

Phase 1 Trial of Gefitinib Plus Sirolimus in Adults with Recurrent Malignant Glioma

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Abstract Purpose: To determine the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of gefitinib, a receptor tyrosine kinase inhibitor of the epidermal growth factor receptor, plus sirolimus, an inhibitor of the mammalian target of rapamycin, among patients with recurrent malignant glioma.

Patients and Methods: Gefitinib and sirolimus were administered on a continuous daily dosing schedule at dose levels that were escalated in successive cohorts of malignant glioma patients at any recurrence who were stratified based on concurrent use of CYP3A-inducing anticonvulsants [enzyme-inducing antiepileptic drugs, (EIAED)]. Pharmacokinetic and archival tumor biomarker data were also assessed.

Results: Thirty-four patients with progressive disease after prior radiation therapy and chemotherapy were enrolled, including 29 (85%) with glioblastoma multiforme and 5 (15%) with anaplastic glioma. The MTD was 500 mg of gefitinib plus 5 mg of sirolimus for patients not on EIAEDs and 1,000 mg of gefitinib plus 10 mg of sirolimus for patients on EIAEDs. DLTs included mucositis, diarrhea, rash, thrombocytopenia, and hypertriglyceridemia. Gefitinib exposure was not affected by sirolimus administration but was significantly lowered by concurrent EIAED use. Two patients (6%) achieved a partial radiographic response, and 13 patients (38%) achieved stable disease.

Conclusion: We show that gefitinib plus sirolimus can be safely coadministered on a continuous, daily dosing schedule, and established the recommended dose level of these agents in combination for future phase 2 clinical trials.

Traditional cytotoxic therapies, including external beam radiotherapy (X-ray therapy) and chemotherapy, provide a modest survival advantage for some patients with newly diagnosed glioblastoma multiforme (1). Salvage therapies are ineffective (2), and nearly all glioblastoma multiforme patients die within 1 to 2 years of diagnosis. Innovative, more effective treatments are desperately needed for this patient population.

Signal transduction pathways, associated with tumor cell proliferation, migration, angiogenesis, and survival, provide multiple potential therapeutic targets currently being evaluated in oncology. Aberrant signaling of the phosphatidylinositol 3'-kinase (PI3K) pathway occurs frequently in glioblastoma multiforme (3) and is associated with poor response to conventional cytotoxic therapy (4). Several molecular mechanisms have been linked to PI3K pathway signaling, including activation of upstream growth factor receptors, such as the epidermal growth factor receptor (EGFR), or loss of function of the PTEN tumor suppressor gene, which normally antagonizes PI3K (5). We recently reported results of a clinical trial with gefitinib, a novel low molecular weight, EGFR tyrosine kinase inhibitor (TKI), in recurrent glioblastoma multiforme patients. Although 9 of 53 patients (17%) remained progression-free for at least 6 months, the majority of patients suffered early disease recurrence (6). Similar, modest antitumor activity has recently been reported among recurrent glioblastoma multiforme patients treated with erlotinib, another EGFR TKI (7). Several possible factors may limit the clinical benefit associated with EGFR TKIs, including compensatory activation of either downstream pathway components or alternative mitogenic/survival pathways, as well as molecular resistance mechanisms (8).

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We recently showed in preclinical studies that the antitumor activity of an EGFR TKI can be enhanced by combination with an inhibitor of the mammalian target of rapamycin (mTOR; ref. 9). mTOR, a downstream target of the PI3K pathway, is a central regulator of several essential cellular processes in both normal and neoplastic cells including nutrient metabolism, cell cycle progression, and protein translation (10, 11). Although the clinical benefit of mTOR inhibitors for malignant glioma patients seems modest (12, 13), the antitumor activity of mTOR antagonists is enhanced by loss of PTEN (14), which occurs commonly in glioblastoma multiforme (15–18). Thus, possible mechanisms of EGFR TKI resistance may be preferentially targeted by mTOR antagonists. To extend this hypothesis to the development of a novel therapeutic approach for malignant glioma patients, we conducted the current phase I study to determine the maximum tolerated dose (MTD) of gefitinib plus sirolimus, an mTOR antagonist, in patients with recurrent malignant glioma. Our report describes the first study of a combinatorial regimen of molecularly targeted agents in the treatment of recurrent malignant glioma patients and specifically includes a combinatorial regimen designed to simultaneously inhibit key upstream and downstream mediators of PI3K signaling.

Patients and Methods

Protocol objectives

The primary objective was to define the MTD and dose-limiting toxicity (DLT) of gefitinib plus sirolimus in adults with recurrent malignant glioma. Secondary objectives included to further define the toxicity of this regimen, to obtain pharmacokinetic data, and to evaluate for antitumor activity relative to clinical, archival tumor biomarker, and pharmacokinetic measures.

Patient eligibility criteria

Patients were required to have histologically confirmed malignant glioma (glioblastoma multiforme, gliosarcoma, anaplastic astrocytoma, anaplastic oligodendroglioma, or anaplastic oligoastrocytoma) that was radiographically progressive following prior radiation or chemotherapy. Additional enrollment criteria included age at least 18 years, Karnofsky performance status $\geq 70\%$, stable corticosteroid dose for at least 1 week before therapy initiation, hematocrit $>29\%$; absolute neutrophil count $>1,000$ cells/ μL ; platelet count $>100,000$ cells/ μL , serum creatinine and bilirubin <1.5 times the institutional upper limit of normal, serum aspartate aminotransferase <2.5 times the institutional upper limit of normal, and carbon monoxide diffusing capacity of $>75\%$ predicted. Patients were required to be at least 3 weeks from prior surgical resection and to have recovered from all toxicities associated with any prior therapy. All patients were informed of the investigational nature of the study and provided informed consent as approved by the Duke University Medical Center Institutional Review Board.

Patients were excluded for any of the following: more than three prior episodes of progressive disease, pregnancy or nursing, refusal to use effective contraception if of reproductive potential, progressive disease following prior treatment directed against either EGFR or mTOR, and acute infection requiring i.v. antibiotics. In addition, patients were not eligible if they received prior stereotactic radiosurgery, radiation implants, or radiolabeled monoclonal antibody therapy, due to the difficulty distinguishing progressive tumor from radionecrosis on magnetic resonance imaging following such therapies. However, patients who had received these therapies were eligible if they had either biopsy confirmation of recurrent tumor, or if they had a new or progressive distant lesion on magnetic resonance imaging.

