Bevacizumab Plus Irinotecan in Recurrent WHO Grade 3 Malignant Gliomas

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Abstract  Purpose: Although patients with newly diagnosed WHO grade 3 malignant glioma have a more favorable prognosis than those with WHO grade 4 malignant glioma, salvage therapies following recurrence offer essentially palliative benefit. We did a phase II trial of bevacizumab, a monoclonal antibody to vascular endothelial growth factor, in combination with irinotecan for patients with recurrent grade 3 malignant glioma.

Experimental Design: Upon documentation of adequate safety among an initial cohort of nine patients treated with bevacizumab (10 mg/kg) and irinotecan every 14 days, a second cohort (n = 24) was treated with bevacizumab (15 mg/kg) every 3 weeks with irinotecan on days 1, 8, 22, and 29 of each 42-day cycle. For both cohorts, the dose of irinotecan was 340 mg/m² for patients on enzyme-inducing antiepileptic drugs (EIAED) and 125 mg/m² for patients not on EIAEDs. After each 6-week cycle, patients were evaluated with a physical examination and magnetic resonance imaging.

Results: The 6-month progression-free survival was 55% (95% confidence interval, 36–70%). The 6-month overall survival was 79% (95% confidence interval, 61–89%). Twenty patients (61%) had at least a partial response. Outcome did not differ between the two treatment cohorts. Significant adverse events were infrequent and included a central nervous system hemorrhage in one patient, and one patient who developed thrombotic thrombocytopenic purpura.

Conclusion: Bevacizumab and irinotecan is an active regimen with acceptable toxicity for patients with recurrent WHO grade 3 malignant glioma.

Malignant gliomas, including WHO grade 4 tumors or glioblastoma multiforme (GBM) and WHO grade 3 tumors such as anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), and anaplastic oligoastrocytoma (AOA), are among the most devastating cancers and account for 0.5% to 1% of all cancers in Western countries (1). Malignant gliomas present unique challenges due to their location, aggressive biological behavior, and diffuse infiltrative growth that lead to profound and progressive disability followed by death in most cases. Notwithstanding surgical and radiation advances in the last 30 years, only temporary control of tumor growth is possible in most cases. Although newly diagnosed grade 3 malignant gliomas show a more favorable response to postsurgical therapy than grade 4 tumors (2–9), at recurrence, salvage therapies offer essentially palliative benefit (10–12).

Vascular proliferation, or neoangiogenesis, is a histopathologic characteristic of malignant gliomas (13, 14). Malignant gliomas overexpress vascular endothelial growth factor (VEGF), the levels of which correlate directly with tumor vascularity and grade, and inversely with prognosis (15–18). VEGF and its receptors are therefore important therapeutic targets (17, 19).

Bevacizumab is a humanized murine monoclonal antibody that binds VEGF-A (20, 21), thereby preventing interaction and activation of VEGF receptor tyrosine kinases VEGFR1 and VEGFR2 (22). Bevacizumab given in combination with conventional chemotherapy significantly improves survival of patients with metastatic colorectal and lung cancer (23, 24) and progression-free survival (PFS) of patients with breast cancer (25). Bevacizumab has been approved by the U.S. Food and Drug Administration for colorectal cancer with irinotecan, as a first-line treatment for non–small cell lung cancer in combination with carboplatin and paclitaxel, and has obtained accelerated approval for metastatic HER2-negative breast cancer patients in combination with paclitaxel.

Irinotecan, a topoisomerase I inhibitor, exhibits modest single-agent activity against recurrent malignant gliomas (26, 27). Based on the activity of bevacizumab plus irinotecan in colorectal cancer, we initiated a phase II trial of this regimen...
Translational Relevance

This article is very important for clinical cancer treatment as there is no standard of care treatment for patients once they present with disease progression or recurrence of their WHO grade 3 malignant glioma. The treatment combination of bevacizumab plus irinotecan shows a response rate as well as an improvement in progression-free and overall survival that has not been seen thus far in this patient population. This information is important to the public as it might change how this type of tumor is treated at the time of recurrence.

for recurrent malignant glioma patients. An initial cohort of 32 patients were treated (28), including 23 patients with grade 4 tumors and 9 patients with grade 3 tumors. Following documentation of adequate safety in this cohort, a second cohort was enrolled to further evaluate this regimen using a more frequent irinotecan schedule and to increase the number of patients with grade 3 malignant glioma to a statistically meaningful level. The preliminary outcome of the initial cohort (28) as well as the outcome of GBM patients in both cohorts (29) have been previously reported. We now present the final outcome of the recurrent grade 3 patients from both cohorts treated in this study.

