Neuro-Oncology 15(2):242–250, 2013.
doi:10.1093/neuonc/nos295
Advance Access publication December 14, 2012

Phase II trial of continuous low-dose temozolomide for patients with recurrent malignant glioma

Antonio Omuro, Timothy A. Chan, Lauren E. Abrey, Mustafa Khasraw, Anne S. Reiner, Thomas J. Kaley, Lisa M. DeAngelis, Andrew B. Lassman, Craig P. Nolan, Igor T. Gavrilovic, Adilia Hormigo, Cynthia Salvant, Adriana Heguy, Andrew Kaufman, Jason T. Huse, Katherine S. Panageas, Andreas F. Hottinger, and Ingo Mellinghoff

Departments of Neurology (A.O., L.E.A., M.K., T.J.K., L.M.D., A.B.L., C.P.N., I.T.G., A.H., C.S., A.F.H., I.M.), Radiation Oncology (T.A.C.), Human Oncology and Pathogenesis Program (T.A.C., A.H., A.K., I.M.), Epidemiology and Biostatistics (A.S.R., K.S.P.), Pathology (J.T.H.), Memorial Sloan-Kettering Cancer Center, New York, New York

Present affiliation: Royal Melbourne and Geelong Hospitals, Australia (M.K.); University of Zurich, Switzerland (L.E.A.); Columbia University Medical Center, New York, New York (A.B.L.); University of Rochester Medical Center, Rochester, New York (A.H.); Centre Hospitalier Universitaire Vaudois and University of Lausanne, Switzerland (A.F.H.); Upstate Medical University, Syracuse, New York (S.C.)

Background. In this phase II trial, we investigated the efficacy of a metronomic temozolomide schedule in the treatment of recurrent malignant gliomas (MGs).

Methods. Eligible patients received daily temozolomide (50 mg/m^2) continuously until progression. The primary endpoint was progression-free survival rate at 6 months in the glioblastoma cohort ($N = 37$). In an exploratory analysis, 10 additional recurrent grade III MG patients were enrolled. Correlative studies included evaluation of 76 frequent mutations in glioblastoma (iPLEX assay, Sequenom) aiming at establishing the frequency of potentially "drugable" mutations in patients entering recurrent MG clinical trials.

Results. Among glioblastoma patients, median age was 56 y; median Karnofsky Performance Score (KPS) was 80; 62% of patients had been treated for $\geq$2 recurrences, including 49% of patients having failed bevacizumab. Treatment was well tolerated; clinical benefit (complete response + partial response + stable disease) was seen in 10 (36%) patients. Progression-free survival rate at 6 months was 19% and median overall survival was 7 months. Patients with previous bevacizumab exposure survived significantly less than bevacizumab-naive patients (median overall survival: 4.3 mo vs 13 mo; hazard ratio = 3.2; $P = .001$), but those patients had lower KPS ($P = .04$) and higher number of recurrences ($P < .0001$). Mutations were found in 13 of the 38 MGs tested, including mutations of *EGFR* ($N = 10$), *IDH1* ($N = 5$), and *ERBB2* ($N = 1$).

Conclusions. In spite of a heavily pretreated population, including nearly half of patients having failed bevacizumab, the primary endpoint was met, suggesting that this regimen deserves further investigation. Results in bevacizumab-naive patients seemed particularly favorable, while results in bevacizumab-failing patients highlight the need to develop further treatment strategies for advanced MG.

Clinical trials.gov identifier. NCT00498927 (available at http://clinicaltrials.gov/ct2/show/NCT00498927)

Keywords: bevacizumab, glioblastoma, malignant glioma, metronomic chemotherapy, temozolomide.

Received August 13, 2012; accepted October 17, 2012.

Corresponding Author: Antonio Omuro, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10065 (omuroa@mskcc.org).

Radiotherapy with concomitant temozolomide followed by adjuvant temozolomide is the standard of care for newly diagnosed glioblastoma,[1] but the optimal regimen for recurrent disease has not been defined. Clinical trials in recurrent glioblastoma have

reported dismal outcomes, with objective response rates (ORRs) of 5%–6%, 6-month progression-free survival rates (PFS6) of 9%–21%, and median overall survival (OS) of 6–7 months.[2–6] Treatment with numerous targeted and nontargeted agents has been attempted without success, with the exception of bevacizumab.[7–9] This agent received accelerated FDA approval for recurrent glioblastoma, based on improved ORRs of 20%–26% seen in 2 phase II trials.[8,9] However, responses tend to be short-lived (median: 4 months), and OS has been modest (median: 8–10 months).