Treatment plan and statistical design

Gefitinib and sirolimus were orally administered on a continuous daily dosing schedule of 28-day cycles (Table 1). For all patients except for those who underwent pharmacokinetic sampling, a loading dose of commercially available sirolimus was administered on the first day of cycle 1 followed by a continuous daily maintenance dose. Gefitinib, provided by AstraZeneca Pharmaceuticals (Wilmington, DE), was taken concurrently with sirolimus. For patients who underwent pharmacokinetic sampling, gefitinib was administered alone for days 1 to 7 of cycle 1. On day 8 of cycle 1, a loading dose of sirolimus was administered. Thereafter, gefitinib and sirolimus were administered concurrently each day. Patients received up to 12 cycles unless unacceptable toxicity or tumor progression occurred.

Gefitinib metabolism is significantly enhanced by concurrent use of CYP3A-inducing antiepileptic drugs (EIAED), including phenytoin, carbamazepine, phenobarbital, oxcarbazepine, and primidone (19). Therefore, patients were accrued independently into two separate strata: stratum A, patients not on EIAEDs; stratum B, patients on EIAEDs. A "3+3" phase I dose escalation design was employed to determine the MTD for each stratum. Inpatient dose escalation was not permitted. The dose level was escalated in successive cohorts of three patients as long as DLT did not occur. If one instance of DLT was observed among the initial three evaluable patients, three additional patients were treated at that dose level. Dose escalation continued as long as no episodes of DLT occurred in the additional three patients. If two instances of DLT were observed at a dose level, the MTD was surpassed and a total of six patients were treated at the previous level. The MTD was defined as the highest dose causing DLT in no more than one of six patients.

DLT was defined as any of the following toxicities that occurred during the first cycle of therapy: grade 4 thrombocytopenia or neutropenia lasting >4 days, grade ≥ 3 nonhematologic toxicities felt to be related to the study regimen excluding grade ≥ 3 nausea or emesis for which inadequate medical therapy was administered, and >14 day delay in treatment due to related toxicity. Toxicities were graded according to the National Cancer Institute's Common Toxicity Criteria version 3.0 and classified as related to the study regimen unless they were attributable to either underlying tumor progression, a concurrent medical condition or a concomitant medication.

Before each cycle, patients underwent a physical examination and full chemistry panel, including fasting cholesterol and triglycerides. A complete blood count with differential was obtained weekly. In addition, before cycle 1, a urinalysis was obtained in all patients, and β -human chorionic gonadotropin was obtained in women with reproductive potential.

Response evaluation was done before each treatment cycle. Determination of overall response was based on radiographic change in tumor size as revealed by computed tomography or magnetic

Table 1. Dose escalation schema

Stratum	Dose level	Gefitinib dose (mg/d)	Sirolimus	
			Loading dose (mg)	Maintenance dose (mg/d)
Stratum A*	1	500	15	5
	2	500	30	10
	3	750	30	10
Stratum B†	1	1,000	15	5
	2	1,000	30	10
	3	1,500	30	10

*Stratum A: patients not on EIAEDs (phenytoin, phenobarbital, carbamazepine, oxcarbazepine, and primidone).

†Stratum B: patients on EIAEDs.

resonance imaging and clinical criteria, including steroid requirement and neurologic examination. Complete response was defined as the disappearance of all enhancing or nonenhancing tumor from baseline on consecutive scans at least 6 weeks apart, with the patient not receiving corticosteroids and neurologically stable or improved. Partial response was defined as $\geq 50\%$ reduction from baseline in the size (measured as the product of the largest perpendicular diameters) of enhancing tumor maintained for at least 6 weeks, use of a stable or reduced corticosteroid dose, and stable or improved neurologic exam. Progressive disease was defined as $>25\%$ increase in size of enhancing or nonenhancing tumor or any new tumor on magnetic resonance imaging scan or neurologic worsening of the patient without a documented nonneurologic etiology while on a stable or increased corticosteroid dose. Stable disease was defined as any other status not meeting the criteria for complete response, partial response, and progressive disease that was observable for more than one course of therapy.

Time to progression and overall survival, measured from the date cycle 1 began, were analyzed by the Kaplan-Meier method, including 95% confidence intervals (95% CI; refs. 20, 21).

Dose modification and retreatment criteria

The criteria for retreatment consisted of the following: absolute neutrophil count $> 1,000$ cells/ μL ; platelets $>100,000$ cells/ μL ; serum aspartate aminotransferase, total bilirubin, and creatinine <1.5 times upper limit of normal and resolution of all related toxicities to grade ≤ 1 except for rash, which was required to improve to grade ≤ 2 . For patients who develop DLT regardless of treatment cycle, the study regimen was reduced to the dose level below that on which the patient was entered. Patients were removed from study for evidence of progressive disease at any time after study initiation, grade 4 nonhematologic toxicity, more than two dose reductions due to toxicity, dose reduction of gefitinib to <250 mg/d, noncompliance, or voluntary withdrawal.

Supportive care

Antiemetic therapy with ondansetron and dexamethasone was permitted if needed. Loperamide was prescribed for diarrhea as previously described (22). Hematopoietic growth factors and blood products were administered as indicated for hematologic DLT or hematologic toxicity that occurred after cycle 1. Lipid lowering agents were permitted if prescribed before study enrollment, or for patients who developed either DLT or hyperlipidemia after cycle 1. Significant rash was treated with over-the-counter acne preparations, antihistamines, and topical clindamycin and/or oral antibiotics (penicillins or cephalosporins) as needed.

Pharmacokinetic analysis

Venous blood samples (4 mL) were collected for gefitinib pharmacokinetic studies from patients on days 7 and 10 of cycle 1 before the daily dose and 1, 2, 4, 6, 8, and 24 hours after the daily dose. For each sample, plasma supernatants were separated by centrifugation ($1,000 \times g$ for 10 minutes at room temperature) and immediately frozen at -20°C . Plasma concentrations of gefitinib were determined by high-pressure liquid chromatography with tandem mass spectrometry detection by the Drug Metabolism and Pharmacokinetics Department at AstraZeneca, Alderley Park, United Kingdom (23).

Steady-state plasma drug concentrations were used to provide a measure of exposure and the pharmacokinetic variables. Maximum steady-state plasma gefitinib concentration during the dosing interval ($C_{ss,max}$), the time to reach maximum gefitinib concentration (T_{max}), and the minimum concentration during the dosing interval at steady state ($C_{ss,min}$), defined as the concentration at 24 hours after dose on each sample day, were obtained directly from the data. The area under the concentration versus time curve at steady state (AUC_{ss}) was calculated by the linear trapezoidal rule using WinNonlin (Pharsight

Corp., Mountain View, CA). Total body clearance of drug from plasma at steady state after an oral dose (CL_{ss}/F) was calculated as daily dose/ AUC_{ss} .

The paired *t* test was used to compare gefitinib alone AUC_{ss} (day 7) to that with sirolimus (day 10) for each stratum and for each dose level. A two-sample *t* test was used to compare dose-normalized, gefitinib AUC_{ss} from day 7 between patients on strata A and B.

