Int. J. Radiation Oncology Biol. Phys., Vol. ■, No. ■, pp. 1–9, 2008

0360-3016/08/$–see front matter

ELSEVIER

doi:10.1016/j.ijrobp.2007.11.068

CLINICAL INVESTIGATION

PHASE II PILOT STUDY OF BEVACIZUMAB IN COMBINATION WITH TEMOZOLOMIDE AND REGIONAL RADIATION THERAPY FOR UP-FRONT TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME: INTERIM ANALYSIS OF SAFETY AND TOLERABILITY

Albert Lai, M.D., Ph.D.,* Emese Filka,* Bruce McGibbon, M.D.,¶ Phioanh Leia Nghiemphu, M.D.,* Carrie Graham,* William H. Yong, M.D.,† Paul Mischel, M.D.,† Linda M. Liau, M.D., Ph.D.,‡ Marvin Bergsneider, M.D.,‡ Whitney Pope, M.D., Ph.D.,§ Michael Selch, M.D.,¶ and Tim Cloughesy, M.D.*

Departments of *Neurology, †Pathology, ‡Neurosurgery, §Radiology, and ¶Radiation Oncology, David Geffen School of Medicine at University of California–Los Angeles, Los Angeles, CA

Purpose: To assess interim safety and tolerability of a 10-patient, Phase II pilot study using bevacizumab (BV) in combination with temozolomide (TMZ) and regional radiation therapy (RT) in the up-front treatment of patients with newly diagnosed glioblastoma.

Methods and Materials: All patients received standard external beam regional RT of 60.0 Gy in 30 fractions started within 3 to 5 weeks after surgery. Concurrently TMZ was given daily at 75 mg/m^2 for 42 days during RT, and BV was given every 2 weeks at 10 mg/kg starting with the first day of RT/TMZ. After a 2-week interval upon completion of RT, the post-RT phase commenced with resumption of TMZ at 150 to 200 mg/m^2 for 5 days every 4 weeks and continuation of BV every 2 weeks.

Results: For these 10 patients, toxicities were compiled until study discontinuation or up to ~40 weeks from initial study treatment for those remaining on-study. In terms of serious immediate or delayed neurotoxicity, 1 patient developed presumed radiation-induced optic neuropathy. Among the toxicities that could be potentially treatment related, relatively high incidences of fatigue, myelotoxicity, wound breakdown, and deep venous thrombosis/pulmonary embolism were observed.

Conclusion: The observed toxicities were acceptable to continue enrollment toward the overall target group of 70 patients. Preliminary efficacy analysis shows encouraging mean progression-free survival. At this time data are not sufficient to encourage routine off-label use of BV combined with TMZ/RT in the setting of newly diagnosed glioblastoma without longer follow-up, enrollment of additional patients, and thorough efficacy assessment. © **2008 Elsevier Inc.**

Bevacizumab, Newly diagnosed glioblastoma, Temozolomide, Radiation, Antiangiogenesis.

INTRODUCTION

The incidence of primary malignant brain tumors in the United States (US) is about 17,000 per year, resulting in approximately 10,000 deaths per year (1). Glioblastoma (GBM) is the most frequent, accounting for ~40% of all primary malignant brain tumors, and is the most aggressive form of brain cancer. Despite optimal treatment with surgery, radiation therapy (RT) and chemotherapy, the prognosis for these patients remains poor. Recently a Phase III randomized trial comparing RT alone vs. combined TMZ and RT followed by six cycles of temozolomide (TMZ) showed that the addition of TMZ increased median survival from 12.1 months to 14.6 months, with a corresponding increase in 2-year survival rates from 10.4% to 26.5% (2). In this study the median progression-free survival was 6.9 months for the RT/TMZ arm. These results have established adjuvant TMZ/RT as the standard of care for newly diagnosed GBM, and serves as the backbone for evaluating other up-front treatment strategies.

In the past year, antiangiogenic therapy using bevacizumab (BV) in combination with irinotecan has emerged as a promising development in the treatment of recurrent GBM (3, 4). The substance BV is a humanized monoclonal

Reprint requests to: Albert Lai, M.D., Ph.D., UCLA Neuro-Oncology Program, Department of Neurology, David Geffen School of Medicine at UCLA, 710 Westwood Plaza, Suite 1-230 RNRC, Los Angeles, CA 90095. Tel: (310) 825-5321; Fax: (310) 825-0644; E-mail: albertlai@mednet.ucla.edu

Conflict of interest: Drs. Lai and Dr. Cloughesy have served on the Scientific Advisory Board of Genentech.

Acknowledgment—This study is an investigator-initiated trial sponsored by Genentech Bio-oncology.

Received Oct 4, 2007, and in revised form Nov 29, 2007. Accepted for publication Nov 30, 2007.

antibody directed against the vascular endothelial growth factor (VEGF). The apparent sensitivity of GBM to BV is consistent with the fact that GBMs are highly vascularized tumors that heavily use proangiogenic factors such as VEGF for new blood vessel formation. Accordingly, high levels of VEGF expression have been detected in glioma tissue and are associated with poor prognosis (5). In addition VEGF may have direct effects (autocrine) on tumor cells (6).

The development of BV as a treatment option for recurrent GBM has raised the possibility that up-front treatment of newly diagnosed GBM with BV may be more advantageous than withholding BV until recurrence. To investigate this hypothesis, our trial design combines BV with the standard-of-care backbone of RT/TMZ to determine whether BV in combination with RT/TMZ is safe and whether BV enhances the efficacy of RT/TMZ. A crucial feature of our trial design is that BV commences with the first dose of RT/TMZ (Fig. 1). This feature enables us to observe any clinically apparent synergistic benefits or toxicities of concurrent BV and RT. The potential for such synergistic benefits has been supported by the ability of antiangiogenic agents to normalize blood vessels, thereby reducing hypoxia via enhanced oxygenation, and to counteract effects of radiation-induced VEGF secretion from tumor cells (6–11). However evidence that radiation-induced VEGF production may play a neuroprotective role for normal irradiated brain tissue raises the possibility that the combination of BV and RT may have deleterious effects (12, 13).

Our Phase II trial included an initial pilot phase consisting of the first 10 patients enrolled out of a projected total of 70 patients. In addition to the expected problems related to BV, TMZ, or RT, we were particularly interested in discerning local effects or neurotoxicities such as wound breakdown, cerebral thromboembolic events, and cerebral microvessel ischemia. In this study we describe interim safety and tolerability observed for these 10 pilot patients.

METHODS AND MATERIALS

Patient eligibility

The protocol was approved by the University of California–Los Angeles institutional review board. Patients or their appointed surrogates signed the approved informed consent form. Patients ≥18 years of age with newly diagnosed, previously untreated GBM or gliosarcoma were eligible. Patients had not received any previous RT to the head or neck regions and had a Karnofsky performance status of ≥60. Therapy began between 3 and 5 weeks of final surgery. At the time of surgery, patients were required to have >200 mg of frozen tissue collected; thus essentially all biopsy patients were excluded unless a subsequent surgery was performed to obtain tissue. Patients had adequate hematologic, renal, and hepatic function. Patients did not have any serious intercurrent illnesses. No exclusions were made for sex, race/ethnicity, minority status, or economic status. Female patients were not pregnant or nursing, and all patients (both men and women) agreed to practice birth control during the study. Patients requiring full-dose anticoagulation were excluded. Patients with history of congestive heart failure (New York Heart Association Grade II), stroke within 6 months, myocardial infarction within 6 months, clinically significant peripheral vascular disease, or major surgical procedure within 28 days of initiating treatment were excluded.

Treatment plan

As indicated in Fig. 1, patients were treated with 10 mg/kg of BV administered intravenously every 2 weeks and 75 mg/m^2 of TMZ administered orally daily during RT. External-beam

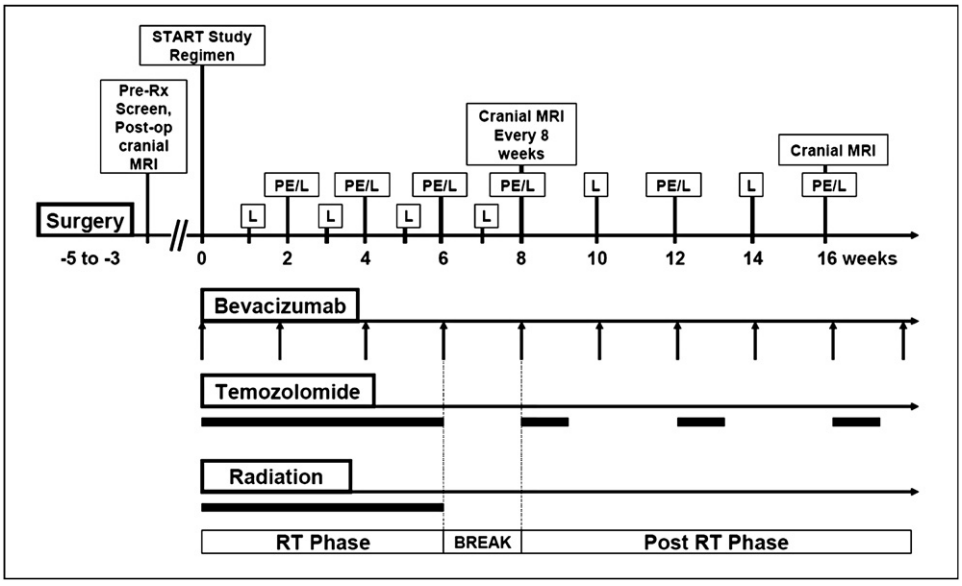

Fig. 1. Study schema. Bevacizumab (BV) is given intravenously at 10 mg/kg every 2 weeks beginning as early as 3 weeks after surgery and no later than 5 weeks. Temozolomide (TMZ) is given daily (75 mg/m^2) during radiation. After a break of 2 weeks, TMZ will be continued for up to 24 cycles given 5 days of 28 at a dose of 150 to 200 mg/m^2 on those days and BV will be continued until progression. If progression occurs after completion of 24 cycles of TMZ, BV will be continued as a single agent. Schedule of physical examination (PE), cranial magnetic resonance imaging (MRI) scans, and laboratory assessments (L) are indicated. Pre-Rx = pretreatment; Post-op = postoperative; RT = radiation therapy.

radiation was started within 3 to 5 weeks after surgery. Each patient received 30 fractions of 200 cGy per fraction, totaling 6,000 cGy. Treatments were given on weekdays with a linear accelerator using either 6-MV or 6/15-MV photons in conformal beam arrangements. A head-holding device and a custom-fit thermoplast mask were used in each case for CT simulation and daily treatments. Target volume was determined by preoperative MRI. If the lesion had surrounding edema, the initial target volume included the contrast-enhancing lesion plus edema plus 2.0 cm, and was treated to 4,600 cGy in 23 fractions. A boost of 1,400 cGy in seven fractions was then given to a cone-down volume of contrast-enhancing tumor plus 2.5 cm. If there was no significant edema, the target volume included contrast-enhancing tumor plus 2.5 cm and was treated to the full dose of 6,000 cGy. If possible (without shielding gross tumor), the following dose limits were applied: optic chiasm ≤6,000 cGy (ideally the dose was limited to ≤5,000 cGy), bilateral retinas ≤5,000 cGy, and brainstem ≤6,000 cGy. After completion of RT, BV was continued every 2 weeks. After a minimum of a 2-week break after the last daily TMZ dose (RT phase), patients were treated with BV every 2 weeks and with TMZ every 4 weeks at a dose of 150 to 200 mg/m^2/day for the first 5 days of every cycle until progression or for a maximum of 24 TMZ cycles. (For patients completing 24 cycles of TMZ, single-agent BV will be continued every 2 weeks until progression.) No dose modifications of BV were allowed. The TMZ doses were adjusted primarily based on hematologic toxicities.

Evaluations

Baseline evaluations were performed within 21 days before initiation of treatment. The evaluations included a complete history, physical examination, vital signs (including blood pressure), neurologic examination, cranial MRI with and without contrast, complete blood count, serum chemistries, and protein/creatinine ratio on spot urinalysis. During the RT phase, a complete blood count was performed weekly; serum chemistries and physical and neurologic examinations were performed every 2 weeks; and a protein/creatinine ratio on spot urinalysis was performed on Week 4 (Fig. 1). During post-RT phase, complete blood count, serum chemistries, and blood pressure were measured every 2 weeks, and physical and neurologic examinations were performed every 4 weeks. All patient evaluations include review of dexamethasone dosage and determination of KPS. No neurocognitive functional assessments were performed. Cranial MRI scans were performed after completion of RT and then every 8 weeks.

Assessment of response and toxicity

Response was evaluated by cranial MRI on a seven-point scale: complete resolution of tumor, 3; tumor definitely smaller, 2; tumor probably smaller, 1; tumor unchanged, 0; tumor probably worse, −1; tumor definitely worse, −2; new lesion, −3. This scale was expected to be advantageous in this study because many newly diagnosed patients may have had gross total resections and thus would not have evaluable disease. After gross total resection, the postoperative MRI is usually scored a +2 and occasionally a +3. After a gross total resection, appearance of new tumor at the local site would be given a −1 or −2, whereas a new tumor at a distant site would be given a −3. In the case of gross total resection, the best possible score is 0. Toxicity was graded using the National Cancer Institute Common Toxicity Criteria (version 3.0).

RESULTS

A total of 10 patients were enrolled in the pilot phase of this study between September 2006 and November 2006, and the study has advanced into the expansion phase (remaining 60 patients) beginning in February 2007. The characteristics of the pilot patients are shown in Table 1. Of the patients, 6 were female and 4 male, with an average age of 54.3 years (range, 42–67 years). The average KPS was 78 (range, 60–90). Interim toxicity assessments were compiled for the 10 patients up to ∼40 weeks on-study. Table 2 shows all Grade 3/4 toxicities observed during both the RT and post-RT phases for each patient. Table 3 shows the frequency of selected toxicities of all grades in the RT and post-RT phases. Table 4 lists treatment characteristics for each patient. At the time of writing, 4 patients remain on-study. Of the 6 patients taken off-study, 1 was taken off for progression, 3 patients were removed because of toxicity, and 2 patients voluntarily withdrew from the study.

Patient 1

This patient tolerated the RT phase without any serious toxicities. During the post-RT phase (Week 13 from initiation), the patient was hospitalized for presumed corticosteroid-induced hyperglycemia and hyponatremia (both Grade 3). At Week 18, the patient required hospitalization for proteinuria with a urinary protein/creatinine ratio of 3.2 (Grade 2) and evidence of congestive heart failure (Grade

Table 1. Characteristics of study patients

Patient no.	Age (y)	Sex	Diagnosis	Presurgery lesion	Extent of resection	Pretreatment KPS
1	42	F	GBM	Ring enhancing	Biopsy (<200 mg frozen tissue obtained)	70
2	48	F	GBM	Mostly NCET	GTR	60
3	52	F	GBM	Ring enhancing	GTR	90
4	55	M	GBM	Ring enhancing	GTR	90
5	53	F	GBM	Bulky enhancing, NCET	STR	80
6	60	F	GBM	Ring enhancing	GTR	90
7	67	M	GBM	Ring enhancing	Biopsy, GTR (tissue obtained)	90
8	46	F	GBM	Multi-focal, Ring enhancing	STR	80
9	66	M	GBM	Ring enhancing	GTR	70
10	54	M	GBM	Ring enhancing	STR	60

Abbreviations: F = female; GBM = glioblastoma; GTR = gross total resection; M = male; KPS = Karnofsky performance status; NCET = non–contrast enhancing tumor; STR = subtotal resection.

Table 2. Observed Grade 3 and 4 toxicities

	RT phase		Post RT phase	
Patient no.	Grade 3	Grade 4	Grade 3	Grade 4
1	0	0	Hyperglycemia, hyponatremia, fatigue, proteinuria, CHF	0
2	Thrombo	0	Fatigue, neutropenia, leukopenia, anemia, thrombo	Thrombo, leukopenia, neutropenia, optic neuropathy
3	0	0	0	0
4	0	0	Thrombo	0
5	Thrombo		ARF, DI, leukopenia, hypokalemia, thrombo, neutropenia, fatigue, wound breakdown	Neutropenia
6	High blood pressure		Focal seizures, DVT	PE
7	0	0	Generalized seizure	0
8	Wound breakdown, CSF leak	0	DVT	PE
9	0	0	Lethargy, encephalopathy	0
10	0	0	DVT	0

Abbreviations: ARF = acute renal failure; DI = diabetes insipidus; CHF = congestive heart failure; DVT = deep venous thrombosis; PE = pulmonary embolism; Thrombo = thrombocytopenia.

3). She voluntarily came off-study at this point. Her last BV dose was given at Week 16. During the post-RT phase, she required a dose reduction of TMZ back down to 150 mg/m^2 after she developed thrombocytopenia (Grade 1) during the first post-RT maintenance cycle at 200 mg/m^2. The patient was stable at 42 weeks after continuing single agent TMZ off-study for 6 months.

Patient 2

The patient developed thrombocytopenia (Grade 3) toward the end of the RT phase. This worsened into pancytopenia (Grade 4) after completion of the RT phase (Week 8). Post-RT treatments of both TMZ and BV were initiated after an interval 7 weeks after recovery of counts (Week 13). The patient developed left optic neuropathy at Week 42 that was presumed to be radiation induced and was removed from the study. Because of the tumor location, the optic apparatus could not be entirely spared from the high-dose region. Ultimately it was decided that, given the size and location of her tumor, there was a higher likelihood of optic apparatus damage secondary to tumor growth than from radiation-induced optic neuropathy. The patient was given a dose of 200 cGy $\times$ 30 fractions delivered to the 90% isodose line. The left optic nerve received a maximum dose of 6,206 cGy (minimum 630 cGy, average 3,800 cGy). The right optic nerve received a maximum dose of 5,946 cGy (minimum 646 cGy, average 3,843 cGy). The optic chiasm received a maximum dose of 6.324 cGy (minimum, 6,082 cGy; average, 6,218 cGy). The last BV dose was Week 41. The post-RT maintenance TMZ doses had been at the reduced dose of 100 mg/m^2. This patient developed thrombocytopenia (Grade 3) at the dose of 150 mg/m^2 and has been stable as of Week 44.

Patient 3

This patient had no serious toxicities. The patient had a 4-week interval between completion of RT and starting post-RT maintenance TMZ and BV because of fatigue (Grade 2). The patient also had anorexia (Grade 1) throughout RT and post-RT phases. The patient achieved a TMZ dose of 150 mg/m^2 that was not increased to 200 mg/m^2 because of fatigue. He voluntarily came off-study at Week 32 to participate in another clinical trial with continuation of off-label BV with TMZ. The patient has been stable as of Week 39.

Patient 4

The patient experienced thrombocytopenia (Grade 2) during the RT phase and missed six daily doses of TMZ. He received two post-RT cycles of TMZ at 150 mg/m^2. Because of thrombocytopenia (Grade 3), a further dose

Table 3. Compiled toxicities (percentages of patients) during and after radiotherapy (RT) phase*

	RT phase		Post-RT phase	
Toxicity	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Blood				
Thrombocytopenia	70	20	40	30
Neutropenia		10		10
Leukopenia		10		
Anemia	20		20	
Cardiac				
Hypertension	30		20	
Constitutional				
Fatigue	100		60	20
Gastrointestinal				
Anorexia	10		10	
Constipation	50		60	
Nausea	60		30	
Vomiting	20		20	
Elevated ALT	10		20	
Bleeding				
Blood in stool	10			
Epistaxis	10		10	
Hemoptysis			10	
Neurology				
Dizziness	20		30	
Mood alteration	30		10	
Seizure	10		20	20
CSF leak		10		
Pain				
Headache	70		30	
Pulmonary				
Cough	20		20	
Sinus infection	10		30	
Vocal changes	30		10	
Infection				
Skin/wound		10		10
Oral thrush	30		20	
Renal/GU				
UTI			20	
Elevated Urine PCR ratio (proteinuria)	10		20	10
ARF		10		
Skin				
Wound dehiscence/healing delay		10		10
Alopecia	60		40	
Scalp erythema	20			
Endocrine				
Diabetes insipidus		10		
Musculoskeletal				
Muscle weakness/myopathy	20		10	
Vascular				
DVT				30
PE				20
Fatal cerebral hemorrhage				0
Visual				
Optic neuropathy				10

Abbreviations: ALT = alanine aminotransferase; ARF = acute renal failure; CSF = cerebrospinal fluid; DVT = deep venous thrombosis; GU = genitourinary; PCR = protein/creatinine ratio; PE = pulmonary embolism; UTI = urinary tract infection.

* For each toxicity, only the highest toxicity per phase is tabulated per patient, such that a patient is counted only once in each phase.

reduction of TMZ to 100 mg/m^2 was required. The patient has been stable as of Week 45.

Patient 5

The patient developed Grade 3 thrombocytopenia (Grade 3) at the end of the RT phase that progressed to thrombocytopenia and neutropenia (Grade 4), requiring cancellation of two BV doses and delay of post-RT TMZ until 6 weeks after completion of RT (Week 12). The patient required a platelet transfusion of 2 units. In addition, the patient developed acute renal failure (Cr 3.1), reversed by hydration and possibly related to use of nonsteroidal anti-inflammatory drugs), diabetes insipidus (treated with DDAVP), and proteinuria (2+ dipstick) requiring hospitalization during post-RT (Week 10); all were Grade 3. In Week 21, the patient developed a wound dehiscence (Grade 3) and infection of the craniotomy site that was successfully treated with cranioplasty and intravenous antibiotics. The TMZ was not held, but the BV was held for 4 weeks after the cranioplasty. The patient's post-RT TMZ dose was maintained at 100 mg/m^2 after she developed thrombocytopenia (Grade 3) at higher doses. She was in stable condition as of Week 43.

Patient 6

During the RT phase, this patient had exacerbation of preexisting HTN (Grade 3) requiring delay of the BV dose as well as thrombocytopenia (Grade 1) requiring delay of first post-RT TMZ dose. During the post-RT phase, she was maintained on a TMZ dose of 200 mg/m^2. The patient was hospitalized for seizures (Grade 3) in Weeks 29 and 35. She developed a deep venous thrombosis (DVT) and pulmonary embolism (PE; Grade 4, Week 34) and was taken off-study. Her last BV dose was at Week 31. This patient was stable as of Week 38.

Patient 7

This patient has had no serious toxicities except one generalized seizure (Grade 3) during the post-RT phase (Week 12). He has been maintained on 200 mg/m^2 TMZ, and has been stable as of Week 41.

Patient 8

This patient experienced wound breakdown and infection (Grade 3) during the RT phase, which was successful treated with cranioplasty at Week 3. Both XRT and TMZ were held for 2 weeks. The BV was held for 4 weeks. The patient developed a DVT and PE (Grade 4) (Week 27) and was taken off-study. Her last BV was given at Week 27. Her TMZ dose was maintained at 200 mg/m^2. The patient had a stable MRI as of 24 weeks but showed progressive disease at Week 31.

Patient 9

This patient had no serious toxicities during the RT phase. However the post-RT maintenance TMZ was never started because of lethargy (Grade 3) and encephalopathy (Grade 3), and the patient received only one dose of post-RT BV at Week 8. His MRI was stable at Week 10 but showed

Table 4. Treatment characteristics of study patients

Patient no.	Status (wk from initiation)	Interval between surgery and treatment initiation (wk)	PFS (wk)	ΔKPS	ΔDex (mg)	Break interval (wk)	Stable maintenance TMZ achieved (mg/m^2)
1	Off-study (18), alive	4.5	42*	0	16 to 6	2	150
2	Off-study (44), alive	4	44*	0	8 to 1	6	100
3	Off-study (32), alive	3.5	39*	0	0 to 0	4	150
4	On-study, alive	3.5	45*	0	1 to 0	2	100
5	On-study, alive	3.5	43*	0	0 to 0	6	133
6	Off-study (34), alive	5	38	−30	1 to 0	3	200
7	On-study, alive	3.5	41*	0	6 to 0	2	200
8	Off-study (28), alive	4	31	0	16 to 3	2	200
9	Off-study (15), deceased (22)	4	15	−10	0 to 0	2	0
10	On-study, alive	3.5	41*	+10	12 to 0	2	100

Abbreviations: ΔDex = change in dexamethasone dose from enrollment to last follow-up on-study; ΔKPS = change in Karnofsky performance status from enrollment to last follow-up on-study; PFS = progression-free survival; TMZ = temozolomide. Break interval signifies weeks between completion of radiotherapy and start of maintenance TMZ.

* No progressive disease yet.

progression at Week 15, and he was taken off-study after this MRI. An MRI at Week 21 showed substantial tumor progression, and the patient died at Week 22.

Patient 10

The patient has had no serious toxicities except the development of a DVT (Grade 3) at Week 17, and was placed on daily Lovenox. His BV was held for 4 weeks and restarted after a petition to allow concurrent anticoagulation with BV treatment. He is being maintained on 150 mg/m^2 TMZ, reduced from 200 mg/m^2 because of fatigue (Grade 2). The patient has been stable as of Week 41.

