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Phase II study of metronomic chemotherapy with bevacizumab for recurrent glioblastoma after progression on bevacizumab therapy

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Abstract

We evaluated the efficacy of metronomic etoposide or temozolomide administered with bevacizumab among recurrent glioblastoma (GBM) patients who progressed on prior bevacizumab therapy in a phase 2, open-label, two-arm trial. Twenty-three patients received bevacizumab (10 mg/kg) every 2 weeks with either oral etoposide (50 mg/m²) daily for 21 consecutive days each month or daily temozolomide (50 mg/m²). The primary endpoint was 6-month progression-free survival (PFS-6) and secondary endpoints included safety and overall survival. Both the etoposide and temozolomide arms of the study closed at the interim analysis due to lack of adequate anti-tumor activity. No radiographic responses were observed. Although 12 patients (52%) achieved stable disease, PFS-6 was 4.4% and the median PFS was 7.3 weeks. The only grade 4 adverse event was reversible neutropenia. Grade 3 toxicities included fatigue ($n = 2$) and infection ($n = 1$). Metronomic etoposide or temozolomide is ineffective when administered with bevacizumab among recurrent GBM patients who have progressed on prior bevacizumab therapy. Alternative treatment strategies remain critically needed for this indication.

Keywords

Glioblastoma; Angiogenesis; Bevacizumab; Vascular endothelial growth factor; Metronomic chemotherapy

Introduction

Outcome for glioblastoma (GBM), the most common malignant primary brain tumor, remains poor with median overall survival of only 14.6 months following current standard therapy with maximum safe resection followed by involved-field radiation with temozolomide and adjuvant temozolomide [1]. Following recurrence, there was no established effective salvage therapy [2–4] until May 2009 when the U.S. Food and Drug Administration granted accelerated approval to bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor (VEGF), based on durable radiographic responses [5, 6]. However, the overall survival benefit following bevacizumab with or without chemotherapy is modest with most patients developing progressive disease within 8–10 months and dying with refractory tumor soon thereafter [5–8]. Thus effective therapy for GBM patients following progression on bevacizumab-based therapy represents a major unmet need in neuro-oncology.

Protracted, daily, low-dose chemotherapy, also referred to as metronomic chemotherapy, has been shown to exert significant anti-tumor effects in preclinical models [9–11]. Nonetheless clinical experience has generated varied, and at best modest, evidence of patient benefit [12–16]. Metronomic chemotherapy offers several potential mechanisms of anti-tumor effect, some of which have been shown to enhance the benefit associated with anti-VEGF therapies [17–22]. Thus we designed the current study to evaluate the hypothesis that bevacizumab combined with metronomic temozolomide or etoposide, two agents with modest therapeutic

benefit when administered using metronomic dosing schedules among recurrent malignant glioma patients [23–28], may be able to rescue recurrent GBM patients who have progressed on prior bevacizumab therapy. Our study, which represents the first reported phase II study designed specifically to evaluate an investigational therapy for recurrent GBM patients who have progressed on prior bevacizumab therapy, demonstrates that metronomic temozolomide or etoposide combined with bevacizumab, is well-tolerated but ineffective for this indication.

Patients and methods

Protocol objectives

Our primary objective was to define the activity of metronomic chemotherapy with either oral etoposide or temozolomide, combined with bevacizumab among adults with recurrent GBM who have progressed on prior bevacizumab therapy. In addition, we evaluated 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 was recurrent including progression on prior bevacizumab based 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 $\geq 60\%$, and were on a stable corticosteroid dose for at least 1 week. Additional enrollment criteria included: hematocrit $> 29\%$; absolute neutrophil count $> 1,500$ 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. There were no limits based on either the number of prior episodes of progression or therapeutic regimens received.

Patients were excluded for: grade ≥ 3 toxicity on prior bevacizumab; progressive disease or grade ≥ 3 toxicity on any prior protracted temozolomide schedule (metronomic temozolomide arm only); progressive disease or grade ≥ 3 toxicity on prior metronomic etoposide (metronomic etoposide arm only); uncontrolled hypertension; therapeutic anticoagulation use; acute hemorrhage on baseline MRI; urine protein:creatinine ratio > 1 ; pregnancy or nursing; active infection requiring intravenous antibiotics; and prior stereotactic radiosurgery, radiation implants, or radiolabeled monoclonal antibody 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 were randomized to one of two treatment arms: metronomic temozolomide plus bevacizumab; or metronomic etoposide plus bevacizumab. Patients with progression or grade ≥ 3 toxicity on prior metronomic temozolomide were enrolled on the metronomic etoposide arm and conversely patients with progressive disease or grade ≥ 3 toxicity on prior metronomic etoposide were enrolled on the metronomic temozolomide arm. Bevacizumab was administered at 10 mg/kg intravenously every 14 days to all patients. Patients on the metronomic temozolomide arm received 50 mg/ m^2 /day orally of temozolomide on a continuous dosing schedule while those on the metronomic etoposide arm took 50 mg/ m^2 of etoposide daily for 21 consecutive days of each 28 day cycle. Study therapy was planned to continue 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 prior to every other treatment cycle. A complete response (CR) required disappearance of all enhancing tumor on consecutive MRIs at least 4 weeks apart, with corticosteroid discontinuation and neurologic stability or improvement. A partial response (PR) required $\geq 50\%$ reduction in size (product of largest perpendicular diameters) of enhancing tumor with stability or improvement of neurologic status and corticosteroids. Complete and partial responses also required stable or improved signal abnormality on fluid-attenuated inversion recovery (FLAIR) sequences. Progressive disease (PD) included $\geq 25\%$ 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 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 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. 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 grade 3, related events resolved to grade $\leq$ grade 1.

Initiation of each cycle required: an ANC $\geq 750/\text{mm}^3$; a platelet count $\geq 75,000/\text{mm}^3$; AST, bilirubin and creatinine $\leq$ twice the institutional upper limit of normal; and resolution of any related grade ≥ 3 event to $\leq$ grade 1.

Statistical considerations

Our primary goal was to evaluate the 6-PFS rate of bevacizumab plus metronomic chemotherapy using either oral etoposide or temozolomide among recurrent GBM patients who had progressed on prior bevacizumab therapy. At the time this study was designed, benchmarks for outcome among recurrent GBM patients who had progressed on prior bevacizumab therapy had not been established, therefore we chose to use the historical benchmark established with temozolomide at first recurrence. Hence, given a 6-PFS rate of 21% among recurrent GBM patients treated with temozolomide at first recurrence [29] a sample size goal of 24 recurrent GBM patients per arm was chosen to allow 88% power to differentiate between 6-PFS rates of 5% and 25% with a type I error rate of 0.03.

An interim efficacy analysis after 12 patients were accrued to each arm was planned a priori. If 8 or more of the these 12 patients progressed or died within 2 months of study initiation, further accrual to that arm would be suspended. Assuming PFS is exponentially distributed, a 6-month PFS rate of 25% implies a 2-month PFS rate of 63%. If the true 2-month PFS was 63%, then the probability that 8 or more of the first 12 enrolled patients had progressed or died after 2 months would be less than 5%.

For this study among heavily pretreated patients with an extremely poor prognosis, rates of unacceptable toxicity, defined as $\geq$ 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 [30] were used to monitor this study after each group of 4 patients. Accrual was not suspended to formally assess the toxicity profile unless the following thresholds of unacceptable toxicity were satisfied: $\geq 3/4$; $\geq 4/8$; $\geq 5/12$; $\geq 6/16$; $\geq 7/20$; $\geq 7/24$. The type I and type II errors associated with this monitoring were 0.09 and 0.09, respectively. These guidelines did not adjust for differential length of follow-up of accrued patients.

Progression-free survival (PFS) and overall survival (OS) were measured from the cycle 1 start date and summarized using Kaplan–Meier estimator including 95% CIs (Fig. 1).

Results

Patient characteristics

Twenty-three patients were enrolled between August 2008 and January 2010 including 10 treated on the daily temozolomide arm and 13 on the metronomic etoposide arm. Characteristics of the patients on both arms were overall comparable (Table 1). Patients were heavily pretreated with 83% having 2 or more episodes of prior progression. All patients had progressed after standard therapy with radiation and temozolomide chemotherapy as well as prior bevacizumab with chemotherapy; none of the patients enrolled following progression on bevacizumab monotherapy. All patients enrolled onto the current study immediately following progression on bevacizumab except for two patients who enrolled 3 and 13 months after bevacizumab progression, respectively. For patients on the temozolomide arm, the median number of prior cycles of standard 5-day temozolomide administered was 6 (range, 0–8). None of the patients on the etoposide arm had received prior etoposide. In addition, 18% had progressed after 2 or more prior bevacizumab-based regimens.

As of July 15, 2010, all patients have discontinued study therapy. Two patients (9%) remain alive including one patient on the temozolomide arm who is alive with progressive disease 32 weeks after starting treatment and one patient who discontinued study therapy on the oral etoposide arm after 2 weeks due to worsened headaches. After terminating study treatment, the second patient completed 12 cycles of daily temozolomide with bevacizumab off-study and remains progression-free 54 weeks after starting treatment. All other patients died within 1 year of treatment initiation.

Study drug administration and safety

Study drug administration and compliance with treatment for the intent-to-treat study population were excellent. A total of 36 cycles of therapy were administered including a median of 1.6 (range, 1–2) cycles for the daily temozolomide arm and 1.8 (range 1–3) cycles for the oral etoposide arm. All patients discontinued study therapy due to progressive disease except for one patient who discontinued treatment due to worsened grade 2 headache, although this symptom was likely attributable to his underlying progressive tumor.

All patients were assessable for toxicity. A summary of toxicity is provided in Table 2. In general, both arms tolerated study therapy well however, duration of treatment was short due to poor efficacy. Significant hematologic toxicity was rare. Grade 3 or greater hematologic toxicity was encountered in only one patient (grade 4 neutropenia). Grade 3 non-hematologic toxicities included fatigue ($n = 2$) and infection ($n = 1$). Remaining significant attributable toxicities were grade 2. There were no study related deaths. One patient

developed an intra-cranial hemorrhage (grade 1) which occurred at the time of progressive disease.

Outcome

Accrual to both arms was discontinued prematurely at the planned interim analysis due to lack of adequate anti-tumor activity. Although the interim analysis was planned after the first 12 patients completed up to 2 months of study therapy, the temozolomide arm was closed to further accrual after only ten patients were enrolled and treated because it was clear at that time that the cut-off required by the interim analysis to permit further accrual would not be met. Specifically, nine of the ten patients on the temozolomide arm progressed within 2 months of study initiation. The remaining patient progressed after four cycles of study therapy. Similarly, 9 of 12 evaluable patients on the etoposide arm progressed within 2 months of study therapy, while the remaining three evaluable patients progressed after three cycles. A 13th patient was enrolled and treated on the oral etoposide arm to replace the patient who discontinued study therapy after 2 weeks due to worsened headache. The median overall survival (OS), progression-free-survival (PFS), 6-month PFS rate, and rate of radiographic response for each treatment arm are summarized in Table 3.

Pattern of failure

Patterns of progression at study enrollment after progression on prior bevacizumab therapy as well as at the time of progression on the current study are summarized in Table 4 using previously defined categories of local, diffuse and distant progression defined previously [31]. Most patients progressed with both enhancing and non-enhancing local disease, and less commonly patients progressed diffusely with either enhancing and non-enhancing disease, or non-enhancing only. Only one patient progressed with distant disease.

Discussion

Although bevacizumab salvage therapy improves progression-free survival for recurrent GBM patients, its overall survival benefit has not been confirmed in a randomized, controlled trial. Therefore effective therapies following progression after bevacizumab salvage therapy are critically needed. We report the first clinical trial dedicated specifically to evaluating outcome among recurrent GBM patients who have progressed on prior bevacizumab therapy. Specifically we performed a phase II study to evaluate the anti-tumor activity of protracted, low-dose, daily (metronomic) schedules of oral etoposide or temozolomide when administered with bevacizumab. Although our results confirm the tolerability of these chemotherapeutics when administered on a metronomic dosing schedule with bevacizumab for this patient population, neither study arm demonstrated sufficient evidence of anti-tumor activity to warrant continued evaluation beyond the planned interim analysis.

The rationale underlying this study was that potential mechanisms of action associated with metronomic chemotherapy combined with bevacizumab may overcome mechanisms of resistance associated with bevacizumab. Bevacizumab inhibits tumor-associated angiogenesis by specifically binding VEGF-A and preventing its activation of VEGFR1 and VEGFR2 [32]. Metronomic chemotherapy has been shown to decrease tumor angiogenesis by a number of alternative mechanisms including induction of endogenous angiogenesis inhibitors such as thrombospondin-1 [17, 33], enhancement of tumor endothelial cell apoptosis [21], and decreased mobilization and viability of circulating endothelial progenitor cells [20]. These mechanisms of action may potentiate the anti-tumor activity of bevacizumab and may also abrogate potential mechanisms of bevacizumab resistance. The rationale for continuing bevacizumab with metronomic chemotherapy in our study was

based on two factors. First, although not corroborated for GBM patients, continuation of bevacizumab after progression on prior bevacizumab therapy has been associated with improved survival compared to discontinuation of bevacizumab for other oncology patient groups [34, 35]. Second, abrupt discontinuation of bevacizumab among recurrent GBM patients has been associated with rapid “rebound” tumor re-growth [36].

The choice of specific chemotherapeutics used in this study was based on findings from preclinical and clinical studies. Metronomic etoposide has been shown to exert preferential cytotoxicity against tumor endothelial cells compared to tumor cells in vitro [37], and prolongs survival when combined with direct anti-VEGF therapy in orthotopic, intracranial U87 GBM xenografts compared to conventionally-dosed chemotherapy with or without anti-angiogenic therapy [19]. Clinically, several studies using metronomic dosing of etoposide have shown evidence of modest activity among recurrent malignant glioma patients [14, 25–27, 38], as well as other cancer patient populations [39–42]. Similarly, metronomic or protracted temozolomide dosing has been shown preclinically to have anti-angiogenic activity [15, 43, 44], and also offers the potential benefit of depleting tumor methylguanine methyltransferase as a strategy to restore temozolomide sensitivity [45]. Protracted temozolomide schedules have been associated with modest anti-tumor benefit clinically among some recurrent GBM patients [23, 24, 46].

Retrospective series have demonstrated that additional bevacizumab-based treatment strategies have negligible anti-tumor activity among recurrent GBM patients who have progressed on prior bevacizumab therapy [47, 48]. Similarly poor results have been observed among patients treated with bevacizumab plus irinotecan after progression on bevacizumab monotherapy [6]. The results of our study are consistent with these prior results and demonstrate that metronomic temozolomide or etoposide is ineffective when combined with bevacizumab following progression on prior bevacizumab with chemotherapy for recurrent GBM patients. Several factors may have contributed to these disappointing results. First, patients in the current study were heavily pretreated. In contrast to the current study in which nearly all patients were enrolled after two or more episodes of prior progression, the “rescue” study which confirmed a clinical benefit associated with metronomic temozolomide in subsets of malignant glioma patients, restricted enrollment to patients at first recurrence [46]. Second, a recent study demonstrated that the addition of metronomic etoposide with bevacizumab achieved anti-tumor benefit comparable to that previously reported for bevacizumab alone among recurrent, bevacizumab-naïve, GBM patients [7]. Thus, the addition of metronomic etoposide does not improve outcome when combined with bevacizumab for patients who are either bevacizumab naïve or who have progressed on prior bevacizumab. Finally, several mechanisms of resistance to bevacizumab therapy in malignant glioma have been proposed including enhanced glioma invasion and infiltration with co-option of normal blood vessels [49–51], increased activity of alternative angiogenic mediators [52–54], recruitment of bone marrow-derived pro-angiogenic cells [55] and adaption of tumor cells to therapy-induced hypoxic microenvironments [56]. Our study data suggest that the use of metronomic chemotherapy with either temozolomide or etoposide in combination with continued bevacizumab is not an effective strategy to overcome bevacizumab resistance in recurrent GBM patients.

Although bevacizumab therapy improves progression-free survival as well as overall survival for recurrent GBM patients, essentially all patients ultimately relapse and effective salvage therapies following progression on bevacizumab-based therapies are desperately needed. Our study results, although limited by small sample size, suggest that metronomic chemotherapy with either daily temozolomide or etoposide when combined with continued bevacizumab is not an effective strategy for recurrent GBM patients who have progressed after bevacizumab combined with chemotherapy. It is possible that our study regimen may

benefit patients who have progressed after bevacizumab monotherapy since our study participants had all progressed after bevacizumab and chemotherapy. Further investigation of alternative strategies to overcome resistance to anti-VEGF therapies should be highly prioritized.

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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

Fig. 1.

a Progression-free survival by treatment arm. **b** Overall survival by treatment arm

Table 1
Patient characteristics

	Temozolomide Arm (<i>n</i> = 10)	Etoposide arm (<i>n</i> = 13)	All patients (<i>n</i> = 23)
Age, median (years)	50.8	52.4	52.4
Gender, male	7 (70)	11 (85)	18 (78)
Diagnosis			
Glioblastoma	10 (100)	12 (92)	22 (96)
Gliosarcoma		1 (8)	1(4)
Karnofsky performance			
90–100	6 (60)	7 (54)	13 (57)
70–80	4 (40)	6 (46)	10 (43)
Time from original diagnosis (weeks)			
Median	76.4	64.3	65.7
Range	44.4–217.9	39.1–159.1	39.1–217.9
Number prior PD			
1	0	2 (15)	4 (17)
2	6 (60)	4 (31)	10 (43)
3	2 (20)	6 (46)	8 (35)
>3	2 (20)	1 (8)	3 (13)
BV regimen with PD at enrollment			
BV monotherapy	0	0	0
BV + irinotecan	7 (70)	12 (92)	19 (83)
BV + 5-day temozolomide	0	0	0
BV + carboplatin + irinotecan	2 (20)	0	2 (9)
BV + temozolomide + irinotecan	1 (10)	1 (8)	2 (9)
Number prior BV regimens with PD prior to enrollment			
1	7 (70)	12 (92)	19 (83)
2	1 (10)	1 (8)	2 (9)
3	2 (20)	0	2 (9)

Numbers in parentheses refer to percentages unless otherwise indicated. *BV* bevacizumab, *PD* progressive disease

Table 2
Number of patients with attributable adverse events grade ≥ 2 per study arm

Study arm	Temozolomide				Etoposide			
	2	3	4		2	3	4	
Event								
Diarrhea					1			
Fatigue	1				2	2		
Infection		1						
Mucositis					2			
Nausea					1			
Neutropenia							1	
Rash					1			

Table 3
Summary of outcome by treatment arm

Study arm	Temozolomide	Etoposide	All
Number of patients	10	13	23
6 month PFS,	0	7.7	4.4
median (%) 95% CI		4.8, 29.2	3.1, 18.2
PFS, median	4.1	8.1	7.3
(weeks) 95% CI	3.0, 7.9	4.1, 12.0	4.0, 8.3
Overall survival, median	12.6	19.0	16.4
(weeks) 95% CI	4.6, 23.3	11.0, 25.7	11.0, 23.3
Radiographic response			
Complete (%)	0	0	0
Partial (%)	0	0	0
Stable disease (%)	4 (40)	8 (62)	12 (52)
Progressive disease (%)	6 (60)	4 (31)	10 (43)
Not evaluable	0	1 (8)	1 (4)

Median overall survival could not be computed because survival probability remained at or above 50%. *CI* confidence interval, *PFS* progression-free survival

Table 4
Patterns of progression on prior bevacizumab (BV) therapy and on study therapy

	Temozolomide arm (n = 10)		Etoposide arm (n = 13)	
	Prior BV therapy	Current study	Prior BV therapy	Current study
Local				
	Enhancing + non-enhancing	7	7	9
	Non-enhancing	0	0	0
Diffuse				
	Enhancing + non-enhancing	2	3	1
	Non-enhancing	1	2	2
Distant				
	Enhancing + non-enhancing	0	1	0
	Non-enhancing	0	0	0
Clinical decline	0	1 ^a	0	0
No PD to date	0	0	0	1

^aThis patient did not undergo MRI assessment but discontinued study therapy due to progressive clinical decline attributable to recurrent tumor