Published in final edited form as:
*J Neurooncol*. 2012 March ; 107(1): 155–164. doi:10.1007/s11060-011-0722-2.

# Phase II study of carboplatin, irinotecan, and bevacizumab for bevacizumab naïve, recurrent glioblastoma

**David A. Reardon**,
Departments of Surgery, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

Departments of Pediatrics, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**Annick Desjardins**,
Departments of Medicine, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**Katherine B. Peters**,
Departments of Medicine, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**Sridharan Gururangan**,
Departments of Surgery, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

Departments of Pediatrics, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**John H. Sampson**,
Departments of Surgery, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**Roger E. McLendon**,
Departments of Pathology, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**James E. Herndon II**,
Departments of Cancer Center Biostatistics, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**Anuradha Bulusu**,
Departments of Cancer Center Biostatistics, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**Stevie Threatt**,
Departments of Surgery, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**Allan H. Friedman**,
Departments of Surgery, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**James J. Vredenburgh**, and



Correspondence to: David A. Reardon, reard003@mc.duke.edu.

Departments of Medicine, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

**Henry S. Friedman**
Departments of Surgery, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

Departments of Pediatrics, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Box 3624, Durham, NC 27710, USA

David A. Reardon: reard003@mc.duke.edu

## Abstract

We evaluated the efficacy of carboplatin, irinotecan, and bevacizumab among bevacizumab-naïve, recurrent glioblastoma (GBM) patients in a phase 2, open-label, single arm trial. Forty eligible patients received carboplatin (area under the plasma curve [AUC] 4 mg/ml-min) on day one, while bevacizumab (10 mg/kg) and irinotecan (340 mg/m$^2$ for patients on CYP3A-enzyme-inducing anti-epileptics [EIAEDs] and 125 mg/m$^2$ for patients not on EIAEDs) were administered on days 1 and 14 of every 28-day cycle. Patients were evaluated after each of the first two cycles and then after every other cycle. Treatment continued until progressive disease, unacceptable toxicity, non-compliance, or voluntary withdrawal. The primary endpoint was progression-free survival at 6 months (PFS-6) and secondary endpoints included safety and median overall survival (OS). All patients had progression after standard therapy. The median age was 51 years. Sixteen patients (40%) had a KPS of 90–100, while 27 (68%) were at first progression. The median time from original diagnosis was 11.4 months. The PFS-6 rate was 46.5% (95% CI: 30.4, 61.0%) and the median OS was 8.3 months [95% confidence interval (CI): 5.9, and 10.7 months]. Grade 4 events were primarily hematologic and included neutropenia and thrombocytopenia in 20 and 10%, respectively. The most common grade 3 events were neutropenia, thrombocytopenia, fatigue, and infection in 25, 20, 13, and 10%, respectively. Eleven patients (28%) discontinued study therapy due to toxicity and 17 patients (43%) required dose modification. One patient died due to treatment-related intestinal perforation. The addition of carboplatin and irinotecan to bevacizumab significantly increases toxicity but does not improve anti-tumor activity to that achieved historically with single-agent bevacizumab among bevacizumab-naïve, recurrent GBM patients. (ClinicalTrials.gov number NCT00953121).

### Keywords

Glioblastoma; Angiogenesis; Bevacizumab; Vascular endothelial growth factor; Irinotecan; Carboplatin

## Introduction

The US Food and Drug Administration (FDA), followed by several other national regulatory agencies, granted approval of single-agent bevacizumab, a humanized monoclonal antibody (MAb) against vascular endothelial growth factor (VEGF), for patients with recurrent GBM based on a rate of radiographic response that was of noteworthy durability among many patients [1]. Others, such as the European medicinal agency, denied bevacizumab approval for several reasons including the lack of a non-bevacizumab control arm [2]. Nonetheless, the rates of overall radiographic response (ORR) and progression-free survival (PFS) achieved with bevacizumab therapy among recurrent GBM patients, 28–38% and 29–50%, respectively [3, 4], are approximately 4-fold greater than those reported historically including meta-analyses of recently completed clinical trials that did not include bevacizumab [5–7]. While these results are compelling, the increment in overall survival

(OS) with bevacizumab has been disproportionally low and is only on the order of 1.5–2 fold greater than that reported historically with non-bevacizumab therapy [3–7]. The explanation underlying the discordance between the degree of ORR/PFS benefit compared to OS is not clear and a matter of ongoing controversy.

Although GBM is one of the most angiogenic of cancers, blood flow within these tumor is often paradoxically deficient due to multiple morphologic and functional abnormalities relative to non-tumor vasculature [8–11]. In preclinical models, inhibition of VEGF signaling can induce a transient window of tumor vessel normalization reflected by decreased tortuosity, dilation, and permeability, as well as thinning of thickened vessel basement membranes and increased pericyte coverage. These morphological changes have been shown to translate into functionally improved blood flow with decreased interstitial pressure, greater oxygenation, and enhanced drug delivery [12–17]. Clinical evidence supporting tumor vessel normalization has also been documented, including colorectal cancer patients treated with bevacizumab [18], as well as GBM patients treated with cediranib, aVEGFR-2 tyrosine kinase inhibitor [19].

In the current study, we hypothesized that an aggressive chemotherapy regimen incorporating agents with potentially complimentary mechanisms of anti-tumor activity, may effectively exploit a window of tumor vessel normalization induced by bevacizumab, and yield greater anti-tumor benefit, particularly with regard to OS, than has been reported with bevacizumab monotherapy or bevacizumab plus other agents. Specifically, we evaluated the DNA crosslinking cytotoxicity of carboplatin when added to irinotecan, a topoisomerase inhibitor, in combination with bevacizumab.

## Patients and methods

### Protocol objectives

Our primary objective was to evaluate anti-tumor activity, defined by PFS at 6 months (PFS-6) of carboplatin and irinotecan combined with bevacizumab among adults with recurrent GBM. In addition, we sought to evaluate the safety of this regimen in this patient population.

### Patient eligibility

Patients were required to have histologic confirmation of WHO grade IV malignant glioma (GBM or gliosarcoma) that progressed after prior radiation and temozolomide therapy. Patients with prior low-grade glioma were eligible if histologic transformation to grade IV malignant glioma was confirmed. Eligible patients were also: at least 18 years of age; had a KPS ≥70; were on a stable corticosteroid dose for at least 1 week; and had no more than three prior episodes of tumor progression. Additional enrollment criteria included: hematocrit >29%; absolute neutrophil count > 1,000 cells/μl; platelet count > 100,000 cells/μl; and serum creatinine, aspartate aminotransferase and bilirubin within 1.5 times the institutional upper limit of normal. At least 4 weeks between surgical resection or chemotherapy, and at least 12 weeks between radiotherapy and enrollment were required. All patients provided informed consent.

Patients were excluded for: prior bevacizumab therapy; progressive disease or grade ≥3 toxicity on prior carboplatin or irinotecan; uncontrolled hypertension; therapeutic anti-coagulation use; acute hemorrhage on baseline MRI; urine protein:creatinine ratio >1; pregnancy or nursing; active infection requiring intravenous antibiotics; therapeutic anti-coagulation with warfarin; and prior stereotactic radiosurgery, radiation implants, or radiolabeled MAb therapy unless there was unequivocal disease progression (such as a new lesion or biopsy-proven recurrence).

### Treatment design

Eligible patients for this open-label phase II study received bevacizumab at 10 mg/kg intravenously every 14 days. Carboplatin was administered at an AUC of 4 on day one of each 28-day cycle. Irinotecan was administered on days 1 and 14 at 340 mg/m$^2$ for patients receiving cytochrome P450 CYP3A enzyme-inducing anti-epileptics (EIAEDs; phenytoin, phenobarbital, carbamazepine, oxcarbazepine, and primidone) and at 125 mg/m$^2$ for those not on EIAEDs. Study therapy continued until progressive disease, unacceptable toxicity, non-compliance with study protocol guidelines or withdrawal of consent.

### Response evaluation

Study investigators determined response by neurologic examination and contrast-enhanced MRI after the first two treatment cycles and then prior to every other cycle based on the recently published response assessment in neuro-oncology criteria [20]. A complete response (CR) required disappearance of all enhancing and non-enhancing tumor on consecutive MRIs at least 4 weeks apart, with corticosteroid discontinuation and neurologic stability or improvement. A partial response (PR) required ≥50% reduction in size (product of largest perpendicular diameters) of enhancing tumor with stability or improvement of neurologic status and corticosteroids. Complete and PRs also required stable or improved signal abnormality on fluid-attenuated inversion recovery (FLAIR) sequences. Progressive disease (PD) included ≥25% increase of enhancing tumor, a new enhancing lesion, significant worsening of non-enhancing tumor including that detected by FLAIR or T2 sequences, or clinical decline. Stable disease (SD) was defined as any assessment not meeting CR, PR, or PD criteria.

### Dose modification and retreatment criteria

Chemotherapy doses were held for grade 3 or 4, related, non-hematologic toxicity, grade 3 thrombocytopenia, grade 4 neutropenia, and fever and neutropenia (any grade) until the event resolved to grade 1 or pre-treatment baseline. Thereafter, chemotherapy doses were reduced by 25%. Chemotherapy doses were also reduced by 25% for any related event that required >2 weeks to satisfy re-treatment criteria. Patients who required more than three chemotherapy dose reductions were allowed to remain on study and receive bevacizumab alone. Dose reductions of bevacizumab were not allowed. Bevacizumab was discontinued for uncontrollable hypertension, grade 2 or greater hemorrhage, arterial thrombosis, wound dehiscence requiring surgical intervention, intestinal perforation, or grade 4 venous thrombosis, proteinuria or congestive heart failure. Bevacizumab was held until other related grade 3 events resolved to grade ≤1.

Initiation of each cycle required: an ANC ≥ 1,000/mm$^3$; a platelet count ≥ 100,000/mm$^3$; creatinine ≤1.5 times the upper limit of normal (ULN×), bilirubin ≤2 × ULN and AST ≤ 2.5 × ULN; proteinuria <3+ on urinalysis or urine protein: creatinine ratio ≤1.0; and resolution of any related grade ≥3 event to grade ≤1.

### Statistical consideration

The study was designed to have adequate power to compare the efficacy of the study regimen to a historical benchmark. The basis for this efficacy assessment was the proportion of patients surviving progression-free for 6-months. Given a 6-month progression-free survival rate of 46% (95% confidence interval [CI]: 32, and 66%) among patients with recurrent GBM treated with bevacizumab and irinotecan [21], a sample size of forty recurrent GBM patients was chosen to allow 90% power to differentiate between PFS-6 rates of 32 and 66% with a type I error of 0.1. If the true 6-month PFS of bevacizumab, irinotecan, and carboplatin were 46% or less, there would be limited interest in developing

this combination further. However, if the true 6-month PFS were 66% or more, there would definitely be interest in further investigation of this treatment regimen. Therefore, within this patient subgroup, the study will be used to differentiate between a 46 and 66% rate of 6-month PFS. Statistically, the hypothesis that will be tested is:

$$H_0: P < 0.46 \text{ vs. } H_1: P > 0.66$$

where $P$ is the proportion of patients who live six or more months without disease progression.

Forty (40) patients were planned to enroll in this single-stage study. If 23 or more of these 40 patients live six or more months without disease progression, the treatment regimen will be considered worthy of further investigation. Otherwise, the treatment regimen will be determined not worthy of further investigation within this patient population. The type I and II error rates associated with this testing are 0.097 and 0.098, respectively.

An interim efficacy analysis after 20 patients were accrued was planned a priori. If 15 or more of these 20 patients progressed or died within 2 months of study initiation, further accrual would be suspended.

For this study among heavily pretreated patients with an extremely poor prognosis, rates of unacceptable toxicity, defined as grade ≥2 CNS hemorrhage or grade 4 or 5 non-hematologic toxicity, of 15% or less were considered desirable, while rates of 40% or greater were considered undesirable. Stopping rules for unacceptable toxicity based upon boundaries proposed by Pocock were used to monitor this study after each group of four patients [22]. Accrual was not suspended to formally assess the toxicity profile unless the following thresholds of unacceptable toxicity were satisfied: ≥ 3/4; ≥ 4/8; ≥ 5/12; ≥ 6/16; ≥ 7/20; ≥ 7/24. The type I and II errors associated with this monitoring were 0.01 and 0.03, respectively. These guidelines did not adjust for differential length of follow-up of accrued patients.

Progression-free survival was defined as the time between the cycle one start date and the date of disease progression or death. PFS was censored at the time of last follow-up if the patient remained alive without disease progression, or at the start of non-study treatment if initiated before disease progression. OS was calculated from the start of therapy until death or last contact if censored. PFS and OS were summarized using Kaplan–Meier estimator including 95% CIs.

## Results

### Patient characteristics

Characteristics of the 40 patients who enrolled on this study between September 2009 and March 2011 are summarized in Table 1. Patients were relatively young (median age, 51 years) and 40% had a KPS of at least 90. All patients had progressed after standard therapy with radiation and temozolomide chemotherapy. The median time from original diagnosis to study enrollment was 11.4 months, and 27 (68%) enrolled at first recurrence.

As of June 1, 2011, five patients (13%) continue to receive study therapy. Three patients (8%) completed planned therapy. Study therapy was discontinued due to PD in 18 patients (45%), toxicity in 11 patients (28%) and withdrawal of consent in three patients (8%). Among patients who discontinued study therapy, four (10%) remain free of progression. Sixteen patients (40%) remain alive while 24 patients (60%) have died.

### Study drug administration and safety

A total of 189 cycles of therapy were administered including a median of 4.7 cycles (range, 1–11) per patient. All patients were assessable for toxicity. Table 2 summarizes the frequency of grade ≥2 adverse events that were at least possibly related to the study regimen per patient. Although most adverse events were grade 2, grade ≥3 events were common and were predominantly hematologic including grade 3 anemia, thrombocytopenia and neutropenia in 3 (8), 8 (20), and 10 (25%) patients. Additional grade 3 events that occurred in three or more patients included fatigue ($n$ = 5, 13%), infection ($n$ = 4, 10%) and nausea ($n$ = 3, 8%). Grade 4 events were primarily hematologic as well, and included neutropenia in eight patients (20%) and thrombocytopenia in four patients (10%). In addition, single patients (3%) developed grade 4 gastrointestinal perforation and venous thrombosis, respectively. Eleven patients (28%) discontinued study therapy due to toxicity and 17 patients (43%) required dose modification. There was one study related death attributed to an intestinal perforation on day 19 of cycle 1. Although this condition stabilized with medical management, this patient developed acute neurologic decline and the family opted to withdraw further anti-tumor therapy and enrolled the patient in hospice.

### Outcome

The median follow-up for all patients was 15.4 months (95% CI: 7.5, 16.8), while the median follow-up for surviving patients is 7.1 months (95% CI: 2.2, 17.4). Outcome analysis was based on the intent-to-treat population and includes the patient who died in cycle one described above. The PFS-6 rate was 46.5% (95% CI: 30.4, 61.0%) and the median OS was 8.3 months (95% CI: 5.9, 10.7 months) (Table 3; Fig. 1). Best radiographic response was PR in 13 patients (33%) (Fig. 2), SD in 21 patients (53%), and PD in five (13%) while the patient who died of toxicity during cycle one was deemed non-evaluable. Among 20 who were on dexamethasone at study initiation, 13 (65%) were able to taper, including eight patients (40%) who were able to completely discontinue dexamethasone. Five patients (25%) required a stable dexamethasone dose and two patients (10%), who had progression at first evaluation, required an increase in dexamethasone. Among patients who progressed after study therapy and underwent MRI evaluation at the time of progression, only one patient had PD at a new, non-contiguous site. All other patients had local evidence of disease progression. Although most patients had evidence of worsened enhancement as well as FLAIR signal abnormality at progression, five patients had progressive FLAIR changes without increased FLAIR at the time of tumor progression (Fig. 3).

## Discussion

A number of chemotherapeutic and molecularly targeted agents have been evaluated with bevacizumab for recurrent GBM and all have achieved outcomes that are no better than bevacizumab monotherapy (Table 3) [3, 21, 23–28]. Although one may argue that these data suggest chemo synergy with bevacizumab may not be possible in recurrent GBM, an alternative explanation is that the anti-tumor activity of agents evaluated in combination with bevacizumab to date is simply insufficient to enhance the benefit of bevacizumab. Of note, for all FDA-approved indications other than GBM, bevacizumab is administered in combination with either a cytotoxic or cytostatic agent, based on improved efficacy compared to bevacizumab monotherapy [29–32]. We conducted the current study to evaluate an aggressive multi-agent chemotherapy regimen in combination with bevacizumab for recurrent GBM [3, 4]. Although carboplatin and irinotecan exhibit modest anti-tumor activity when administered separately among recurrent malignant glioma patients [33–38], we reasoned that their ability to impair DNA replication via potentially complementary mechanisms including the introduction of alkyl groups by carboplatin and the inhibition of topoisomerase 1 activity by irinotecan, may lead to greater anti-tumor activity when

administered in combination. Such enhanced activity has been demonstrated in preclinical GBM models [39–42]. Furthermore, based on data supporting tumor vasculature normalization associated with anti-VEGF therapy [14, 43], we hypothesized that bevacizumab may enhance delivery of these two cytotoxins, thereby further augmenting their anti-tumor activity. We predicted adequate safety with this regimen based on the non-overlapping primary toxicities of each of the agents, although the agents do share secondary toxicities. Furthermore, we did not observe unexpected toxicity with this combination among bevacizumab-naïve, recurrent malignant glioma patients treated off study at our institution as a "best clinical management" strategy, as well as among a cohort of bevacizumab-resistant patients treated on a formal phase II study [44].

Nonetheless, the results of the current study failed to demonstrate an improvement in outcome compared to the historical precedent set by single-agent bevacizumab. Several factors may have contributed to the disappointing outcome observed on our study. First, it appears that in the clinical setting of GBM that has progressed after radiotherapy and temozolomide, the chemotherapy regimen employed in the current study was insufficiently active, despite its incorporation of potentially complementary mechanisms of anti-tumor activity. Second, bevacizumab may have failed to adequately normalize tumor vasculature and therefore did not enhance delivery and associated cytotoxicity of the chemotherapy regimen. A missed opportunity of the current study is that it did not incorporate imaging assessments to determine the impact of bevacizumab on tumor blood flow and as a correlate, potential chemotherapy delivery. Specifically we did not assess whether bevacizumab was able to effectively normalize tumor vasculature. Third, it is possible that bevacizumab may actually diminish tumor blood vessel permeability to a sufficient degree to limit intratumoral chemotherapy exposure as has been demonstrated in some GBM models [45]. The rapid and marked decrease in tumor contrast uptake typically observed after bevacizumab therapy attests to its potent anti-permeability action. Fourth, potent anti-angiogenic therapy such as bevacizumab may select for resistance mechanisms within a subpopulation of the tumor that enhance the tumor's intrinsic aggressiveness. One example of such an adaptation is the increased ability of tumor cells to invade and infiltrate widely [46–52]. Preclinical studies in orthotopic GBM models that accurately reflect the behavior of GBM in patients are critically needed to evaluate the specific mechanism of action of bevacizumab and to assess adaptive responses of the tumor to bevacizumab exposure.