Patient Characteristics. Eligible patients included adults with histologically confirmed grade 3 malignant glioma (AA, AO, or AOA), who received prior radiation therapy and temozolomide. All patients had tumor progression at enrollment, measurable disease on contrast-enhanced magnetic resonance imaging (MRI), and adequate recovery from prior treatment. An interval of at least 6 wk from surgery, and at least 4 wk from prior radiation therapy or chemotherapy were also required, unless tumor progression was confirmed by either histology, positron-emission tomography, magnetic resonance spectroscopy, or progressive changes on consecutive MRIs. Additional eligibility criteria included a Karnofsky performance status of ≥60%: satisfactory hematologic (hematocrit ≥29%, absolute neutrophil count ≥1,500 cells/μL, platelets ≥125,000 cells/μL), renal (serum creatinine ≤1.5 mg/dL), and hepatic (serum aspartate aminotransferase and bilirubin <1.5× upper limit of normal) functions; and stable corticosteroid dose for 1 wk. Exclusion criteria included evidence of hemorrhage on baseline MRI, more than three prior recurrences, previous bevacizumab therapy, pregnancy or breast feeding, and use of therapeutic anticoagulation. Contraceptive measures were required for sexually active patients. The protocol was approved by the U.S. Food and Drug Administration and the Duke University Institutional Review Board. Each patient provided informed consent.

Treatment. There were two cohorts of treated patients. The first nine patients received both bevacizumab (10 mg/kg) and irinotecan every 14 d (cohort 1). After these patients were assessed and the regimen was determined to be active and safe, a second cohort, including 24 patients, was treated for two reasons: (a) to have an adequate number of grade 3 malignant glioma patients to make meaningful conclusions and (b) to change the irinotecan schedule to an accepted malignant glioma treatment of four doses in 6 wk in anticipation of a randomized phase III trial (30–32). The second cohort received bevacizumab every 21 d (15 mg/kg) and irinotecan on days 1, 8, 22, and 29 of a 42-d cycle. For both cohorts, irinotecan was administered before bevacizumab at a dose of 340 mg/m² for patients on enzyme-inducing antiepileptic drugs and 125 mg/m² for patients not on enzyme-inducing antiepileptic drugs (30). In both cohorts, patients received dexamethasone 10 mg i.v. and ondansetron 24 mg i.v. as premedication. Diarrhea was treated symptomatically with atropine 1 mg i.v., oral loperamide, or an oral combination of diphenoxylate and atropine on an as-needed basis. For each cohort of patients 1 cycle was 42 d long. To be considered has having completed therapy, a patient had to have completed 9 cycles of 42 d each of therapy.

Before each infusion, patients had to have adequate hematologic recovery (absolute neutrophil count ≥1,000 and platelets ≥100,000), an aspartate aminotransferase ≤2.5× normal, and a creatinine <1.5× normal. For patients in cohort 1, the spot urine protein:creatinine ratio had to be ≤1.0 before retreatment. Based on the lack of significant nephrotoxicity observed in cohort 1, the spot urine to protein:creatinine ratio was required to be <3.5 before retreatment for the second cohort.

The dose of irinotecan was allowed to decrease by 25% for grade 3 or 4 gastrointestinal or hematologic toxicity. If grade 4 gastrointestinal or hematologic toxicity occurred following an initial dose reduction, treatment was discontinued. Protocol treatment was also discontinued for disease progression, withdrawal of consent, grade 2 or greater central nervous system hemorrhage, grade 4 nonhematologic toxicity, arterial thrombosis, gastrointestinal perforation, wound dehiscence requiring medical or surgical intervention, inability of the subject to comply with study requirements, or determination by the investigator that it was no longer safe for the subject to continue therapy.

Patient evaluations. Within 14 d of initiating treatment, patients had a full medical history and physical exam, Karnofsky performance status determination, complete blood count with differential, prothrombin time and partial thromboplastin time, serum metabolic panel, urine protein:creatinine ratio analysis every 2 wk for the second cohort; and a pregnancy test for women of child-bearing potential. A baseline brain MRI was obtained within 1 wk of starting treatment. Evaluations during treatment included complete blood count with differential, serum metabolic panel, and urine protein:creatinine ratio analysis every 2 wk for the first cohort; and complete blood count with differential and serum metabolic panel before each irinotecan infusion, and urine protein:creatinine ratio before each bevacizumab infusion for the second cohort. A full medical history and physical examination, including neurologic examination, and brain MRI were completed every 6 wk for both cohorts. The National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0, was used to evaluate toxicity.

