

Phase II Trial of Bevacizumab and Irinotecan in Recurrent Malignant Glioma

James J. Vredenburgh,^{1,2,3} Annick Desjardins,^{1,2,3} James E. Herndon II,⁴ Jeannette M. Dowell,⁴ David A. Reardon,^{1,2,5} Jennifer A. Quinn,^{1,2,3} Jeremy N. Rich,^{1,2,3,6} Sith Sathornsumetee,^{1,2,3} Sridharan Gururangan,^{1,2,5} Melissa Wagner,^{1,2} Darell D. Bigner,^{1,2,7} Allan H. Friedman,^{1,2} and Henry S. Friedman^{1,2,3,5}

Abstract Purpose: Recurrent grade III-IV gliomas have a dismal prognosis with minimal improvements in survival seen following currently available salvage therapy. This study was conducted to determine if the combination of a novel antiangiogenic therapy, bevacizumab, and a cytotoxic agent, irinotecan, is safe and effective for patients with recurrent grade III-IV glioma.

Experimental Design: We conducted a phase II trial of bevacizumab and irinotecan in adults with recurrent grade III-IV glioma. Patients with evidence of intracranial hemorrhage on initial brain magnetic resonance imaging were excluded. Patients were scheduled to receive bevacizumab and irinotecan i.v. every 2 weeks of a 6-week cycle. Bevacizumab was administered at 10 mg/kg. The dose of irinotecan was determined based on antiepileptic use: patients taking enzyme-inducing antiepileptic drugs received 340 mg/m², whereas patients not taking enzyme-inducing antiepileptic drugs received 125 mg/m². Toxicity and response were assessed.

Results: Thirty-two patients were assessed (23 with grade IV glioma and 9 with grade III glioma). Radiographic responses were noted in 63% (20 of 32) of patients (14 of 23 grade IV patients and 6 of 9 grade III patients). The median progression-free survival was 23 weeks for all patients (95% confidence interval, 15-30 weeks; 20 weeks for grade IV patients and 30 weeks for grade III patients). The 6-month progression-free survival probability was 38% and the 6-month overall survival probability was 72%. No central nervous system hemorrhages occurred, but three patients developed deep venous thromboses or pulmonary emboli, and one patient had an arterial ischemic stroke.

Conclusions: The combination of bevacizumab and irinotecan is an active regimen for recurrent grade III-IV glioma with acceptable toxicity.

The prognosis for patients diagnosed with WHO grade III glioma (anaplastic astrocytoma, anaplastic oligoastrocytoma, and anaplastic oligodendroglioma) and grade IV glioma (glioblastoma multiforme) remains extremely grave. The median survival for patients with newly diagnosed glioblastoma is 8 to 15 months (1, 2). The prognosis for newly diagnosed grade III glioma is only slightly better, with a median survival of 12 to 24 months (3, 4). The standard of care for patients with glioma

blastoma was defined in a recent report of a phase III randomized trial as radiation therapy with concurrent temozolomide followed by 6 months of temozolomide (1).

The prognosis for recurrent malignant gliomas is even worse, with median survivals of 3 to 9 months (5, 6). Several chemotherapeutic agents or biological agents may palliate patients, but the agents that have been used produce only minimal increases in survival (6). The putative gold standards, temozolomide and nitrosoureas, produce only modest benefits, even in previously chemotherapy-naïve patients, and disease recurs (5, 6).

The overwhelming need for improved treatment efficacy has driven the development of novel therapeutic approaches to target mechanisms that underlie glioma development and growth.

In grades II-IV gliomas, which are highly vascular, solid cancers, widespread expression of the proangiogenic vascular endothelial growth factor (VEGF) has been observed (7, 8). There are five members of the VEGF family in mammals: VEGF-A (hereafter called VEGF), VEGF-B, VEGF-C, VEGF-D, and placental growth factor (9). In addition, multiple isoforms are generated through alternative exon splicing and protease cleavage. VEGF is produced by tumor cells and by stromal elements, including invading inflammatory cells. VEGF binds to its putative receptors, which include three receptor tyrosine kinases, VEGF receptor (VEGFR)-1, VEGFR-2, and VEGFR-3, as

Authors' Affiliations: ¹The Preston Robert Tisch Brain Tumor Center and Departments of ²Surgery, ³Medicine, ⁴Biostatistics, ⁵Pediatrics, ⁶Neurobiology, and ⁷Pathology, Duke University Medical Center, Durham, North Carolina
Received 9/18/06; revised 11/6/06; accepted 11/15/06.

Grant support: NIH Department of Health and Human Services (Bethesda, MD) grants 5 P50 CA108786, 5 P50 NS20023, and 4 R37 CA11898. Preston Robert Tisch Brain Tumor Research Fund and Bryan Cless Research Fund.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: J.N. Rich is a Damon Runyon-Lilly Clinical Investigator.

Requests for reprints: James J. Vredenburgh, Duke University Medical Center, Box 3624, Durham, NC 27710. Phone: 919-681-3824; Fax: 919-684-6674; E-mail: vrede001@mc.duke.edu.

© 2007 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-06-2309

well as to coreceptors, including the neuropilins. VEGFRs are expressed primarily by endothelial cells, but other nonneoplastic cells and tumor cells may also express these receptors. VEGF promotes endothelial cell proliferation and migration in support of normal as well as pathologic angiogenesis (10, 11), and increased VEGF expression in human patient glioma specimens has been associated with a poor prognosis (12). Preclinical studies have suggested that targeting VEGF function through neutralizing antibodies to VEGF can inhibit the growth of malignant glioma (13).