An important aspect of response assessment associated with the current study was the incorporation of the RANO criteria which includes the assessment of non-enhancing as well as enhancing disease [20] rather than solely relying on measurement of enhancing disease as has been utilized traditionally via the Macdonald criteria [53]. In fact 13% of the patients treated on our study had PD documented solely by increasing T2/FLAIR abnormality without increased contrast enhancement (Fig. 3). These patients would have not been assessed as progressive using the conventional Macdonald criteria, thus highlighting the importance of the RANO criteria for the assessment of response particularly among patients treated with anti-angiogenic agents.

Although the primary toxicities of irinotecan and carboplatin differ, we observed that irinotecan and carboplatin when combined with bevacizumab is associated with significant toxicity among recurrent GBM patients. The overlapping secondary toxicities of these agents coupled with the general debility of recurrent GBM patients likely contributed to these adverse events. Although most grade ≥3 toxicity was hematologic, a spectrum of significant non-hematologic toxicities was also observed including one death attributable to intestinal perforation. In addition, eleven patients (28%) discontinued study therapy due to toxicity and 17 patients (43%) required dose modification. Our study findings are analogous to those reported for the addition of irinotecan to bevacizumab in a recently reported

randomized phase II study in which patients treated with combinatorial therapy had greater toxicity than those treated with single-agent bevacizumab [3].

Our study findings do not support further evaluation of irinotecan, carboplatin, and bevacizumab for bevacizumab naïve, recurrent GBM patients because this regimen appears to have similar anti-tumor activity as bevacizumab monotherapy, but significantly greater toxicity. Future studies should incorporate imaging and circulating biomarkers to better delineate the specific mechanism of action associated with bevacizumab therapy as well as the adaptive responses by the tumor and associated microenvironment that evolve with therapeutic resistance.

## Acknowledgments

This study was supported by NIH Grants 5P50-NS-20023 and 5 R37 CA11898; and a grant from Genentech Pharmaceuticals. The investigators wish to thank Wendy Gentry for her assistance in the preparation of this manuscript.

## Abbreviations

| | |
|---|---|
| **MG** | Malignant glioma |
| **ITT** | Intent-to treat |
| **ANC** | Absolute neutrophil count |
| **AST** | Aspartate aminotransferase |
| **CNS** | Central nervous system |
| **CR** | Complete response |
| **GBM** | Glioblastoma |
| **KPS** | Karnofsky performance status |
| **ORR** | Overall response rate |
| **OS** | Overall survival |
| **PD** | Progressive disease |
| **PFS** | Progression-free survival |
| **PR** | Partial response |
| **SD** | Stable disease |
| **VEGF** | Vascular endothelial growth factor |

## References

1. Cohen MH, Shen YL, Keegan P, Pazdur R. FDA drug approval summary: bevacizumab (Avastin) as treatment of recurrent glioblastoma multiforme. Oncologist. 2009; 14:1131–1138. [PubMed: 19897538]
2. Wick W, Weller M, van den Bent M, Stupp R. Bevacizumab and recurrent malignant gliomas: a European perspective. J Clin Oncol. 2010; 28:e188–e189. author reply e190–182. [PubMed: 20159801]
3. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, Yung WK, Paleologos N, Nicholas MK, Jensen R, Vredenburgh J, Huang J, Zheng M, Cloughesy T. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol. 2009; 27:4733–4740. [PubMed: 19720927]

4. Kreisl TN, Kim L, Moore K, Duic P, Royce C, Stroud I, Garren N, Mackey M, Butman JA, Camphausen K, Park J, Albert PS, Fine HA. Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J Clin Oncol. 2009; 27:740–745. [PubMed: 19114704]
5. Lamborn KR, Chang SM, Prados MD. Prognostic factors for survival of patients with glioblastoma: recursive partitioning analysis. Neuro-oncol. 2004; 6:227–235. [PubMed: 15279715]
6. Ballman KV, Buckner JC, Brown PD, Giannini C, Flynn PJ, LaPlant BR, Jaeckle KA. The relationship between six-month progression-free survival and 12-month overall survival end points for phase II trials in patients with glioblastoma multiforme. Neuro-oncology. 2007; 9:29–38. [PubMed: 17108063]
7. Wu W, Lamborn KR, Buckner JC, Novotny PJ, Chang SM, O'Fallon JR, Jaeckle KA, Prados MD. Joint NCCTG and NABTC prognostic factors analysis for high-grade recurrent glioma. Neuro-oncology. 2010; 12:164–172. [PubMed: 20150383]
8. 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; 359:845–848. [PubMed: 1279432]
9. Millauer B, Shawver LK, Plate KH, Risau W, Ullrich A. Glioblastoma growth inhibited in vivo by a dominant-negative Flk-1 mutant. Nature. 1994; 367:576–579. [PubMed: 8107827]
10. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000; 407:249–257. [PubMed: 11001068]
11. Dvorak HF. Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy. J Clin Oncol. 2002; 20:4368–4380. [PubMed: 12409337]
12. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005; 307:58–62. [PubMed: 15637262]
13. Winkler F, Kozin SV, Tong RT, Chae SS, Booth MF, Garkavtsev I, Xu L, Hicklin DJ, Fukumura D, di Tomaso E, Munn LL, Jain RK. Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell. 2004; 6:553–563. [PubMed: 15607960]
14. 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; 64:3731–3736. [PubMed: 15172975]
15. Lee CG, Heijn M, di Tomaso E, Griffon-Etienne G, Ancukiewicz M, Koike C, Park KR, Ferrara N, Jain RK, Suit HD, Boucher Y. Anti-Vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. Cancer Res. 2000; 60:5565–5570. [PubMed: 11034104]
16. Inai T, Mancuso M, Hashizume H, Baffert F, Haskell A, Baluk P, Hu-Lowe DD, Shalinsky DR, Thurston G, Yancopoulos GD, McDonald DM. Inhibition of vascular endothelial growth factor (VEGF) signaling in cancer causes loss of endothelial fenestrations, regression of tumor vessels, and appearance of basement membrane ghosts. Am J Pathol. 2004; 165:35–52. [PubMed: 15215160]
17. Wildiers H, Guetens G, De Boeck G, Verbeken E, Landuyt B, Landuyt W, de Bruijn EA, van Oosterom AT. Effect of antivascular endothelial growth factor treatment on the intratumoral uptake of CPT-11. Br J Cancer. 2003; 88:1979–1986. [PubMed: 12799646]
18. Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, Chung DC, Sahani DV, Kalva SP, Kozin SV, Mino M, Cohen KS, Scadden DT, Hartford AC, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Chen HX, Shellito PC, Lauwers GY, Jain RK. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med. 2004; 10:145–147. [PubMed: 14745444]
19. Batchelor TT, Sorensen AG, di Tomaso E, Zhang WT, Duda DG, Cohen KS, Kozak KR, Cahill DP, Chen PJ, Zhu M, Ancukiewicz M, Mrugala MM, Plotkin S, Drappatz J, Louis DN, Ivy P, Scadden DT, Benner T, Loeffler JS, Wen PY, Jain RK. AZD2171, a Pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell. 2007; 11:83–95. [PubMed: 17222792]

20. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, Degroot J, Wick W, Gilbert MR, Lassman AB, Tsien C, Mikkelsen T, Wong ET, Chamberlain MC, Stupp R, Lamborn KR, Vogelbaum MA, van den Bent MJ, Chang SM. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J Clin Oncol. 2010; 28:1963–1972. [PubMed: 20231676]
21. Vredenburgh JJ, Desjardins A, Herndon JE 2nd, Marcello J, Reardon DA, Quinn JA, Rich JN, Sathornsumetee S, Gururangan S, Sampson J, Wagner M, Bailey L, Bigner DD, Friedman AH, Friedman HS. Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol. 2007; 25:4722–4729. [PubMed: 17947719]
22. Pocock SJ. Interim analyses for randomized clinical trials: the group sequential approach. Biometrics. 1982; 38:153–162. [PubMed: 7082757]
23. Vredenburgh JJ, Desjardins A, Herndon JE 2nd, Dowell JM, Reardon DA, Quinn JA, Rich JN, Sathornsumetee S, Gururangan S, Wagner M, Bigner DD, Friedman AH, Friedman HS. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. Clin Cancer Res. 2007; 13:1253–1259. [PubMed: 17317837]
24. Raizer JJ, Grimm S, Chamberlain MC, Nicholas MK, Chandler JP, Muro K, Dubner S, Rademaker AW, Renfrow J, Bredel M. A phase 2 trial of single-agent bevacizumab given in an every-3-week schedule for patients with recurrent high-grade gliomas. Cancer. 2010; 116:5297–5305. [PubMed: 20665891]
25. Reardon DA, Desjardins A, Vredenburgh JJ, Gururangan S, Sampson JH, Sathornsumetee S, McLendon RE, Herndon JE 2nd, Marcello JE, Norfleet J, Friedman AH, Bigner DD, Friedman HS. Metronomic chemotherapy with daily, oral etoposide plus bevacizumab for recurrent malignant glioma: a phase II study. Br J Cancer. 2009; 101:1986–1994. [PubMed: 19920819]
26. Hasselbalch B, Lassen U, Hansen S, Holmberg M, Sorensen M, Kosteljanetz M, Broholm H, Stockhausen MT, Poulsen HS. Cetuximab, bevacizumab, and irinotecan for patients with primary glioblastoma and progression after radiation therapy and temozolomide: a phase II trial. Neuro-oncology. 2010; 12:508–516. [PubMed: 20406901]
27. Gutin PH, Iwamoto FM, Beal K, Mohile NA, Karimi S, Hou BL, Lymberis S, Yamada Y, Chang J, Abrey LE. Safety and efficacy of bevacizumab with hypofractionated stereotactic irradiation for recurrent malignant gliomas. Int J Radiat Oncol Biol Phys. 2009; 75:156–163. [PubMed: 19167838]
28. Desjardins A, Reardon DA, Coan A, Marcello J, Herndon JE 2nd, Bailey L, Peters KB, Friedman HS, Vredenburgh JJ. Bevacizumab and daily temozolomide for recurrent glioblastoma. Cancer. 2011
29. Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, Steinberg SM, Chen HX, Rosenberg SA. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med. 2003; 349:427–434. [PubMed: 12890841]
30. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004; 350:2335–2342. [PubMed: 15175435]
31. Miller K, Wang M, Gralow J, Dickler M, Cobleigh M, Perez EA, Shenkier T, Cella D, Davidson NE. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N Engl J Med. 2007; 357:2666–2676. [PubMed: 18160686]
32. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, Lilenbaum R, Johnson DH. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N Engl J Med. 2006; 355:2542–2550. [PubMed: 17167137]
33. Friedman HS, Petros WP, Friedman AH, Schaaf LJ, Kerby T, Lawyer J, Parry M, Houghton PJ, Lovell S, Rasheed K, Cloughsey T, Stewart ES, Colvin OM, Provenzale JM, McLendon RE, Bigner DD, Cokgor I, Haglund M, Rich J, Ashley D, Malczyn J, Elfring GL, Miller LL. Irinotecan therapy in adults with recurrent or progressive malignant glioma. J Clin Oncol. 1999; 17:1516–1525. [PubMed: 10334539]
34. Prados MD, Warnick RE, Mack EE, Chandler KL, Rabbitt J, Page M, Malec M. Intravenous carboplatin for recurrent gliomas. A dose-escalating phase II trial. Am J Clin Oncol. 1996; 19:609–612. [PubMed: 8931682]

35. Yung WK, Mechtler L, Gleason MJ. Intravenous carboplatin for recurrent malignant glioma: a phase II study. J Clin Oncol. 1991; 9:860–864. [PubMed: 1849986]
36. Batchelor TT, Gilbert MR, Supko JG, Carson KA, Nabors LB, Grossman SA, Lesser GJ, Mikkelsen T, Phuphanich S. Phase 2 study of weekly irinotecan in adults with recurrent malignant glioma: final report of NABTT 97–11. Neuro-oncol. 2004; 6:21–27. [PubMed: 14769136]
37. Gilbert MR, Supko JG, Batchelor T, Lesser G, Fisher JD, Piantadosi S, Grossman S. Phase I clinical and pharmacokinetic study of irinotecan in adults with recurrent malignant glioma. Clin Cancer Res. 2003; 9:2940–2949. [PubMed: 12912940]
38. Santisteban M, Buckner JC, Reid JM, Wu W, Scheithauer BW, Ames MM, Felten SJ, Nikcevich DA, Wiesenfeld M, Jaeckle KA, Galanis E. Phase II trial of two different irinotecan schedules with pharmacokinetic analysis in patients with recurrent glioma: north central cancer treatment group results. J Neurooncol. 2009; 92(2):165–175. [PubMed: 19066728]
39. Coggins CA, Elion GB, Houghton PJ, Hare CB, Keir S, Colvin OM, Bigner DD, Friedman HS. Enhancement of irinotecan (CPT-11) activity against central nervous system tumor xenografts by alkylating agents. Cancer Chemother Pharmacol. 1998; 41:485–490. [PubMed: 9554593]
40. Patel VJ, Elion GB, Houghton PJ, Keir S, Pegg AE, Johnson SP, Dolan ME, Bigner DD, Friedman HS. Schedule-dependent activity of temozolomide plus CPT-11 against a human central nervous system tumor-derived xenograft. Clin Cancer Res. 2000; 6:4154–4157. [PubMed: 11051270]
41. Houghton PJ, Stewart CF, Cheshire PJ, Richmond LB, Kirstein MN, Poquette CA, Tan M, Friedman HS, Brent TP. Antitumor activity of temozolomide combined with irinotecan is partly independent of O6-methylguanine-DNA methyltransferase and mismatch repair phenotypes in xenograft models. Clin Cancer Res. 2000; 6:4110–4118. [PubMed: 11051264]
42. Castellino RC, Elion GB, Keir ST, Houghton PJ, Johnson SP, Bigner DD, Friedman HS. Schedule-dependent activity of irinotecan plus BCNU against malignant glioma xenografts. Cancer Chemother Pharmacol. 2000; 45:345–349. [PubMed: 10755324]
43. Jain RK, Tong RT, Munn LL. Effect of vascular normalization by antiangiogenic therapy on interstitial hypertension, peritumor edema, and lymphatic metastasis: insights from a mathematical model. Cancer Res. 2007; 67:2729–2735. [PubMed: 17363594]
44. Reardon DA, Desjardins A, Peters KB, Vredenburgh JJ, Gururangan S, Sampson JH, McLendon RE, Herndon IJE, Coan A, Threatt S, Friedman AH, Friedman HS. Phase II study of carboplatin, irinotecan and bevacizumab for recurrent glioblastoma after progression on bevacizumab therapy. Cancer. 2011 (in press).
45. Claes A, Wesseling P, Jeuken J, Maass C, Heerschap A, Leenders WP. Antiangiogenic compounds interfere with chemotherapy of brain tumors due to vessel normalization. Mol Cancer Ther. 2008; 7:71–78. [PubMed: 18187807]
46. Lamszus K, Kunkel P, Westphal M. Invasion as limitation to anti-angiogenic glioma therapy. Acta Neurochir Suppl. 2003; 88:169–177. [PubMed: 14531575]
47. Rubenstein JL, Kim J, Ozawa T, Zhang M, Westphal M, Deen DF, Shuman MA. Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. Neoplasia. 2000; 2:306–314. [PubMed: 11005565]
48. Ebos JM, Lee CR, Cruz-Munoz W, Bjarnason GA, Christensen JG, Kerbel RS. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. Cancer Cell. 2009; 15:232–239. [PubMed: 19249681]
49. Paez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Vinals F, Inoue M, Bergers G, Hanahan D, Casanovas O. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer Cell. 2009; 15:220–231. [PubMed: 19249680]
50. de Groot JF, Fuller G, Kumar AJ, Piao Y, Eterovic K, Ji Y, Conrad CA. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. Neuro-oncology. 2010; 12:233–242. [PubMed: 20167811]
51. Iwamoto FM, Abrey LE, Beal K, Gutin PH, Rosenblum MK, Reuter VE, DeAngelis LM, Lassman AB. Patterns of relapse and prognosis after bevacizumab failure in recurrent glioblastoma. Neurology. 2009; 73:1200–1206. [PubMed: 19822869]
52. Keunen O, Johansson M, Oudin A, Sanzey M, Rahim SA, Fack F, Thorsen F, Taxt T, Bartos M, Jirik R, Miletic H, Wang J, Stieber D, Stuhr L, Moen I, Rygh CB, Bjerkvig R, Niclou SP. Anti-

VEGF treatment reduces blood supply and increases tumor cell invasion in glioblastoma. Proc Natl Acad Sci U S A. 2011; 108:3749–3754. [PubMed: 21321221]

53. Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol. 1990; 8:1277–1280. [PubMed: 2358840]

54. Sathornsumetee S, Desjardins A, Vredenburgh JJ, McLendon RE, Marcello J, Herndon JE, Mathe A, Hamilton M, Rich JN, Norfleet JA, Gururangan S, Friedman HS, Reardon DA. Phase II trial of bevacizumab and erlotinib in patients with recurrent malignant glioma. Neuro Oncol. 2010; 12(12):1300–1310. [PubMed: 20716591]

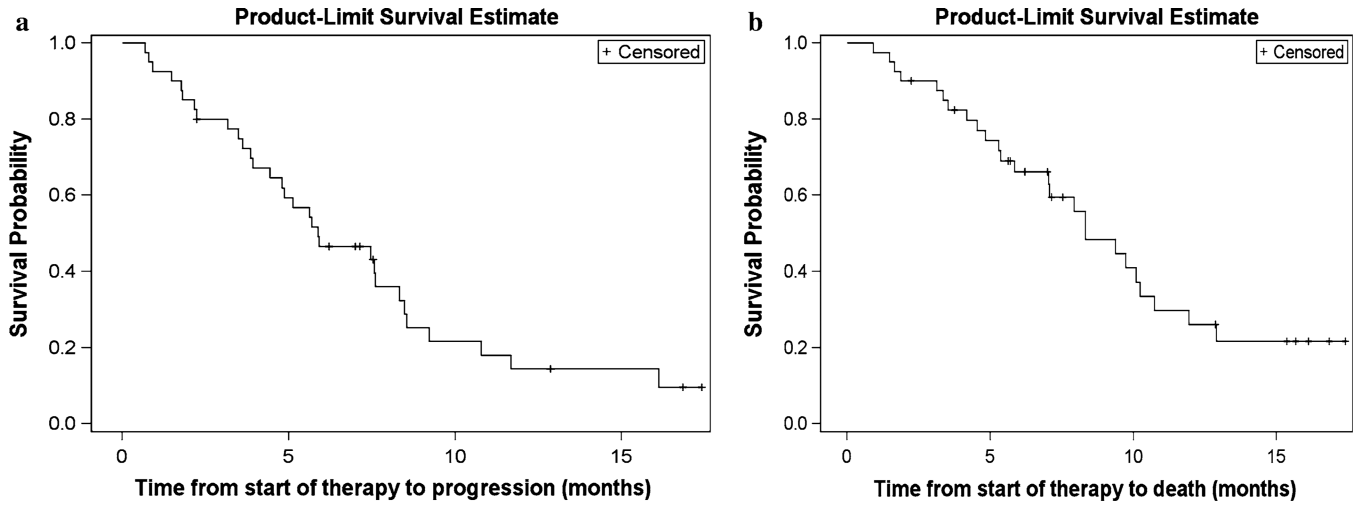


**Fig. 1.**

Kaplan–Meier estimate of time to progression (**a**) and overall survival (**b**) for patients treated on the current study

