clin Cancer Res* 13(24): 7401-6.

Lamborn, K. R., W. K. Yung, *et al.* (2008). Progression-free survival: an important end point in evaluating therapy

for recurrent high-grade gliomas." *Neuro Oncol* **10**(2): 162-70.

Lassman, A. B., M. R. Rossi, *et al.* (2005). Molecular study of malignant gliomas treated with epidermal growth factor receptor inhibitors: tissue analysis from north american brain tumor consortium trials 01-03 and 00-01 [published correction appears in Clin Cancer Res. 2005;12(1): 322]. *Clin Cancer Res* 11(21): 7841-50.

Lassman, A. B. and E. C. Holland (2006). Molecular biology and genetic models of gliomas and medulloblastomas. Russell and Rubinstein's Pathology of Tumors of the Nervous System 7th Edition. R. E. McLendon, D. D. Bigner, M. R. Rosenblum and J. M. Bruner. London, Arnold Health Sciences: 1039-1091.

Lassman, A. B., F. M. Iwamoto, *et al.* (2008). Patterns of relapse and prognosis after bevacizumab (BEV) failure in recurrent glioblastoma (GBM) [abstract 2028]. *J Clin Oncol* 26(May 20 suppl): Abstract 2028.

Lieberman, F. S., T. Cloughesy, *et al.* (2003). Phase I-II study of ZD-1839 for recurrent malignant gliomas and meningiomas progressing after radiation therapy [abstract]. *J Clin Oncol* 22: 105 (Abst 421).

Lyustikman, Y. and A. B. Lassman (2006). Glioma Oncogenesis and Animal Models of Glioma Formation. *Hematol Oncol Clin North Am* 20(6): 1193-1214.

Macdonald, D. R., T. L. Cascino, *et al.* (1990). Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 8(7): 1277-80.

Mellinghoff, I. K., M. Y. Wang, et al. (2005). Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med* 353(19): 2012-24.

Momota, H., E. Nerio, et al. (2005). Perifosine inhibits multiple signaling pathways in glial progenitors and cooperates with temozolomide to arrest cell proliferation in gliomas in vivo. *Cancer Res* 65(16): 7429-35.

Maly, K., F. Uberall, C. Schubert, *et al*. (1995). Interference of new alkylphospholipid analogues with mitogenic signal transduction. *Anti-Cancer Drug Design*. 10:411-425.

Margolin, K., Longmate, J., Baratta, T., *et al.* (2005) CCI-779 in metastatic melanoma: a phase II trial of the California Cancer Consortium. *Cancer*. 104(5):1045-8.

Mills, G.B., Y. Lu, and E.C. Kohn. (2001). Linking molecular therapeutics to molecular diagnostics: Inhibition of the FRAP/RAFT/TOR component of the PI3K pathway preferentially blocks PTEN mutant cells *in vitro* and *in vivo*. *Proc Natl Acad Sci USA*. 98:10031-10033.

Monga, M., R.A. Messmann, D. Headlee, *et al*. (2002). A phase I trial of oral perifosine in patients with refractory neoplasms. *Proc Am Soc Clin Oncol*. Abstract 1837.

Motzer, R.J., J. Bacik, B.A. Murphy, *et al*. (2002). Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. *J Clin Oncol*. 20:289-296.

Neshat, M.S., I.K. Mellinghoff, C. Tran, *et al*. (2001). Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci USA*. 98:10314-10319.

O'Reilly, K. E., F. Rojo, *et al.* (2006). mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 66(3): 1500-8.

Oza, A.M., Elit, L., Biagi, J., *et al.* (2006)Molecular correlates associated with a phase II study of temsirolimus (CCI-779) in patients with metastatic or recurrent endometrial cancer--NCIC IND 160. *J Clin Oncol 2006 ASCO*

*Annual Meeting Proceedings Part 1*24(18S (June Suppl)):Abstract 3003.