A trough serum sirolimus level was measured after day 10 of cycle 1. Two-way ANOVA was used in a generalized linear model framework to examine the effect of dose and strata on blood levels of sirolimus. This analytic approach assumed measurement errors to be normally distributed, and repeated measures of sirolimus levels within a subject were correlated.

Archival tumor biomarker assessment

Archival tumor samples from either initial diagnosis or after prior therapy were analyzed for phospho-p44/42 mitogen-activated protein kinase (p-MAPK), p-S6 ribosomal protein, p-AKT, PTEN, and EGFR using immunohistochemistry reagents and methods as described below. Similarly, archival tumor samples were analyzed by fluorescence *in situ* hybridization (FISH) for EGFR and PTEN DNA locus copy number using reagents and methods as described below. Primary antibodies for immunohistochemical staining included rabbit monoclonal p-MAPK (Thr²⁰²/Tyr²⁰⁴, clone E10), rabbit polyclonal p-S6 ribosomal protein (Ser²³⁵/Ser²³⁶), rabbit polyclonal p-AKT (Ser⁴⁷³; Cell Signaling Technology, Boston, MA), and mouse monoclonal PTEN (clone 6H2.1; Cascade Bioscience, Inc., Winchester, MA). The EGFRpharmDx kit (DAKO Corp., Carpinteria, CA) was used for EGFR wild-type immunostaining.

Primary antibodies were used at the following dilutions and incubations: p-MAPK, 1:100 overnight at 4°C ; p-S6 ribosomal protein, 1:100 for 1 hour at room temperature; p-AKT, 1:50 overnight at 4°C ; and PTEN, 1:1000 overnight at 4°C . The EGFR antibody was provided at a predetermined dilution, and immunohistochemistry was done according to the Food and Drug Administration–approved manufacturer's protocol for the DAKO EGFRpharmDx kit.

Immunohistochemistry. For all immunohistochemistry assays, 5- μm sections were cut from paraffin-embedded, formalin-fixed brain tissue, placed on silanized slides, deparaffinized with a series of xylenes, cleared in a series of alcohols, and rehydrated. Endogenous peroxidase was quenched using 0.3% H_2O_2 .

Antigen retrieval was done by one of several methods. For p-S6 and p-AKT, a solution of 10 mmol/L EDTA was used in a decloaking chamber for 5 minutes at 120°C . For p-MAPK and PTEN, a sodium citrate solution (pH 6.0) was used in a Black and Decker steamer for 20 minutes at 95°C .

Following antigen retrieval, slides were washed in TBS with 0.1% Tween 20, and nonspecific protein binding was blocked with 5% normal goat serum for 15 minutes at room temperature. For p-AKT and p-S6, a 30-minute incubation with goat anti-rabbit secondary antibody was followed by detection with avidin-biotin complex Elite kit (Vector Laboratories, Burlingame, CA). For PTEN, a 30-minute incubation with goat anti-mouse supersensitive link was followed by detection with Super Sensitive Detection Kit (Biogenex, San Ramon, CA). For MAPK, a 30-minute incubation with goat anti-rabbit secondary antibody was followed by detection with the Multilink Detection kit (Biogenex, San Ramon, CA). Nuclear counterstaining was done using Harris' modified hematoxylin. The intensity of cytoplasmic/membranous staining detected by immunohistochemistry was scored on a scale of 0 to 4+, and the distribution was defined as the percentage of cells with any level of expression. Immunohistochemical staining was defined as "high" for tumors expressing 2 to 4+ intensity in $\geq 25\%$ of tumor cells and as "low" for tumors expressing either 0 to 1+ staining in any percentage of tumor cells or 2 to 4+ intensity in $<25\%$ of tumor cells (3).

FISH. Dual-color FISH was done on formalin-fixed, paraffin-embedded tissue specimens using the EGFR/CEP 7, CEP 10/CEP 2

(Vysis, Downers Grove, IL), and CEP 10/PTEN (Human BAC CITB library clone 265N13, Research Genetics, Huntsville, AL) probe combinations (using three separate slides) for each patient sample. CEP 2 was chosen as an internal control for the loss of chromosome 10 (23). The EGFR probe does not discriminate between wild-type EGFR and any of its variants.

Paraffin sections were cut at 5 μ m onto silanized slides. Control and patient slides were baked overnight at 56°C. Formalin-fixed, paraffin-embedded control cell lines, showing the locus of interest, were used as control slides for each FISH test.

Slides were deparaffinized, pretreated with 0.2 N HCl at room temperature for 20 minutes, then washed in deionized water and 2 \times SSC for 3 minutes each. They were then placed in Pretreatment Solution (Vysis) at 80°C for 30 minutes and washed with two changes of 2 \times SSC for 5 minutes each. Sections were subjected to digestion with protease at 37°C for 20 to 23 minutes. Slides were washed in two changes of 2 \times SSC for 5 minutes each and air-dried, then were denatured in a 70% formamide/2 \times SSC solution at 72°C for 5 minutes and immediately dehydrated in 70%, 85%, and 100% ethanol for 1 minute each. Subsequently, the probe was denatured at 75°C for 5 minutes. Fluoresceinated probe was applied to each slide, sealed with rubber cement, and then placed in a humidified chamber at 37°C for an overnight incubation. After overnight incubation, slides were then washed in 2 \times SSC/0.3% NP40 at room temperature and then at 72°C for 2 minutes. 4',6-Diamidino-2-phenylindole counterstain and a coverslip were applied to the hybridization area.

Slides were viewed using an Olympus BX-60 fluorescent microscope. The number of green and orange signals was enumerated in 100 intact, nonoverlapping nuclei per slide. With regard to chromosomal gain, the cutoff value was set at 20%, meaning that >20% of the enumerated nuclei must show more than two copies of the respective probe. For chromosomal loss, the cutoff value was set at 30% for definitive loss and 20% to 30% for indeterminate loss. EGFR gene amplification was defined as an EGFR/chromosome 7 centromere ratio of >2.0. Definitive PTEN loss was defined as tumors in which \geq 30% of nuclei exhibited less than two copies of the PTEN locus and two copies of CEP 2 control. Indeterminate PTEN loss refers to tumors in which 20% to 30% of enumerated nuclei had less than two copies of the PTEN locus and two copies of CEP 2 control.