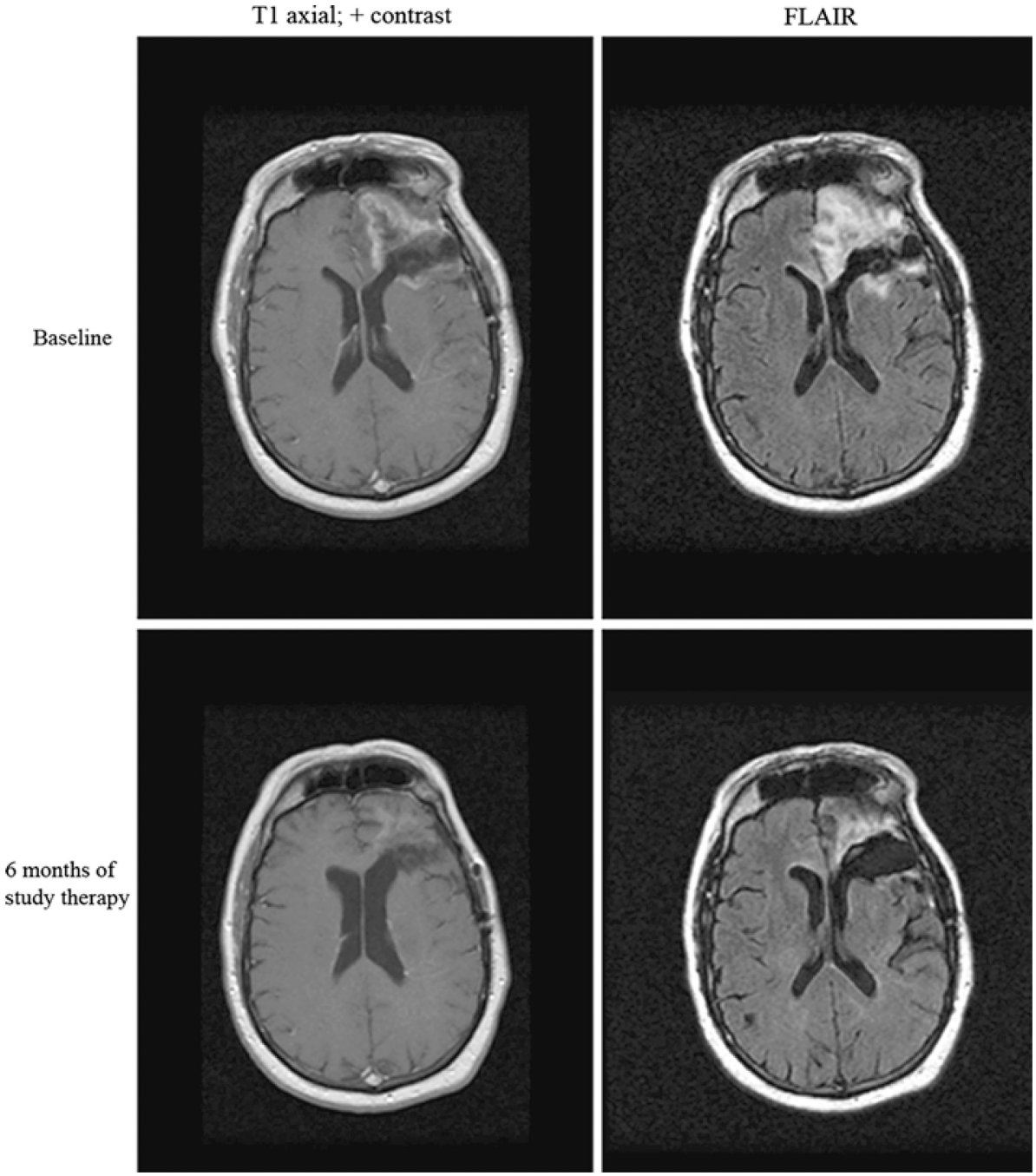


**Fig. 2.**
Representative partial radiographic response of a recurrent GBM patient after six cycles of treatment with bevacizumab, irinotecan, and carboplatin that includes both diminished contrast uptake as well as FLAIR signal abnormality

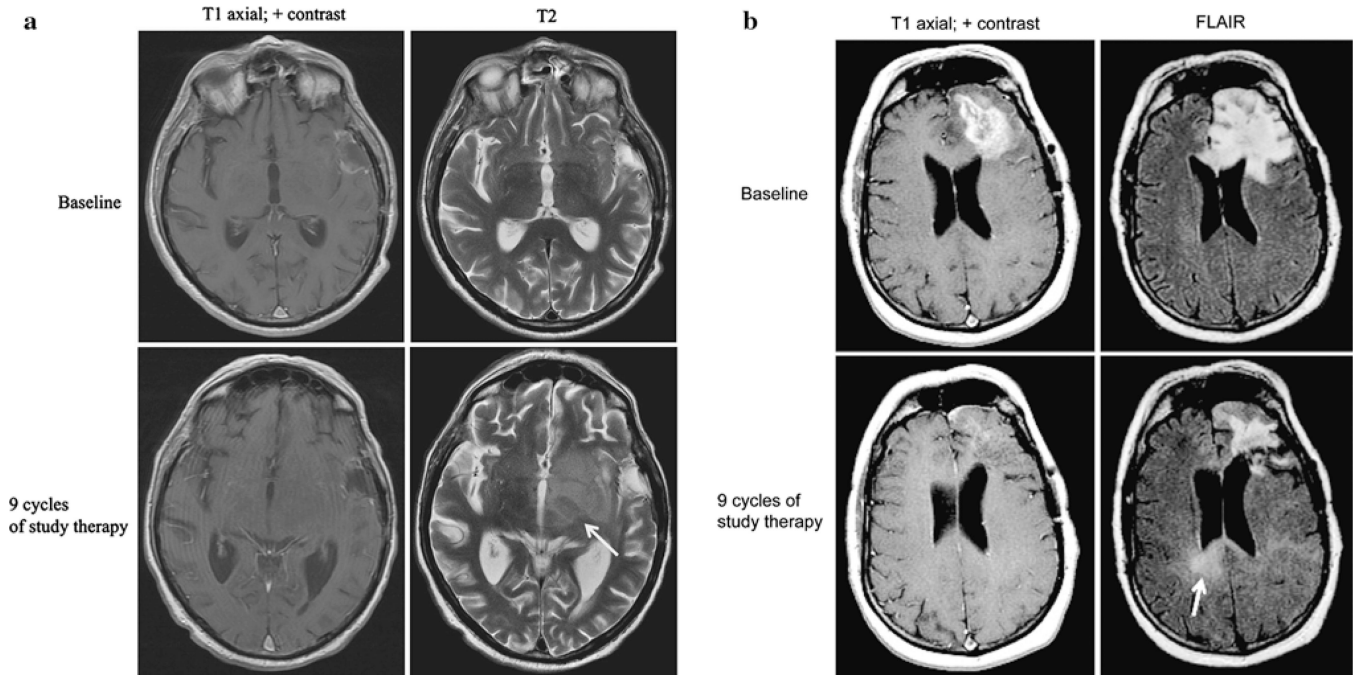


**Fig. 3.**

Representative examples of patients with PD documented by increased T2/FLAIR signal abnormality. In both cases, the contrast-enhancing disease continued to improve. In **a**, the T2 signal abnormality increased significantly medially (*arrow*) and in **b**, new FLAIR signal abnormality developed in the contralateral hemisphere (*arrow*)

**Table 1**

Patient Characteristic

| | |
|---|---|
| Age (years) | |
| Median | 51.0 |
| Range | 25.2–72.0 |
| Gender (%) | |
| Male | 30 (75) |
| Female | 10 (25) |
| KPS | |
| 90–100 | 16 (40) |
| 80 | 20 (50) |
| 70 | 4 (10) |
| EIAED | |
| Yes | 5 (13) |
| No | 35 (87) |
| Time from original diagnosis | |
| Median (months) | 11.4 |
| Range | 3.2–80.1 |
| Surgery prior to enrollment | |
| Biopsy | 3 (8) |
| STR | 2 (5) |
| GTR | 1 (3) |
| None | 34 (85) |
| Prior treatment | |
| XRT | 40 (100) |
| Prior chemotherapy regimens | |
| 1 | 24 (60) |
| 2 | 12 (30) |
| 3 | 4 (10) |
| Number prior PD | |
| 1 | 27 (68) |
| 2 | 11 (28) |
| 3 | 2 (5) |

**Table 2**

Number of patients with adverse events (number in parentheses equals % of study population)

| Toxicity | Grade 2 | Grade 3 | Grade 4 | Grade 5 |
|---|---|---|---|---|
| Anemia | 5 (13) | 3 (8) | | |
| Anorexia | 2 (5) | | | |
| Diarrhea | 3 (8) | 2 (5) | | |
| Dyspnea | | | | |
| Edema | | | | |
| Fatigue | 13 (33) | 5 (13) | | |
| GI perforation/fistula | | 1 (3) | 1 (3) | 1 (3) |
| Hemorrhage (GI) | | 1 (3) | | |
| Hypertension | 2 (5) | | | |
| Hypocalcemia | 1 (3) | | | |
| Hyponatremia | | 2 (5) | | |
| Hypokalemia | | 1 (3) | | |
| Hypophosphatemia | | 1 (3) | | |
| Infection | 5 (13) | 4 (10) | | |
| Microangiopathic hemolytic anemia | | 1 (3) | | |
| Mucositis | 1 (3) | | | |
| Nausea | 4 (10) | 3 (8) | | |
| Neutropenia | 6 (15) | 10 (25) | 8 (20) | |
| Proteinuria | 1 (3) | 2 (5) | | |
| Rash | | | | |
| Skin/wound breakdown | 1 (3) | 1 (3) | | |
| Thrombocytopenia | 2 (5) | 8 (20) | 4 (10) | |
| Thrombosis | | 1 (3) | 1 (3) | |
| Transaminase elevation | 1 (3) | 2 (5) | | |

**Table 3**

Summary of outcome in the current study relative to previously reported prospective studies of bevacizumab therapy among bevacizumab-naïve, recurrent GBM patients

| Study design | Regimen | Number of patients | Median PFS, months (95% CI) | PFS-6, % (95% CI) | Median OS, months (95% CI) | Number of patients discontinued therapy due to toxicity (%) | Citation |
|---|---|---|---|---|---|---|---|
| Single arm | BV + carboplatin + CPT-11 | 40 | 5.9 (4.4, 8.3) | 46.5 (30.4, 61.0) | 8.3 (5.9, 10.7) | 11 (28) | Current study |
| Randomized | BV | 85 | 4.2 (2.9, 5.8) | 42.6 (97.5% CI: 29.6, 55.5) | 9.2 (8.2, 10.7) | 4 (5) | Friedman [3] |
| Randomized | BV + CPT-11 | 82 | 5.6 (4.4, 6.2) | 50.3 (97.5% CI: 36.8, 63.9) | 8.7 (7.8, 10.9) | 14 (18) | Friedman [3] |
| Single arm | BV | 48 | 4.0 (3.0, 6.5) | 29 (18, 48) | 7.8 (5.3, 13.5) | 6 (13) | Kreisl [4] |
| Single arm | BV + CPT-11 | 35 | NR | 46 (32, 66) | 10.5 (8.8, 15) | 11 (31) | Vredenburgh [21] |
| Single arm | BV + erlotinib | 25 | 4.5 (3.0, 6.0) | 29.2 (13.0, 47.6) | 11.2 (7.1, 17.2) | 8 (14) | Sathornsumetee [54] |
| Single arm | BV + VP-16 | 27 | 4.5 (3, 10) | 44 (26, 62) | 12 (6, 18) | 3 (11) | Reardon [25] |
| Single arm | BV + SRS | 20 | 7.3 (4.4, 8.9) | 65.0 (40.0–82.0) | 12.5 (6.9, 22.8) | 3 (12) | Gutin [27] |
| Single arm | BV (3-week schedule) | 50 | 2.8 | 25 | 6.5 | 2 (3) | Raizer [24] |
| Single arm | BV + CPT-11 + cetuximab | 43 | 4.0 (3.3, 5.0) | 33 (19, 48) | 7.5 (5.8, 9.3) | 6 (14) | Hasselbalch [26] |
| Single arm | BV + daily TMZ | 32 | 4.0 | 18.8 (7.6, 33.7) | 9.3 | 2 (6) | Desjardins [28] |

*BV*, bevacizumab; *CI*, confidence interval; *CPT-11*, irinotecan; *GBM*, glioblastoma; *NR*, not reported; *OS*, overall survival; *PFS*, progression-free survival; *SRS*, stereotactic radiosurgery; *TMZ*, temozolomide; *VP-16*, etoposide