Bevacizumab represents the first antiangiogenic therapy that has been approved for use in human cancer patients. Bevacizumab is a humanized monoclonal IgG1 antibody that binds to and inhibits the biological activity of human VEGF-A. Bevacizumab had acceptable toxicity in a phase I study (14) and it showed a significant survival advantage in a phase III trial of patients with metastatic colorectal cancer comparing bevacizumab plus irinotecan/5-fluorouracil/leucovorin versus irinotecan/5-fluorouracil/leucovorin alone (15). In addition, compared with placebo, bevacizumab prolonged the time-to-progression for patients with metastatic renal cell cancer (16).

Bevacizumab has generally been used in combination with cytotoxic agents for the treatment of solid malignancies. Irinotecan is a topoisomerase 1 inhibitor that has been used in combination with bevacizumab in prior studies for other solid malignancies and has also been used for patients with malignant glioma. Irinotecan has activity against gastrointestinal malignancies, particularly colorectal carcinoma (17), and it readily passes through the blood-brain barrier. Weekly administration or every 3-week administration has shown activity against malignant gliomas, with response rates between 0% and 15% (18–22). On the weekly schedule, irinotecan is administered for 4 weeks followed by 2 weeks off. In a phase II trial of glioblastoma multiforme patients with a fixed dose of irinotecan of 125 mg/m², 15% of the patients had a confirmed partial response (18). The trial included pharmacokinetic evaluations, which documented low concentrations of irinotecan and the active metabolite SN-38 in patients on enzyme-inducing antiepileptic drugs (EIAED) and corticosteroids, likely secondary to increased clearance due to these agents. In another phase II study, a fixed dose of 300 mg/m² was administered every 3 weeks, and there was a similar, confirmed partial response rate of 14% (19). Other glioma trials have produced response rates <10% (20–22).

A preliminary report of the combination of bevacizumab and irinotecan as therapy for 21 patients with malignant gliomas described an encouraging response rate of 43%, with two treatment-related deaths, one due to intracranial hemorrhage and one due to intestinal perforation (23). Here, we report the first prospectively designed phase II trial of bevacizumab and irinotecan for malignant gliomas.

Patients and Methods

Patient selection. Adult patients (age ≥18 years) with histologically proven grade III-IV glioma that was progressive or recurrent after radiation therapy were eligible for the study. Conditions required for entry into the study included the following: (a) measurable disease by contrast-enhanced magnetic resonance imaging (MRI) or computed tomography; (b) Karnofsky performance status ≥60%; and (c) life expectancy >2 months. Minimum permitted time intervals from prior

treatments were 6 weeks for intracranial surgery, 6 weeks for chloroethylnitrosoureas, and 4 weeks for radiation and all other chemotherapeutic agents unless there was unequivocal evidence of tumor progression after radiotherapy or chemotherapy. Full recovery from the effects of any earlier intervention was required. Eligibility also required showing acceptable hematologic variables (absolute neutrophil count ≥1,500/μL; platelet count ≥125,000/μL; hematocrit >29%), renal function (serum creatinine ≤1.5 mg/dL), and hepatic function (bilirubin ≤1.5 mg/dL; serum levels of aspartate aminotransferase <1.5 × the upper limit of normal). Exclusion criteria included the following: (a) a prior malignancy other than curatively treated basal cell carcinoma or cervical carcinoma *in situ*; (b) previous treatment with bevacizumab; (c) a serious concurrent infection, illness, or medical condition; (d) females who were pregnant or nursing; and (e) any other condition that would compromise treatment with reasonable safety. Agreement to practice adequate birth control methods was required for fertile patients. The protocol for this clinical study was reviewed and approved by the National Cancer Institute (Bethesda, MD) and the Duke University Institutional Review Board. Each patient signed a written informed consent document, satisfying all federal and institutional policies and regulations, as a condition of registering for participation in the study.

Drug administration. Patients were assigned to one of two treatment groups based on preexisting use of antiepileptic drugs. Patients taking phenytoin, carbamazepine, phenobarbital, primidone, and oxcarbazepine were assigned to the EIAED group. Patients assigned to the non-EIAED group were either not being treated with an antiepileptic drug or taking one that does not significantly induce hepatic enzymes, including gabapentin, lamotrigine, valproic acid, felbamate, levetiracetam, tiagabine, topiramate, and zonisamide. Inclusion in the non-EIAED group also required discontinuation of any EIAEDs for at least 14 days. A clinically appropriate daily dose of a corticosteroid, such as dexamethasone, was determined for each patient before beginning the first cycle of therapy. The dose was required to have been stable for at least 7 days before treatment initiation, and efforts were made to maintain the same dose until the radiographic tumor measurement was done after completing the second cycle.

Bevacizumab was supplied by Genentech (South San Francisco, CA) and irinotecan was obtained commercially. Each agent was administered *i.v.*, once every 2 weeks: days 1, 15, and 29 of a 6-week cycle. Irinotecan was administered over 90 min before the bevacizumab infusion. Irinotecan is highly metabolized by the hepatic CYP3A4 enzymes, which are induced in response to EIAEDs that include phenytoin, carbamazepine, oxcarbazepine, primidone, and phenobarbital (24). We based dosage on prior pharmacokinetic studies in clinical trials, including brain tumor patients taking EIAEDs, and we determined that irinotecan was to be dosed at 340 mg/m² for patients taking an EIAED and 125 mg/m² for patients not taking antiepileptic drugs or taking non-EIAEDs (levetiracetam, lamotrigine, topiramate, and valproic acid). Bevacizumab was to be dosed at 10 mg/kg, with the first dose administered over 90 min. If the patient had no adverse reactions, the second dose was to be administered over 60 min, and all subsequent doses were to be administered over 30 min. The patients were required to have an absolute neutrophil count >1,000, platelets >100,000, aspartate aminotransferase <2.5 × normal, creatinine <1.5 × normal, and spot urine protein/creatinine ratio <1.0 before retreatment. Appropriate antiemetics were permitted. Treatment with any other approved or investigational chemotherapeutic agents was not permitted.