Treatment response evaluation. Response to therapy was determined by two investigators independently using the Macdonald criteria (33). The investigators also evaluated noncontrast T1, T2, and fluid-attenuated inversion recovery images. A partial response was assessed if the contrasted images showed a >50% decrease in the bidimensional-enhancing tumor product and stable or decreased T2 and fluid-attenuated inversion recovery signal, provided the patient was on a stable or decreased dose of dexamethasone and was stable or improved neurologically. A complete response required resolution of all measurable enhancing abnormalities, with stable or decreased changes on T2 and fluid-attenuated inversion recovery images as well as a stable or decreased dexamethasone dose and stable or improved neurologically. Progressive disease was defined as a >25% increase in bidimensional-enhancing tumor product, the appearance of any new enhancing lesion, or deterioration in clinical status deemed related to tumor progression. Patients were defined as having a stable disease when the radiographic criteria for a partial response, complete response, or progressive disease were not met and if there was no sign of clinical progression.

Statistical considerations. Among patients with first relapse grade 3 malignant glioma treated with temozolomide, Yung reported a 6-mo progression-free survival (PFS) rate of 46% with 95% confidence intervals (95% CI) between 38% and 54% (34). Our patient population was expected to have a poorer prognosis than the patient population reported by Yung, due to the allowance of more than one previous
disease progression. Nevertheless, Yung’s data were used as the historical basis for the design of this study. If the 6-mo PFS rate with bevacizumab and irinotecan was at least comparable with the lower CI reported by Yung ($\geq 40\%$), there would be interest in further investigation of this combination. However, if the 6-mo PFS rate was $<20\%$, there would be no interest in further pursuing this regimen for this patient population. With a sample size of 36 patients, the study was designed to differentiate between 6-mo PFS rates of 20% and 40% with type I and II error rates of 0.089 and 0.090, respectively. If $\geq 11$ of these 36 patients lived 6 mo without disease progression, the treatment regimen would be considered worthy of further investigation.

Early stopping rules for unacceptable toxicity, defined as grade 2 or worse central nervous system hemorrhage or grade 4 or 5 non-hematologic toxicity related to the treatment, were incorporated. Specifically, an unacceptable toxicity rate of $\leq 15\%$ was considered desirable, whereas rates of $\geq 40\%$ were considered undesirable. A statistical hypothesis differentiating between a 15% and 40% rate of unacceptable toxicity was tested with type I and II error rates of 0.040 and 0.043, respectively.

**Results**

**Patient characteristics.** From April 5, 2005, to February 17, 2006, 36 adult patients were enrolled. Upon central pathology review, however, three patients had GBM. Thus, 33 grade 3 patients are included in this analysis. Demographic and prior treatment characteristics did not differ between the two cohorts (Table 1). The median age was 43 years (range, 22-62 years). Twenty-five patients had AA (76%) and eight had AO (24%). Patients received a median of three prior chemotherapy regimens (range, 1-7), and 61% had two or three previous episodes of progression. Although eligibility criteria specified no more than three prior episodes of progression, some patients received more than three chemotherapy regimens that may have been discontinued due to a variety of reasons other than progression, including completion of planned therapy and toxicity. Patients with prior diagnosis of low-grade glioma were considered as having their first grade 3 progression at the time of transformation to WHO grade 3. As of December 2, 2007, 22 patients have died (67%). All surviving patients have discontinued protocol treatment and four remain free of disease progression.

**Progression-free survival.** With a median follow-up of 106 weeks, the 6-month PFS for all patients was 55% (95% CI, 36-70%). The median PFS was 30 weeks (95% CI, 21-60 weeks; Table 2; Fig. 1). Although outcome did not differ between treatment cohorts, AO patients trended toward a better outcome than AA patients. Specifically, AO patients had a 6-month PFS of 62% (95% CI, 23-86%) and a median PFS of 50 weeks (95% CI, 21-undetermined) compared with a 6-month PFS of 52% (95% CI, 31-69%) and a median PFS of 28 weeks (95% CI, 17-60 weeks) for AA patients.