Results

Patient characteristics. Thirty-four patients with recurrent malignant glioma were enrolled at the Duke University Medical Center between August 2004 and February 2005 (Table 2). Twenty-nine patients had glioblastoma multiforme (85%) and 5 (15%) had anaplastic astrocytoma. Fifteen patients (44%) were not on EIAEDs (stratum A) and 19 (56%) were on EIAEDs (stratum B). Patient characteristics did not differ substantially based on EIAED status. Twenty-three patients (68%) were male. The median age was 49.9 years

Table 2. Patient characteristics

Characteristic	Stratum A, not on EIAED* (n = 15)	Stratum B, on EIAED (n = 19)	All patients (n = 34)
Age (y)			
Median	56.3	48.8	49.9
Range	32.8-76.8	32.9-65.8	32.8-76.8
Sex			
Male	7 (47%)	16 (84%)	23 (68%)
Female	8 (53%)	3 (16%)	11 (32%)
Histology			
GBM/GS	12 (80%)	17 (87%)	29 (85%)
AA	3 (20%)	2 (11%)	5 (15%)
KPS			
90-100	9 (60%)	13 (68%)	22 (65%)
70-80	6 (40%)	6 (32%)	12 (35%)
Prior XRT	15 (100%)	19 (100%)	34 (100%)
Prior chemotherapy	15 (100%)	19 (100%)	34 (100%)
No. prior chemotherapy agents			
1	4 (27%)	8 (42%)	12 (35%)
2	4 (27%)	5 (26%)	9 (26%)
\geq 3	7 (47%)	6 (32%)	13 (38%)
Median	2	2	2
No. prior progressions			
1	4 (27%)	9 (47%)	13 (38%)
2	8 (54%)	7 (37%)	15 (44%)
3	3 (20%)	3 (16%)	6 (18%)
Median	2	2	2
Median time from diagnosis to initiation (wk)	30.7 (range, 7.3-179.6)	28.9 (range, 10.9-248.0)	29.8 (range, 7.3-248.0)

Abbreviations: GBM, glioblastoma multiforme; GS, gliosarcoma; AA, anaplastic astrocytoma; KPS, Karnofsky performance status; XRT, X-ray therapy.

*EIAEDs: phenytoin, carbamazepine, phenobarbital, oxcarbazepine, and primidone.

(range, 32.8-76.8 years). All patients had a Karnofsky performance status of at least 70%.

All patients had received prior X-ray therapy and chemotherapy. The median number of prior chemotherapeutic agents administered per patient was 2 (range, 1-6). The median number of prior episodes of progressive disease per patient was 2 (range, 1-3). The median time from original diagnosis to initiation of study treatment was 29.8 weeks (range, 7.3-248.0 weeks).

As of September 15, 2005, five patients continue to receive treatment on study with stable disease. Twenty-one patients have died.

Dose-limiting toxicity. Table 3 summarizes the frequency and type of DLT observed at each dose level per stratum. One group A patient developed fulminant progressive disease and discontinued study treatment after <2 weeks of cycle 1. Although this patient did not experience a DLT, they were replaced in the cohort for MTD determination. However, this patient was included in overall toxicity assessment. One additional patient was added at dose level one for stratum A and provided additional safety and pharmacokinetic data. Three patients (one in dose level 2 of stratum A and two in dose level 3 of stratum B) decreased or interrupted dosing during cycle 1 due to miscommunication or noncompliance with administration guidelines. Although these patients were assessable for DLT, they were not assessable for dose escalation within each cohort and were therefore replaced.

For stratum A, one of seven patients treated at dose level 1 experienced DLT (grade 3 mucositis), whereas two of seven patients treated at dose level 2 experienced DLT, including one patient with grade 3 thrombocytopenia that required >2 weeks to resolve to retreatment criteria, and one patient with grade 3 rash and mucositis. For stratum B, none of the patients experienced DLT at dose level 1. However, one of six patients treated at dose level 2 developed dose-limiting hypertriglyceridemia, whereas two of eight patients treated at dose level 3 developed DLT, including one patient with grade 3 diarrhea and one patient with grade 3 mucositis.

Non-DLT. One hundred courses of gefitinib plus sirolimus have been administered to date, including 44 courses to patients on stratum A and 56 courses to patients on stratum B. Table 4 summarizes the most frequent toxicities stratified by toxicity grade and treatment stratum.

Diarrhea, mucositis, and rash were the most common toxicities as expected. Hematologic toxicity and fasting cholesterol or triglyceride elevations were also noted, primarily as low-grade events, and also as infrequent grade 3 or 4 events. Grade 1 or 2 infections, most frequently involving the skin and nailbeds, were also noted. Two serious infections occurred among patients on study and included episodes of grade 3 and 4 pneumonia, respectively. The episode of grade 4 pneumonia was most likely due to aspiration following a seizure. Both events resolved with i.v. antibiotics and hospitalization. One patient, treated with gefitinib plus sirolimus for 7 months, developed disseminated *Aspergillus* ~2 months following study discontinuation while receiving an alternative, salvage therapy. Of note, there were no grade 5 toxicities.

Pharmacokinetic analyses. Ten patients from stratum A and nine patients from stratum B underwent plasma gefitinib pharmacokinetic analysis (Table 5).

Table 3. DLTs encountered

Dose level	No. patients	No. DLTs	Type DLT
Stratum A*			
1	7 [†]	1	Mucositis
2	7 ^{‡,§}	3	Thrombocytopenia, rash; mucositis
Stratum B [†]			
1	5	0	—
2	6	1	Hypertriglyceridemia
3	8	2	Diarrhea; mucositis

*Stratum A: patients not on EIAEDs (phenytoin, phenobarbital, carbamazepine, oxcarbazepine, and primidone).

[†] One additional patient was treated at the MTD (dose level 1) for stratum A for additional safety and pk data.

[‡] One patient treated at dose level 2 of stratum A was not eligible for MTD determination due to fulminant progressive disease.

[§] One patient treated at dose level 2 of stratum A and two patients treated at dose level 3 of stratum B interrupted dosing during cycle 1 and were replaced for MTD determination.

^{||} Stratum B: patients on EIAEDs (phenytoin, phenobarbital, carbamazepine, oxcarbazepine, and primidone).

Limited sampling from two stratum B patients treated on dose level 3 was available and is therefore not included. Comparison of day 7 (gefitinib alone) and day 10 (gefitinib plus sirolimus) measures revealed that sirolimus, a known substrate for CYP3A4, did not affect gefitinib metabolism. However, gefitinib exposure was significantly reduced by concurrent EIAED use. Specifically, the dose-normalized, geometric mean of AUC_{ss} for patients not on (stratum A) and for those on EIAEDs (stratum B) were 19.9 and 7.91 ng h/mL, respectively ($P = 0.003$).

Trough sirolimus data was available on 23 patients, including 12 patients from stratum A and 11 patients from stratum B. The mean trough sirolimus level for patients treated with 5 mg/d (7.0) was significantly less than that of patients treated with 10 mg/d (16.7; $P < 0.0001$); however, trough sirolimus levels did not differ based on stratum ($P = 0.136$).