Treatment with the same doses of bevacizumab and irinotecan was repeated every 2 weeks until the occurrence of grade 3 or 4 toxicity or tumor progression. Patients were allowed a one-time 25% dose reduction of irinotecan for grade 3 or 4 gastrointestinal toxicity or grade 4 hematologic toxicity, but no patients required a dose reduction. Patients were also removed from the study because of disease progression, circumstances for which continued treatment could be detrimental to the health of a patient, patient withdrawal of consent, or noncompliance.

Patient evaluations. Evaluations done within 14 days of initiating therapy included a medical history, physical and neurologic examinations, vital signs, performance status determination, complete blood count with differential and platelet counts, blood coagulation variables, serum chemistry profile, urine protein/creatinine ratio, and pregnancy test for women of child-bearing potential. A contrast and a noncontrast brain MRI were done within 7 days of starting treatment and every 6 weeks after starting treatment. The bevacizumab and irinotecan were given on days 1, 15, and 29, and the MRI was done on day 39, 40, 41, or 42 of each cycle. A complete blood count with differentials and platelet count, chemistry panel, and urine protein/creatinine ratio was repeated every 2 weeks. Histories and physical examinations were repeated every 6 weeks. Toxicities were evaluated during each cycle and graded according to the National Cancer Institute Common Toxicity Criteria, version 3.0.

Response evaluation and criteria. Response to therapy was determined by MRI and neurologic examinations. Two investigators (J.J.V. and A.D.) measured the tumors independently and noted any discrepancies. MRIs were evaluated according to the MacDonald criteria (25). The criteria use the largest cross-sectional area of the postcontrast images and take into account steroid use and clinical findings. In addition, the investigators evaluated the T1, T2, and fluid-attenuated inversion recovery images. For a patient to be scored as showing a partial response, the contrasted images showed a >50% decrease in the area of enhancement and stable or decreased disease on T2 and fluid-attenuated inversion recovery images, and the patient was on stable or decreased steroid dose and was determined to have a stable or improved clinical status. Disease progression was defined as a >25% increase in the area of enhancement, defined as the product of the largest perpendicular diameters, appearance of a new lesion, or deterioration in the clinical status, likely secondary to tumor progression.

Statistical considerations. The historical control group used in the design of this phase II study assessing the activity of a combination of bevacizumab and irinotecan was a group of patients with recurrent glioblastoma multiforme or grade III tumors (AA) described by Wong et al. (6). That article reports for glioblastoma multiforme a 6-month progression-free survival (PFS) rate of 15%, with 95% confidence interval (95% CI) ranging between 10% and 19%, and for AA a 6-month PFS rate of 31%, with 95% CI of 24% to 39% (6).

Within the glioblastoma multiforme patient population, a true 6-month PFS rate with bevacizumab and irinotecan of 25% or more would definitely merit further investigation of the combination chemotherapy treatment. However, if the true 6-month PFS rate was no greater than 2.5%, there would be limited interest in pursuing the development of this treatment regimen in this patient population. With a sample size of 23 patients, the study was able to differentiate between a 2.5% and a 25% rate of 6-month PFS, with type I and II error rates of 0.019 and 0.059, respectively. Thus, if 3 or more of the 32 patients lived 6 or more months without disease progression, we determined that the treatment regimen would be considered worthy of further investigation for patients with glioblastoma multiforme.

Within the AA patient population, a true 6-month PFS rate with bevacizumab and irinotecan of 50% or more would definitely merit further investigation of the combination chemotherapy treatment. However, if the true 6-month PFS rate was 10% or less, there would be limited interest in pursuing the development of this treatment regimen in this patient population. With a sample size of nine patients, the study was able to differentiate between a 10% and a 50% rate of 6-month PFS, with type I and II error rates of 0.053 and 0.090, respectively. Thus, if three or more of the nine patients lived 6 or more months without disease progression, we determined that the treatment regimen would be considered worthy of further investigation for patients with AA.

Patients with recurrent grade III or IV tumors were eligible according to the Food and Drug Administration review of this investigator-initiated study. Patients removed from study because of toxicity were followed for progression or death and were included in the PFS and overall survival analyses. The patients were offered alternative therapies,

and the date of progression following removal from the study was used as the date of progression for the study.

In addition, there were early stopping rules for unacceptable toxicity, defined as the occurrence of \geq grade 2 central nervous system hemorrhage or grade 4 or 5 nonhematologic toxicity, probably due to the treatment. Unacceptable toxicity rates of 15% or less were considered desirable, whereas rates of 40% or greater were considered undesirable. The statistical hypothesis that was tested differentiated between a 15% and a 40% rate of unacceptable toxicity. After 16 patients with recurrent malignant glioma were treated, an interim analysis was conducted. If three or more patients experienced unacceptable toxicity, accrual of patients would be terminated. Otherwise, patient accrual was to continue. If 4 or more of the total 32 patients experienced unacceptable toxicity, the treatment regimen would be considered to have an unacceptable toxicity profile. The type I and type II error rates associated with this testing are 0.053 and 0.053, respectively.