**Overall survival.** The median overall survival (OS) for all patients was 65 weeks (Table 2; Fig. 2). We observed a 6-month OS of 79% (95% CI, 61-89%). Patients with AO had a 6-month OS of 62% (95% CI, 23-86%) and a median OS of 50 weeks (95% CI, 21-undetermined) compared with a 6-month OS of 52% (95% CI, 31-69%) and a median OS of 28 weeks (95% CI, 17-60 weeks) for AA patients.

**Possible prognostic factors.** Cox regression analysis failed to show an effect of histology, baseline Karnofsky performance
status, number of episodes of prior progression, age, or time from initial diagnosis on OS and PFS.

The outcome of the 5 patients who accrued to this study within 12 weeks of completing radiation therapy and temozolomide did not differ with regard to PFS ($P = 0.2$) and OS ($P = 0.6$) when compared with patients who accrued more than 12 weeks after radiation therapy and temozolomide (Table 2).

**Response.** Three patients achieved a complete response and 17 achieved a partial response, yielding an overall response rate of 61%. Eleven patients achieved a best response of stable disease, whereas only two patients had progressive disease upon initial evaluation at six weeks (Table 2). Radiographic response did not correlate with PFS ($P = 0.09$) and OS ($P = 0.1$; Table 3). Of 12 patients on dexamethasone at enrollment, 8 (67%) decreased their dose of dexamethasone (median daily start dose, 6.75 mg; median nadir dose, 2 mg) and 5 (42%) were able to completely discontinue dexamethasone. Five patients required increased dexamethasone while on study, and all had progressive disease. Seven patients (19%) completed a year of therapy. Of note, fluorodeoxyglucose positron emission tomography scans were hypometabolic among six of these patients. Among the seven patients who completed a year of therapy, two have died due to progressive disease, two are alive and undergoing treatment for progressive disease, and three remain free of disease recurrence. Additional information on the reason for treatment discontinuation and subsequent treatments received by the patients after recurrence are available in the Supplementary Tables S1 and S2.

**1p19q status.** Five of eight patients with AO had known 1p19q status, including three patients with 1p19q deletion, one patient with intact 1p and 19q deletion, and one patient with intact 1p and 19q. Seven AO patients had a partial response and one achieved stable disease.

**Toxicity.** Overall, 137 cycles were administered. Toxicities were more common among patients in cohort 2, and were most likely due to the more intensive irinotecan schedule (see Supplementary Table S3). Five patients in cohort 2 necessitated dose reduction due to gastrointestinal toxicity or neutropenia. No patient in cohort 1 required dose reduction. Four patients in cohort 2 withdrew consent due to fatigue or gastrointestinal toxicity. One patient developed a central nervous system hemorrhage in the 9th cycle of therapy, 17 days after having initiated low molecular weight heparin for a lower extremity deep venous thrombosis. This patient required hospitalization, high-dose dexamethasone, and study discontinuation, but made a full recovery following rehabilitative therapy. One patient developed thrombotic thrombocytopenic purpura.

**Fig. 1.** A, Kaplan-Meier PFS. B, Kaplan-Meier OS.

**Fig. 2.** Baseline and posttreatment magnetic resonance imaging of a patient with anaplastic astrocytoma treated with bevacizumab and irinotecan. Axial postcontrast T1-weighted magnetic resonance scans at baseline (A) and after eight cycles (B). Axial precontrast axial T1-weighted magnetic resonance scans at baseline (C) and after eight cycles (D). Axial fluid-attenuated inversion recovery (FLAIR) images at baseline (E) and after eight cycles (F).
(TTP) after the second bevacizumab and irinotecan infusion of cycle 1, requiring study discontinuation. Although this patient continues to undergo peritoneal dialysis, she remains without evidence of tumor progression 21 months later (for additional follow-up, see Supplementary Table S1).

**Discussion**

We report the first phase II trial of bevacizumab and irinotecan for the treatment of patients with recurrent grade 3 malignant glioma. This study shows that treatment with bevacizumab and irinotecan is associated with a prolonged PFS and OS compared with historical controls treated with alternative salvage therapy, in association with a high radiographic response rate.

Overall, the study regimen had acceptable toxicity. However, the more frequent irinotecan dosing schedule administered to patients in cohort 2 was associated with increased toxicity that often required dose reduction or study discontinuation. Because efficacy was comparable between both cohorts, but cohort 2 patients had greater toxicity, the irinotecan dosing schedule used in cohort 1 patients is preferable and will be used in future studies.