Pandya, K.J., Levy, D.E., Hidalgo, M., *et al.* (2005) A randomized, phase II ECOG trial of two dose levels of temsirolimus (CCI-779) in patients with extensive stage small cell lung cancer in remission after induction chemotherapy. A preliminary report. *Proc Am Soc Clin Oncol.* 24:Abstract 2005. Note Correction in presentation found at:
http://www.asco.org/portal/site/ASCO/menuitem.34d60f5624ba07fd506fe310ee37a01d/?vgnextoid=76f8201eb61a7010VgnVCM100000ed730ad1RCRD&vmview=abst_detail_view&confID=34&abstractID=30785

Pao, W., V. Miller, *et al.* (2004). EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc Natl Acad Sci U S A* 101(36): 13306-11.

Patel, V., T. Lahusen, T. Sy, *et al*. (2002). Perifosine, a novel alkylphospholipid, induced $p21^{WAF1}$ expression in squamous carcinoma cells through a p53-independent pathway, leading to loss in cyclin-dependent kinase activity and cell cycle arrest. *Cancer Res*. 62:1401-1409.

Perifosine Investigator's Brochure, 1998. Zentaris AG.

Phillips, H. S., S. Kharbanda, et al. (2006). Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* 9(3): 157-73.

Podsypanina, K., R.T. Lee, C. Politis, *et al*., (2001). An inhibitor of mTOR reduces neoplasia and normalizes p70/S6 kinase activity in $Pten^{+/-}$ mice. *Proc Natl Acad Sci USA*. 98:10320-10325.

Quant E., A.D. Norden, J. Drappatz J, *et al*., (2001) Role of a second chemotherapy in recurrent malignant glioma patients who progress on a bevacizumab-containing regimen [abstract 2008]. *J Clin Oncol* 2008, 26:abstract 2008

Raizer, J. J., L. E. Abrey, *et al.* (2004). A phase II trial of erlotinib (OSI-774) in patients with recurrent malignant gliomas not on EIAEDs [abstract]. *J Clin Oncol* 22(14S): 1502.

Raymond, E., J.Alexandre, S. Faivre, *et al*. (2004). Safety and pharmacokinetics of escalated doses of weekly intravenous infusion of CCI-779, a novel mTOR inhibitor, in patients with cancer. *J Clin Oncol*. 22:2336-2347.

Rich, J. N., D. A. Reardon, *et al.* (2004). Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* 22(1): 133-42.

Ruiter, G.A., S.F. Zerp. H. Bartelink, *et al*. (1999). Alkyl-lysophospholipids activate the SAPK/JNK pathway and enhance radiation-induced apoptosis. *Cancer Res.* 59:2457-2463.

Schmelzle, T., and M.N. Hall. (2000). TOR, a central controller of cell growth. *Cell*. 103:253-262.

Sekulic, A., C.C. Hudson, J.L. Homme, *et al*., (2000). A direct linkage between the phosphoinositide 3-kinase-AKT signaling pathway and the mammalian target of rapamycin in mitogen-stimulated and transformed cells. *Cancer Res*. 60:3504-3513.

Shi, Y., J. Gera, L. Hu, *et al*., (2002). Enhanced sensitivity of multiple myeloma cells containing PTEN mutations to CCI-779. *Cancer Res*. 62:5027-5034.

Smith, S.G., C.N. Trinh, L.J. Inge, *et al*., (2002). PTEN expression status predicts glioblastoma cell sensitivity to CCI-779. *Proc Am Assoc Cancer Res*. Abstract 1661.

Smith, S., Pro, B., Smith, S., *et al.* (2006) Molecular Inhibition of mTOR with Temsirolimus (TORISEL , CCI-779) Is a Promising Strategy in Relapsed NHL: The University of Chicago Phase II Consortium. The American Society of Hematology 48th Annual Meeting and Exposition; December 9-12, 2006; *Blood.* 108(11):Abstract 2483.

Stupp, R., W. P. Mason, *et al.* (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 352(10): 987-96.

Sun, S. Y., L. M. Rosenberg, *et al.* (2005). Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. *Cancer Res* 65(16): 7052-8.

Terada, N., H.R. Patel, K. Takase, *et al*., (1994). Rapamycin selectively inhibits translation of mRNAs encoding elongation factors and ribosomal proteins. *Proc Natl Acad Sci USA*. 91:11477-11481.

The Cancer Genome Atlas (TCGA) Research Network. Comprehensive genomic characterization defines novel cancer genes and core pathways in human glioblastomas (submitted).

