Neuro-Oncology

Neuro-Oncology 16(4), 567–578, 2014
doi:10.1093/neuonc/not247
Advance Access date 26 January 2014

Phase I/II study of erlotinib and temsirolimus for patients with recurrent malignant gliomas: North American Brain Tumor Consortium trial 04-02

Patrick Y. Wen, Susan M. Chang, Kathleen R. Lamborn, John G. Kuhn, Andrew D. Norden, Timothy F. Cloughesy, H. Ian Robins, Frank S. Lieberman, Mark R. Gilbert, Minesh P. Mehta, Jan Drappatz, Morris D. Groves, Sandro Santagata, Azra H. Ligon, W.K. Alfred Yung, John J. Wright, Janet Dancey*, Kenneth D. Aldape, Michael D. Prados, and Keith L. Ligon

Center for Neuro-Oncology, Dana Farber/Brigham and Women's Cancer Center, Boston, Massachusetts (P.Y.W., A.D.N., J.D.); Department of Neurosurgery, University of California, San Francisco, San Francisco, California (S.M.C., K.R.L., M.D.P.); University of Texas Health Science Center, San Antonio, Texas (J.G.K.); Division of Neuro-Oncology, Department of Neurology, University of California, Los Angeles, Los Angeles, California (T.F.C.); University of Wisconsin, Madison Wisconsin (H.I.R., M.P.M.); Neurooncology Program, Division of Hematology/Oncology, University of Pittsburgh Medical Center Cancer Pavilion, Pittsburgh, Pennsylvania (F.S.L.); Division of Neuro-Oncology, MD Anderson Cancer Center, Houston, Texas (M.R.G., M.D.G., W.K.A.Y., K.D.A.); Center for Molecular Oncologic Pathology, Dana Farber/Brigham and Women's Cancer Center, Boston, Massachusetts (S.S., A.H.L.); Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts (S.S., A.H.L., K.L.L.); Investigational Drug Branch, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Maryland (J.D., J.J.W.)*

Corresponding Author: Patrick Y. Wen, MD, Center for Neuro-Oncology, Dana Farber/Brigham and Women's Cancer Center, 450 Brookline Avenue, Boston, MA 02215 (pwen@partners.org).

Background. Inhibition of epidermal growth factor receptor (EGFR) and the mechanistic target of rapamycin (mTOR) may have synergistic antitumor effects in high-grade glioma patients.

Methods. We conducted a phase I/II study of the EGFR inhibitor erlotinib (150 mg/day) and the mTOR inhibitor temsirolimus. Patients initially received temsirolimus 50 mg weekly, and the dose adjusted based on toxicities. In the phase II component, the primary endpoint was 6-month progression-free survival (PFS6) among glioblastoma patients.

Results. Twenty-two patients enrolled in phase I, 47 in phase II. Twelve phase I patients treated at the maximum tolerated dosage were included in the phase II cohort for analysis. The maximum tolerated dosage was 15 mg temsirolimus weekly with erlotinib 150 mg daily. Dose-limiting toxicities were rash and mucositis. Among 42 evaluable glioblastoma patients, 12 (29%) achieved stable disease, but there were no responses, and PFS6 was 13%. Among 16 anaplastic glioma patients, 1 (6%) achieved complete response, 1 (6%) partial response, and 2 (12.5%) stable disease, with PFS6 of 8%. Tumor levels of both drugs were low, and posttreatment tissue in 3 patients showed no reduction in the mTOR target phosphorylated (phospho-)S6$^{S235/236}$ but possible compensatory increase in phospho-Akt^{S473}. Presence of EGFR variant III, phospho-EGFR, and EGFR amplification did not correlate with survival, but patients with elevated phospho–extracellular signal-regulated kinase or reduced phosphatase and tensin homolog protein expression had decreased progression-free survival at 4 months.

Conclusion. Because of increased toxicity, the maximum tolerated dosage of temsirolimus in combination with erlotinib proved lower than expected. Insufficient tumor drug levels and redundant signaling pathways may partly explain the minimal antitumor activity noted.

Keywords: anaplastic glioma, clinical trial, epidermal growth factor, erlotinib, glioblastoma, temsirolimus.

High-grade gliomas are the most common type of brain tumor in adults.[1] Despite optimal therapy, patients with glioblastoma (GBM) have a median survival of only 14–19 months, while those with anaplastic astrocytoma (AA) have a median survival of 24–36 months.[2] For patients with high-grade glioma whose tumors recur, the median time to tumor progression is only 9–13 weeks.[3] There is a need for more effective therapies based on novel mechanisms of action.

Epidermal growth factor receptor (EGFR) is amplified and overexpressed in 40%–50% of GBM,[4] and nearly half of these tumors

Received 13 October 2013; accepted 11 November 2013

have a constitutively activated mutation known as EGFR variant (v)III.[5,6] Genomic alterations (deletions or mutations) of phosphatase and tensin homolog (PTEN) lead to protein loss or reduction in 30%–40% of GBM.[6–10] These molecular abnormalities activate the pathways for mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt/mechanistic target of rapamycin (mTOR), resulting in tumor proliferation, angiogenesis, and inhibition of apoptosis.

Several phase II trials evaluated the benefit of EGFR inhibitors in patients with recurrent malignant glioma. Objective response rates ranged from 0% to 26%, but there was no apparent survival benefit.[11–13] Glioblastomas with EGFRvIII and wild-type PTEN[14] and tumors with low levels of phospho-Akt[15] appear to be sensitive to EGFR inhibitors, suggesting that mTOR inhibition may overcome resistance to these drugs.[16] Other studies showed no relationship between EGFR or PTEN genotype and response.[17–20] Temsirolimus (CCI-779, Torisel, Wyeth) is metabolized to sirolimus (rapamycin), an mTOR inhibitor. Although temsirolimus inhibits growth in malignant glioma cell lines,[21] phase II trials of temsirolimus in recurrent GBM have shown minimal antitumor activity.[22,23]

In preclinical studies, sirolimus and the EGFR inhibitor EKI-785 achieved synergistic antitumor effects in GBM cell lines.[24] Sirolimus and erlotinib also demonstrated synergistic activity, regardless of PTEN status.[16,25] A phase I study of gefitinib and sirolimus in recurrent malignant glioma demonstrated acceptable toxicity,[26] and a pilot study of gefitinib or erlotinib and sirolimus in heavily pretreated recurrent GBM patients found a 19% partial response (PR) rate and 25% 6-month progression-free survival (PFS6).[27] Given the potential synergy of EGFR/mTOR therapy, the North American Brain Tumor Consortium conducted a phase I/II study of erlotinib and temsirolimus in recurrent high-grade glioma.

Materials and Methods

Patient Eligibility

Eligibility criteria were the same in the phase I and phase II components, except as we will note. Adults with histologically confirmed supratentorial high-grade gliomas with tumor recurrence on MRI were eligible. A baseline MRI was performed within 2 weeks of registration on a stable steroid dosage for ≥5 days. Patients had an interval of ≥12 weeks from the completion of radiotherapy to study entry. There was no limitation to the number of prior therapies for patients enrolled in the phase I component, whereas phase II patients were permitted to have treatment for ≤2 prior relapses. Phase II patients were required to have tumor tissue from a prior surgery. The protocol permitted up to 12 phase II patients to receive treatment with both study drugs prior to surgery. Tissue obtained at surgery provided data on tumor drug concentrations and pharmacodynamic effects. Patients enrolled in this surgical arm resumed treatment upon recovery from surgery.