The risks of arterial and venous thrombosis with bevacizumab are well known. In our study, one patient developed an arterial stroke and four patients had venous thrombosis, for an incidence of 3% and 12%, respectively. Given the small number of patients in our study, and the inherent risk of thrombosis in brain tumor patients (35, 36), additional studies will be necessary to further determine the incidence of arterial and venous thrombosis among malignant glioma patients treated with bevacizumab.

To our knowledge, no cases of TTP have been previously observed among patients treated with bevacizumab. In fact, a case of combined central retinal artery occlusion and central retinal vein occlusion secondary to TTP responding to intravitreal bevacizumab has been described (37). However, the risks of the intravitreal administration of bevacizumab are different from the risks of its i.v. administration. However, one case report of a patient who developed thrombotic microangiopathy likely associated with bevacizumab was published in *Lancet Oncology* (38). Thrombotic microangiopathy may present as TTP. Also, chemotherapy is known to increase the risk of TTP. The etiology of TTP in our study patient remains uncertain, between bevacizumab and irinotecan.

No established therapy for disease progression after radiation therapy and temozolomide is available for patients with grade 3 malignant glioma. Our results compare favorably with those of other studies among recurrent grade 3 malignant glioma patients. Among 162 AA and AOA patients treated with temozolomide at first recurrence, Yung reported a radiographic response rate, median PFS, and 6-month PFS rate of 35%, 21.6 weeks, and 46%, respectively (34). Our results, including a radiographic response rate, median PFS, and 6-month PFS rate of 61%, 30 weeks, and 55%, respectively, suggest an advantage of the combination of bevacizumab and irinotecan, particularly because our patient population had progressive disease after prior temozolomide therapy and was more heavily pretreated than those reported by Yung (34). Our results also compare favorably with those presented by Wong who reported a radiographic response rate, median PFS, and 6-month PFS rate of 14%, 13 weeks, and 31%, respectively, for 150 recurrent AA patients treated on 8 consecutive salvage regimens (39).

The small number of patients with AO histology in our study precludes a meaningful conclusion of potential differences in outcome based on grade 3 histology. Nonetheless, we observed a trend toward a better 6-month PFS (62% for AO versus 52% for AA) and median PFS (50 weeks for AO versus 28 weeks for AA), but this did not translate into an improved OS.

The absence of correlation with OS and PFS for well-known prognostic factors of grade 3 malignant glioma, mostly baseline Karnofsky performance status and age, can be explained by the small number of patients in our study and by the very homogeneous distribution of the demographics of our study population. It is also possible that a subtle difference might have been cancelled by the treatment itself.

New therapeutic regimens with mechanisms of action different from DNA alkylation are imperative because nearly all grade 3 malignant glioma patients will develop disease recurrence following temozolomide, implicating the evolution of chemoresistance as an important mediator of treatment failure. Methylguanine methyltransferase is a key mediator of temozolomide resistance (40, 41). Irinotecan, a topoisomerase I inhibitor, is not affected by methylguanine methyltransferase. However, the antitumor effect of single-agent irinotecan is disappointing, with response rates of 0% to 17% and 6-month PFS inferior to 25% in malignant glioma patients, without major differences between recurrent GBM and grade 3 malignant glioma patients (26, 27, 42). In contrast, our current study and prior reports among recurrent GBM patients show that the combination of irinotecan plus bevacizumab has significant, durable antitumor benefit (29). The shown durable antitumor benefit, comparative to tumor response rate only, is primordial when evaluating VEGF-directed therapies, due to the known reduction in vascular permeability induced by VEGF inhibition, which can account for the radiographic improvement, but not for the prolonged PFS and OS. The reason for the greater efficacy observed with the combination of bevacizumab and irinotecan compared with irinotecan alone is unclear. One possibility is that normalization of tumor vasculature observed with VEGF-directed therapies can decrease interstitial pressure, improve tissue oxygenation, and increase delivery of irinotecan to the tumor (43–45). Another potential mechanism of action

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Table 3. Tumor response versus PFS and OS
of this regimen may involve the suppression of angiogenesis linked to glioma stem cells by bevacizumab (46, 47).

In conclusion, we show that the combination of bevacizumab and irinotecan has significant efficacy in patients with grade 3 recurrent malignant glioma. This regimen achieved a dramatic rate of radiographic response, but more importantly, grade 3 recurrent malignant glioma. This regimen achieved a...