Verheij, M., G. Ruiter, S. Zerp, *et al*. (2002). Low doses of alkyl-lysophospholipids induce MAPK/ERK activation by ligand-independent endocytosis of the epidermal growth factor receptor. *Proc Am Assoc Cancer Res*. Abstract 1002.

Vogelbaum, M. A., G. Peerboom*, et al*. (2004). Phase II trial of the EGFR tyrosine kinase inhibitor erlotinib for single agent therapy of recurrent Glioblastoma Multiforme: Interim results [abstract]. *J Clin Oncol* 22(14S): 1558.

Watling, C. J., D. H. Lee, *et al.* (1994). Corticosteroid-induced magnetic resonance imaging changes in patients with recurrent malignant glioma. *J Clin Oncol* 12(9): 1886-9.

Witzig, T.E., Geyer, S.M., Ghobrial, I., *et al.* (2005) Phase II trial of single-agent temsirolimus (CCI-779) for relapsed mantle cell lymphoma. *J Clin Oncol*. 23(23):5347-56.

Wong, E. T., K. R. Hess, *et al.* (1999). Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Onco*l 17(8): 2572-8.

Yamada, K.M., and M. Araki. (2001). Tumor suppressor PTEN: modulator of cell signaling, growth, migration and apoptosis. *J Cell Sci*. 114:2375-2382.

Yung, A., J. Vredenburgh, *et al.* (2004). Erlotinib HCL for glioblastoma multiforme in first relapse, a phase II trial [abstract]. *J Clin Oncol* 22(14S): 1555

Zhang, X., L. Shu, H. Hosio, *et al*., (2002). Predominant nuclear localization of mammalian target of rapamycin in normal and malignant cells in culture. *J Biol Chem*. 277:28127-28134.

APPENDIX A: Performance Status Criteria

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 B: EIAEDs and Non-EIAEDs

EIAEDs:

Carbamazipine	(Tegretol, Tegretol XR, Carbatrol)
Oxcarbazepine	(Trileptal)
Phenytoin	(Dilantin, Phenytek)
Fosphenytoin	(Cerebyx)
Phenobarbital	(Solfoton, and Luminal)
Primidone	(Mysoline)

Non-EIAEDs:

Valproic acid	(Depakote, Depakene)
Gabapentin	(Neurontin)
Lamotrigine	(Lamictil)
Topriamate	(Topamax)
Tiagabine	(Gabatril)
Zonisamide	(Zonegran)
Levatriacetam	(Keppra)
Clonazepam	(Klonopin)
Clobazam	(Frisium)

APPENDIX C: Drugs Metabolized by Selected CYP450 Isoenzymes

Selected drugs known to be metabolized by CYP2D6

CYP2D6					
SUBSTRATES		INHIBITORS		INDUCERS	
Generic Name	**Trade Name**	**Generic Name**	**Trade Name**	**Generic Name**	**Trade Name**
Methylphenidate	Ritalin	Methadone	Dolophine	None known	
Antidepressants: *e.g.*		Antidepressants: *e.g.*			
Amitriptyline	Elavil	Clomipramine	Thorazine		
Clomipramine	Anafranil	Paroxetine	Paxil		
Paroxetine	Paxil	Sertraline	Zoloft		
Antipsychotic agents: *e.g.*		Antipsychotic agents: *e.g.*			
Chlorpromazine	Thorazine	Chlorpromazine	Thorazine		
Haloperidol	Haldol	Haloperidol	Haldol		
Analgesics: *e.g.*		Antimalarials: *e.g.*			
Codeine	--	Chloroquine	Aralen		
Oxycodone	Oxycontin	Quinine	Legatrin		
Anit-hypertensives: *e.g.*		Antifungals: *e.g.*			
Captopril	Capoten	Ketoconazole	Nizoral		
Metoprolol	Lopressor	Miconazole	Lotramin		
Anti-neoplastics: *e.g.*		Antihistamines: *e.g.*			
Doxorubicin	Adriamycin	Diphenhydramine	Benadryl		
Tamoxifen	Nolvadex	Tripelennamine	Di-delamine		
Methamphetamine	Desoxyn	Anti-virals: *e.g.*			
		Delavirdine	Rescriptor		
		Ritonavir	Norvir		
Lidocaine	Xylocaine	Lidocaine	Xylocaine		
Mexiletine	Mexitil	Isoniazid	Nydrazid		
Tolterodine	Detrol	Amiodarone	Cordarone		
Dextromethorphan	Tussin	Methimazole	Tapazole		
Chloroquine	Aralen	Cimetadine	Tagamet		
		Cocaine			