DISCUSSION

Bevacizumab (BV) has recently emerged as a promising treatment option for recurrent GBM (3). The present study addresses whether BV can be safely used up-front in GBM patients. We completed enrollment of the pilot phase of a Phase II clinical trial examining the safety and efficacy of the combination of BV with RT/TMZ in the up-front treatment of GBM. This trial adds BV to the current recently established standard of care for the treatment of newly diagnosed GBM (2). We report results of the interim toxicity analysis of 10 pilot patients. A major goal of this analysis was to discern any unexpected neurotoxicities related to RT and BV during either the RT phase or post-RT phase. Preliminarily, no obvious white matter abnormalities were noted on serial MRI scans to indicate increased toxicity from the combination of RT/BV. The only unexpected neurotoxicity was observed in Patient 2, who developed optic neuropathy resulting in nearly complete blindness occurring at Week 42. Emami *et al.* conducted an extensive literature search and compiled a table of normal tissue tolerances in terms of total dose (TD) 5/5 (the probability of 5% complication within 5 years from treatment) and TD 50/5 (the probability of 50% complication within 5 years) (14). In that publication, the optic nerve and optic chiasm TD 5/5 was 5,000 cGy and the TD 50/5 was 6,500 cGy, with the selected endpoint being blindness. Parsons *et al.* investigated the risk of radiation-induced optic neuropathy after fractionated external-beam irradiation for primary extracranial head-and-neck tumors (15). The clinical endpoint was visual acuity of 20/100 or worse as a result of optic nerve injury. Of 215 optic nerves that were infield in 131 patients, anterior ischemic optic neuropathy developed in five nerves (median, 30 months; range, 2–4 years), and retrobulbar optic neuropathy developed in 12 nerves (median, 28 months; range, 1–14 years). No injuries were seen in the 106 nerves that received <5,900 cGy. The 15-year actuarial risk of optic neuropathy after doses ≥6,000 cGy was 11% if the fraction size was <190 cGy, compared with 47% if the fraction size was ≥190 cGy. In our study, the left optic nerve in Patient 2 received a maximum dose of 206.85 cGy per fraction (6,206 cGy total). Therefore it is likely that the left optic neuropathy observed was caused by radiation. However the injury onset was sooner than would be expected. This raises the possibility that BV may have been responsible for enhancing or hastening radiation damage. In contrast, the right optic nerve received only a slightly lower dose (and the optic chiasm received slightly more), without apparent injury to date. One possible modification would be to use a lower dose per fraction (1.8 Gy/fraction) in cases in which tumor location requires administration of higher doses than the standard tolerance doses to critical structures.

Another important goal of this analysis was to determine whether the combination of BV and RT/TMZ was associated with increased rates of expected toxicities. The major observed toxicities that could be treatment related were fatigue, myelotoxicity, wound breakdown, and venous thrombosis. Although our sample size was clearly too small to allow conclusions to be drawn regarding whether the combination enhanced these toxicities, our observations raise that possibility.

Fatigue

In terms of Grade 1 and 2 fatigue, all 10 patients noted fatigue; fatigue was also common in the post-RT phase (8/10), with

2 patients experiencing Grade 3 fatigue (defined as ''severe fatigue interfering with activities of daily living (ADL)''). Because this frequency of Grade 3 fatigue was higher than that reported for RT/TMZ alone (7%), these results suggest that the combination of BV and TMZ may contribute to increased fatigue compared with TMZ alone (2).

Myelotoxicity

We observed thrombocytopenia of all grades in 8 patients, with 3 patients developing Grade 3/4 thrombocytopenia. In 2 patients, Grade 4 pancytopenia developed near the end of the concomitant RT/TMZ phase or shortly thereafter, and both patients required transfusions. All patients were eventually able to continue TMZ. For comparison, the myelotoxicity (Grade 3/4) in the original prospective study was reported to be 16% over the entire study period, with 7% arising during RT/TMZ and 14% of patient in the post-RT phase (2). Specifically the occurrence of thrombocytopenia was 3% during RT/TMZ, 11% during adjuvant TMZ, and 12% total. These values are lower than the frequency of Grade 3/4 thrombocytopenia (30%) observed in our patient group. However in a retrospective single-institution analysis of 52 patients with high-grade gliomas treated up-front with RT/TMZ. 19% of patients had Grade 3/4 thrombocytopenia and 10% had severe neutropenia; myelotoxicity usually occurred toward the end of RT/TMZ or shortly thereafter; and thrombocytopenia appeared to be more common in female than male patients (16). Given the disparity between the two previously published experiences with concomitant RT/TMZ, it is difficult to conclude that the addition of BV to RT/TMZ enhances the potential for myelotoxicity compared with RT/TMZ alone. Of note, in colorectal cancer patients, the addition of BV to chemotherapy appeared to be associated with a modest increase in the incidence of myelosuppression (17). As another possible indicator of increased toxicity with addition of BV to TMZ, 67% of patients that started on the adjuvant phase had their dose escalated to the full 200 mg/m^2 of TMZ (2). By comparison, we were able to dose escalate 30% of the patients to the full TMZ dose of 200 mg/m^2.

Wound healing problems

Another major issue that occurred in 2 patients was wound breakdown and infection. In both cases, the wound problems were successfully treated with debridement and cranioplasty, and both patients were able to resume BV treatments without further wound complications. This issue was anticipated during the design of the trial, and we required that the beginning of treatment commence no earlier than 3 weeks from the surgery. This decision was based in part on the experience of using BV for colorectal cancer postoperatively, in which only a slightly increased incidence of wound healing complications (1.5%) was observed if BV was started between 28 and 60 days after surgery (18). Of note, these patients did not receive radiation. We also required that treatment not be started more than 5 weeks from surgery to minimize potential tumor growth during this interval. The RT protocol did not attempt to reduce the skin dose, and both patients had nearly the full prescribed dose delivered to their wound site. The finding of 2 of 10 patients with wound problems appears increased compared with historical rates of wound complications for craniotomies. For example, in an analysis of perioperative (first 21 days after surgery) complications in the Glioma Outcome Project, wound infections were observed at 0.5% in the first craniotomy group vs. 1.1% in the second craniotomy group (19). These rates are similar to a retrospective study of neurosurgeries performed in Australia, which reported a 1% rate of wound complications (20). Interestingly these figures contrast with reports of a single-institution Gliadel experience (21). Because all of these patients were likely to have had extensive resections, this study may be more relevant to our study, as the frozen tissue requirement in our study resulted in a predominance of extensive resections. However the problems associated with local carmustine infiltration prevent direct comparison. Nonetheless, in this study, they reported 2 wound complications out of 18 in the placebo arm, and 3 wound complications out of 18 in Gliadel arm. Another factor that needs to be considered is the role of dexamethasone in impairing wound healing. At the time of the wound dehiscence, Patient 5 was off decadron, whereas Patient 8 was on 16 mg of decadron. Two Phase I studies have been published regarding combinations of BV, RT, and chemotherapy in non–central nervous system tumor types. In a rectal cancer study, 1 of 11 patients developed a postoperative abscess, although it is not clear when the initial surgery was performed (22). In the locally advanced pancreatic cancer study, in which treatment began no earlier than 28 days after surgery, 4 of 48 patients had problems within the RT field (ulcer/fistula/bleeding/gastrointestinal perforation) (23). In addition it has recently been observed that 2 of 29 lung cancer patients treated with BV combined with RT and chemotherapy developed tracheoesophageal fistula during maintenance BV phase (http://www.fda.gov/medwatch/safety/2007/Avastin_DHCP_TEF_Final_April2007.pdf). Taken together with our results, these studies suggest that the combination of BV with RT/chemotherapy may potentiate local wound healing problems.

Venous thromboembolism

We observed 3 patients who developed DVTs, with 2 of these patients developing PEs that required discontinuation from the study. Although BV is not considered to increase the risk of verous thromboembolism, one study observed increased rate of venous thromboembolism associated with BV noted in the treatment of metastatic colorectal cancer (17). In this study, thrombotic events (primarily venous) were observed in 9% of the control arm (chemotherapy only), in 26% (14% Grade 3/4) of the BV dosing arm of 5 mg/kg plus chemotherapy and in 13% (6% Grade 3/4) of patients receiving the BV dose of 10 mg/kg plus chemotherapy. Venous thromboembolism frequently occurs in the primary brain tumor population. In a large recent retrospective analysis of patients with malignant gliomas, a 2-year incidence of 7.5% was reported (24). However, in a recent retrospective study,

24% of high-grade–glioma patients developed venous thromboembolism; and of those, 60% developed pulmonary embolism (25). Similar results were reported in another study of high-grade gliomas (26). Although our results indicate similarly high rates of DVT and PE, we cannot exclude the possibility that the addition of BV could enhance the development of venous thromboembolic disease. Higher patient numbers are required to determine whether BV increases the already high rate of thromboembolic disease observed in this patient population.

CONCLUSION

Analysis of toxicity data from this small patient group receiving BV, RT, and TMZ did not reveal any serious unexpected neurotoxicities except optic neuropathy developing in 1 patient. Assuming that this is indeed related to RT, possible alterations in the regimen include stricter dose limitation on optic apparatus and other critical structures, and/or reduction of the dose per fraction to 180 cGy. We observed three serious toxicities with a frequency of at least 20%: myelotoxicity, wound healing complications, and venous thromboembolic events. Our results are clearly limited by the small sample size. Regardless of whether the relatively high incidences of these toxicities are related to the combination of BV with RT/TMZ, our observations indicate that these toxicities need to be anticipated and watched for carefully. Analysis of toxicities from patients enrolled in the expansion phase is expected to help determine the contribution of BV to these toxicities. If BV is linked to increasing the incidences of these toxicities, not only will it be important to anticipate these problems, but it will also be necessary to adjust future protocols using BV in this context. Because the incidences of BV associated hypertension and proteinuria may be related to cumulative BV dose, it may be more tolerable to decrease the dose of B,V given that both 10-mg/kg dosing and 5-mg/kg dosing have been effective in the treatment of recurrent GBM (3, 27). Alternatively, decreasing the frequency of BV to every 3 weeks might be considered. Regarding impaired wound healing, it may be necessary to commence BV therapy later in the treatment, such as midway into the course of RT, to enable more complete wound healing. However this may not optimize the possible synergistic benefit of RT and BV. As an alternative, the time from surgery to RT can be increased, and dosing to the skin can be reduced. In terms of risk of developing DVT/PE, it may be important to prescreen for venous thromboembolism or give prophylactic anticoagulation (24, 28). Although it is too early to determine whether the BV/RT/TMZ regimen results in increased overall survival, our results in terms of progression-free survival (>8.8 months) are promising. Nonetheless, given the enthusiasm for the use of BV for GBM, we believe that it is premature to begin using BV in the up-front treatment of GBM patients until more safety and efficacy data become available.

REFERENCES

1. Wrensch M, Minn Y, Chew T, *et al*. Epidemiology of primary brain tumors: Current concepts and review of the literature. *Neuro-Oncol* 2002;4:278–299.
2. Stupp R, Mason WP, van den Bent MJ, *et al*. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–996.
3. Vredenburgh JJ, Desjardins A, Herndon JE 2nd, *et al*. Phase II trial of bevacizumab and irinotecan in recurrent malignant glioma. *Clin Cancer Res* 2007;13:1253–1259.
4. Pope WB, Lai A, Nghiemphu P, *et al*. MRI in patients with high-grade gliomas treated with bevacizumab and chemotherapy. *Neurology* 2006;66:1258–1260.
5. Carlson MR, Pope WB, Horvath S, *et al*. Relationship between survival and edema in malignant gliomas: Role of vascular endothelial growth factor and neuronal pentraxin 2. *Clin Cancer Res* 2007;13:2592–2598.
6. Steiner H-H, Karcher S, Mueller MM, *et al*. Autocrine pathways of the vascular endothelial growth factor (VEGF) in glioblastoma multiforme: Clinical relevance of radiation-induced increase of VEGF levels. *J Neuro-Oncol* 2004;66:129–138.
7. Senan S, Smit EF. Design of clinical trials of radiation combined with antiangiogenic therapy. *Oncologist* 2007;12:465–477.
8. Hovinga K, Stalpers L, Bree C, *et al*. Radiation-enhanced vascular endothelial growth factor (VEGF) secretion in glioblastoma multiforme cell lines—a clue to radioresistance? *J Neuro-Oncol* 2005;74:99–103.
9. Wachsberger PR, Burd R, Cardi C, *et al*. VEGF trap in combination with radiotherapy improves tumor control in u87 glioblastoma. *Int J Radiat Oncol Biol Phys* 2007;67:1526–1537.
10. Nieder C, Wiedenmann N, Andratschke N, *et al*. Current status of angiogenesis inhibitors combined with radiation therapy. *Cancer Treat Rev* 2006;32:348–364.
11. 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;6:553–563.
12. Wong CS, Van der Kogel AJ. Mechanisms of radiation injury to the central nervous system: Implications for neuroprotection. *Mol Interv* 2004;4:273–284.
13. Zachary I. Neuroprotective role of vascular endothelial growth factor: Signalling mechanisms, biological function, and therapeutic potential. *Neurosignals* 2005;14:207–221.
14. Emami B, Lyman J, Brown A, *et al*. Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys* 1991;21:109–122.
15. Parsons JT, Bova FJ, Fitzgerald CR, *et al*. Radiation retinopathy after external-beam irradiation: Analysis of time-dose factors. *Int J Radiat Oncol Biol Phys* 1994;30:765–773.
16. Gerber DE, Grossman SA, Zeltzman M, *et al*. The impact of thrombocytopenia from temozolomide and radiation in newly diagnosed adults with high-grade gliomas. *Neuro-Oncol* 2007;9:47–52.
17. Kabbinavar F, Hurwitz HI, Fehrenbacher L, *et al*. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003;21:60–65.
18. Scappaticci FA, Fehrenbacher L, Cartwright T, *et al*. Surgical wound healing complications in metastatic colorectal cancer patients treated with bevacizumab. *J Surg Oncol* 2005;91:173–180.

19. Chang SM, Parney IF, McDermott M, *et al*. Perioperative complications and neurological outcomes of first and second craniotomies among patients enrolled in the Glioma Outcome Project. *J Neurosurg* 2003;98:1175–1181.
20. Smith SF, Simpson JM, Sekhon LH. What progress has been made in surgical management of patients with astrocytoma and oligodendroglioma in Australia over the last two decades? *J Clin Neurosci* 2005;12:915–920; discussion 921.
21. Lawson HC, Sampath P, Bohan E, *et al*. Interstitial chemotherapy for malignant gliomas: The Johns Hopkins experience. *J Neurooncol* 2007;83:61–70.
22. Czito BG, Bendell JC, Willett CG, *et al*. Bevacizumab, oxaliplatin, and capecitabine with radiation therapy in rectal cancer: Phase I trial results. *Int J Radiat Oncol Biol Phys* 2007;68:472–478.
23. Crane CH, Ellis LM, Abbruzzese JL, *et al*. Phase I trial evaluating the safety of bevacizumab with concurrent radiotherapy and capecitabine in locally advanced pancreatic cancer. *J Clin Oncol* 2006;24:1145–1151.
24. Semrad TJ, O'Donnell R, Wun T, *et al*. Epidemiology of venous thromboembolism in 9489 patients with malignant glioma. *J Neurosurg* 2007;106:601–608.
25. Simanek R, Vormittag R, Hassler M, *et al*. Venous thromboembolism and survival in patients with high-grade glioma. *Neuro-Oncol* 2007;9:89–95.
26. 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;33:1592–1596.
27. Stark-Vance V. Bevacizumab (Avastin) and CPT-11 (Camptosar) in the treatment of relapsed malignant glioma. World Federation of NeuroOncology, Edinburgh, UK, May 5–8, 2005 (abstract 342).
28. 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;89:640–646.