Patients who had been previously treated with EGFR or mTOR inhibitors were excluded. Patients were required to have recovered from the toxic effects of prior therapy. Enzyme-inducing antiepileptic drugs (EIAEDs) that induce cytochrome P450 enzymes were not permitted because of potential drug interactions. Additional eligibility criteria included KPS ≥60, life expectancy ≥8 weeks, adequate bone marrow function (absolute neutrophil count ≥1500/mm^3, platelet count ≥100 000/mm^3, hemoglobin ≥10/dL), adequate liver function (alanine aminotransferase and alkaline phosphatase ≤2 times the upper limit of normal [ULN]; bilirubin <1.5 mg/dL), and adequate renal function (blood urea nitrogen or creatinine ≤1.5 times ULN). Contraception was required for patients of childbearing potential. Pregnant women and patients with serious intercurrent medical illnesses were excluded.

The study was approved by the local institutional review boards (IRBs) and conducted in accordance with institutional and federal guidelines for human investigations. Patients were informed of the investigational nature of the study and signed IRB-approved informed consent forms prior to enrollment.

Evaluation During Study

For both phase I and phase II patients, history and physical examination were performed at baseline and then at the start of each 4-week cycle. Complete blood count, routine serum chemistries, and lipid tests were obtained weekly for the first 4 weeks and then every 2 weeks. MRI was performed at baseline and then prior to every other cycle. Determination of response or progression was made using the Macdonald criteria.[28] Responses had to be present on 2 consecutive scans and were centrally reviewed. A neuropathologist (K.A.) conducted a central review of pathology.

Treatment Plan

Erlotinib and temsirolimus were supplied by the National Cancer Institute's Division of Cancer Treatment and Diagnosis Cancer Therapy Evaluation Program under a Cooperative Research and Development Agreement with OSI Pharmaceuticals and Wyeth Pharmaceuticals. Patients received oral erlotinib on an empty stomach at 150 mg once daily. In the phase I component, the starting dose of temsirolimus was 50 mg intravenously once weekly, with a plan to escalate toward the single-agent dose of 170 mg once weekly.[22] Three patients were to be treated at each dose level, with an additional 3 patients added to the cohort if any subject developed a dose-limiting toxicity (DLT). Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria v3.0 (http://ctep.info.nih.gov/reporting/index.html). Dose-limiting toxicities were defined as grade 3 thrombocytopenia, grade 4 anemia and neutropenia, grade ≥3 nonhematologic toxicity, and failure to recover from toxicities to be eligible for retreatment within 2 weeks of the last doses of either drug. The maximum tolerated dose (MTD) was based on the first 4 weeks of treatment. Due to greater than expected incidences of rash and mucositis, the definition of DLT was modified to include grade 3 nonhematologic toxicities only if they were refractory to maximal medical therapy. The MTD was defined as the dose at which fewer than one-third of patients experienced a DLT.

In the phase II component, all participants began therapy at 150 mg erlotinib and the MTD of temsirolimus. After 4 weeks of treatment, patients who tolerated treatment well were permitted to increase the dose of erlotinib to 175 mg daily for 2 weeks. A second dose escalation to 200 mg daily was permitted. Patients in the surgical arm received erlotinib 150 mg daily for 5–7 days prior to surgery and temsirolimus at the MTD 3–24 h prior to surgery. Treatment resumed upon recovery from surgery.

Pharmacokinetic Studies

Sample collection

In the phase I portion, blood samples were obtained before and after erlotinib and temsirolimus administration on days 1 and 2 of cycles 1 and 2. On these days, patients took erlotinib at the start of the 30-min temsirolimus infusion. For measurement of temsirolimus levels, whole blood (5 mL) was collected in EDTA-containing tubes. For measurement of erlotinib and OSI-420 levels, a second specimen of whole blood (5 mL) was collected in sodium- or lithium-containing tubes. Blood specimens were collected prior to administration of either drug; at the end of the temsirolimus infusion; and at 1, 2, 4, 6, and 24 h after erlotinib administration but prior to the next day's erlotinib dose.

Prior to taking erlotinib on day 1 of cycles 1 and 2, whole blood (2 mL) was collected in a red-top tube and allowed to clot for 30 min prior to

centrifugation for the analysis of alpha-1-acid glycoprotein (AGP). All whole blood, plasma, and serum samples were stored at or below −20°C until analysis. For surgical patients, blood was drawn intraoperatively. Tumor tissue was flash frozen in liquid nitrogen and stored at or below −20°C. Prior to analysis, tissue was weighed and homogenized with 1 mL of analytical grade methanol.

Analytical methods

Analytical grade erlotinib, OSI-420, and the internal standard CP-396-059 were obtained from OSI Pharmaceuticals. Because the liquid chromatography/mass spectrometry technique cannot distinguish between the isomeric forms of OSI-413 and OSI-420, they are collectively referred to as OSI-420.[29] Concentrations of erlotinib and its O-demethylated metabolite OSI-420 in plasma and tissue were analyzed using liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization in the positive ion mode as previously described.[20] Selected ion monitoring was used for the fragment ion: erlotinib (394.5 → 278.0 m/z), OSI-420 (380.3 → 278.0 m/z), and CP-396-059 (408.4 → 292.0 m/z). The lower limit of detection of erlotinib and OSI-420 was 1 ng/mL. The interday precision proportions for erlotinib/OSI-420 were 8.3%/10.7% and 5.9%/8.3% for the low and high quality-control samples, respectively.

Analytical standards for temsirolimus and its deuterated internal standard and sirolimus and its internal standard (desmethoxyrapamycin) were obtained from Wyeth-Ayerst Research. Analysis of temsirolimus and sirolimus in whole blood and tissue was performed by 2 high-performance liquid chromatography assays using electrospray ionization mass spectrometry as previously reported.[30] Selected ion monitoring was used for the determination of the sodium adducts [M + Na] and the compound's respective fragment ion: temsirolimus (1052.3 → 1020.4 m/z), d7-temsirolimus (1057.3 → 1027.3 m/z), sirolimus (936.5 → 904.3 m/z), and desmethoxyrapamycin (906.4 → 874.4 m/z). The lower limit of detection was 3 ng/mL for both temsirolimus and sirolimus. The interday precision proportions for temsirolimus/sirolimus were 1.7%/12.1% and 10.5%/6.7%, respectively, for the low and high quality-control samples. A radial immunodiffusion assay (Bindarid) was used for the measurement of AGP in serum.

Pharmacokinetic analyses

Noncompartmental analysis was used. Peak concentrations (C_{max}) were determined by inspection of each individual's concentration-time curve. Terminal disposition rate constants were estimated by linear regression analysis of the log-concentration versus time. Terminal half-lives ($t_{1/2}$) were calculated by dividing 0.693 by the elimination rate constants. The area under the concentration-time curve (AUC) was calculated using the linear trapezoidal rule up to the last measurable time point (AUC_{0-24}), then extrapolated to infinity (AUC). Systemic clearance (CL) was determined by dividing the dose by AUC. The apparent volume of distribution at steady state (Vd_{ss}) was determined by the following relationship: $Vd_{ss} = (\text{Dose} \times \text{AUMC}/\text{AUC}^2) - (\text{Dose} \times \text{Duration of Infusion})/(2 \times \text{AUC})$, where AUMC is the area under the moment curve extrapolated to infinity. A metabolic ratio estimated as the ratio of the AUC metabolite to the AUC parent was used as a measure of the relative extent of conversion of temsirolimus to its metabolite, sirolimus. AUC_{sum} represents the aggregate of the parent and active metabolites AUC. The relative tumor tissue concentrations (ng/mL) were normalized to nanogram per gram dry weight.