When drugs classified as 'substrates' are co-administered with temsirolimus, there is the potential for higher concentrations of the 'substrate'. When temsirolimus is co-administered with compounds classified as 'inhibitors', increased plasma concentrations of temsirolimus is the potential outcome. The coadministration of 'inducers' would potentially lower plasma temsirolimus concentrations.

.

Comprehensive list of drugs that may have potential interactions with CYP2D6

CYP2D6

Substrates			
Amitryptilene	Dextromethorphan	Maprotiline	Promethazine
Amoxapine	Dihydrocodeine	Methamphetamine	Propafenone
Aripiprazole	Doxepin	Methylphenidate	Propranolol
Atomoxetine	Doxorubicin	Metoprolol	Risperidone
Betaxolol	Flecainide	Mexiletine	Ritonavir
Captopril	Fluoxetine	Mirtazapine	Tamoxifen
Carvedilol	Fluphenazine	Nefazodone	Tamsulosin
Chloroquine	Fluvoxamine	Nortriptyline	Thioridazine
Chlorpromazine	Haloperidol Hydrocodone	Oxycodone	Timolol
Clomipramine	Imipramine	Paroxetine	Tolterodine
Codeine	Labetalol	Perphenazine	Tramadol
Desipramine	Lidocaine	Pindolol	Trimipramine
Dextroamphetamine	Lomustine	Procainamide	Venlafaxine

Inhibitors			
Acebutolol	Dolasetron	Metoclopramide	Rabeprazole
Amiodarone	Doxorubicin	Metoprolol	Ranitidine
Amitriptyline	Entacapone	Miconazole	Risperidone
Amlodipine	Escitalopram	Mifepristone	Ritonavir
Amphetamine	Felodipine	Nefazodone	Ropinirole
Azelastine	Fexofenadine	Nelfinavir	Rosiglitazone
Bepridil	Flecainide	Nevirapine	Saquinavir
Betaxolol	Fluoxetine	Nicardipine	Selegiline
Biperiden	Fluphenazine	Nifedipine	Sertraline
Bortezomib	Fluvastatin	Nortriptyline	Sildenafil
Buprenorphine	Fluvoxamine	Olanzapine	Simvastatin
Bupropion	Gefitinib	Omeprazole	Sulconazole
Celecoxib	Halofantrine	Ondansetron	Telithromycin
Chloroquine	Haloperidol	Orphenadrine	Terbinafine
Chlorpheniramine	Hydroxyzine	Oxybutynin	Thioridazine
Chlorpromazine	Imatinib	Paroxetine	Thiothixene
Cholecalciferol/Vitamin D_3	Imipramine	Pentamidine	Ticlopidine
Cimetidine	Indinavir	Pergolide	Timolol
Cisapride	Irbesartan	Perphenazine	Tioconazole
Citalopram	Isoniazid	Pimozide	Tranylcypromine
Clemastine	Ketoconazole	Pindolol	Trazodone
Clomipramine	Labetalol	Pioglitazone	Tripelennamine
Clotrimazole	Lansoprazole	Pravastatin	Triprolidine
Clozapine	Lidocaine	Praziquantel	Valproic acid
Cocaine	Lomustine	Primaquine	Venlafaxine
Codeine	Loratadine	Promethazine	Verapamil
Delavirdine	Lovastatin	Propafenone	Vinblastine
Desipramine	Mefloquine	Propofol	Vinorelbine
Dexmedetomidine	Methadone	Propoxyphene	Yohimbine
Dextromethorphan	Methimazole	Propanolol	Zafirlukast
Diltiazem	Methotrimeprazine	Pyrimethamine	Ziprasidone
Diphenhydramine	Methoxsalen	Quinidine	
Disulfiram	Methylphenidate	Quinine	

Note: There are no known CYP2D6 inducers.