Archival tumor biomarker analysis. Archival tumor material was available for 14 patients (Table 6). FISH analysis revealed that 6 patients had *EGFR* amplification (43%) and 7 patients had evidence of *PTEN* loss (50%). "High" levels of *EGFR*, p-S6, p-MAPK, and p-AKT were detected by immunohistochemistry in 90% (9 of 10), 60% (6 of 10), 60% (6 of 10), and 90% (9 of 10) of assessable patients, respectively. A good correlation was observed between *EGFR* amplification detected by FISH and *EGFR* expression by immunohistochemistry. All four tumors with *EGFR* amplification by FISH showed 3 to 4+ *EGFR* expression in 90% to 100% of cells by immunohistochemistry. Of note, three of four tumors with evidence of *PTEN* loss by FISH had elevated p-AKT expression by immunohistochemistry.

Outcome. All 34 patients were evaluable for response. Two patients achieved a partial radiographic response, including one patient treated at dose level 1 on stratum A (Fig. 1) and another patient treated at dose level 3 on stratum B. Thirteen patients (38%) achieved stable disease, including 7 patients on stratum A (47%) and 6 patients on stratum B (32%). By

Table 4. Most frequent toxicities stratified by grade and patient stratum

Stratum A* (15 patients, 44 cycles)				Stratum B† (19 patients, 56 cycles)			
Grade 1-2‡		Grade 3-4§		Grade 1-2‡		Grade 3-4§	
Toxicity	No. events¶ (%¶)	Toxicity	No. events¶ (%¶)	Toxicity	No. events¶ (%¶)	Toxicity	No. events¶ (%¶)
Hemoglobin	9 (60)	Mucositis	5 (33)	Rash	14 (74)	Diarrhea	6 (32)
Rash	9 (60)	Diarrhea	3 (20)	Mucositis	13 (68)	AST/ALT	2 (11)
Diarrhea	6 (40)	Seizures	3 (20)	Diarrhea	8 (42)	Hypertriglyceridemia	2 (11)
Infection	6 (40)	Infection	2 (13)	Fatigue	7 (37)	Hypercholesterolemia	1 (5)
Thrombocytopenia	6 (40)	Thrombocytopenia	2 (13)	Hemoglobin	6 (32)	Mucositis	1 (5)
Hypertriglyceridemia	5 (33)	Thrombosis	2 (13)	Leukopenia	6 (32)	Seizures	1 (5)
Fatigue	4 (27)	Hypercholesterolemia	1 (4)	Infection	5 (26)	Thrombosis	1 (5)
Leukopenia	4 (27)	Dehydration	1 (4)	Hypercholesterolemia	4 (21)		
Hypercholesterolemia	3 (20)	Nausea/emesis	1 (4)	Hypertriglyceridemia	4 (21)		
		Rash	1 (4)	Thrombocytopenia	4 (21)		
		Weight loss	1 (4)	AST/ALT	3 (16)		
				Nausea/emesis	3 (16)		

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase.
*Stratum A: patients not on EIAEDs (phenytoin, carbamazepine, oxcarbazepine, phenobarbital, and primidone).
†Stratum B: patients on EIAEDs (phenytoin, carbamazepine, oxcarbazepine, phenobarbital, and primidone).
‡Includes only grade 1 and 2 events that occurred at a minimum of three times.
§Includes all grade 3 and 4 events including those reported as DLT.
¶Cumulative number of events observed among all cycles of therapy.
¶Percentage of all patients in stratum.

histology, 12 patients with glioblastoma multiforme (41%) and 1 with recurrent anaplastic glioma (20%) achieved stable disease.

With a median follow-up of 35.2 weeks (95% CI, 27.7-42.4), the median progression-free disease (PFS) and 6-month PFS rate for all patients were 8.2 weeks (95% CI, 7.5-18.6 weeks) and 23.5% (95% CI, 12.8-43.1%), respectively. Median PFS and 6-month PFS did not differ significantly by histology or EIAED status. Among patients who achieved at least stable disease, the median PFS was 27.4 weeks (95% CI, 18.7-30.6

weeks). Analysis of possible associations among clinical, pharmacokinetic, and archival tumor biomarker variables with outcome was limited by study accrual and the dose escalation design (Table 7).

Discussion

The rationale for the study regimen of gefitinib plus sirolimus is that simultaneously targeting key upstream and downstream mediators of PI3K signaling may produce greater antitumor

Table 5. Gefitinib pharmacokinetic variables

Dose level	Day	n	AUC _{ss} (ng h/mL)		C _{ss,max} (ng/mL)		T _{max} (h)	C _{ss,min} (ng/mL)		CL _{ss} /F (mL/min)	
			G _{mean}	CV (%)	G _{mean}	CV (%)		G _{mean}	CV (%)	G _{mean}	CV (%)
Stratum A: no EIAEDs*											
1	7	4	11,600	78.7	640	94.2	6	375	73.6	720	78.7
	10	4	11,700	67.9	607	83.0	4	372	54.9	715	67.9
2	7	5	9,060	89.8	542	71.7	4	272	103	921	89.7
	10	5	9,670	66.2	591	48.4	4	274	89.6	862	66.3
Stratum B: on EIAEDs*											
1	7	3	9,110	35.9	537	30.9	2	276	43.6	1,830	35.6
	10	3	9,280	14.2	635	16.7	4	214	21.6	1,800	14.3
2	7	3	5,650	79.0	387	103	4	138	85.7	2,950	79.1
	10	3	5,380	92.6	320	84.1	4	151	99.8	3,100	92.8

Abbreviations: G_{mean}, geometric mean; CV%, coefficient of variation.
*EIAEDs: phenytoin, carbamazepine, oxcarbazepine, phenobarbital, primidone.

activity than that achieved when either mediator is targeted separately. Results of preclinical studies confirm that such combinatorial regimens are capable of synergistic antitumor activity (9, 24–27). Furthermore, such combinatorial regimens may be less vulnerable to resistance mechanisms against targeted therapeutics. Although current understanding of such resistance mechanisms is limited (28), insights can be gained from both clinical and preclinical studies. For example, the majority of patients exhibit resistance to EGFR TKIs, although most glioblastoma multiforme tumors express EGFR, suggesting that compensatory mechanisms can overcome EGFR inhibition. One such compensatory mechanism may be increased activity of additional growth factor receptors. Growth factors reported to be overexpressed in malignant glioma include platelet-derived growth factor receptor (29, 30), vascular endothelial growth factor (31), fibroblast growth factor receptor (32), and insulin-like growth factor-I receptor (33). Furthermore, some EGFR-resistant tumors exhibit activation of alternative growth factor receptor pathways, suggesting that either tumor mitogenesis or induction of angiogenesis may act to compensate for EGFR signaling loss (26, 33–36). EGFR TKI resistance has also been associated with increased activity of intracellular mediators. For example, loss of the PTEN tumor suppressor, which constitutively activates AKT (36, 37), is linked to resistance to EGFR-based therapies (25, 38–40). In a recent glioblastoma multiforme trial, elevated levels of p-AKT correlated with erlotinib resistance (7). These data suggest that in response to upstream growth factor TKI therapy, enhanced activity of either

downstream signaling mediators or alternative signaling pathways may provide compensatory proliferative and survival capability.