Correlation of Tumor Genenotype With Benefit

Pathology

Tumors were collected with IRB approval of each institution from archival and surgical samples using consent and waiver of consent as appropriate. All samples were independently re-reviewed by board-certified neuropathologists (K.A., K.L.L., S.S.) using World Health Organization 2007 histologic grading criteria.

Immunohistochemistry

Formalin-fixed paraffin-embedded sections of patient tumors were utilized for immunohistochemical analysis using a Biogenix autostainer according to standard manufacturer methods. Heat antigen retrieval (citrate buffer) was used for all antibodies studied. The following antibodies and conditions were used: phosphorylated (p)EGFR (1:100; Santa Cruz Biotechnology), $pAkt^{S473}$ (1:50; #4060, Cell Signaling Technology [CST]), phosphorylated extracellular signal-regulated kinase (pERK)$1/2^{T202/Y204}$ (1:100; #4370, CST), $pS6^{S235/236}$ (1:100; #2211, CST), Stathmin (1:100; #3352, CST), and PTEN (1:100; #9559, CST). The percentage of tumor cells with any level of positive staining was scored according to the following protocols: Stathmin, pS6, pAKT: 0 = no positive cells, 1 = 0%–10%, 2 = 11%–30%, 3 = 31%–50%, 4 = 51%–80%, and 5 = 81%–100%; pEGFR, pERK: 0 = no positive cells, 1 = 0%–50%, and 2 = 51%–100%; PTEN: 0 = no positive cells, 1 = 1%–10%, 2 = 11%–50%, 3 = 51%–80%, 4 = 81%–90%, and 5 = 91%–100%. The average intensity of staining within tumor cells and relative to the most intense staining seen across the cohort for each marker was scored as 0 = no staining, 1 = low staining, 2 = medium staining, and 3 = strong staining. PTEN staining was also given an integrated score to identify cases where more than 25% of tumor cells exhibited reduced staining intensity (score of 0 or 1) relative to internal control positive signal in vessels (assigned intensity score 2).[14]

EGFRvIII detection

Testing for EGFRvIII RNA was performed by reverse transcription (RT)–PCR on RNA extracted from 5 formalin-fixed paraffin-embedded 4-μm sections (Qiagen RNeasy) using primers and assay conditions previously described.[31]

Cytogenetics

Fluorescence in situ hybridization (FISH) evaluation for 1p/19q deletion status was performed according to previously published methods using the Vysis 1p36/1q25 and 19q13/19p13 FISH Probes (Abbott Molecular).[32] Colorimetric in situ hybridization (CISH) for EGFR amplification detection was performed using Life Technologies EGFR SPoTLight Probe for EGFR alone (no chromosome enumeration probe detection), and samples were scored manually by a neuropathologist into the following categories: normal (2 signals per nucleus), abnormal (3–10 signals per nucleus, suggestive of polysomy 7), and high amplification (>10 signals per nucleus). Cases that were normal and abnormal were considered to be not amplified.

Statistical Considerations

The primary endpoints of the phase I component were: (i) to determine the MTD, (ii) to describe the toxicities, and (iii) to characterize the pharmacokinetics (PK). Differences in PK variables were evaluated using the unpaired 2-tailed *t*-test. The primary endpoint of the phase II component was PFS6 from the date of registration; for patients in the surgical arm, PFS6 was measured from the first postsurgery treatment date. In an analysis of 8 negative phase II trials in recurrent malignant glioma, PFS6 was 15% for GBM and 31% for anaplastic glioma (AG).[3] In this study, the AG cohort was considered exploratory. The sample size was chosen to discriminate between 15% and 35% PFS6 rates for the GBM patients. With accrual of 32 GBM patients, the trial would be considered successful if 8 achieved PFS6. This yields 0.92 power to detect a 35% PFS6 rate, with 0.90 probability of rejecting the treatment regimen if the PFS6 rate is only 15%. Patients treated in the phase I component at the MTD were included in efficacy analyses if they met phase II eligibility criteria.

Because most patients had progressed by 6 months, it was decided to use 4-month PFS status for purposes of evaluation of tumor markers that might predict outcome (PFS $>$ 4 vs PFS $\leq$ 4). The Cochran-Mantel-Haenszel test with ranks was used to compare the tumor marker characteristics between those with a positive versus negative outcome by this metric. Since the intent of these analyses was to identify markers for potential future studies, no adjustment was made for multiple comparisons.

Results

Phase I

Patient characteristics

Twenty-two eligible patients enrolled in the phase I component. One patient never received therapy because of a rapid decline and was excluded from the analysis. Twelve patients were treated at the MTD and are considered part of the phase II study analysis. Characteristics of the 9 remaining phase I patients are summarized in Table 1.

Maximum tolerated doses and toxicities

The MTD of temsirolimus was 15 mg once weekly in combination with erlotinib 150 mg daily. The temsirolimus dose was varied, while the erlotinib dose was held constant. The first 3 patients received temsirolimus 50 mg weekly. One patient developed intolerable grade 2 rash and mucositis. Another had grade 2 mucositis, dehydration, and hypotension; grade 3 elevations of alanine aminotransferase and aspartate aminotransferase; and grade 4 cardiac ischemia. Six patients were then treated at 25 mg weekly. One had grade 3 rash, one grade 3 mucositis and infection, and one grade 3 rash, diarrhea, and dehydration. Twelve patients were then treated at 15 mg weekly. In the first 6 patients, 2 developed grade 3 rash. The protocol was subsequently amended such that grade 3 nonhematologic toxicities were classified as DLTs if they were refractory to maximal medical therapy. Six additional patients were treated at the same dose; one experienced grade 3 hypophosphatemia in the first treatment cycle, but no other DLTs were observed. Treatment-related toxicities reported during the first cycle are summarized in Table 3.

Response data

Of the phase I patients treated above the MTD, one was not evaluable because of progression prior to the first scheduled scan. One patient with AA achieved PR. Three patients had stable disease (SD) at 8 weeks (2 GBM, 1 AA).

Pharmacokinetic results

The PK parameters for erlotinib/OSI-420 are summarized in Table 4. Course 2 PK parameters for erlotinib/OSI-420 reflect steady-state concentrations. AUC accumulation ratios between course 1 and course 2 for erlotinib and OSI-420 were 3.6 and 4.6, respectively. For comparison, we provide first course PK data[12] and steady-state PK parameters[33] for non-EIAED patients receiving 150 mg erlotinib monotherapy. Alpha-1-acid glycoprotein was elevated (normal 73 mg/dL) in the combined non-EIAED group ($n = 93$; average 101 ± 36.7 mg/dL) receiving erlotinib. There was a significant ($P < .05$) albeit poor positive correlation ($R_s = 0.1$) between AGP levels and the C_{max} and AUC values for both erlotinib and OSI-420. Fifteen patients had AGP levels obtained on both course 1 and course 2 for comparison. Course 1 AGP levels (101 ± 23 mg/dL) were lower than course 2 levels (132 ± 24 mg/dL; $P < .001$).