Results

Patients. Characteristics of the 32 patients enrolled in the study are summarized in Table 1. There were 21 males and 12 females, and the median age was 49 years. Twenty-three patients had WHO grade IV glioma, and nine had WHO grade III glioma. Every patient had undergone prior surgical resection and external beam radiation therapy with concurrent temozolomide. Of note, many patients had poor prognostic factors, such as age >50 years ($n = 14$) and more than one progression ($n = 17$). Four of the patients had progressed <12 weeks after radiation therapy, and all four had progression outside the radiation field; thus, no patient had pseudoprogression. Two of the four had grade III tumors, and the other two had grade IV tumors. The response rate and PFS of the study are changed only slightly if these four patients are excluded [response rate of 61% (17 of 28) and 6-month PFS of 36%]. EIAEDs were used by 44% of the 32 patients. There were no differences in the

Table 1. Patient characteristics

No. patients	32
Age (y)	
Median (range)	49 (27-66)
Gender	
Male	21
Female	11
Karnofsky performance status	
<70%	2
70-80%	19
90-100%	11
Histologic diagnosis	
Glioblastoma multiforme	23 (72%)
Anaplastic astrocytoma	7 (22%)
Anaplastic oligodendroglioma	2 (6%)
Concomitant medications	
EIAED	14
Non-EIAED	14
No AEDs	4
Dexamethasone	19
No. progressions	
Median (range)	2 (1-5)
Time from diagnosis (mo)	
Median (range)	14 (3-66)

NOTE: Unless indicated otherwise, data are reported as number of patients.
Abbreviation: AED, antiepileptic drug.

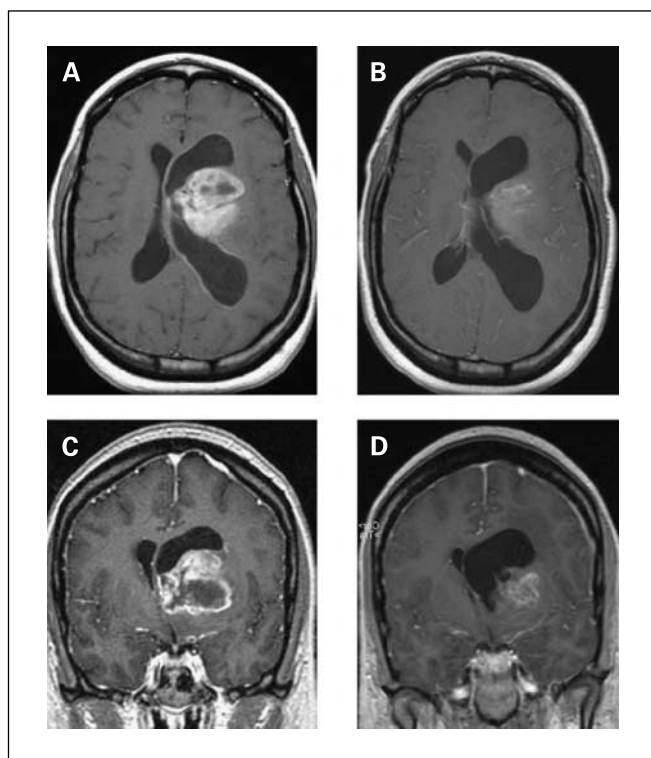


Fig. 1. Baseline and posttreatment MRIs of patient treated with bevacizumab and irinotecan. Postcontrast axial and coronal T1-weighted magnetic resonance scans in a patient with an anaplastic astrocytoma at baseline (A and C) and after four cycles of irinotecan/bevacizumab (B and D).

response rate, PFS, or overall survival in the EIAED group versus the non-EIAED group.

Toxicity. Importantly, there were no central nervous system hemorrhages. For safety purposes, we excluded the ~30% of the malignant glioma patients who have required therapeutic anticoagulation. The patients had a MRI every 6 weeks, and the noncontrast T1 and T2 images showed that no patient had evidence of hemorrhage throughout the study. We observed two treatment-associated deaths, one from a pulmonary embolus and the other from an arterial ischemic stroke. Four patients developed thromboembolic complications, two with pulmonary emboli, one with a deep venous thrombus, and one with an arterial ischemic stroke. One patient with a pulmonary embolus died 2 weeks after the event with rapid neurologic deterioration, and the patient with an arterial ischemic stroke died 3 weeks after the stroke. Both patients elected hospice care after the events. The four patients with thromboembolic complications were taken off study. Given the potential risks of administering anticoagulant therapy to the patients and continuing irinotecan and bevacizumab, the investigators decided that it was prudent to discontinue protocol therapy. Two patients developed grade 2 proteinuria, for which the protocol mandated cessation of irinotecan and bevacizumab. Both patients were asymptomatic, and the proteinuria resolved within 4 weeks of stopping bevacizumab and irinotecan. Three patients required surgery for unrelated reasons, appendectomy, stabilization of a fractured hip, and repair of anal fissures, and they were taken off study. One patient was removed from study after he developed an anaphylactic reaction to irinotecan during his fourth cycle. Two other patients withdrew from the

trial after one cycle, and both patients cited extreme fatigue as the reason. No patient developed grade 4 hematologic toxicity or >grade 3 nonhematologic toxicity except as noted above. The interim analysis was done for the first 16 patients, and there was one unacceptable toxicity. The patient with the arterial ischemic stroke had a grade IV nonhematologic toxicity that was probably due to the treatment. Toxicity was not different in patients receiving EIAEDs and those not receiving EIAEDs.

Response rate. Twenty of the 32 (63%) patients had a radiographic response. One was a complete response, and 19 were partial responses, as determined by at least a 50% decrease in the cross-sectional area of the enhancing tumor, with no clinical deterioration or increase in the corticosteroid dose. All the responses were associated with decreased T2-weighted and fluid-attenuated inversion recovery abnormalities. Figures 1 and 2 show two patients' MRIs that are representative of the marked activity that was seen in this trial. Of the nine patients with grade III gliomas, six had partial responses and three had stable disease. Of the 23 patients with grade IV gliomas, 1 patient had a complete response, 13 patients had partial responses, 8 patients had stable disease, and 1 patient had disease progression as the best response. The patients who responded were able to taper their steroids and improved neurologically. No responder required increased steroids, and none deteriorated neurologically.