Our phase I study achieved its primary objective of establishing the MTD of a continuous daily dosing regimen of gefitinib plus sirolimus for patients with recurrent malignant glioma. Specifically, the MTD is 500 mg of gefitinib plus 5 mg of sirolimus for patients not on EIAEDs, and 1,000 mg of gefitinib plus 10 mg of sirolimus for those on EIAEDs. Furthermore, we show that these agents can be safely combined at doses used in monotherapy dosing schedules (6, 41, 42). There were no unexpected toxicities and the spectrum of observed toxicities, including DLTs, was similar to those previously reported in monotherapy studies (6, 12, 13, 41, 42). Although not observed among enrolled patients, opportunistic infections pose an appropriate concern with this regimen due to the immunosuppressive activity of sirolimus, particularly because malignant glioma patients are inherently immunocompromised (43, 44) and are frequently on immunosuppressive corticosteroids.

Secondary objectives of this study included the evaluation of pharmacokinetic end points, the assessment of biomarkers from archival tumor specimens of enrolled patients, and the determination of evidence of antitumor activity. Our pharmacokinetic studies confirmed that gefitinib exposure is significantly affected by concurrent EIAED use and provided reassurance that sirolimus does not affect gefitinib metabolism.

The analysis of our immunohistochemistry and FISH findings was limited by specimen availability and the dose

Table 6. Archival tumor biomarker analysis

Patient treatment data					FISH*				Immunohistochemistry†							
Pt ID no.	Stratum‡	Dose level	Best response	TTP (months)	EGFR		PTEN		EGFR		p-S6		p-MAPK		p-AKT	
					Copy number	Status	Copy number	Status	I	D	I	D	I	D	I	D
102	A	1	PR	5.5	35.8	Amplified	1.7	Loss	4+	100	4+	90	3+	80	1	70
103	A	1	PD	2.2	30.2	Amplified	1.8	Indeterminate	ND	ND	ND	ND	ND	ND	ND	ND
107	A	1	PD	1.8	3	Intact	1.7	Indeterminate	3+	50	3+	10	2+	30	ND	ND
113	A	2	SD	4.5	15.6	Amplified	1.1	Loss	ND	ND	ND	ND	ND	ND	3	80
114	A	2	SD	3.1	21.6	Amplified	1.3	Loss	4+	100	4+	50	2+	50	ND	ND
115	A	2	SD	4.6	3.2	Polysomy	1.1	Loss	ND	ND	ND	ND	ND	ND	3	90
204	B	1	SD	6.0	3.3	Polysomy	1.8	Intact	2+	35	2+	10	3+	80	3	50
205	B	1	PD	2.0	50	Amplified	1.2	Loss	3+	100	3+	25	2+	50	3	90
211	B	2	PD	1.0	50	Amplified	1.9	Intact	4+	90	3+	1	Focal	50	3	70
212	B	2	PD	2.1	3.6	Polysomy	2.9	Polysomy	4+	75%	3+	25	Focal	80	1	1
221	B	3	PD	1.3	4.3	Polysomy	1.8	Intact	3+	30	4+	75	2+	90	3	50
225	B	3	PD	1.0	1.9	Intact	1.6	Loss	ND	ND	ND	ND	ND	ND	ND	ND
226	B	3	SD	5.0	4.7	Polysomy	2.5	Polysomy	3+	10	4+	10	Focal	30	3	70
222	B	3	PD	2.5	3.7	Polysomy	1.3	Loss	2+	75	4+	75	Focal	10	3	70

Abbreviations: ND, not done; PR: partial response; SD: stable disease; PD: progressive disease; TTP: time to progression; I, intensity; D, distribution.
 * FISH: amplified *EGFR*: *EGFR*/chromosome 7 ratio >2.0; polysomy *EGFR*: *EGFR*/chromosome 7 ratio 1-2; *PTEN* loss: <2 copies *PTEN* with 2 copies of CEP 2 control in ≥30% nuclei; *PTEN* indeterminate: <2 copies *PTEN* with 2 copies of CEP 2 control in 20% to 30% nuclei.
 † Immunohistochemistry: wild-type EGFR, p-S6 ribosomal protein, p-p44/42 MAPK, p-AKT. I, most common staining pattern present overall (0+, no staining; 1+, minimal cytoplasmic/membraneous staining; 2+, mild cytoplasmic/membraneous staining; 3+, moderate cytoplasmic/membraneous staining; 4+, strong cytoplasmic/membraneous staining). D, percent positive cells; focal refers to heterogeneous, regional, or sporadic staining of <25% of evaluated tumor cells.
 ‡ Stratum A: no EIAEDs; stratum B, on EIAEDs.

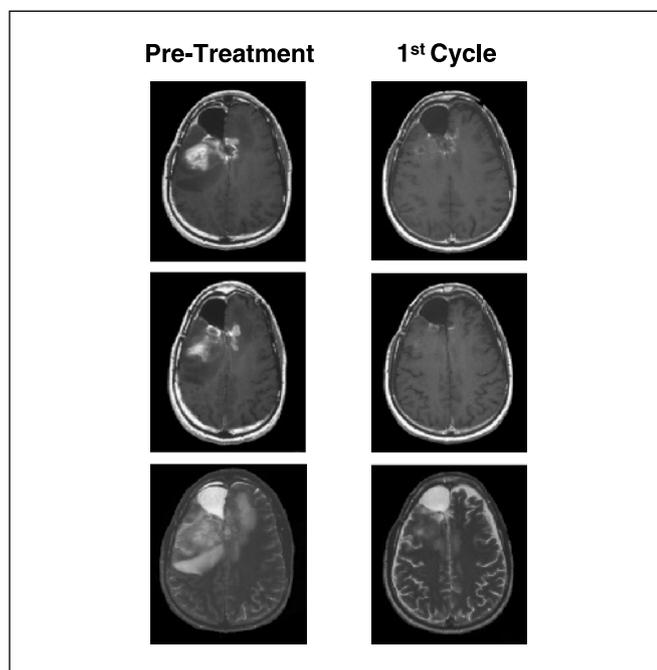


Fig. 1. Partial response to gefitinib plus sirolimus. Top and middle, axial T₁-weighted sequences following gadolinium administration; bottom, T₂-weighted sequences. After one cycle of treatment, a marked reduction of both contrast-enhancing tumor and associated edema was observed. The radiographic response, accompanied by marked clinical improvement, was maintained for 4 months, at which point a progressive tumor developed.

escalation design of this study. Furthermore, tumor samples evaluated in our trial were obtained at either initial diagnosis or after prior therapy and therefore may not have reflected the actual molecular genetic profile of the tumor at study entry. Nonetheless, the potential of such assays to prospectively identify appropriate cohorts of malignant glioma patients for treatment with selected targeted therapeutics was recently shown (7). In this analysis, patients with archival tumor samples showing p-AKT and EGFR amplification had a significantly greater likelihood of response to the EGFR TKI erlotinib.