Table 1. Patient characteristics

	Phase I Patients, $n = 9$	Phase II Patients	
		Anaplastic Glioma, $n = 16$	Glioblastoma, $n = 43$
KPS			
Median	90	90	90
Range	70–100	60–100	60–100
Age, y			
Median	57	47	50
Range	33–74	29–72	20–69
Gender M/F, *n*	5/4	12/4	31/12
Prior chemotherapy treatments, *n*			
Median	2	1	1
Range	0–3	1–3	0–3
Histology, *n*			
Anaplastic glioma	3		
Glioblastoma	6		

Table 2. Molecular characteristics of patient tumors

All Tumors	*n*	%
Diagnosis	44	
Glioblastoma grade IV	31	70
Astrocytoma grade III	6	14
Oligodendroglioma grade III	6	14
Oligoastrocytoma grade III	1	2
PTEN protein reduction (IHC)	39	
Reduced	26	67
No reduction	13	33
1p/19q codeletion (FISH)	43	
Codeleted	2**	5
Not codeleted	41	95
Glioblastoma		
EGFR amplification (CISH)	23	
Amplified	11	48
Amplified + EGFRvIII	7	30
Not amplified	12	52
EGFRvIII RNA (RT-PCR)	31	
Positive	14	45
Negative	17	55
PTEN protein reduction (IHC)	27	
Reduced	16	59
No reduction	11	41

Abbreviation: IHC, immunohistochemistry.
**Detected in only oligodendrogliomas.

There were no significant differences in whole blood PK parameters for either temsirolimus or sirolimus (Tables 5 and 6) between courses 1 and 2. The PK values were consistent with reported single-agent data,[34] suggesting that there was no significant interaction with erlotinib. Clearance and volume of distribution values for temsirolimus were dose dependent, as reported for temsirolimus monotherapy.[35]

Phase II

Patient characteristics

Fifty-nine patients were enrolled in the phase II component (43 GBM and 16 AG). Twelve phase I patients who were treated at the MTD are considered part of the phase II cohort for purposes of analysis. Patient characteristics are summarized in Tables 1 and 2. Five patients were enrolled in the surgical arm: 1 AG and 4 GBM.

Table 3. Cycle 1 adverse events related to therapy with erlotinib or temsirolimus (phase I) in 21 evaluable patients

Adverse Event	Grade 2	Grade 3	Grade 4
Hematologic			
Anemia	0	0	0
Leukopenia	2	0	0
Granulocytopenia	0	0	0
Thrombocytopenia	3	1	0
Nonhematologic			
Anorexia	2	0	0
Cardiac ischemia	0	0	1
Dehydration	2	1	0
Diarrhea	1	1	0
Fatigue	2	0	0
Hypercholesterolemia	1	0	0
Hypertriglyceridemia	1	1	0
Hypocalcemia	2	0	0
Hypophosphatemia	4	1	0
Hypotension	2	0	0
Infection	0	1	0
Liver function test abnormality	2	1	0
Mucositis	5	1	0
Nausea	1	0	0
Pruritis	2	0	0
Rash	4	4	0
Vomiting	1	0	0

Toxicities

Treatment was moderately well tolerated. Six patients (11%) came off study due to toxicity, and 2 of these refused further tumor-directed therapy. Treatment-related grades 3 and 4 adverse events are summarized in Table 7. Grade 1 or 2 diarrhea (44%), mucositis (29%), and rash (58%) were the most common adverse events.

Response and progression-free survival data

One of the GBM patients in the surgical arm had only necrosis at surgery, did not receive postsurgery treatment, and is excluded from the efficacy analysis.

Among the 16 AG patients, there was 1 (6%) PR and 1 (6%) complete response. Two (12.5%) achieved SD. One patient was censored at 12 weeks because he stopped treatment for toxicity. Median PFS was 8 weeks (95% confidence interval [CI], 4–11 wk). The Kaplan–Meier estimate of PFS6 is 8% (Fig. 1). Among the 42 evaluable GBM patients, 12 (29%) achieved SD. There were no responses. Two patients were censored prior to 26 weeks because they stopped treatment for toxicity. Median PFS was 8 weeks (95% CI, 8–10 wk). The Kaplan–Meier estimate of PFS6 is 13% (Fig. 1).

Tumor tissue concentrations

Three of 5 surgical patient's' tumors were available for drug concentration analysis. Limited tumor tissue size (31–58 mg) restricted the analysis to either erlotinib/OSI-420 or temsirolimus. Study drug concentrations in tumor and plasma are reported in Table 8.

Results of tumor genotyping

Of the 59 patients in the study, 44 (31 GBM, 13 AG) had evaluable tissue for inclusion in correlative studies. The main cause for exclusion from correlative studies was lack of sufficient tissue available for analysis. Given their impact on interpretation of survival, we identified patients with codeletion of 1p/19q by FISH and noted that 2 cases in the AG cohort harbored codeletion and were histologically classified as grade III anaplastic oligodendrogliomas. We also assessed EGFR amplification by CISH and EGFRvIII mutation by RT-PCR using previously described methods,[31,36] because of the potential relevance of EGFR alterations for interpretation of results. Both markers were tightly associated with grade IV histology (GBM) as expected based on prior results. EGFR was highly amplified in 48% of GBM patients (11/23). Furthermore we identified combined EGFRvIII and EGFR amplification in 30% of GBM where combined results were available (7/23). The study cohort

Table 4. Erlotinib pharmacokinetic parameters

	Erlotinib 150 mg				OSI-420		
Course	C_1 ($n=11$)	C_1^{50} ($n=76$)	C_2 ($n=7$)	C_2^{33} ($n=3$)	C_1 ($n=11$)	C_1^{50} ($n=76$)	C_2 ($n=7$)
Cp_{max} (ng/mL)	642 (±283)	872 (±399)	2150 (±335)	2120 (±152)	49 (±33)	68 (±45)	175 (±71)
AUC_{0-24} (μg × hr/mL)	11 (±4.78)	12 (±5.01)	39 (±6.46)	38 (±30)	0.72 (±0.43)	0.84 (±0.48)	3.33 (±0.82)

Abbreviations: C_1, course 1; C_2, course 2.

Table 5. Temsirolimus pharmacokinetic parameters

Dose	C_{max} (ng/mL)		$t_{1/2}$* (hr)		AUC (μg × hr/mL)		CL (L/hr)		Vd_{ss} (L)	
Course	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
15 mg (C_1 $n = 11$; C_2 $n = 11$)	460 (±219)	416 (±234)	12.0 (±3.11)	12.3 (±2.43)	1.68 (±0.53)	1.53 (±0.37)	9.7 (±2.92)	10.3 (±2.66)	126 (±44.8)	129 (±41.5)
25 mg (C_1 $n = 6$; C_2 $n = 4$)	428 (±115)	544 (±100)	12.1 (±1.91)	12.2 (±4.82)	1.63 (±0.26)	1.89 (±0.39)	15.0 (±2.82)	13.6 (±2.86)	192 (±40)	183 (±49)
25 mg ($n = 4$)	595 (±102)	–	12.8 (±1.09)	–	1.58 (±0.27)	–	16.1 (±2.51)	–	232 (±36)	–
50 mg (C_1 $n = 3$; C_2 $n = 1$)	451 (±281)	261	12.0 (±3.80)	10.5	1.92 (±1.29)	0.50	43.3 (±40.6)	99.0	567 (±493)	944

Abbreviations: C_1, course 1; C_2, course 2. *Harmonic mean.

therefore generally matched previously published cohorts with respect to clinical and molecular phenotypes.