PFS. The 6-month PFS was 38% (95% CI, 24-59%) for the entire group of patients. As expected, the 6-month PFS was better in the WHO grade III glioma patients, 56% (95% CI, 31-99%) versus 30% (95% CI, 16-57%) for the WHO grade IV glioma patients. The median PFS was 23 weeks (95% CI, 15-30 weeks), with a range of 6 to 46+ weeks. The median PFS was 20 weeks in

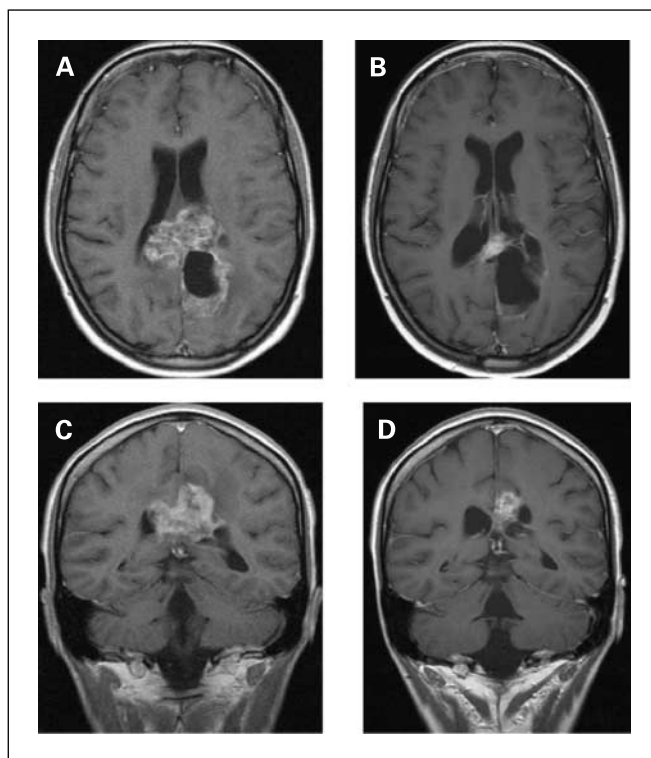
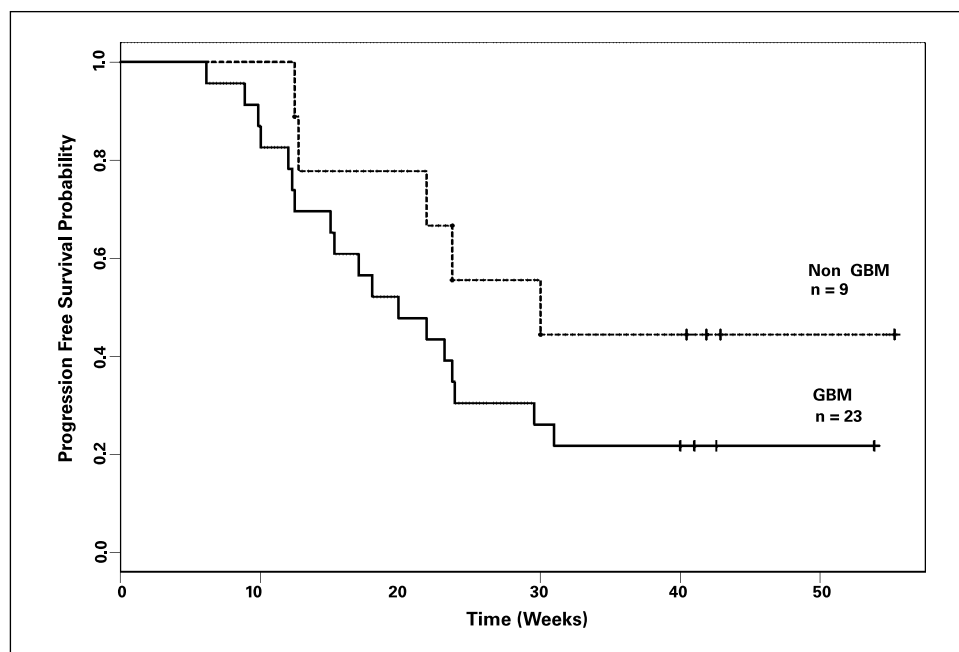


Fig. 2. Baseline and posttreatment MRIs of a second patient treated with bevacizumab and irinotecan. Postcontrast axial and coronal T1-weighted magnetic resonance scans in a patient with glioblastoma multiforme at baseline (A and C) and after four cycles of irinotecan/bevacizumab (B and D).

Fig. 3. Kaplan-Meier PFS grade III versus grade IV tumors.



grade IV patients and 30 weeks in grade III patients. Figure 3 is the Kaplan-Meier PFS curve. Twelve patients had not progressed when they came off study secondary to toxicity, surgery for an unrelated condition, or patient decision, and all of these patients subsequently progressed rapidly, at a median of 4 weeks, leading us to predict that a much higher PFS would have been seen had less restrictive toxicity criteria mandating cessation of therapy been used. The 12 patients who were removed secondary to toxicity were followed and their date of progression was used in the analysis.

Overall survival. The 6-month overall survival is 72% (95% CI, 58-89%). The median overall survival is 40 weeks in grade IV patients and has not been reached in grade III patients. Sixteen of the 32 patients have died, 14 of tumor progression and 2 of toxicity, and 16 patients remain alive after 48 to 64 weeks from the time of enrollment.