The rate of radiographic response on the current study was comparable with that observed among glioblastoma multiforme patients treated with temozolomide at first recurrence (45). However, PFS on the current study was similar to that achieved on our prior phase II study with gefitinib alone (6). Although the assessment of antitumor activity is limited in any phase I study, several additional factors may have affected our study's outcome. First, patients were heavily pretreated, having enrolled following treatment with a median of two prior chemotherapy agents (range, 1-6) and a median of two prior recurrences (range, 1-3). Second, nearly all patients on the current study had bulky measurable tumor, whereas only 11 of 53 patients (21%) enrolled on our prior phase 2 study had measurable tumor (6). Third, EGFRvIII expression, which was unable to be assessed in the current study due to technical factors with the EGFRvIII immunohistochemistry assay, may have also affected response (7, 46). Fourth, our pharmacokinetic studies confirm that concurrent use of EIAEDs markedly diminish gefitinib exposure. Finally, and perhaps most importantly, pharmaco-

dynamic measures to assess the study regimen's effect on intratumoral PI3K and mTOR signaling was not assessed. Therefore, confirmation that either study agent was successfully delivered at dose levels required to inhibit the intended intracellular target was not obtained. Ongoing and planned clinical trials with EGFR and mTOR inhibitors that incorporate pharmacodynamic evaluations of tumor cell targets may clarify this critical issue. Finally, it is possible that suppressing both EGFR and mTOR may not be sufficient to effectively treat some glioblastoma multiforme tumors due to aberrant activation of alternative downstream PI3K mediators or other growth factor/survival pathways. The identification of several signal transduction pathways commonly altered in malignant glioma suggests that targeting pathways in parallel may also contribute to effective therapeutic synergy.

In conclusion, we report the first clinical trial incorporating a combinatorial regimen of signal transduction inhibitors for malignant glioma patients. In addition to establishing the MTD of this regimen, we confirm that therapeutics targeting EGFR and mTOR can be safely coadministered to malignant glioma patients. Phase 2 trials to evaluate the antitumor activity of EGFR and mTOR targeting regimens are under way for recurrent malignant glioma patients. Combinatorial regimens, including those designed to simultaneously target key upstream and downstream signaling mediators, represent an important advance in the evaluation of targeted therapeutics for cancer patients. The therapeutic potential of such combinatorial approaches for future studies is noteworthy but critically hinges on the comprehensive integration of clinical, pretreatment tumor biomarker, pharmacokinetic and intratumoral pharmacodynamic measures.

Table 7. Association of FISH and immunohistochemistry data and outcome

	Best radiographic response		
	PR/SD (%)*	PD (%)	Total (%)*
FISH			
EGFR amplified [†]	3 (50)	3 (50)	6 (43)
EGFR not amplified	3 (38)	5 (63)	8 (57)
PTEN loss [‡]	4 (57)	3 (43)	7 (50)
PTEN no loss	2 (29)	5 (71)	7 (50)
Immunohistochemistry[§]			
EGFR high	3 (33)	6 (67)	9 (90)
EGFR low	1 (100)	0	1 (10)
p-S6 high	2 (33)	4 (67)	6 (60)
p-S6 low	2 (50)	2 (50)	4 (40)
p-MAPK high	3 (50)	3 (50)	6 (60)
p-MAPK low	1 (25)	3 (75)	4 (40)
p-AKT high	4 (44)	5 (56)	9 (90)
p-AKT low	0	1 (10)	1 (10)

Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease.

*% Patients with an evaluable assay.

[†]Amplified: EGFR/chromosome 7 centromere ratio of >2.0.

[‡]Loss: 30% of nuclei with definitive loss and 20-30% with indeterminate loss.

[§]High, 1-4+ intensity in ≥25% of tumor cells; low, either no staining or 1-4+ intensity in <25% of tumor cells.