Pharmacodynamic and posttreatment assessment of drug effects

Of the 5 surgery arm patients, sufficient tissue was obtained following treatment with drug on 3 patients (GBM.69-EGFR amplified, GBM.64-EGFR abnormal but not amplified, AG.60 anaplastic oligodendroglioma with 1p/19q codeletion) to allow them to be analyzed for pharmacodynamic response to drug. All 3 evaluable patients showed the presence of active tumor but with significant effects of treatment. Evaluation of $pS6^{S235/236}$ staining as a measure of both EGFR and mTOR inhibition showed activation at moderate levels in pre- and posttreatment samples with no qualitative change in percentage or intensity of tumor or normal brain following treatment with erlotinib and temsirolimus (Fig. 2). Similarly, measurement of $pAkt^{S473}$ levels as a read-out of upstream EGFR inhibition also showed no evidence of reduction, but instead exhibited some evidence for mildly increased levels in 2 tumors (Fig. 2, GBM.64, AG.60).

Molecular correlations with outcome

Prior studies have suggested that response to EGFR inhibitors in GBM and other AGs might be correlated with molecular evidence for activation of EGFR signaling in the presence of retained PTEN activity. To examine this possibility, we correlated EGFR amplification and EGFRvIII mutation status in combination with retained PTEN protein to survival using methods previously reported.[14] A similar prevalence of PTEN protein expression by immunohistochemistry was seen in our overall cohort compared with prior studies. Given that few patients survived longer than 6 months as a clinical endpoint, we utilized PFS >4 months (PFS4) for all molecular correlation analyses. We examined all phase II patients regardless of histology, and GBM patients separately.

Ten patients in the study (7 with GBM and 3 with anaplastic oligodendrogliomas) had PFS4 and some available tumor marker information. Tumors from these patients were associated with the presence of significantly retained PTEN protein expression when compared against the whole cohort (5/6 evaluable patients, $P = .02$). However, while GBM patients with PFS4 retained significant PTEN expression, this was not statistically significant (4/4, $P = .11$). The presence of EGFR amplification or EGFRvIII did not independently correlate with survival. Likewise, the direct activation status of EGFR using immunohistochemistry for pEGFR was not significantly associated with survival despite all tumors showing some level of activity for the receptor (Table 9).[37] In GBM patients the presence of EGFRvIII or EGFR amplification in combination with retained PTEN expression did not reach statistical significance ($Ps = .33$ and .63, respectively) for correlation with PFS4, albeit patient numbers were small (3/7 patients with EGFRvIII, 1/3 patients amplified).

Downstream effectors of EGFR activation within the PI3K/Akt/mTOR pathway were also evaluated using immunohistochemical detection of $pAkt^{S473}$ and Stathmin1.[38] Overall most patients, including GBM patients, showed significant activation of these downstream effectors in the de novo tumors, but no statistically significant correlation with outcome measures was identified using multiple parameters.

Table 6. Sirolimus pharmacokinetic parameters

Dose	C_{max} (ng/mL)		AUC_{0-t} (μg × hr/mL)		AUC_{0-t} Ratio (μg × hr/mL)		AUC_{sum} (μg × hr/mL)	
Course	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
15 mg (C_1 $n = 11$; C_2 $n = 11$)	19.42 (±8.60)	23.17 (±8.50)	0.32 (±0.08)	0.37 (±0.15)	0.25 (±0.09)	0.31 (±0.10)	1.65 (±0.40)	1.58 (±0.39)
25 mg (C_1 $n = 6$; C_2 $n = 3$)	45.80 (±23)	81.60 (±51)	0.79 (±0.47)	1.17 (±0.64)	0.57 (±0.34)	0.83 (±0.51)	2.15 (±0.59)	2.64 (±0.49)
50 mg (C_1 $n = 3$; C_2 $n = 1$)	66.60 (±27.2)	46.70	1.43 (±0.64)	0.91	1.10 (±0.64)	2.11	3.22 (±1.98)	1.34

Abbreviations: C_1, course 1; C_2, course 2.

Table 7. Number of patients with grades 3 and 4 adverse events related to therapy with erlotinib or temsirolimus (phase II)

Adverse Event	Grade 3	Grade 4
Dry skin	1	0
Fatigue	2	0
Hypercholesterolemia	1	0
Hyperglycemia	1	1
Hypertriglyceridemia	1	0
Hypocalcemia	1	0
Hyponatremia	1	0
Hypophosphatemia	4	0
Liver function test elevation	1	0
Lymphopenia	4	1
Mucositis	5	0
Pain in limb	1	0
Rash	10	0
Retinopathy	0	1
Thrombocytopenia	2	0
Weakness	1	0

To measure mTOR activity and its potential correlation with effects of temsirolimus and time to progression, we assessed phosphorylation of S6 ribosomal protein, a direct substrate of the mTOR downstream effector S6 kinase 1, by immunohistochemistry. We utilized the phospho-specific antibody against $pS6^{S235/236}$ given prior evidence suggesting its correlation with response to mTOR inhibition in GBM.[39] Both GBM and AG patients expressed high levels of $pS6^{S235/236}$, but no significant correlation was noted with PFS4 as an independent variable in GBM patients.

Resistance to PI3K/Akt/mTOR inhibition and specifically EGFR inhibition may be mediated by MAPK pathway activation in GBM and other cancers. We therefore evaluated whether evidence of such an escape mechanism might explain the lack of response seen in this study. Examination of GBM patients for the presence of $pERK1/2^{T202/Y204}$, a downstream biomarker of MAPK pathway activation, showed that qualitatively $pERK1/2^{T202/Y204}$ expression was present in a lower percentage of tumor cells in patients with PFS4 ($P = .04$). Examination to determine whether preferential activation of the PI3K/Akt pathway compared with the MAPK pathway in individual tumors might also correlate with survival also was not significant (pAkt intensity >1 with pERK percentage score <2; $P = .13$).

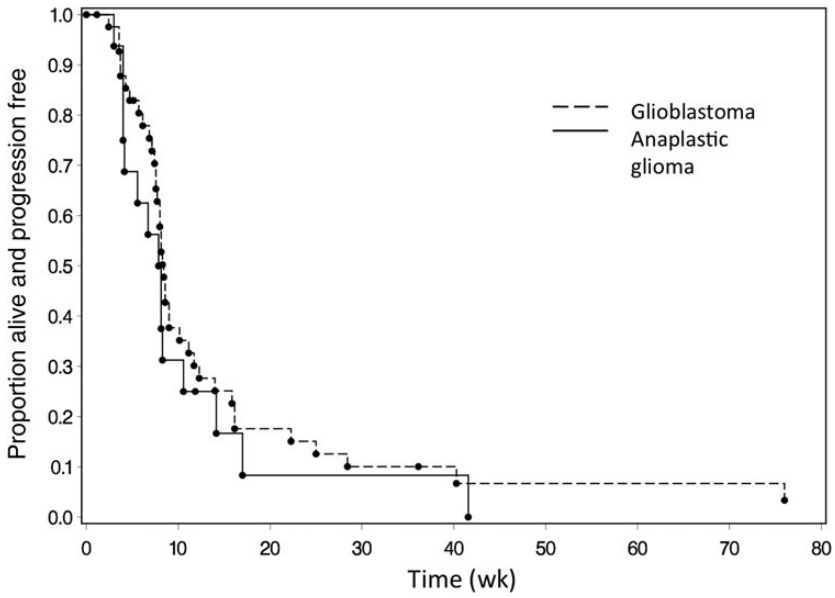

Fig. 1. Kaplan–Meier survival plot stratified by histology.