Patient follow-up. Eight of the 32 patients continue on bevacizumab and irinotecan >48 weeks after starting therapy. The reasons that the other 24 patients discontinued the study are listed in Table 2. Twelve patients had progressive disease, seven came off study because of toxicity, three required surgery for unrelated problems, and two withdrew consent and opted for comfort care.

Discussion

Grade III-IV gliomas are among the greatest challenges facing oncology, with near-universal lethality. The failure of current therapies can be linked to multiple factors: inherent tumor resistance to standard cytotoxic therapies, early invasion of tumor cells into normal brain preventing surgical resection, and limitations of drug delivery due to the blood-brain barrier. Few cytotoxic agents effectively penetrate into the central nervous system, and even fewer agents have activity against gliomas. Alkylating agents, such as the nitrosoureas, 1-(2-chloroethyl)-3-cyclohexyl-L-nitrosourea and 1,3-bis(2-chloroethyl)-1-nitrosourea, carboplatin, procarbazine, and temozolomide, are the most active agents (5, 26, 27). However, for patients with

recurrent disease after external beam radiation therapy, response rates are <20%, and the median PFS is 6 to 12 weeks. Our trial was designed to compare the observed activity of bevacizumab and irinotecan with the results detailed by Wong et al. (6) for a study that represents patients with recurrent glioblastoma multiforme or grade III gliomas who were treated on one of eight different chemotherapy trials. The current results for patients with glioblastoma multiforme show a 6-month PFS and median PFS of 30% and 20 weeks, respectively, compared with the Wong et al. (6) results of 15% and 9 weeks. In patients with grade III gliomas, we observed a 6-month PFS of 56% and median PFS of 30 weeks compared with the Wong et al. (6) results of 31% and 13 weeks, respectively.

Irinotecan has an excellent central nervous system penetration and a unique mechanism of action: inhibition of topoisomerase 1, an enzyme critical to DNA unwinding and cell division. Irinotecan is one of the few agents that have activity against recurrent malignant glioma, but response rates are 0% to 15%, and median PFS is 6 to 12 weeks (18-22). The addition of the anti-VEGF monoclonal antibody bevacizumab to the irinotecan therapy resulted in markedly increased activity, improved PFS, and modest toxicity. Four patients developed thromboembolic complications, which is of concern, although not unexpected, as malignant glioma patients have an increased incidence of thromboembolic complications, with up to 30% of patients having a thrombus over the course of their illness (28, 29).

Our results with bevacizumab and irinotecan seem superior to those reported for other glioma trials of antiangiogenic therapies in terms of response rate. Thalidomide has shown antiangiogenic activity (30), and a trial of high-dose thalidomide for recurrent high-grade gliomas showed a 6% response rate, a median time-to-progression of 10 weeks, and a median survival of 28 weeks (31). The addition of carmustine to thalidomide improved the response rate to 24% and the median PFS to 14 weeks (32). Vatalanib (PTK787/ZK222584) is an oral VEGFR tyrosine kinase inhibitor that has been studied alone and in combination with chemotherapy for

Table 2. Reasons for patient discontinuation from the study with duration on study

Reason for discontinuation	No. patients	Duration on study (wk)
Disease progression	12	6-36
Thromboembolic complications	4	6-12
Required surgery for unrelated conditions	3	6-14
Proteinuria	2	6, 8
Grade 2 fatigue and withdrew consent	2	6, 8
Allergic reaction to irinotecan	1	20

NOTE: Eight of 32 patients remain on study >48 weeks after treatment initiation.

recurrent glioblastoma multiforme (33, 34). Vatalanib monotherapy produced a 6% response rate and a median time-to-progression of 12 weeks (33). Vatalanib in combination with temozolomide or lomustine resulted in an 8% response rate and a median time-to-progression of 16 weeks (34). In addition, cell signal transduction pathways other than VEGF contribute to glioma angiogenesis. For example, glioblastomas display inappropriate activation of the epidermal growth factor receptor pathway through overexpression, amplification, or mutation of the receptor. Inhibitors of epidermal growth factor receptor tyrosine kinase activity have been studied in recurrent glioblastoma. Gefitinib and erlotinib produced response rates of 0% to 14% and a 6-month PFS of 13% to 17% (35, 36). A phase II trial of enzastaurin, a protein kinase C inhibitor that may primarily inhibit glycogen synthase kinase-3 β , produced a response rate of 18%, but PFS was not reported (37).

Although we have studied bevacizumab only in combination with irinotecan, we expect that bevacizumab will be more effective in combination with other agents than as monotherapy, for reasons that are delineated below. Although the mechanisms underlying the activities of bevacizumab remain unclear, the targeting of VEGF by bevacizumab may affect tumor physiology in multiple ways. Thus, bevacizumab may improve the efficacy of other agents through several effects including (a) direct antiangiogenic effects of bevacizumab and cytotoxics against endothelial cells and other stromal elements, (b) direct effects of bevacizumab on tumor cells that express VEGFRs, (c) augmenting the efficacy of vasculature to improve the delivery of other agents ("forced normalization") and thus effect a reversal of hypoxia, a condition associated with tumor cell resistance to cytotoxic therapies (38), and (d) direct effects on stem cell-like glioma cells (39). Additionally, in the maintenance of angiogenesis suppression, bevacizumab monotherapy will likely face challenges, as it targets only one VEGF family member, which suggests that other family members may compensate or that other angiogenic factors (fibroblast growth factors, platelet-derived growth factors, etc.) may compensate for the loss of VEGF-A activities under bevacizumab treatment. We therefore expect that bevacizumab will require an additional agent for sustained activity against grade III-IV glioma.