(Adapted from Cytochrome P-450 Enzymes and Drug metabolism. In: Lacy CF, Armstrong LL, Goldman MP, Lance LL eds. Drug Information Handbook 12TH ed. Hudson, OH; LexiComp Inc. 2004: 1619-1631.)

Selected drugs known to be metabolized by CYP3A4

CYP3A4					
SUBSTRATES		**INHIBITORS**		**INDUCERS**	
Generic Name	**Trade Name**	**Generic Name**	**Trade Name**	**Generic Name**	**Trade Name**
Anti-neoplastics: *e.g.* Docetaxel Gefitinib Irinotecan	 Taxotere Iressa Camptosar	Anti-arrhythmics: *e.g.* Amiodarone Diltiazem Quinidine	 Cordarone, Pacerone Cardizem, Dilacor XR Cardioquin	Aminoglutethimide	Cytadren
Anti-virals: *e.g.* Amprenivir Rifampin	 Agenerase Rifadin	Anti-virals: *e.g.* Amprenavir Indinavir Nelfinavir Ritonavir	 Agenerase Crixivan Viracept Norvir	Antibiotics: *e.g.* Rifabutin Rifampin	 Rifadin Mycobutin
Anxiolytics: *e.g.* Diazepam Sertraline	 Valium Zoloft	Cimetadine	Tagamet	Anticonvulsants: *e.g.* Carbamazapine Phenytoin Pentobarbital Phenobarbital	 Tegretol Dilantin Nembutal Luminal
Cyclosporine	Sandimmune	Cyclosporine	Sandimmune	*Hypericum perforatum* (2)	St. John's Wort
Anti-infectives: *e.g.* Erythromycin Tetracycline	 Erythrocin Sumycin	Antibiotics: *e.g.* Ciprofloxacin Clarithromycin Doxycycline Enoxacin Isoniazid Telithromycin	 Cipro, Ciloxan Biaxin Adoxa, Periostat Penetrex Nydrazid, INH Ketek		
Steroids: *e.g.* Estrogens, conjugated Estradiol Progesterone	 Premarin Climara Crinone	Imatinib	Gleevec		
Haloperidol	Haldol	Haloperidol	Haldol		
Cardiovascular agents: *e.g.* Digitoxin Quinidine	 Crystodigin Cardioquin	Diclofenac	Cataflam, Voltaren		
Anti-hypertensives: *e.g.* Nicardipine Verapamil	 Cardene Calan, Chronovera	Vasodilators: *e.g.* Nicardipine Verapamil	 Cardene Calan, Chronovera		
Anesthetics: *e.g.* Ketamine Lidocaine	 Xylocaine Diprivan	Anesthetics: *e.g.* Lidocaine Propofol	 Xylocaine Diprivan		
Nefazodone	Serzone	Anti-depressants: *e.g.* Nefazodone Sertraline	 Serzone Zoloft		
Cocaine		Anti-fungals: *e.g.* Itraconazole Ketoconazole Miconazole	 Sporanox Nizoral Lotrimin, Monistat		
Ketoconazole	Nizoral	Caffeine			
Sildenafil	Viagra	Grapefruit juice (1)			
Albuterol	Ventolin				
Carbamazapine	Tegretol				
Lovastatin	Mevacor				

When drugs classified as 'substrates' are co-administered with temsirolimus, there is the potential for higher concentrations of the 'substrate'. When temsirolimus is co-administered with compounds classified as 'inhibitors', increased plasma concentrations of temsirolimus is the potential outcome. The coadministration of 'inducers' would potentially lower plasma temsirolimus concentrations.