Discussion

Despite a strong preclinical rationale and early clinical evidence that combination therapy with EGFR and mTOR inhibitors is effective against recurrent high-grade glioma, we found minimal antitumor activity in this phase I/II trial of erlotinib and temsirolimus. Two smaller studies combining EGFR and mTOR inhibitors showed a similar lack of efficacy. In a study of 22 recurrent GBM patients treated with gefitinib and the mTOR inhibitor everolimus, 14% achieved PR, but PFS6 was <5%.[40] A phase II study of erlotinib and sirolimus in 32 recurrent GBM patients found no radiographic responses, but 15 patients (47%) achieved SD.[41]

Several explanations may account for these disappointing results. First, the combination proved more toxic than expected, with frequent rash, diarrhea, and mucositis. This required temsirolimus dose reductions instead of the planned escalations. The MTD of temsirolimus in combination with erlotinib was 15 mg weekly, which is <10% of the single-agent dose of 170 mg weekly.[22] Even at the MTD, 10 of 59 patients (17%) experienced grade 3 rash, and 5 (8.5%) experienced grade 3 mucositis. Finally, recent studies suggest that the lack of activity of EGFR inhibition in this study and others of GBM may be due in part to the limited activity of erlotinib and other type I EGFR kinase inhibitors against the inactive conformation of EGFR most commonly found in GBM.[42] We detected no correlation of survival or response with markers previously suspected as predictors, such as EGFR amplification, EGFRvIII mutation, and PTEN status. Our study therefore lends further evidence that inhibition of EGFR with existing type I classes of drugs will likely not be effective even in rational combinations, and evaluation of type II and other novel classes of EGFR inhibitors is warranted in the clinical trial setting for GBM.

Although 5 patients underwent surgery after treatment with erlotinib and temsirolimus, measurement of drug levels in plasma and tumor tissue was possible only in 3 patients, too few to allow any firm conclusions to be drawn. However, the limited pharmacokinetic and pharmacodynamic data suggest relatively poor penetration into the enhancing tumor by either erlotinib or temsirolimus. Pharmacokinetic assessments showed no evidence of significant interaction between erlotinib and temsirolimus. The poor accrual into this arm of the study and the inadequate sample collection and tissue preservation provided an invaluable lesson for subsequent surgical trials conducted by the consortium. In these latter studies, patients were eligible only if the surgeon felt that it was possible to resect the required amount of tumor for the proposed studies, much greater attention was paid to collecting and preserving tumor tissue appropriately, additional tissue for pharmacodynamic studies to complement information regarding drug concentrations was collected, and allocation of tissue for the proposed studies was strictly prioritized. In addition, whenever possible, tumor from nonenhancing areas of tumor where the blood–brain barrier was relatively intact was collected to provide additional information on the penetration of drugs into these areas. These refinements have made subsequent surgical studies much more informative.

Because our study had a limited amount of posttreatment tissue, we were unable to make solid conclusions about pre- and posttreatment Akt activation in the immediate posttreatment interval. Pharmacodynamic studies of $pAkt^{473}$ and $pS6^{S235/236}$ showed no evidence of significant pathway inhibition, and 2 of the 3 patients may have had slight increases in $pAkt^{473}$ levels

Table 8. Study drug concentrations in tumor tissue and blood

Sample		Erlotinib	OSI-420	Temsirolimus
1	Plasma (ng/mL)	515	50	–
	Tumor (ng/g)	386	51	–
2	Plasma (ng/mL)	–	–	–
	Tumor (ng/g)	743	81	–
3	Blood (ng/mL)	–	–	12
	Tumor (ng/g)	–	–	65

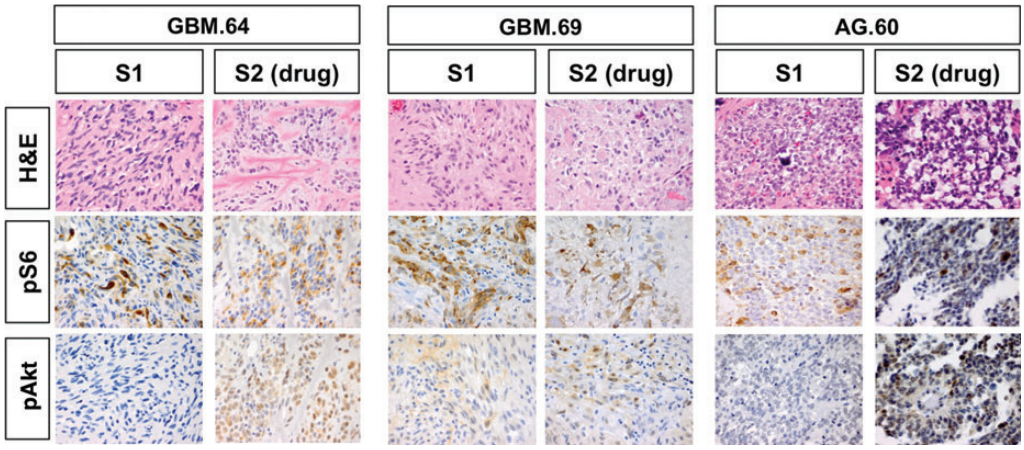

Fig. 2. Pharmacodynamic analysis of surgical biopsy tissue from patients treated with erlotinib and temsirolimus. Tissue samples taken from 3 patients prior to treatment with erlotinib and temsirolimus (S1) were compared with those taken after treatment for 7 days (S2 drug). Two GBM patients showed no evidence of histologic progression (GBM.64 and GBM.69), while an AG patient (AG.60) showed increased atypia, density consistent with histologic progression. No significant change in $pS6^{S235/236}$ staining was noted in paired samples. Qualitative increase in $pAkt^{S473}$ staining was noted in GBM.64 and AG.60 compared with pretreatment biopsies. H&E, hematoxylin and eosin.

Table 9. Molecular correlates and progression-free survival (subjects with PFS in boldface achieved PFS 4)

Subject #	Phase	Diagnosis	Surgery Arm	PFS (wk)	Clinical Response	EGFR (Copy Number)	EGFRvIII (RNA)	pEGFR (%)	pEGFR (Intensity)	PTEN (IHC level)	pAkt (Intensity)	pS6 (Intensity)	pERK (%)	pERK (Intensity)
1	I/II	A4	No	**157.7**	NR	–	Pos	–	–	–	3	3	1	3
2	II	A4	No	**76.0**	NR	–	Pos	1	2	Not reduced	1	3	1	1
3	I/II	A4	No	**40.3**	NR	No Amp	Neg	–	–	–	1	1	1	3
4	I/II	A4	No	**36.1**	NR	–	Neg	–	–	–	1	3	2	2
5	II	A4	No	**28.4**	NR	–	Pos	2	3	Not reduced	2	1	1	3
6	II	A4	No	**22.3**	NR	No Amp	Neg	–	–	Not reduced	2	3	2	1
7	II	A4	No	**16.1**	NR	Amplified	Neg	1	2	Not reduced	1	1	2	2
8	II	A4	No	15.9	NR	No Amp	Neg	1	2	Not reduced	2	3	2	2
9	II	A4	Yes	14.0	NR	No Amp	Neg	–	–	Not reduced	1	2	2	2
10	II	A4	No	11.7	NR	Amplified	Pos	1	2	Not reduced	3	2	–	–
11	II	A4	No	9.0	NR	Amplified	Pos	1	2	Reduced	2	2	2	3
12	II	A4	No	8.4	NR	–	Neg	0	0	Reduced	2	3	1	2
13	II	A4	Yes	8.3	NR	No Amp	Neg	–	–	Reduced	0	3	0	0
14	II	A4	No	8.1	NR	Amplified	Pos	2	3	Reduced	3	2	2	2
15	II	A4	No	8.0	NR	Amplified	Pos	–	–	–	2	3	–	–
16	II	A4	No	8.0	NR	No Amp	Pos	1	2	Not reduced	0	1	0	0
17	II	A4	No	7.7	NR	No Amp	Neg	0	0	Reduced	3	3	2	1
18	II	A4	No	7.6	NR	Amplified	Neg	2	1	Reduced	3	3	2	2
19	II	A4	No	7.4	NR	–	Pos	0	0	Reduced	1	3	2	2
20	II	A4	No	7.1	NR	No Amp	Neg	0	0	Not reduced	2	2	2	2
21	II	A4	No	6.9	NR	Amplified	Pos	1	2	Reduced	2	3	1	1
22	II	A4	No	6.1	NR	No Amp	Neg	1	1	Reduced	2	2	2	2
23	II	A4	No	5.7	NR	Amplified	Pos	1	2	Not reduced	2	3	2	3
24	II	A4	No	4.7	NR	No Amp	Pos	0	0	Reduced	2	3	2	2
25	II	A4	No	4.3	NR	Amplified	Pos	1	2	Reduced	1	1	2	1
26	II	A4	Yes	3.7	NR	Amplified	Neg	–	–	Not reduced	2	3	2	3
27	II	A4	No	3.7	NR	Amplified	Neg	1	1	Reduced	3	2	2	2
28	II	A4	No	3.6	NR	No Amp	Pos	0	0	Not reduced	2	2	2	2
29	II	A4	No	3.6	NR	–	Neg	0	0	Not reduced	3	2	2	2
30	II	A4	No	2.4	NR	No Amp	Neg	0	0	Not reduced	2	3	2	1
31	II	A4	No	1.1	NR	–	Neg	0	0	Not reduced	3	3	2	3
32	II	O3	No	**41.6**	NR			0	0	Not reduced	1	1	2	2
33	II	O3	No	**25.0**	NR			–	–	–	2	1	2	3
34	II	O3	Yes	**17.0**	PR			0	0	Reduced	1	2	–	–
35	II	A3	No	14.1	CR			1	1	Reduced	1	1	2	2
36	II	A3	No	10.6	NR			–	–	Reduced	0	3	2	2
37	II	A3	No	8.1	NR			0	0	Reduced	0	1	1	3
38	II	A3	No	8.1	NR			0	0	Not reduced	1	1	2	3
39	II	A3	No	6.7	NR			0	0	Reduced	1	2	2	1
40	II	O3	No	4.1	NR			2	3	Reduced	1	1	2	3
41	II	M3	No	4.0	NR			0	0	Reduced	1	1	1	3

Continued

Table 9. *Continued*

Subject #	Phase	Diagnosis	Surgery Arm	PFS (wk)	Clinical Response	EGFR (Copy Number)	EGFRvIII (RNA)	pEGFR (%)	pEGFR (Intensity)	PTEN (IHC level)	pAkt (Intensity)	pS6 (Intensity)	pERK (%)	pERK (Intensity)
42	II	O3	No	4.0	NR			–	–	Reduced	2	2	2	3
43	II	O3	No	4.0	NR			–	–	Reduced	1	3	1	3
44	II	A3	No	3.0	NR			0	0	Not reduced	1	1	1	2

Abbreviations: IHC, immunohistochemistry;A4, glioblastoma; O3, anaplastic oligodendroglioma; A3, anaplastic astrocytoma; NR, nonresponse; CR, complete response.

after treatment. These results could be consistent with poor drug penetration or, if drug was penetrant, would support a concern that mTOR inhibition may promote pAkt^{473} and mTORC2 activation.[43,44] Recent data indicate that mTOR inhibition may eliminate feedback attenuation of other survival signaling pathways, including that of MAPK.[45,46] The mTOR effector S6 kinase 1 activates insulin receptor substrate 1, which serves to regulate PI3K/Akt signaling. S6 kinase 1 also regulates expression of platelet-derived growth factor receptor, which drives PI3K/Akt signaling.[47] In a phase I study of recurrent PTEN-deficient GBM patients who underwent surgical resection, the mTOR inhibitor sirolimus significantly increased pAkt activation in 50% of patients.[39] Akt activation in this setting was associated with a shorter time to progression, but we did not see such associations within our study set.

Although the combination of erlotinib and temsirolimus was not active in this study, the paradigm of combination therapy with targeted molecular drugs for high-grade glioma is scientifically compelling. Future trials may need to take into account the recent observation that EGFR inhibition may not reliably reduce pAkt or pERK in the setting of EGFR extracellular domain mutations.[42] Strategies designed to improve prophylaxis and management of toxicities are needed, as targeted therapies may have additive or synergistic toxicity profiles. Use of more potent and specific agents with fewer off-target effects may result in reduced toxicity when used in combination. The optimal approach to overcome loss of negative feedback due to mTOR inhibition has not been determined. Preclinical data suggest that inhibiting the sirolimus-insensitive mTOR complex 2 in addition to the sirolimus-sensitive mTOR complex 1 may be fruitful.[48] Agents that inhibit PI3K or Akt,[49] in combination with agents that inhibit the MAPK pathway, may prove valuable as well. Our finding that a trend in pERK activation was present in patients with shorter PFS lends support to this concept. As we develop clinical trials to test these new strategies, incorporation of molecular endpoints will be critical in order to understand why success is often elusive.

Funding

This study was funded by U01 CA137443. K.L.L. acknowledges support from P01CA095616, the Ivy Foundation, and the Sontag Foundation.

Acknowledgments

We gratefully acknowledge the help of the NABTC Data Management Center (Dianna Merwin, Pam Peterson, Lisa Hughes, and Janelle Hibbert).

Conflict of interest statement. None declared.

References

1. *CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2004–2007*. Hinsdale, IL: Central Brain Tumor Registry of the United States; 2011.
2. Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med*. 2008;359:492–507.

3. 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;17:2572–2578.
4. Nicholas MK, Lukas RV, Jafri NF, et al. Epidermal growth factor receptor–mediated signal transduction in the development and therapy of gliomas. *Clin Cancer Res*. 2006;12:7261–7270.
5. Frederick L, Wang XY, Eley G, et al. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. *Cancer Res*. 2000;60:1383–1387.
6. Dunn GP, Rinne ML, Wykosky J, et al. Emerging insights into the molecular and cellular basis of glioblastoma. *Genes Dev*. 2012;26:756–784.
7. Bostrom J, Cobbers JM, Wolter M, et al. Mutation of the PTEN (MMAC1) tumor suppressor gene in a subset of glioblastomas but not in meningiomas with loss of chromosome arm 10q. *Cancer Res*. 1998;58:29–33.
8. Wang SI, Puc J, Li J, et al. Somatic mutations of PTEN in glioblastoma multiforme. *Cancer Res*. 1997;57:4183–4186.
9. 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;275:1943–1947.
10. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008;455:1061–1068.
11. Omuro AM, Faivre S, Raymond E. Lessons learned in the development of targeted therapy for malignant gliomas. *Mol Cancer Ther*. 2007;6:1909–1919.
12. Raizer JJ, Abrey LE, Lassman AB, et al. A phase II trial of erlotinib in patients with recurrent malignant gliomas and nonprogressive glioblastoma multiforme postradiation therapy. *Neuro Oncol*. 2010;12:95–103.
13. Yung WK, Vredenburgh JJ, Cloughesy TF, et al. Safety and efficacy of erlotinib in first-relapse glioblastoma: a phase II open-label study. *Neuro Oncol*. 2010;12:1061–1070.
14. Mellinghoff IK, Wang MY, Vivanco I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med*. 2005;353:2012–2024.
15. 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;97:880–887.
16. Wang MY, Lu KV, Zhu S, et al. Mammalian target of rapamycin inhibition promotes response to epidermal growth factor receptor kinase inhibitors in PTEN-deficient and PTEN-intact glioblastoma cells. *Cancer Res*. 2006;66:7864–7869.
17. van den Bent MJ, Brandes AA, Rampling R, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. *J Clin Oncol*. 2009;27:1268–1274.
18. de Groot JF, Gilbert MR, Aldape K, et al. Phase II study of carboplatin and erlotinib (Tarceva, OSI-774) in patients with recurrent glioblastoma. *J Neurooncol*. 2008;90:89–97.
19. Franceschi E, Cavallo G, Lonardi S, et al. Gefitinib in patients with progressive high-grade gliomas: a multicentre phase II study by Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO). *Br J Cancer*. 2007;96:1047–1051.
20. Lassman AB, Rossi MR, Raizer JJ, et al. 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. *Clin Cancer Res*. 2005;11:7841–7850.
21. Geoerger B, Kerr K, Tang CB, et al. Antitumor activity of the rapamycin analog CCI-779 in human primitive neuroectodermal tumor/medulloblastoma models as single agent and in combination chemotherapy. *Cancer Res*. 2001;61:1527–1532.
22. Chang SM, Wen P, Cloughesy T, et al. Phase II study of CCI-779 in patients with recurrent glioblastoma multiforme. *Invest New Drugs*. 2005;23:357–361.
23. Galanis E, Buckner JC, Maurer MJ, et al. Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. *J Clin Oncol*. 2005;23:5294–5304.
24. Rao RD, Mladek AC, Lamont JD, et al. Disruption of parallel and converging signaling pathways contributes to the synergistic antitumor effects of simultaneous mTOR and EGFR inhibition in GBM cells. *Neoplasia*. 2005;7:921–929.
25. Fan QW, Cheng CK, Nicolaides TP, et al. A dual phosphoinositide-3-kinase alpha/mTOR inhibitor cooperates with blockade of epidermal growth factor receptor in PTEN-mutant glioma. *Cancer Res*. 2007;67:7960–7965.
26. Reardon DA, Quinn JA, Vredenburgh JJ, et al. Phase 1 trial of gefitinib plus sirolimus in adults with recurrent malignant glioma. *Clin Cancer Res*. 2006;12:860–868.
27. Doherty L, Gigas DC, Kesari S, et al. Pilot study of the combination of EGFR and mTOR inhibitors in recurrent malignant gliomas. *Neurology*. 2006;67:156–158.
28. Macdonald DR, Cascino TL, Schold SC Jr, et al. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol*. 1990;8:1277–1280.
29. Hamilton M, Wolf JL, Rusk J, et al. Effects of smoking on the pharmacokinetics of erlotinib. *Clin Cancer Res*. 2006;12:2166–2171.
30. Kuhn JG, Chang SM, Wen PY, et al. Pharmacokinetic and tumor distribution characteristics of temsirolimus in patients with recurrent malignant glioma. *Clin Cancer Res*. 2007;13:7401–7406.
31. Yoshimoto K, Dang J, Zhu S, et al. Development of a real-time RT-PCR assay for detecting EGFRvIII in glioblastoma samples. *Clin Cancer Res*. 2008;14:488–493.
32. Snuderl M, Eichler AF, Ligon KL, et al. Polysomy for chromosomes 1 and 19 predicts earlier recurrence in anaplastic oligodendrogliomas with concurrent 1p/19q loss. *Clin Cancer Res*. 2009;15:6430–6437.
33. Hidalgo M, Siu LL, Nemunaitis J, et al. Phase I and pharmacologic study of OSI-774, an epidermal growth factor receptor tyrosine kinase inhibitor, in patients with advanced solid malignancies. *J Clin Oncol*. 2001;19:3267–3279.
34. Atkins MB, Hidalgo M, Stadler WM, et al. Randomized phase II 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*. 2004;22:909–918.
35. Hidalgo M, Buckner JC, Erlichman C, et al. A phase I and pharmacokinetic study of temsirolimus (CCI-779) administered intravenously daily for 5 days every 2 weeks to patients with advanced cancer. *Clin Cancer Res*. 2006;12:5755–5763.
36. Fischer I, de la Cruz C, Rivera AL, Aldape K. Utility of chromogenic in situ hybridization (CISH) for detection of EGFR amplification in glioblastoma: comparison with fluorescence in situ hybridization (FISH). *Diagn Mol Pathol*. 2008;17:227–230.
37. Stommel JM, Kimmelman AC, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science*. 2007;318:287–290.
38. Salvesen HB, Carter SL, Mannelqvist M, et al. Integrated genomic profiling of endometrial carcinoma associates aggressive tumors with indicators of PI3 kinase activation. *Proc Natl Acad Sci U S A*. 2009;106:4834–4839.
39. Cloughesy TF, Yoshimoto K, Nghiemphu P, et al. Antitumor activity of rapamycin in a phase I trial for patients with recurrent PTEN-deficient glioblastoma. *PLoS Med*. 2008;5:e8.

40. Kreisl TN, Lassman AB, Mischel PS, et al. A pilot study of everolimus and gefitinib in the treatment of recurrent glioblastoma (GBM). *J Neurooncol*. 2009;92:99–105.
41. Reardon DA, Desjardins A, Vredenburgh JJ, et al. Phase 2 trial of erlotinib plus sirolimus in adults with recurrent glioblastoma. *J Neurooncol*. 2010;96:219–230.
42. Vivanco I, Robins HI, Rohle D, et al. Differential sensitivity of glioma- versus lung cancer–specific EGFR mutations to EGFR kinase inhibitors. *Cancer Discov*. 2012;2:458–471.
43. O'Reilly KE, Rojo F, She QB, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res*. 2006;66:1500–1508.
44. Sun SY, Rosenberg LM, Wang X, et al. Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. *Cancer Res*. 2005;65:7052–7058.
45. Carracedo A, Ma L, Teruya-Feldstein J, et al. Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J Clin Invest*. 2008;118:3065–3074.
46. Wang X, Hawk N, Yue P, et al. Overcoming mTOR inhibition–induced paradoxical activation of survival signaling pathways enhances mTOR inhibitors' anticancer efficacy. *Cancer Biol Ther*. 2008;7:1952–1958.
47. Zhang H, Bajraszewski N, Wu E, et al. PDGFRs are critical for PI3 K/Akt activation and negatively regulated by mTOR. *J Clin Invest*. 2007;117:730–738.
48. Sarbassov DD, Guertin DA, Ali SM, et al. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*. 2005;307:1098–1101.
49. Wen PY, Lee EQ, Reardon DA, et al. Current clinical development of PI3 K pathway inhibitors in glioblastoma. *Neuro Oncol*. 2012;14:819–829.