Because bevacizumab targets VEGF, it may reverse many of the abnormalities of brain tumor vasculature, which include increased vessel density, permeability, and tortuosity, with resultant hypoxia. Bevacizumab treatment results in decreased microvessel

density (40). For example, renal cell carcinoma is another highly vascular tumor with high levels of VEGF. In patients with renal cell carcinoma, bevacizumab alone significantly improved survival when compared with placebo, possibly secondary to the direct antiangiogenic effect of bevacizumab (16). Our studies used radiographic response as one measure of clinical efficacy. Contrast enhancement on brain MRI is regulated by the vascular permeability of cerebral vasculature. VEGF is also known as vascular permeability factor, and targeting VEGF through bevacizumab administration may decrease enhancement directly. However, the radiographic responses in our study were associated with clinical improvement as reported by patients and showed on neurological examination. The decrease in vascular permeability may have additional benefits in decreased cerebral edema, but the durability of the responses suggests that our results are likely secondary to tumor reduction and not just bevacizumab affecting the blood-brain barrier. The fact that the responding patients had an improved PFS compared with the nonresponding patients further corroborates that the responses were true tumor responses and not just a dexamethasone-like effect. Additionally, 8 of the 32 patients (five patients with recurrent glioblastoma multiforme) completed a full year of therapy, a result inconsistent with only a dexamethasone-like effect on the MRIs.

Through another potential mechanism of action, bevacizumab may act independently against VEGF to enhance the therapeutic effects of irinotecan. Malignant gliomas have some of the highest concentrations of VEGF, and VEGF directly stimulates many intracellular growth-signaling pathways (41). VEGF-mediated intracellular signaling results in proliferation, migration, cell survival, vascular permeability, and angiogenesis. VEGFR-2 signaling is linked to the integrins (42). VEGFR-2 and integrins are physically and functionally linked so that they influence communication between cells and the extracellular matrix. Cell-cell adhesion is affected by VEGF via an association with cadherins that enables the regulation of cellular junctions (43). Bevacizumab may directly inhibit many of the stimulatory effects of VEGF within and between tumor cells.

An additional explanation for the high response rates and suggestion of an improved PFS is increased irinotecan delivery to the tumor secondary to bevacizumab-induced decreased interstitial pressure and vascular normalization as well as improved cytotoxic efficacy of irinotecan from decreased hypoxia. Tumor vasculature is both structurally and functionally abnormal (44). As brain tumor cells proliferate, the interstitial pressure rises, which leads to an abnormal microenvironment with hypoxia and acidosis (45). The elevated interstitial pressure interferes with the delivery of cytotoxic agents to the tumor, and hypoxia makes tumor cells resistant to the agent (46). Bevacizumab abrogates VEGF-induced increased vascular permeability, with a resultant decreased interstitial pressure and improved oxygenation. The normalization of tumor vasculature and increased delivery of cytotoxics to the entire tumor explains the broad synergy of bevacizumab and a variety of chemotherapies. Moreover, recent results by Bao et al. (39) suggest that bevacizumab may have preferential activity against stem cell-like glioma cells derived from human glioblastoma clinical specimens and xenografts. Therefore, irinotecan delivery may have been preferentially enhanced to this cancer cell population.

The combination of bevacizumab and irinotecan resulted in significant antitumor activity against recurrent grade III-IV

glioma albeit with significant toxicity. Bevacizumab disrupts critical tumor processes by blocking the multiple abnormalities resulting from VEGF. We are participating in a multi-institutional, randomized phase II study of bevacizumab, alone and in combination with irinotecan, in an effort to corroborate

these results. In future studies, we plan to further elucidate the mechanisms of potential combinatorial benefit with chemotherapy, and we will also study bevacizumab in the setting of newly diagnosed patients, where the largest effect on overall survival would be expected.