Comprehensive list of drugs that may have potential interactions with CYP3A4

CYP3A4

Substrates			
Albuterol	Docetaxel	Ketoconazole	Quetiapine
Alfentanil	Doxepin	Lansoprazole	Quinidine
Alprazolam	Doxorubicin	Letrozole	Rabeprazole
Amlodipine	Doxycycline	Levomethadyl acetate hydrochloride	Repaglinide
Amprenavir	Efavirenz	Levonorgestrel	Rifabutin
Aprepitant	Eletriptan	Lidocaine	Rifampin
Aripiprazole	Enalapril	Losartan	Ritonavir
Atazanavir	Eplerenone	Lovastatin	Saquinavir
Atorvastatin	Ergoloid mesylates	Medroxyprogesterone Mefloquine	Sertraline
Benzphetamine	Ergonovine	Mestranol	Sibutramine
Bisoprolol	Ergotamine	Methadone	Sildenafil
Bortezomib	Erythromycin	Methylergonovine	Simvastatin
Bosentan	Escitalopram	Methysergide	Sirolimus
Bromazepam	Estradiol	Miconazole	Sufentanil
Bromocriptine	Estrogens, conj., synthetic	Midazolam	Tacrolimus
Buprenorphine	Estrogens, conj., equine	Miglustat	Tamoxifen
Buspirone	Estrogens, conj., esterified	Mirtazapine	Tamsulosin
Busulfan	Estrone	Modafinil	Telithromycin
Carbamazapine	Estropipate	Montelukast	Teniposide
Cerivastatin	Ethinyl estradiol	Moricizine	Terbinafine
Chlordiazepoxide	Ethosuximide	Nateglinide	Tetracycline
Chloroquine	Etoposide	Nefazodone	Theophylline
Chlorpheniramine	Felbamate	Nelfinavir	Tiagabine
Cisapride	Felodipine	Nevirapine	Ticlopidine
Citalopram	Fentanyl	Nicardipine	Tolterodine
Clarithromycin	Flurazepam	Nifedipine	Toremifene
Clobazam	Flutamide	Nimodipine	Trazodone
Clonazepam	Fosamprenavir	Nisoldipine	Triazolam
Clorazepate	Fulvestrant	Nitrendipine	Trimethoprim
Cocaine	Gefitinib	Norethindrone	Trimipramine
Colchicine	Halofantrine	Norgestrel	Troleandomycin
Cyclophosphamide	Haloperidol	Ondansetron	Vardenafil
Cyclosporine	Ifosfamide	Paclitaxel	Venlafaxine
Dantrolene	Imatinib	Pergolide	Verapamil
Dapsone	Indinavir	Phencyclidine	Vinblastine
Delavirdine	Irinotecan	Pimozide	Vincristine
Diazepam	Isosorbide dinitrate	Pioglitazone	Vinorelbine
Digitoxin	Isosorbide mononitrate	Primaquine	Zolpidem
Dihydroergotamine	Isradipine	Progesterone	Zonisamide
Diltiazem	Itraconazole		Zopiclone
Disopyramide	Ketamine		

CYP3A4

Inhibitors			
Acetominophen	Diltiazem	Lovastatin	Progesterone
Acetazolamide	Disulfiram	Mefloquine	Propofol
Amioderone	Docetaxel	Mestranol	Propoxyphene
Amlodipine	Doxorubicin	Methadone	Quinidine
Amprenavir	Doxycycline	Methimazole	Quinine
Anastrozole	Drospirenone	Methoxsalen	Quinupristin
Aprepitant	Efavirenz	Methylprednisolone	Rabeprazole
Atazanavir	Enoxacin	Metronidazole	Risperidone
Atorvastatin	Entacapone	Miconazole	Ritonavir
Azelastine	Ergotamine	Midazolam	Saquinavir
Azithromycin	Erythromycin	Mifepristone	Selegiline
Betamethasone	Ethinyl estradiol	Mirtazapine	Sertraline
Bortezomib	Etoposide	Mitoxantrone	Sildenafil
Bromocriptine	Felodipine	Modafinil	Sirolimus
Caffiene	Fentanyl	Nefazodone	Sulconazole
Cerivastatin	Fluconazole	Nelfinavir	Tacrolimus
Chloramphenicol	Fluoxetine	Nevirapine	Tamoxifen
Chlorzoxazone	Fluvastatin	Nicardipine	Telithromycin
Cimetadine	Fluvoxamine	Nifedipine	Teniposide
Ciprofloxacin	Fosamprenavir	Nisoldipine	Testosterone
Cisapride	Glyburide	Nitrendipine	Tetracycline
Clarithromycin	Grapefruit juice	Nizatidine	Ticlopidine
Clemastine	Haloperidol	Norfloxacin	Tranylcypromine
Clofazimine	Hydralazine	Olanzapine	Trazodone
Clotrimazole	Ifosfamide	Omeprazole	Troleandomycin
Clozapine	Imatinib	Orphenadrine	Valproic acid
Cocaine	Indinavir	Oxybutynin	Venlafaxine
Cyclophosphamide	Irbesartan	Paroxetine	Verapimil
Cyclosporine	Isoniazid	Pentamidine	Vinblastine
Danazol	Isradapine	Pergolide	Vincristine
Delavirdine	Itraconazole	Phencyclidine	Vinorelbine
Desipramine	Ketoconazole	Pilocarpine	Zafirlukast
Dexmedetomidine	Lansoprazole	Pimozide	Ziprasidone
Diazepam	Lidocaine	Pravastatin	
Diclofenac	Lomustine	Prednisolone	
Dihydroergotamine	Losartan	Primaquine	