References

1. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–96.
2. Wong ET, Hess KR, Gleason MJ, et al. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol* 1999;17:2572–8.
3. Choe G, Horvath S, Cloughesy TF, et al. Analysis of the phosphatidylinositol 3'-kinase signaling pathway in glioblastoma patients *in vivo*. *Cancer Res* 2003;63:2742–6.
4. Chakravarti A, Zhai G, Suzuki Y, et al. The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. *J Clin Oncol* 2004;22:1926–33.
5. Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase/AKT pathway in human cancer. *Nat Rev Cancer* 2002;2:489–501.
6. Rich JN, Reardon DA, Peery T, et al. Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* 2004;22:133–42.
7. Haas-Kogan DA, Prados MD, Tihan T, et al. Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. *J Natl Cancer Inst* 2005;97:880–7.
8. Camp ER, Summy J, Bauer TW, et al. Molecular mechanisms of resistance to therapies targeting the epidermal growth factor receptor. *Clin Cancer Res* 2005;11:397–405.
9. Goudar RK, Shi Q, Hjelmeland MD, et al. Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. *Mol Cancer Ther* 2005;4:101–12.
10. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. *Nat Rev Cancer* 2004;4:335–48.
11. Schmelzle T, Hall MN. TOR, a central controller of cell growth. *Cell* 2000;103:253–62.
12. Chang SM, Kuhn J, Wen P, et al. Phase I/pharmacokinetic study of CCI-779 in patients with recurrent malignant glioma on enzyme-inducing antiepileptic drugs. *Invest New Drugs* 2004;22:427–35.
13. Galanis E, Buckner JC, Maurer MJ, et al. Phase II trial of Temsirolimus (CCI-779) in recurrent glioblastoma multiforme: North Central Cancer Treatment Group. *J Clin Oncol* 2005;23:5294–304.
14. Neshat MS, Mellinghoff IK, Tran C, et al. Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. *Proc Natl Acad Sci U S A* 2001;98:10314–9.
15. Duerr EM, Rollbrocker B, Hayashi Y, et al. PTEN mutations in gliomas and glioneuronal tumors. *Oncogene* 1998;16:2259–64.
16. Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997;275:1943–7.
17. Rasheed BK, Stenzel TT, McLendon RE, et al. PTEN gene mutations are seen in high-grade but not in low-grade gliomas. *Cancer Res* 1997;57:4187–90.
18. Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumour suppressor gene, MIMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* 1997;15:356–62.
19. Swaisland H, Smith R, Farebrother J, Laight A. The effect of the induction and inhibition of CYP3A4 on the pharmacokinetics of single oral doses of ZD1839 ('Iressa'), a selective epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), in healthy male volunteers. In: *Proc Am Soc Clin Oncol*. Orlando, FL; May 18–21, 2002 2002, p. 83a.
20. Brookmeyer R, Crowley J. A confidence interval for the median survival time. *Biometrics* 1982;38:29–41.
21. Klein JP. Small sample moments of some estimators of the variance of the Kaplan-Meier and Nelson-Aalen estimators. *Scand J Stat* 1991;18:333–40.
22. Friedman HS, Petros WP, Friedman AH, et al. Irinotecan therapy in adults with recurrent or progressive malignant glioma. *J Clin Oncol* 1999;17:1516–25.
23. Wiltshire RN, Herndon JE II, Lloyd A, et al. Comparative genomic hybridization analysis of astrocytomas: prognostic and diagnostic implications. *J Mol Diagn* 2004;6:166–79.
24. Amador ML, Maitra A, Gruenewald V, Peralba JM, Hidalgo M. Determinants of resistance to OSI-774 in billiary tract carcinoma cell lines. In: *Proc Am Soc Clin Oncol*, Chicago IL, May 31-June 3, 2003 2003, pp. 213.
25. Fan QW, Specht KM, Zhang C, et al. Combinatorial efficacy achieved through two-point blockade within a signaling pathway—a chemical genetic approach. *Cancer Res* 2003;63:8930–8.
26. Perez-Soler R, Ling Y, Lia M, et al. Molecular mechanisms of resistance to the HER1/EGFR tyrosine kinase inhibitor erlotinib HCl in human cell lines. In: *Proc Am Soc Clin Oncol*, Chicago IL, May 31-June 3, 2003 2003, pp. 190.
27. Rao R, Sarkaria J, Frederick L, Erlichman C, CD J. Synergistic inhibition of glioma cell growth upon combination therapy with the mTOR inhibitor, rapamycin, and the epidermal growth factor receptor inhibitor, EKI-785. In: *American Association of Cancer Research Annual Meeting*, Toronto, Ontario, Canada, April 5–9, 2003 2003, pp. 164.
28. Vitoria-Petit AM, Kerbel RS. Acquired resistance to EGFR inhibitors: mechanisms and prevention strategies. *Int J Radiat Oncol Biol Phys* 2004;58:914–26.
29. Fleming TP, Saxena A, Clark WC, et al. Amplification and/or overexpression of platelet-derived growth factor receptors and epidermal growth factor receptor in human glial tumors. *Cancer Res* 1992;52:4550–3.
30. Hermanson M, Funa K, Hartman M, et al. Platelet-derived growth factor and its receptors in human glioma tissue: expression of messenger RNA and protein suggests the presence of autocrine and paracrine loops. *Cancer Res* 1992;52:3213–9.
31. Takano S, Yoshii Y, Kondo S, et al. Concentration of vascular endothelial growth factor in the serum and tumor tissue of brain tumor patients. *Cancer Res* 1996;56:2185–90.
32. Brem S, Tsanaclis AM, Gately S, Gross JL, Herblin WF. Immunolocalization of basic fibroblast growth factor to the microvasculature of human brain tumors. *Cancer* 1992;70:2673–80.
33. Chakravarti A, Loeffler JS, Dyson NJ. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. *Cancer Res* 2002;62:200–7.
34. Natale R, Shak S, Aronson N, et al. Quantitative gene expression in non-small cell lung cancer from paraffin-embedded tissue specimens: Predicting response to gefitinib, an EGFR kinase inhibitor. In: *Proc Am Soc Clin Oncol*, Chicago, Illinois, May 31-June 2, 2003 2003, pp. 190.
35. Haas-Kogan D, Shalev N, Wong M, et al. Protein kinase B (PKB/Akt) activity is elevated in glioblastoma cells due to mutation of the tumor suppressor PTEN/MMAC. *Curr Biol* 1998;8:1195–8.
36. Davies MA, Lu Y, Sano T, et al. Adenoviral transgene expression of MMAC/PTEN in human glioma cells inhibits Akt activation and induces anoikis. *Cancer Res* 1998;58:5285–90.
37. Li B, Chang CM, Yuan M, McKenna WG, Shu HK. Resistance to small molecule inhibitors of epidermal growth factor receptor in malignant gliomas. *Cancer Res* 2003;63:7443–50.
38. She QB, Solit D, Basso A, Moasser MM. Resistance to gefitinib in PTEN-null HER-overexpressing tumor cells can be overcome through restoration of PTEN function or pharmacologic modulation of constitutive phosphatidylinositol 3'-kinase/Akt pathway signaling. *Clin Cancer Res* 2003;9:4340–6.
39. Bianco R, Shin I, Ritter CA, et al. Loss of PTEN/MMAC1/TEP in EGF receptor-expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. *Oncogene* 2003;22:2812–22.
40. Kokubo Y, Gemma A, Noro R, et al. Reduction of PTEN protein and loss of epidermal growth factor receptor gene mutation in lung cancer with natural resistance to gefitinib (IRESSA). *Br J Cancer* 2005;92:1711–9.
41. Giaccone G. Epidermal growth factor receptor inhibitors in the treatment of non-small-cell lung cancer. *J Clin Oncol* 2005;23:3235–42.
42. Kuypers DR. Benefit-risk assessment of sirolimus in renal transplantation. *Drug Saf* 2005;28:153–81.
43. Mahaley MS, Jr., Brooks WH, Roszman TL, et al. Immunobiology of primary intracranial tumors. Part 1: studies of the cellular and humoral general immune competence of brain-tumor patients. *J Neurosurg* 1977;46:467–76.
44. Roszman TL, Elliott LH, Brooks WH. Proliferative potential of T-cell lymphocytes from gliomas. *J Neurosurg* 1992;77:820–1.
45. Yung WK, Albright RE, Olson J, et al. A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. *Br J Cancer* 2000;83:588–93.
46. Learn CA, Hartzell TL, Wikstrand CJ, et al. Resistance to tyrosine kinase inhibition by mutant epidermal growth factor receptor variant III contributes to the neoplastic phenotype of glioblastoma multiforme. *Clin Cancer Res* 2004;10:3216–24.

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