References

- Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–96.
- Grossman SA, O'Neill A, Grunnet M, et al. Phase III study comparing three cycles of infusional carmustine and cisplatin followed by radiation therapy with radiation therapy and concurrent carmustine in patients with newly diagnosed supratentorial glioblastoma multiforme: Eastern Cooperative Oncology Group Trial 2394. *J Clin Oncol* 2003;21:1485–91.
- Buckner JC. Factors influencing survival in high-grade gliomas. *Semin Oncol* 2003;30:10–4.
- Curran WJ, Jr., Scott CB, Horton J, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. *J Natl Cancer Inst* 1993;85:704–10.
- Yung WK, Albright RE, Olson J, et al. A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. *Br J Cancer* 2000;83:588–93.
- Wong ET, Hess KR, Gleason MJ, et al. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol* 1999;17:2572–8.
- Salmaggi A, Eoli M, Frigerio S, et al. Intracavitary VEGF, bFGF, IL-8, IL-12 levels in primary and recurrent malignant glioma. *J Neurooncol* 2003;62:297–303.
- Lamszus K, Ulbricht U, Matschke J, Brockmann MA, Fillbrandt R, Westphal M. Levels of soluble vascular endothelial growth factor (VEGF) receptor 1 in astrocytic tumors and its relation to malignancy, vascularity, and VEGF-A. *Clin Cancer Res* 2003;9:1399–405.
- Kowanetz M, Ferrara N. Vascular endothelial growth factor signaling pathways: therapeutic perspective. *Clin Cancer Res* 2006;12:5018–22.
- Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003;9:669–76.
- Carmeliet P, Ferreira V, Breier G, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 1996;380:435–9.
- Godard S, Getz G, Delorenzi M, et al. Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. *Cancer Res* 2003;63:6613–25.
- Stefanik DF, Fellows WK, Rizkalla LR, et al. Monoclonal antibodies to vascular endothelial growth factor (VEGF) and the VEGF receptor, FLT-1, inhibit the growth of C6 glioma in a mouse xenograft. *J Neurooncol* 2001;55:91–100.
- Cobleigh MA, Langmuir VK, Sledge GW, et al. A phase I/II dose-escalation trial of bevacizumab in previously treated metastatic breast cancer. *Semin Oncol* 2003;30:117–24.
- Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335–42.
- Yang JC, Haworth L, Sherry RM, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N Engl J Med* 2003;349:427–34.
- Saltz LB, Cox JV, Blanke C, et al. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. *N Engl J Med* 2000;343:905–14.
- Friedman HS, Petros WP, Friedman AH, et al. Irinotecan therapy in adults with recurrent or progressive malignant glioma. *J Clin Oncol* 1999;17:1516–25.
- Cloughesy TF, Filka E, Kuhn J, et al. Two studies evaluating irinotecan treatment for recurrent malignant glioma using an every-3-week regimen. *Cancer* 2003;97:2381–6.
- Chamberlain MC. Salvage chemotherapy with CPT-11 for recurrent glioblastoma multiforme. *J Neurooncol* 2002;56:183–8.
- Prados MD, Lamborn K, Yung WK, et al. A phase 2 trial of irinotecan (CPT-11) in patients with recurrent malignant glioma: a North American Brain Tumor Consortium study. *Neurooncol* 2006;8:189–93.
- Batchelor TT, Gilbert MR, Supko JG, et al. Phase 2 study of weekly irinotecan in adults with recurrent malignant glioma: final report of NABTT 97-11. *Neurooncol* 2004;6:21–7.
- Stark-Vance V. Bevacizumab and CPT-11 in the treatment of relapsed malignant glioma. In: World Federation of Neuro-Oncology Meeting; 2005 May; 2005. p. 91.
- Kuhn JG. Influence of anticonvulsants on the metabolism and elimination of irinotecan. A North American Brain Tumor Consortium preliminary report. *Oncology (Huntingt)* 2002;16:33–40.
- Macdonald DR, Cascino TL, Schold SC, Jr., Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol* 1990;8:1277–80.
- Huncharek M, Muscat J, Geschwind JF. Multi-drug versus single agent chemotherapy for high grade astrocytoma; results of a meta-analysis. *Anticancer Res* 1998;18:4693–7.
- Vinolas N, Gil M, Verger E, et al. Pre-irradiation semi-intensive chemotherapy with carboplatin and cyclophosphamide in malignant glioma: a phase II study. *Anticancer Drugs* 2002;13:163–7.
- Marras LC, Geerts WH, Perry JR. The risk of venous thromboembolism is increased throughout the course of malignant glioma: an evidence-based review. *Cancer* 2000;89:640–6.
- Brandes AA, Scelzi E, Salmistraro G, et al. Incidence of risk of thromboembolism during treatment high-grade gliomas: a prospective study. *Eur J Cancer* 1997;33:1592–6.
- D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci U S A* 1994;91:4082–5.
- Fine HA, Figg WD, Jaeckle K, et al. Phase II trial of the antiangiogenic agent thalidomide in patients with recurrent high-grade gliomas. *J Clin Oncol* 2000;18:708–15.
- Fine HA, Wen PY, Maher EA, et al. Phase II trial of thalidomide and carmustine for patients with recurrent high-grade gliomas. *J Clin Oncol* 2003;21:2299–304.
- Conrad C, Friedman HS, Reardon DA, et al. A phase I/II trial of single agent PTK 787/ZK 222584 (PTK/ZK), a novel, oral angiogenesis inhibitor, in patients with recurrent glioblastoma multiforme (GBM). 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition). *J Clin Oncol* 2004;22(14S; July 15 Supplement):1512.
- Reardon DA, Friedman HS, Yung WK, et al. A phase I/II trial of PTK787/ZK 22584 (PTK/ZK), a novel, oral angiogenesis inhibitor, in combination with either temozolomide or lomustine for patients with recurrent glioblastoma multiforme (GBM) [abstract 1513]. *Proc Am Soc Clin Oncol* 2004;23:110.
- Rich JN, Reardon DA, Peery T, et al. Phase II trial of gefitinib in recurrent glioblastoma. *J Clin Oncol* 2004;22:133–42.
- 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. *Neurooncol* 2006;8:67–78.
- Fine HA, Kim L, Royce C, et al. Results from phase II trial of enzastaurin (LY317615) in patients with recurrent high grade gliomas [abstract 1504]. *J Clin Oncol* 2005;23:115.
- Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. *Nature* 2005;437:497–504.
- Bao S, Wu Q, Sathornsumetee S, et al. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. *Cancer Res* 2006;66:7843–8.
- Willett CG, Boucher Y, di Tomaso E, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 2004;10:145–7.
- Wu HM, Yuan Y, Zawieja DC, Tinsley J, Granger HJ. Role of phospholipase C, protein kinase C, and calcium in VEGF-induced venular hyperpermeability. *Am J Physiol* 1999;276:H535–42.
- Friedlander M, Brooks PC, Shaffer RW, Kincaid CM, Varner JA, Cheresh DA. Definition of two angiogenic pathways by distinct α_v integrins. *Science* 1995;270:1500–2.
- Esser S, Lampugnani MG, Corada M, Dejana E, Risau W. Vascular endothelial growth factor induces VE-cadherin tyrosine phosphorylation in endothelial cells. *J Cell Sci* 1998;111:1853–65.
- Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249–57.
- Winkler F, Kozin SV, Tong RT, et al. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. *Cancer Cell* 2004;6:553–63.
- Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. *Cancer Res* 2004;64:3731–6.