Inducers			
Aminoglutethimide	Nevirapine	Phenytoin	Rifapentine
Carbamazapine	Oxcarbazepine	Primidone	
Fosphenytoin	Pentobarbital	Rifabutin	
St. John's wort	Phenobarbital	Rifampin	

(Adapted from Cytochrome P-450 Enzymes and Drug metabolism. In: Lacy CF, Armstrong LL, Goldman MP, Lance LL eds. Drug Information Handbook 12TH ed. Hudson, OH; LexiComp Inc. 2004: 1619-1631.)

(1) Malhorta *et al*. (2000). Clin Pharmacol Ther. 69:14-23

(2) Mathijssen *et al*. (2002). J Natl Cancer Inst. 94:1247-1249
Frye *et al*. (2004). Clin Pharmacol Ther. 76:323-329

APPENDIX D: Patient Pill Diary

NAME	INITIALS	MRN	CYCLE	For Optical Scanning: Document Code: **RS3 – Research Patient Diaries**
DRUG NAME: ____________ **DAILY DOSE:** ____________		DATE DIARY DISTRIBUTED / /	DATE DIARY RETURNED / /	

Special Instructions: Take your medication as directed; when taking *study drug*, indicate your dose taken per day, and the time the pills were taken. Circle "no" if dose was missed.

Day:	Day:	Day:	Day:	Day:	Day:	Day:
Date: **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM
Date: **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** YES | NO **Daily Dose:** ______ **Time:**___:___ AM | PM

Page 1 of 2

Confirm that you took the perifosine with food : Yes / No

Patient Signature: ____________________ **Date:** ________

APPENDIX D: Patient Pill Diary

NAME		INITIALS	MRN	CYCLE	For Optical Scanning: Document Code: **RS3 – Research Patient Diaries**
DRUG NAME: ____________ DAILY DOSE: ____________			DATE DIARY DISTRIBUTED / /	DATE DIARY RETURNED / /	

Special Instructions: Take your medication as directed; when taking *study drug*, indicate your dose taken per day, and the time the pills were taken. Circle "no" if dose was missed.

Day:	Day:	Day:	Day:	Day:	Day:	Day:
Date: **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM
Date: **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM	**Date:** **Pills Taken:** **YES | NO** **Daily Dose:** ______ **Time:**___:___ AM | PM

Page 2 of 2

Confirm that you took the perifosine with food : Yes / No **Patient Signature:** ____________________ **Date:** ________

APPENDIX E: Drug Reconciliation Form

Name		MRN		Cycle	Dates Pills Taken / / - / /
Pill Diary Returned YES | NO	**Pill Diary Completed** YES | NO	**Drug Name**	**Drug Lot Number**		**Number of Pills Dispensed**
Pills Returned To PATIENT | PHARMACY	**Pill Bottle Returned** YES | NO	**Number of Pills Returned**	**Number of Pills Taken**		**Number of Pills Missed**

Comments (If patient does not complete or return pill diary, explain and confirm patient's compliance in the area provided)	
RN Name (please print):	
RN signature:	
Date/Time:	Date: Time: