Neuro-Oncology 12(12):1300–1310, 2010.
doi:10.1093/neuonc/noq099
Advance Access publication August 17, 2010

Phase II trial of bevacizumab and erlotinib in patients with recurrent malignant glioma

Sith Sathornsumetee, Annick Desjardins, James J. Vredenburgh, Roger E. McLendon, Jennifer Marcello, James E. Herndon, Alyssa Mathe, Marta Hamilton, Jeremy N. Rich, Julie A. Norfleet, Sridharan Gururangan, Henry S. Friedman, and David A. Reardon

The Preston Robert Tisch Brain Tumor Center, Department of Medicine (S.S., A.D., J.J.V., H.S.F.), Department of Surgery (S.S., A.D., J.J.V., A.M., J.A.N., S.G., H.S.F., D.A.R.), Department of Pathology (R.E.M., H.S.F.), Department of Cancer Biostatistics (J.M., J.E.H.), Department of Pediatrics (S.G., H.S.F., D.A.R.), Duke University Medical Center, Durham, North Carolina; Department of Medicine (Neurology) and Department of Stem Cell Biology and Regenerative Medicine, Cleveland Clinic, Cleveland, Ohio (J.N.R.); Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand (S.S.); OSI Pharmaceuticals, Inc., Melville, New York (M.H.)

Vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR) signaling are established contributors to malignant glioma (MG) biology. We, therefore, evaluated bevacizumab, a humanized anti-VEGF monoclonal antibody, in combination with the EGFR tyrosine kinase inhibitor erlotinib, in this phase 2 study for recurrent MG patients (www.ClinicalTrials.gov, NCT00671970). Fifty-seven patients ($n = 25$, glioblastoma [GBM]; $n = 32$, anaplastic glioma [AG]) were enrolled. The primary endpoint was 6-month progression-free survival (PFS-6). Overall survival (OS), radiographic response, pharmacokinetics, and correlative biomarkers were the secondary endpoints. Patients were stratified based on the concurrent use of enzyme-inducing antiepileptic drugs (EIAEDs). Bevacizumab (10 mg/kg) was given intravenously every 2 weeks. Erlotinib was orally administered daily at 200 mg/day for patients not on EIAEDs and 500 mg/day for patients on EIAEDs. PFS-6 and median OS were 28% and 42 weeks for GBM patients and 44% and 71 weeks for AG patients, respectively. Twelve (48%) GBM patients and 10 (31%) AG patients achieved a radiographic response. Erlotinib pharmacokinetic exposures were comparable between EIAED and non-EIAED groups. Rash, mucositis, diarrhea, and fatigue were common but mostly grades 1 and 2. Among GBM patients, grade 3 rash, observed in 32%, was associated with survival benefit, whereas elevated hypoxia-inducible factor-2 alpha and VEGF receptor-2 levels were associated with poor survival. Bevacizumab plus erlotinib was adequately tolerated in recurrent MG patients. However, this regimen was associated with similar PFS benefit and radiographic response when compared with other historical bevacizumab-containing regimens.

Keywords: antiangiogenesis, bevacizumab, EGFR, glioblastoma, malignant glioma, VEGF.

Malignant glioma (MG), a highly lethal, common primary adult brain tumor, remain a major therapeutic challenge. Overall survival (OS) of World Health Organization (WHO) classified grade 3 (anaplastic glioma [AG]) and grade 4 (glioblastoma [GBM]) MG is 2–5 years and 12–15 months, respectively, despite multimodality therapy.[1] Historically, salvage therapies are ineffective, with 6-month progression-free survival (PFS-6) rates of 28%–31% for AG and 15%–16% for GBM.[2–4]

Angiogenesis, driven predominantly by vascular endothelial growth factor (VEGF), is markedly upregulated in MG.[5] Bevacizumab (Avastin®, Genentech-Roche), a monoclonal antibody against VEGF, was granted accelerated approval by the US Food and Drug Administration (FDA) for progressive GBM based on durable radiographic responses.[6,7] Bevacizumab ± chemotherapy has also demonstrated encouraging antitumor benefit for recurrent AG patients.[8–10]

Amplification of the epidermal growth factor receptor (*EGFR*) gene occurs in approximately 40% of GBMs,[11,12] and EGFR is overexpressed in many MGs

Received April 9, 2010; accepted July 2, 2010.

Corresponding Author: David A. Reardon, MD, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624 Med Ctr, 047 Baker House, Durham, NC 27710 (reard003@mc.duke.edu).

independent of amplification status.[13,14] Dysregulation of cell signaling pathways associated with EGFR activation contributes to GBM proliferation, survival, and angiogenesis.[15,16] Erlotinib (Tarceva®, OSI-774; Genentech-Roche), a reversible kinase inhibitor of EGFR, has undergone clinical testing in MG. In a recent randomized phase II trial of erlotinib vs temozolomide (TMZ) or carmustine among recurrent GBM patients, PFS-6 was only 11.4% in the erlotinib group, compared with 24% in the TMZ and carmustine groups.[17] Additional phase II trials of reversible EGFR inhibitors have demonstrated similar results with no impact on PFS or OS rates.[18–20] Taken together, these data suggest that erlotinib monotherapy has no appreciable antiglioma activity among unselected patients. Strategies such as enriching trial populations for patients more likely to respond, evaluating potentially more effective agents such as irreversible inhibitors, or combinations with other targeted therapeutics may be needed to improve clinical benefit.

Dual targeting of EGFR and VEGFR signaling by multitargeted kinase inhibitors such as vandetanib (Zactima™, AstraZenaca) demonstrates antiglioma efficacy in preclinical models in which EGFR monotherapy was ineffective.[21] Furthermore, preclinical studies of cell lines and xenografts have identified compensatory activation of the VEGF pathway as a prominent tumor-related resistance mechanism to EGFR inhibitors.[22,23] We therefore hypothesized that a combinatorial regimen designed to concurrently inhibit VEGF and EGFR signaling would be associated with significant antitumor benefit among recurrent MG patients. Our study is the first to evaluate such a regimen in this patient population.

Methods

Patient Eligibility

Patients with recurrent WHO grade III (anaplastic astrocytoma, oligodendroglioma, oligoastrocytoma or pleomorphic xanthoastrocytoma) or WHO grade IV MG (GBM or gliosarcoma) who met the following criteria were eligible: age ≥18 years; ≥4 weeks from craniotomy (1 week for biopsy), prior radiotherapy, or chemotherapy (6 weeks for a nitrosourea); Karnofsky performance status (KPS) score ≥60; and adequate hematologic, hepatic, and renal function. Key exclusion criteria included: prior therapy with bevacizumab or EGFR inhibitor; greater than 3 prior recurrences; evidence of CNS hemorrhage; concurrent therapeutic anticoagulation; significant intercurrent illness; another primary malignancy that required treatment within 1 year; history of myocardial infarction or stroke within 6 months; or urine protein to creatinine ratio ≥1.

Study Design and Treatments

This open-label phase II study was conducted at Duke University Medical Center (ClinicalTrials.gov: NCT00671970) following the institutional review board approval. Bevacizumab (10 mg/kg) was administered intravenously every 2 weeks. Erlotinib was orally administered, once daily for each 42-day treatment cycle at 200 mg/day for patients not on CYP3A4 enzyme-inducing antiepileptic drugs (EIAEDs: phenytoin, phenobarbital, primidone, carbamazepine, and oxcarbazepine) and 500 mg/day for patients on EIAEDs as established previously.[24] Treatment was continued until progressive disease (PD) or unacceptable toxicity. Dose modification followed previously published guidelines (Supplementary Methods). The Macdonald criteria[25] were the basis for response classification. In addition, changes noted on T2 and fluid-attenuated inversion recovery (FLAIR) sequences were also incorporated in the determination of radiographic response. Specifically, patients were classified as either a complete or partial response only if the T2 and FLAIR sequences were at least stable. Patients were also defined as progressive if they had significant worsening of T2 or FLAIR sequences, regardless of changes with contrast enhancement.

Pharmacokinetic Analysis

Pharmacokinetic studies of erlotinib and its active metabolite, OSI-420, were performed as described previously.[24] Plasma samples were collected on days 1 and 42 of cycle 1 before treatment and at 0.5, 2, 6, 8, and 24 hours after erlotinib administration. These data were analyzed by noncompartmental analysis using the WinNonlin® Pro V.5.2 program (Pharsight).

Tumor Biomarker Study

Immunohistochemical staining of paraffin-embedded tumor samples from GBM patients obtained at original diagnosis was performed as previously described for various biomarkers including VEGF, carbonic anhydrase (CA)-9, hypoxia-inducible factor-2 alpha (HIF-2A), O^6-methylguanine-DNA methyltransferase (MGMT), EGFR (wild-type), EGFR-vIII, phosphatase and tension homolog (PTEN), phosphorylated S6 (pS6), phosphorylated AKT (pAKT), phosphorylated mitogen-activated protein kinase (pMAPK), CD31, and VEGF receptor-2 (VEGFR-2).[26,27] Expression of each marker was scored as previously described by a neuropathologist (R.E.M.), who was unaware of patient treatment status or response.

Statistical Considerations

The primary objective of this single-stage phase II study was to evaluate the PFS-6 rate. Previous meta-analyses demonstrated that PFS-6 is an adequate indicator of antitumor benefit for salvage GBM clinical trials.[3,4] With Yung et al.[28] reporting a PFS-6 of 21% (95% confidence interval [CI], 13%–29%) among GBM patients treated at first relapse with TMZ, a sample size of 25 recurrent GBM patients was chosen to differentiate

between a 5% and a 25% PFS-6 rate with type I and II error rates of 0.034 and 0.10, respectively. Yung et al.[29] also reported a PFS-6 of 46% (95% CI, 38%–54%) for recurrent AG patients. With some patients in the targeted patient population having more than 1 relapse, an accrual goal of 32 recurrent AG patients was planned to differentiate between PFS-6 of 20% and 40% with type I and II error rates of 0.09 and 0.12, respectively. Of note, the benchmark set by TMZ was chosen as the historical comparator for our study rather than outcome reported on prior bevacizumab studies because the latter had not been reported or validated in a multi-institutional setting when this study was designed.[30]

PFS was defined as the time between treatment initiation and first occurrence of disease progression or death; PFS was censored at last follow-up if the patient remained alive without disease progression. OS was determined from the time of treatment initiation until the time of death, with OS being censored at last follow-up if the patient remained alive. The Kaplan–Meier curves were used to graphically describe PFS and OS and to estimate the median and 6-month estimates.

For pharmacokinetic comparisons between patients on and not on EIAEDs, a *t*-test was used. Results were confirmed using the nonparametric Wilcoxon rank-sum test.

The Cox proportional hazard model was used to explore the individual effect of age, KPS, and biomarker status on OS and PFS. For continuous biomarkers (CD31, VEGF, and VEGFR-2), hazard ratios are reported, with a 95% CI, that represent the effect of a 10- or 20-unit increase in the biomarker. For categorical biomarkers (CA-9, HIF-2A, EGFR, EGFR-vIII, PTEN, pS6, pAKT, pMAPK, and CD31), hazard ratios were reported comparing the expression level of each biomarker to the others. However, no adjustment for multiple testing was done given the exploratory nature of the analyses. The Kaplan–Meier method was used to describe OS and PFS within patient subgroups defined by these predictors. To estimate the effect that biomarkers had on the dichotomous outcomes of PFS-6, 1-year survival, and response, Fisher's exact test was used for the categorical predictors and the Wilcoxon rank-sum test was used for continuous biomarkers. Statistical analysis was performed, and graphs were generated with a SAS program (SAS Institute). *P*-values of $<.05$ were considered statistically significant.

Role of the Funding Source

Funding was provided to support study conduct and for bevacizumab and erlotinib treatments. The sponsors had no role in study conduct, data collection, analysis (except for performance of erlotinib pharmacokinetic measurements), interpretation, or manuscript preparation. The corresponding author had full access to all study data and final responsibility to submit for publication.

Results

Patient Characteristics

Between February 2007 and May 2008, 25 patients with recurrent GBM and 32 patients with recurrent AG were enrolled in this study (Table 1). All patients had measurable disease on MRI at enrollment. Eighty-eight percent of GBM patients and 81% of AG patients had a KPS score of 80 or higher. All patients had undergone radiation therapy and all but 2 patients (with AG) had received prior TMZ treatment. Of note, this study was designed prior to wide awareness of radiographic "pseudoprogression" that occurs most frequently within

Table 1. Patients characteristics ($N = 57$)

Tumor grade	WHO grade 3 (AG; $n = 32$)	WHO grade 4 (GBM; $n = 25$)
Age		
Median	47.5	52.4
Range	26.0–72.2	24.1–70.4
Gender		
Male	24 (75%)	13 (52%)
Female	8 (25%)	12 (48%)
Histology		
GBM		25 (100%)
Anaplastic astrocytoma	24 (75%)	
Anaplastic oligodendroglioma	7 (22%)	
Pleomorphic xanthoastrocytoma with anaplastic features	1 (3%)	
KPS		
90–100	16 (50%)	14 (56%)
80–89	10 (31%)	8 (32%)
70–79	6 (19%)	3 (12%)
Time from diagnosis, median (wks)	71	34
Range	(1–450)	(2–149)
Prior episodes of progression		
1	19 (59%)	13 (52%)
2	6 (19%)	9 (36%)
3	7 (22%)	3 (12%)
Prior radiotherapy	32 (100%)	25 (100%)
Number of prior chemotherapies		
1	17 (53%)	10 (40%)
2	11 (34%)	7 (28%)
3	4 (13%)	4 (16%)
≥4	0	4 (16%)
Concurrent medications		
EIAED	11 (34%)	10 (40%)
Non-EIAED	15 (47%)	8 (32%)
No AED	6 (19%)	7 (28%)
Corticosteroids	11 (34%)	17 (68%)

Abbreviations: AG, anaplastic glioma; EIAED, enzyme-inducing antiepileptic drug; GBM, glioblastoma; KPS, Karnofsky performance status.

Table 2. Summary of outcome by histological grade

Tumor grade	WHO grade 3 (AG; $n = 32$)	WHO grade 4 (GBM; $n = 25$)
Number of evaluable patients	32	24
Number of patients enrolled >3 months postradiotherapy	32	20
Follow-up (wks), median (95% CI)	103 (91.7–127.9)	141.8 (141–142.6)
Six-month PFS (%), median (95% CI)		
All patients	43.8 (26.5–59.8)	29.2 (13.0–47.6)
Patients enrolled >3 months postradiotherapy	43.8 (26.5–59.8)	26.3 (9.6–46.8)
PFS (wks), median (95% CI)		
All patients	23.4 (18.1–36.1)	18 (12.0–23.9)
Patients enrolled >3 months postradiotherapy	23.4 (18.1–36.1)	17 (11.9–23.9)
OS (wks), median (95% CI)		
All patients	71.3 (44.7–123.6)	44.6 (28.4–68.7)
Patients enrolled >3 months postradiotherapy	71.3 (44.7–123.6)	42.4 (28.4–55.1)
Radiographic response		
Complete (%)	1 (3)	1 (4)
Partial (%)	9 (28)	11 (46)
Stable disease (%)	14 (44)	10 (42)
Progressive disease (%)	7 (22)	2 (8)
Not evaluable (%)	1 (3)	0 (0)

Abbreviations: CI, confidence interval; OS, overall survival; AG, anaplastic glioma; GBM, glioblastoma; PFS, progression-free survival.

3 months of completing radiotherapy and concurrent TMZ.[31] Five GBM patients enrolled within this time frame, whereas all others enrolled greater than 12 weeks from completing radiotherapy with concurrent TMZ. Patients were significantly pretreated with 48% and 41% of GBM and AG patients, respectively, enrolling at either 2nd or 3rd progression. Sixty percent of GBM patients and 47% of AG patients received 2 or more prior chemotherapeutic agents.

Efficacy

A total of 145 and 90 cycles were administered to GBM and AG patients, respectively. The median follow-up time for GBM was 142 weeks and that for AG was 103 weeks. The PFS-6 and radiographic response rates, as well as PFS and OS data, are summarized in Table 2 and Fig. 1. There were no significant differences in OS and PFS between patients on EIAEDs and those not on EIAEDs (Supplementary Material, Fig. SA). Upon the exclusion of 5 GBM patients, who may have had "pseudoprogression," that is, enrolled less than 3 months postradiotherapy, the PFS and OS rates of the remaining patients were not significantly different from those of all patients (Table 2).

All 32 patients were evaluated for outcome assessment. Twelve (48%) GBM and 10 (31%) AG patients experienced radiographic (complete and partial) responses (Fig. 2 and Supplementary Material, Fig. SB). Radiographic responses were associated with significant prolongation of PFS but not OS in GBM patients (Fig. 3A and C), whereas they were associated with benefit in both PFS and OS in patients with AG (Fig. 3B and D).

Four (24%) of 17 GBM patients who were on dexamethasone at study initiation discontinued it during the study, 6 (35%) patients were able to reduce their doses, and 7 (41%) patients continued on the same dose. Four (36%) of 11 AG patients discontinued dexamethasone, 4 (36%) patients were able to reduce their doses, 1 (9%) patient continued on the same dose, and 2 (18%) patients increased their doses during the study. Two GBM patients and 2 AG patients required dexamethasone initiation during study therapy for symptom treatment.

Toxicity

Treatment-related toxicities are listed in Table 3. Common side effects, including rash (93%), diarrhea (57%), and fatigue (66%), were mainly grade 1 or 2; however, 22 (39%) patients developed grade 3 rash. Eighteen patients (31.5%) required erlotinib dose reduction including 12 on EIAEDs (57%) and 6 not on EIAEDs (17%). Reasons for dose reduction included rash ($n = 12$), diarrhea ($n = 4$), hypophosphatemia ($n = 1$), and thrombocytopenia ($n = 1$).

Bevacizumab was held in only 1 patient during the study and was resumed after 4 weeks upon the resolution of proteinuria to retreatment criteria. Eight (14%) patients (2 GBMs and 6 AGs) discontinued study therapy due to toxicity, including 2 patients with persistent rash (grade 3), and single patients with ischemic stroke (grade 4), CNS hemorrhage (grade 1), GI perforation (grade 3), GI hemorrhage (grade 3), hypophosphatemia (grade 3), and wound infection (grade 3).

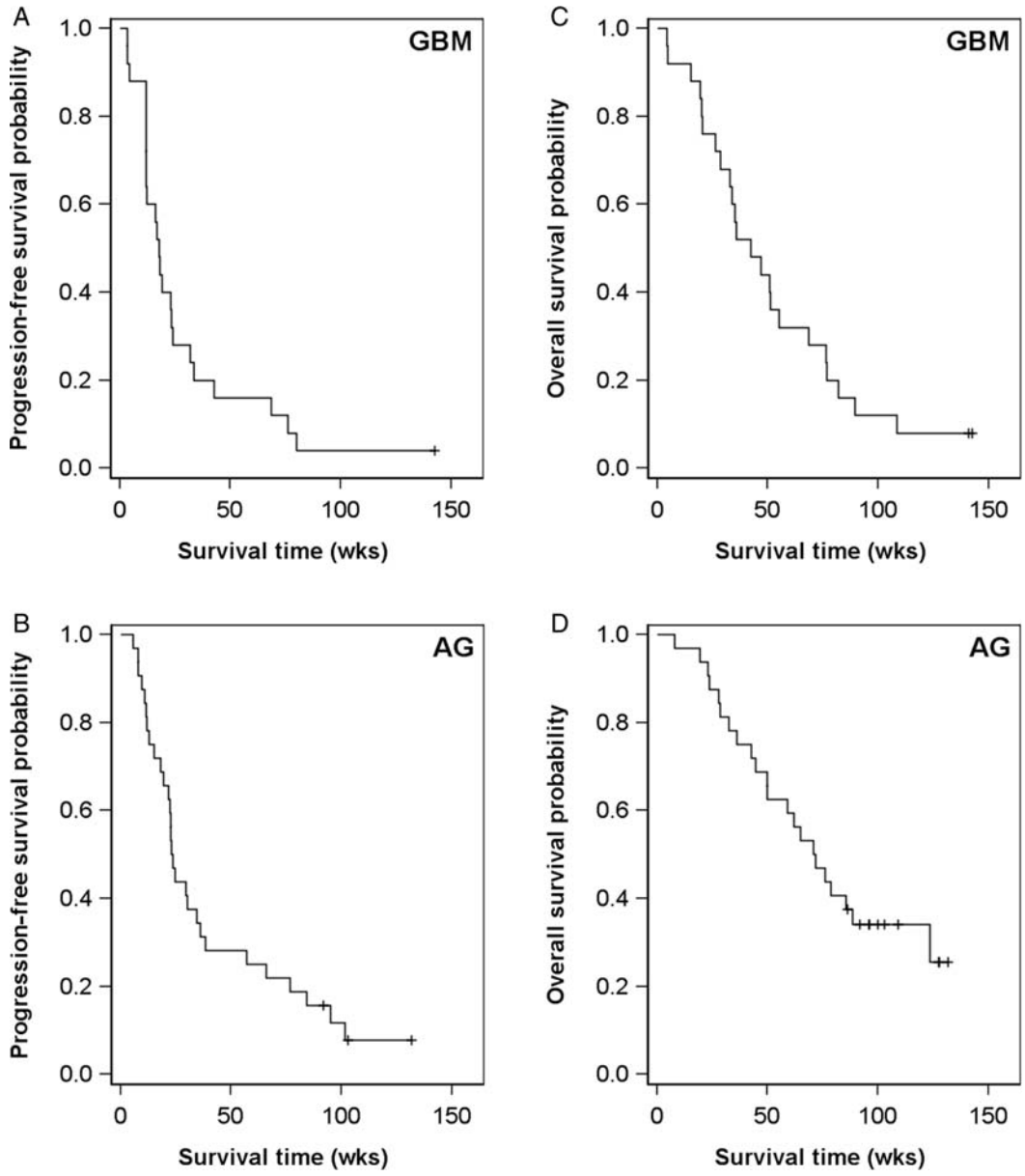

Fig. 1. PFS and OS. (A) PFS of GBM patients. (B) PFS of AG patients. (C) OS of GBM patients. (D) OS of AG patients.

Pharmacokinetics

Pharmacokinetic studies of erlotinib were performed in 22 GBM patients (Table 4 and Supplementary Material, Fig. SC). Our results confirm previously described interpatient variability among MG patients receiving erlotinib.[24] Erlotinib exposures were comparable between EIAED (erlotinib 500 mg/day) and non-EIAED (erlotinib 200 mg/day) groups as demonstrated by similar AUC_{0-24} levels ($P = .9$) despite markedly increased clearance among patients on EIAED ($P = .004$). The C_{max} and AUC_{0-24} levels of OSI-420, an erlotinib metabolite, were significantly higher in the EIAED group ($P < .05$). Furthermore, the ratio of OSI-420 AUC_{0-24} to erlotinib when 500 mg/day of erlotinib was coadministered with EIAED (33%) was higher than that when 200 mg/day of erlotinib was administered without EIAED (9%).

Pattern of Failure

All GBM patients developed PD, including 23 based on radiographic criteria and 2 with a significant clinical decline who did not undergo MRI evaluation at study discontinuation. Local progression with both increased gadolinium-enhanced T1 weighted signal as well as T2/FLAIR abnormalities was noted in 20 (87%) patients. Single patients (4%) had local progression only on T2/FLAIR sequences, or distant progression with local disease control or synchronous local progression with a new distant site (Supplementary Material, Table S1).

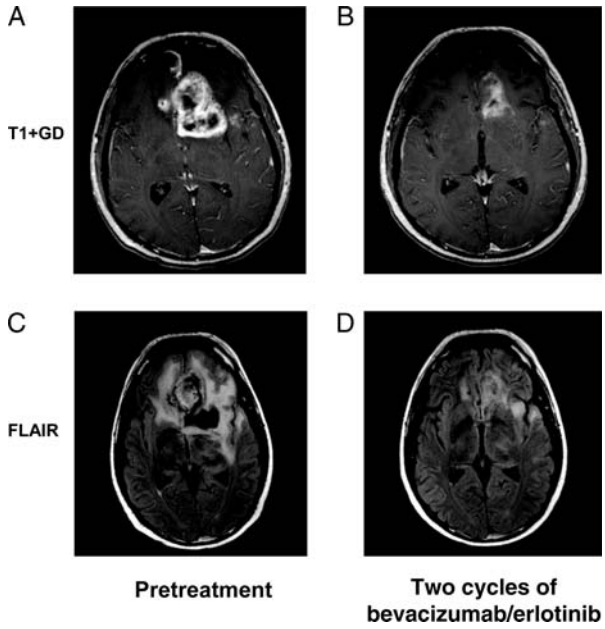

Fig. 2. Representative radiographic response associated with bevacizumab and erlotinib in recurrent GBM.

Thirty (94%) of 32 AG patients experienced radiographic progression. Two patients (6%) completed study therapy and remain progression-free as of December 2009. At study failure, all patients had evidence of local radiographic progression and 1 patient also developed a new synchronous site of disease. Thirteen (43%) patients had progression only on T2/FLAIR sequences (Supplementary Material, Table S1).

Salvage Treatments Following Progression

Seventeen (71%) of 24 patients with GBM received salvage therapy (Supplementary Material, Table S2). None of these patients achieved a radiographic response. Six (35%) of 17 GBM patients treated with bevacizumab-containing regimens as their first salvage therapy achieved a median PFS of 12 weeks, including 3 patients with stable disease (SD) for 15 (bevacizumab + irinotecan and metronomic TMZ), 36, and 48 (bevacizumab + irinotecan) weeks. Eleven GBM patients who received nonbevacizumab salvage regimens achieved a median PFS of 8 weeks. Ten GBM patients received second salvage treatments, including 8 (80%) patients treated with an additional bevacizumab-containing regimen. Four of these patients (1 bevacizumab alone and 3 bevacizumab + irinotecan) experienced SD as their best response with a median PFS of 26 weeks. Four patients received third bevacizumab-containing salvage regimens with PD as their best response.

Twenty-five (78%) of 32 patients with AG received salvage treatment following progression on study (Supplementary Material, Table S2). Thirteen patients received bevacizumab-containing regimens with a median PFS of 13 weeks. Seven patients (2 bevacizumab + metronomic TMZ, 2 bevacizumab + irinotecan, 2 bevacizumab + irinotecan + carboplatin, and 1 bevacizumab + etoposide) had SD and 6 patients had PD as their best responses. Twelve patients received nonbevacizumab salvage regimens with a median PFS of 33.6 weeks. Ten patients received second salvage therapies. Eight of these patients received bevacizumab-containing regimens with a median PFS of 15.1 weeks, whereas 2 patients received nonbevacizumab regimens with a median PFS of 19 weeks.

Clinical Predictors of Therapeutic Benefit

In the GBM cohort, patients with a KPS of less than 90 had a 2.9 times greater risk of progression than patients with a KPS of 90 or higher (Wald χ^2, $P = .029$), whereas neither age (≥ 50 or < 50) nor multifocal disease (Supplementary Material, Fig. SE) was associated with either PFS or OS.

Interestingly, GBM patients who developed grade 3 rash and AG patients who developed hypertension achieved significantly longer PFS, when compared with patients on each arm who did not develop these toxicities (Supplementary Material, Fig. SD). Diarrhea was not associated with outcome.

Tumor Biomarker Profile

Tumors from 22 (out of 24) GBM patients underwent immunohistochemical staining to identify potential biomarkers of response or survival benefit (Fig. 4 and Supplementary Material, Table S3). Among the 22 patients included in this analysis, median OS was 44.6 weeks and median PFS was 18.6 weeks. Thirty-two percent (95% CI: 14%–51%) remained progression-free for at least 6 months, 36% (95% CI: 17%–56%) lived for at least 1 year after initiating treatment, and 55% (95% CI: 32%–76%) experienced complete or partial response. MGMT expression was not associated with response or survival benefit. Patients with positive parenchymal HIF-2A had 2.7 times greater risk of death and 2.5 times greater risk of progression than patients with negative parenchymal HIF-2A (OS: $P = .058$; PFS: $P = .077$). Patients with positive pS6 had a 3.4 times greater risk of progression compared with patients with negative pS6 ($P = .05$). Patients with lower values for VEGFR-2 were more likely to survive more than 1 year than those with high values of VEGFR-2 ($P = .0079$; Supplementary Material, Table S3). Results from the Cox model predicting OS with VEGFR-2 expression support this result: every 20-point increase in the VEGFR-2 score was associated with a 1.3-fold increased risk of death ($P = .021$; Table 3).

Discussion

To our knowledge, we provide the first report of the combination of an EGFR inhibitor, erlotinib, with bevacizumab in patients with recurrent/progressive MG. Although we show that the combination of bevacizumab and erlotinib has activity, we observed PFS-6 and radiographic response rates that were comparable or inferior

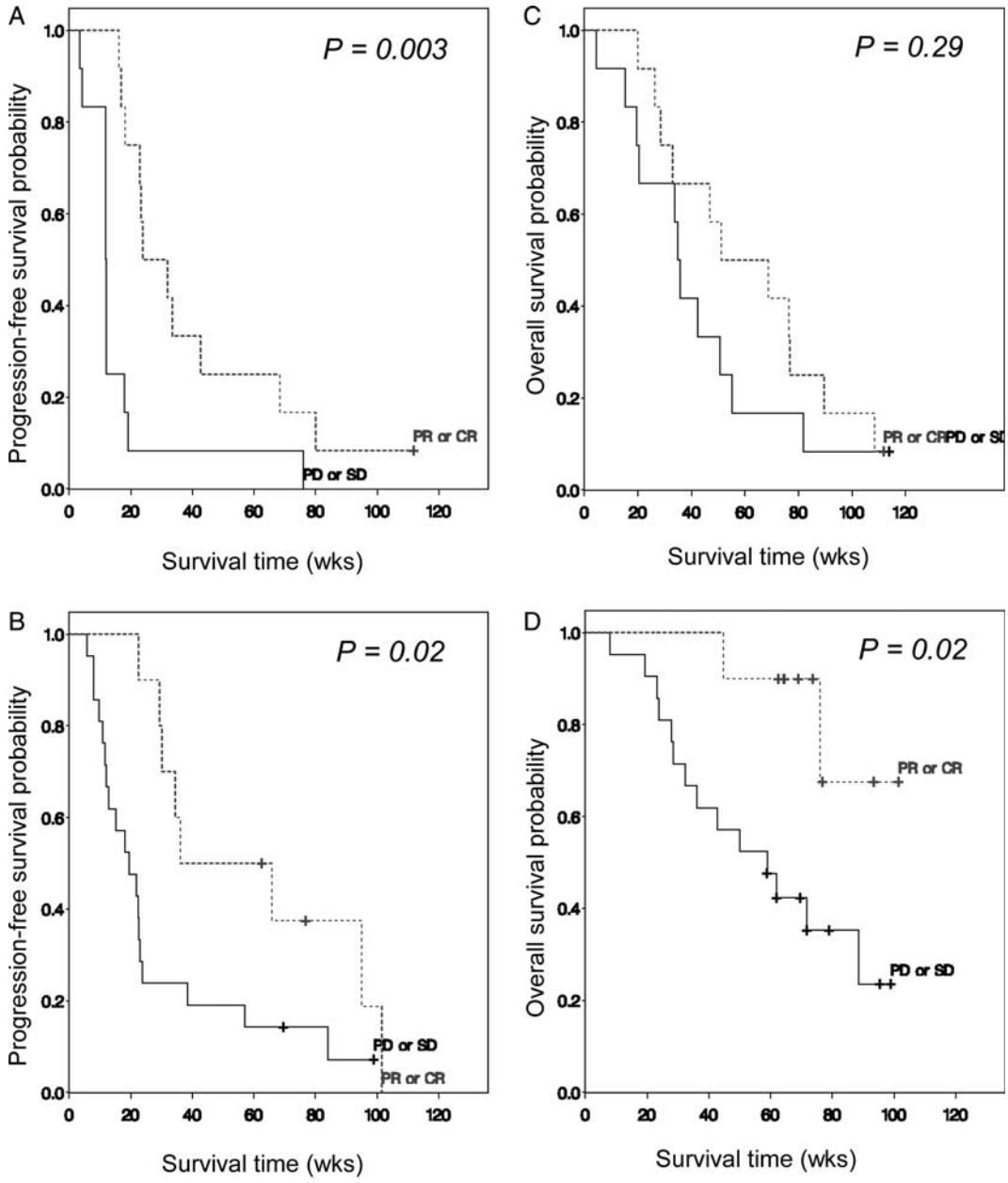

Fig. 3. PFS and OS stratified by response. (A) PFS of GBM patients. (B) PFS of AG patients. (C) OS of GBM patients. (D) OS of AG patients.

to other bevacizumab-containing salvage regimens (Supplementary Material, Table S4). Of note, the addition of EGFR monoclonal antibodies to bevacizumab plus chemotherapy also yielded unexpectedly poor outcome compared with bevacizumab plus chemotherapy among colorectal cancer patients.[32,33] The mechanism underlying a potentially detrimental interaction between EGFR and VEGF therapeutics is unclear but did not appear to be due to increased toxicity and decreased treatment dose intensity in these studies. Similarly, the adverse event profile observed on our study, with the exception of grade 3 rash, was similar to that reported with other bevacizumab salvage regimens for recurrent MG patients.[6,7,30,34] Furthermore, only 14% of patients required erlotinib dose reduction and 3% required treatment discontinuation due to this complication. In addition, GBM patients who developed grade 3 rash had better PFS compared with those who did not. This may reflect possible enhanced activity for both efficacy and toxicity when bevacizumab is combined with erlotinib in some patients. However, an alternative explanation is that many GBM patients progressed early and thus did not stay on study long enough to develop severe rash. In any case, it is unlikely that decreased treatment dose intensity due to erlotinib toxicity contributed to poor outcome on our study. Further preclinical studies are needed to elucidate potential mechanisms of in vivo interaction between EGFR inhibitors and bevacizumab.

One explanation for the frequency of grade 3 rash observed on our study is that higher administered

Table 3. Treatment-related toxicities ($N = 56$)

Toxicity	Grade 1 or 2		Grade 3		Grade 4	
	n	Percent	*n*	Percent	*n*	Percent
Rash	30	54	22	39	0	0
Mucositis	15	27	0	0	0	0
Diarrhea	26	46	6	11	0	0
Hypertension	21	38	0	0	0	0
Fatigue	28	50	9	16	1	2
Abnormal liver functions	12	21	2	4	0	0
Leukopenia	7	13	0	0	0	0
Anemia	8	14	1	2	0	0
Thrombocytopenia	4	7	2	4	0	0
Nausea/vomiting	15	27	5	9	0	0
Hyponatremia	5	9	1	2	0	0
Hypokalemia	9	16	1	2	0	0
Hypophosphatemia	1	2	2	4	0	0
Proteinuria	4	7	0	0	0	0
Infection	6	11	6	11	0	0
CNS hemorrhage	1	2	0	0	0	0
Ischemic stroke	0	0	0	0	1	2
Deep venous thrombosis	0	0	1	2	0	0
Pulmonary embolism	0	0	1	2	1	2
GI perforation	0	0	1	2	0	0
Nasal septal perforation	0	0	1	2	0	0
Bacterial meningitis	0	0	0	0	1	2
Bleeding	5	9	1	2	0	0
Avascular necrosis	0	0	1	2	0	0
Wound dehiscence	0	0	1	2	0	0

erlotinib doses, which were based on a prior phase I/II trial of erlotinib in MG,[24] may have contributed. Nonetheless, our pharmacokinetic data do not support higher erlotinib exposures when compared with previous clinical trials.[24] Alternatively, bevacizumab may potentiate toxicity and/or delay healing of erlotinib-associated rash.

Pharmacokinetic analyses did not demonstrate differences in erlotinib exposure between EIAED and non-EIAED groups. Survival and toxicity rates were also similar between these 2 groups. The ratio of AUC_{0-24} of OSI-420 to erlotinib in the EIAED group was significantly higher than that in the non-EIAED group. This confirmed previous findings and likely represents a decrease in the erlotinib level due to increased OSI-420 metabolism induced by EIAED.[24] In addition, our study demonstrates that bevacizumab did not impact erlotinib or OSI-420 levels when compared with historical data with erlotinib monotherapy in recurrent MG patients.[24]

OS on our study was comparable with that reported for other bevacizumab-containing regimens. Eleven (46%) of 24 GBM patients received bevacizumab-containing regimens as salvage treatment after on-study progression (Supplementary Material, Table S3). Of note, 6 of these 11 patients (54%), all of whom received chemotherapy with bevacizumab as subsequent salvage therapy, experienced SD as their best responses for more than 6 months. This finding contrasts with results of recent studies, which demonstrate a median PFS of 2 months or less among GBM patients treated with additional bevacizumab-based therapy following progression on an initial bevacizumab regimen.[35,36] Of note, none of the patients who received nonbevacizumab salvage therapy following on-study progression achieved SD for more than 3 months. However, these results should be interpreted cautiously given the small number of patients involved and the risk of selection bias.

The pattern of disease progression for our patients also varied from other bevacizumab studies in GBM. We noted local progression on both enhanced and T2/FLAIR images for most GBM and AG patients. Progression manifest solely by nonenhancing radiographic progression, which may represent invasive microscopic tumor, was found in only 2 GBM patients (8%) in our study compared with 15%–36% in other GBM series.[35–37] In addition, we observed new distant PD in only 3 patients (5%; 2 GBM and 1 AG), whereas other bevacizumab studies have reported such progression in 15%–16%.[35,37] Nonetheless, our results further support a need for new response and outcome criteria for assessing MG patients receiving antiangiogenic therapy that incorporate assessment of both enhancing as well as nonenhancing tumor such as the Response Assessment in Neuro-Oncology (RANO) criteria.[38,39]

Identification of biomarkers of response or resistance to antiangiogenic agents is currently a paramount priority.[40] We performed immunohistochemical profiling of archival tumor samples from the GBM patients in the current study to attempt to identify potential informative biomarkers. However, these results should be interpreted cautiously given the small sample size of our study and the lack of adjustment for multiple testing. Furthermore, our analysis was based on archival tumor material due to difficulty obtaining multiple tissue specimens from CNS tumor patients, and it is unclear to what degree findings from archival tumor samples reflect findings at recurrence. Thus, our findings warrant further investigation in future studies. Nonetheless, we noted an association between high expression of parenchymal HIF-2A and poor survival. This result supports our previous findings linking hypoxia and poor prognosis in MG patients treated with bevacizumab + irinotecan, although high expression of CA-9, a hypoxia-inducible transmembrane enzyme, was not associated with survival in the current study.[26] Of interest, HIF-2A has recently been demonstrated to be an important regulator of glioma stem cells, which contribute to angiogenesis and therapeutic resistance,[41] and *HIF-2A* gene upregulation was shown to predict poor prognosis in a GBM series.[41] Taken together, these data suggest that hypoxia serves as a potential mechanism of resistance to bevacizumab and that adding

Table 4. Median pharmacokinetic parameters of erlotinib by dose stratum

Parameter	Erlotinib dose stratum			
	Erlotinib		OSI-420	
Day 1	200 mg ($n = 12$)	500 mg ($n = 10$)	200 mg ($n = 12$)	500 mg ($n = 10$)
T_{max} (range; h)	2 (2–24)	2 (0.5–8)	2 (2–24)	2 (2–6)
C_{max} (range; ng/mL)	794 (524–2200)	1323 (317–2990)	79.2 (46.4–222)	280 (64.1–755)
T_{last} (range; h)	24 (8–24)	24 (24)	24 (8–24)	24 (24)
C_{last} (range; ng/mL)	393 (157–1031)	410 (194–1280)	34 (13.7–147)	80.2 (38.3–247)
AUC_{0-24} (range; ng/mL·h)	11 072 (7850–30 029)	15 611 (5295–40 110)	991 (655–2461)	3697 (993–9207)
Day 42	200 mg ($n = 9$)	500 mg ($n = 9$)	200 mg ($n = 9$)	500 mg ($n = 9$)
T_{max} (range; h)	2 (0.5–24)	6 (0.5–24)	2 (2–24)	6 (2–24)
C_{max} (range; ng/mL)	1320 (525–2940)	1400 (716–3955)	149 (52.4–464)	522 (223–1975)
C_{ave} (range; ng/mL)	1086 (346–1787)	893 (487–2332)	95 (32–243)	291 (109–1081)
AUC_{0-24} (range; ng/mL·h)	26 072 (8308–42 878)	21 421 (11 680–55 960)	2287 (763–5828)	6976 (2617–25 948)
Clss/F (range; mL/h)	7671 (4664–24 073)	23 342 (8935–42 808)	N/A	N/A
T_{last} (range; h)	24 (24)	24 (24)	24 (24)	24 (24)
T_{min} (range; h)	2 (0–24)	0.5 (0–24)	2 (0–24)	0.5 (0–24)
C_{min} (range; ng/mL)	558 (118–1125)	327 (191–1170)	50 (9.96–203)	84.3 (34.2–417)

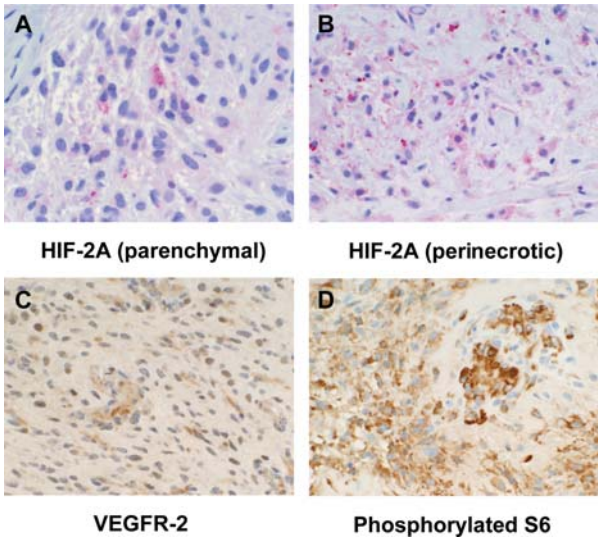

Fig. 4. Representative immunohistochemical detection of biomarkers (×40). (A) HIF-2A in non-necrotic parenchyma of the tumor. (B) HIF-2A in the majority of the tumor cells in the perinecrotic area. (C) VEGFR-2 in both the endothelial cells of the small vessel and in the adjacent tumor cells. (D) Phosphorylated S6 in both the proliferative endothelial cells and the adjacent tumor cells.

antihypoxic agents may represent a new therapeutic approach to overcome resistance to antiangiogenic therapy. Of note, high expression of VEGFR-2 was also associated with poor outcome. None of the GBM patients in the current study had coexpression of EGFR-vIII and wild-type PTEN, which were demonstrated in 1 study to predict response to EGFR inhibitors in GBM.[42]

In conclusion, although erlotinib + bevacizumab was adequately tolerated in recurrent MG patients, it was not associated with improved PFS benefit or radiographic response, when compared with historical bevacizumab-salvage therapy.

Supplementary Material

Supplementary Material is available at *Neuro-Oncology* online.

Conflict of interest statement. J.J.V. has a paid consulting relationship with Genentech. M.H. is an employee of OSI Pharmaceuticals. H.S.F. served on the Advisory Board of Genentech and was a paid consultant for Genentech. D.A.R. is a paid consultant and speaker for Genentech, Schering Plough, and EMD Serono.

Funding

This work was supported by the National Institutes of Health (P50-NS-020023 and NCI SPORE P50 CA 108786) and a grant from Genentech Pharmaceuticals.

References

1. Wen PY, Kesari S. Malignant gliomas in adults. *N Engl J Med*. 2008;359(5):492–507.
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(8):2572–2578.
3. Lamborn KR, Yung WK, Chang SM, et al. Progression-free survival: an important end point in evaluating therapy for recurrent high-grade gliomas. *Neurooncology*. 2008;10(2):162–170.
4. Ballman KV, Buckner JC, Brown PD, et al. The relationship between six-month progression-free survival and 12-month overall survival end points for phase II trials in patients with glioblastoma multiforme. *Neurooncology*. 2007;9(1):29–38.
5. Plate KH, Breier G, Weich HA, Risau W. Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. *Nature*. 1992;359(6398):845–848.
6. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol*. 2009;27(28):4733–4740.
7. Kreisl TN, Kim L, Moore K, et al. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. *J Clin Oncol*. 2009;27(5):740–745.
8. Desjardins A, Reardon DA, Herndon JE, 2nd, et al. Bevacizumab plus irinotecan in recurrent WHO grade 3 malignant gliomas. *Clin Cancer Res*. 2008;14(21):7068–7073.
9. Chamberlain MC, Johnston S. Salvage chemotherapy with bevacizumab for recurrent alkylator-refractory anaplastic astrocytoma. *J Neurooncol*. 2009;91(3):359–367.
10. Reardon DA, Desjardins A, Vredenburgh JJ, et al. Metronomic chemotherapy with daily, oral etoposide plus bevacizumab for recurrent malignant glioma: a phase II study. *Br J Cancer*. 2009;101(12):1986–1994.
11. Libermann TA, Nusbaum HR, Razon N, et al. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. *Nature*. 1985; 313(5998):144–147.
12. Wong H, Bigner SH, Bigner DD, Kinzler KW, Hamilton SR, Vogelstein B. Increased expression of the epidermal growth factor gene in malignant gliomas is invariably related with gene amplification. *Proc Natl AcadSciUSA*. 1987;84:6899–6903.
13. Ekstrand AJ, James CD, Cavenee WK, Seliger B, Pettersson RF, Collins VP. Genes for epidermal growth factor receptor, transforming growth factor alpha, and epidermal growth factor and their expression in human gliomas in vivo. *Cancer Res*. 1991;51(8):2164–2172.
14. 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(12):880–887.
15. Nicholas MK, Lukas RV, Jafri NF, Faoro L, Salgia R. Epidermal growth factor receptor—mediated signal transduction in the development and therapy of gliomas. *Clin Cancer Res*. 2006;12(24):7261–7270.
16. Guillamo JS, de Bouard S, Valable S, et al. Molecular mechanisms underlying effects of epidermal growth factor receptor inhibition on invasion, proliferation, and angiogenesis in experimental glioma. *Clin Cancer Res*. 2009;15(11):3697–3704.
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(8):1268–1274.
18. 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. *Neurooncology*. 2010;12(1): 95–103.
19. Vogelbaum MA, Peereboom D, Stevens G, Barnett G, Brewer C. Phase II trial of the EGFR tyrosine kinase inhibitor erlotinib for single agent therapy of recurrent Glioblastoma Multiforme: Interim results. Paper presented at: Proceedings of American Society of Clinical Oncology; June 5–8; 2004; New Orleans, LA.
20. Rich JN, Reardon DA, Peery T, et al. Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol*. 2004;22(1):133–142.
21. Rich JN, Sathornsumetee S, Keir ST, et al. ZD6474, a novel tyrosine kinase inhibitor of vascular endothelial growth factor receptor and epidermal growth factor receptor, inhibits tumor growth of multiple nervous system tumors. *Clin Cancer Res*. 2005;11(22):8145–8157.
22. Naumov GN, Nilsson MB, Cascone T, et al. Combined vascular endothelial growth factor receptor and epidermal growth factor receptor (EGFR) blockade inhibits tumor growth in xenograft models of EGFR inhibitor resistance. *Clin Cancer Res*. 2009;15(10):3484–3494.
23. Viloria-Petit A, Crombet T, Jothy S, et al. Acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies in vivo: a role for altered tumor angiogenesis. *Cancer Res*. 2001;61(13): 5090–5101.
24. Prados MD, Lamborn KR, Chang S, et al. Phase 1 study of erlotinib HCl alone and combined with temozolomide in patients with stable or recurrent malignant glioma. *Neurooncology*. 2006;8(1):67–78.
25. Macdonald DR, Cascino TL, Schold SC, Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol*. 1990;8(7):1277–1280.
26. Sathornsumetee S, Cao Y, Marcello JE, et al. Tumor angiogenic and hypoxic profiles predict radiographic response and survival in malignant astrocytoma patients treated with bevacizumab and irinotecan. *J Clin Oncol*. 2008;26(2):271–278.
27. 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(3 pt 1):860–868.
28. 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(5):588–593.
29. Yung WK, Prados MD, Yaya-Tur R, et al. Multicenter phase II trial of temozolomide in patients with anaplastic astrocytoma or anaplastic oligoastrocytoma at first relapse. Temodal Brain Tumor Group. *J Clin Oncol*. 1999;17(9):2762–2771.
30. Vredenburgh JJ, Desjardins A, Herndon JE, 2nd, et al. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. *Clin Cancer Res*. 2007;13(4):1253–1259.
31. Brandsma D, Stalpers L, Taal W, Sminia P, van den Bent MJ. Clinical features, mechanisms, management of pseudoprogression in malignant gliomas. *Lancet Oncol*. 2008;9(5):453–461.
32. Hecht JR, Mitchell E, Chidiac T, et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. *J Clin Oncol*. 2009;27(5):672–680.
33. Tol J, Koopman M, Cats A, et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med*. 2009;360(6):563–572.

34. Vredenburgh JJ, Desjardins A, Herndon JE, 2nd, et al. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. *J Clin Oncol*. 2007;25(30):4722–4729.
35. Iwamoto FM, Abrey LE, Beal K, et al. Patterns of relapse and prognosis after bevacizumab failure in recurrent glioblastoma. *Neurology*. 2009;73(15):1200–1206.
36. Quant E, Norden AD, Drappatz J, et al. Role of a second chemotherapy in recurrent malignant glioma patients who progress on a bevacizumab-containing regimen. Paper presented at: American Society of Clinical Oncology Annual Meeting Proceedings; 2008; Chicago, IL.
37. Norden AD, Young GS, Setayesh K, et al. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. *Neurology*. 2008;70(10):779–787.
38. van den Bent MJ, Vogelbaum MA, Wen PY, Macdonald DR, Chang SM. End point assessment in gliomas: novel treatments limit usefulness of classical Macdonald's criteria. *J Clin Oncol*. 2009;27(18):2905–2908.
39. Wen P, Macdonald D, Reardon D, et al. Updated response assessment criteria for high-grade gliomas: response assessment in Neuro-Oncology (RANO) Working Group. *J Clin Oncol*. 2010;28(11): 1963–1972.
40. Jain RK, Duda DG, Willett CG, et al. Biomarkers of response and resistance to antiangiogenic therapy. *Nat Rev Clin Oncol*. 2009;6(6):327–338.
41. Li Z, Bao S, Wu Q, et al. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. *Cancer Cell*. 2009;15(6):501–513.
42. 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(19):2012–2024.