Metronomic temozolomide has emerged as a well-tolerated salvage approach in recurrent glioblastoma.[10–15] Preclinical studies have suggested that metronomic temozolomide may result in increased activity through depletion of the O^6-methylguanine-DNA methyltransferase (MGMT) protein[16] and anti-angiogenic properties through targeting of endothelial cells.[17,18] A phase II study[19] using metronomic, daily temozolomide (50 mg/m^2/d) for glioblastoma at first relapse investigated 3 preestablished cohorts: patients with progression before completion of 6 adjuvant cycles ("early" group), patients with progression after 6 cycles while still on temozolomide ("extended" group), and patients who discontinued temozolomide after completing 6 cycles without progression ("rechallenge" group). The extended group did not seem to benefit (PFS6: 7%), but the early and rechallenge groups achieved PFS6 of 27% and 36%, respectively. However, these results are difficult to interpret because there are no historical controls for either of these cohorts. Moreover, results in the early group could have represented inclusion of patients with pseudoprogression, while patients in the rechallenge group could have harbored tumors with a different biology. It is also unclear how these results apply to current treatment trends in the United States, where temozolomide tends to be continued beyond 6 cycles and the use of bevacizumab is frequent.

We report a phase II trial using a similar regimen of temozolomide 50 mg/m^2 daily but encompassing eligibility criteria similar to available historical controls and typical clinical trials in glioblastoma,[4,20] with no restriction on timing of failure with respect to previous temozolomide use. We also performed an exploratory, comprehensive disease-specific mutational analysis to characterize the typical population of patients entering studies of recurrent malignant gliomas (MGs) from a molecular perspective.

Patients and Methods

This was a prospective, phase II trial conducted at Memorial Sloan-Kettering Cancer Center from July 2007 to April 2010. The main cohort comprised patients with progressive/recurrent glioblastoma; patients with recurrent World Health Organization grade III anaplastic astrocytoma, anaplastic oligoastrocytoma, or anaplastic oligodendroglioma were enrolled in a separate, exploratory cohort.

Inclusion criteria consisted of histologically confirmed diagnosis of MG, previous exposure to temozolomide and radiotherapy, unequivocal tumor recurrence at enrollment, age ≥18 years, Karnofsky Performance Score (KPS) ≥60, and adequate bone marrow and organ function. Patients with other, concurrent active malignancy or serious medical illness were excluded. There was no limit on number of previous recurrences or chemotherapy regimens.

Treatment consisted of temozolomide given at a dose of 50 mg/m^2 daily, without interruption, until progression or development of unacceptable side effects. One cycle was defined as 28 days. Anti-emetic therapy and pneumocystosis prophylaxis were prescribed at the discretion of treating physicians. Treatment was held for toxicity grades ≥3. Once resumed, the dose of temozolomide could be reduced by 25% as clinically indicated, but if further reductions were necessary, patients were removed from the study. Complete blood counts were repeated weekly, and comprehensive chemistry panels were obtained monthly. MRIs and clinical exams were performed every 2 months. Responses were evaluated using Macdonald criteria.[21] The study was approved by the institutional review board; written informed consent was obtained from all patients.

Statistics

The primary endpoint was PFS6 in glioblastoma patients. A Simon 2-stage design was used. In the first stage, 12 patients were accrued. If at least one of those patients did not progress at the 6-month mark, then an additional 25 patients (for a total of 37) would be accrued to the second stage; otherwise, the trial would be terminated. At the end of the trial, if fewer than 4 patients were progression free at 6 months, the study would be declared negative. This design yields at least a .90 probability of a positive result if the true PFS6 was at least 20% and a .90 probability of a negative result if the true PFS6 was 5%.

Secondary endpoints were median PFS, OS, ORR, and toxicity as per the National Cancer Institute's Common Terminology Criteria of Adverse Events version 3.0. Survival was calculated from registration date (Kaplan–Meier methodology), and log-rank statistic was utilized for group comparisons. An additional 10 patients with grade III MG were enrolled in an exploratory cohort, and a descriptive analysis was performed.

MGMT Promoter Methylation Status and Evaluation of Relevant Glioblastoma Mutations

Available formalin-fixed paraffin-embedded (FFPE) tissue samples were evaluated for MGMT promoter methylation status by real-time methylation-specific PCR as described previously,[22] performed by MDxHealthC. FFPE samples were also subjected to a comprehensive mutation screening using mass spectrometry genotyping/iPLEX assay (Sequenom) in genomic DNA, looking for a panel of 76 mutations described in glioblastoma (Supplementary material, Appendix S1). The iPLEX assay was performed as described by the manufacturer (Supplementary material, Appendix S2). Primers are listed in Supplementary material, Appendix S3.

Results

Patient Characteristics

Accrual was completed as planned, with all 37 glioblastoma patients enrolled, constituting the main study cohort. Ten additional grade III recurrent MG patients were accrued to the exploratory cohort. Patient characteristics are summarized in Table 1.

Among the glioblastoma patients, 32 (86%) had recurrence of a de novo glioblastoma and 5 (14%) had recurrent secondary glioblastoma (ie, initial diagnosis was of a grade 2 or 3 glioma, which recurred as a glioblastoma histologically proven).

Patients were heavily pretreated by the time of enrollment (Table 1). All glioblastoma patients had received radiotherapy with concomitant temozolomide. Thirty (81%) patients had a history of tumor progression diagnosed during adjuvant temozolomide, and 7 (19%) had never progressed during temozolomide, having previously received a median of 12 cycles. The number of prior recurrences was 1 in 14 (38%) patients, 2 in 11 (30%), and 3 or more in 12 (32%). Other prior chemotherapies consisted of bevacizumab in 18 (49%) patients, other cytotoxic agents in 9 (24%), and experimental targeted therapies in 8 (21%). In all patients with bevacizumab exposure, bevacizumab had been given as salvage treatment, and all of those patients experienced progression while on bevacizumab.

Glioblastoma Cohort—Response, PFS, and OS

Patients who had measurable tumor and completed at least one cycle of treatment were considered evaluable for response ($N = 28$). A partial response (PR) was seen in 3 (11%) patients, stable disease (SD) in 7 (25%), and progressive disease (PD) in 18 (64%); clinical benefit (CR + PR + SD) was seen in 10 (36%) patients. Among the 9 patients considered nonevaluable for response, 1 had no measurable disease due to surgical resection and 8 did not complete the first cycle.

All patients ($N = 37$) were included in the PFS and OS analysis, in an intent-to-treat fashion. A total of 7 patients were progression free and alive at 6 months, and

Table 1. Patient characteristics

	Glioblastoma ($N = 37$)	Grade III Malignant Gliomas ($N = 10$)
Gender		
Men	12 (32%)	6 (60%)
Women	25 (68%)	4 (40%)
Median age (range)	56 (31–81)	58 (29–73)
Median KPS (range)	80 (60–100)	80 (60–100)
Histology at time of enrollment		
De novo glioblastoma	32 (86%)	–
Secondary glioblastoma	5 (14%)	–
Grade III anaplastic astrocytoma	–	4 (40%)
Grade III anaplastic oligodendroglioma	–	5 (50%)
Grade III anaplastic oligoastrocytoma	–	1 (10%)
Median time between current histologic diagnosis and enrollment (range)	20 mo (0.3–58)	18 mo (0.5–177)
Number of previous recurrences		
1	14 (38%)	4 (40%)
2	11 (30%)	3 (30%)
$\geq$3	12 (32%)	3 (30%)
Prior salvage therapies		
Bevacizumab	18 (49%)	0
Other cytotoxic agents	9 (24%)	4 (40%)
Experimental targeted therapies	8 (21%)	3 (30%)
Progression while on previous adjuvant temozolomide		
Progression during first 6 adjuvant cycles	21 (57%)	NA
Progression after >6 adjuvant cycles	9 (24%)	NA
Completed adjuvant cycles without progression	7 (19%; median of 12 adjuvant cycles)	NA
MGMT promoter methylation status		
Methylated	3 (8%)	2 (20%)
Unmethylated	20 (54%)	3 (30%)
Invalid MGMT results	10 (27%)	4 (40%)
Tissue unavailable	4 (11%)	1 (10%)

Abbreviation: NA, not applicable to grade 3 tumors (patients received variable chemotherapy regimens at diagnosis).

therefore the primary endpoint was met. PFS6 was 19% ($N = 37$; 95% confidence interval [CI]: 6–32), and median PFS was 2 months (95% CI: 1–4) (Fig. 1). Median OS was 7 months (95% CI: 5–12), and 1-year OS was 35% (95% CI: 20–51) (Fig. 1). Median follow-up for survivors was 19 months.

Given the previously reported poor prognosis observed after bevacizumab discontinuation/failure in retrospective studies,[23–27] we analyzed the impact of prior bevacizumab exposure on outcomes. Patients with bevacizumab failure survived significantly less (median OS: 4.3 months; 6-months OS: 28%) than bevacizumab-naive patients (median OS: 13 months; 6-months OS: 84%); hazard ratio (HR) = 3.2; $P = .001$ (Fig. 2). PFS6 in the bevacizumab-failure group was 11.1% versus 26.3% in the bevacizumab-naive group ($P = .07$) (Fig. 3). Responses were seen in 3/15 (20%) evaluable bevacizumab-naive patients, and no responses were observed in the bevacizumab-failure group. Among the bevacizumab-naive group, bevacizumab was given to 9 (47%) patients after progression on this trial.

In an exploratory fashion, we sought to investigate whether differences in pretreatment characteristics could explain the differences in outcomes according to previous bevacizumab exposure (Supplementary material, Appendix S4). In a post-hoc analysis, we evaluated the distribution of potential prognostic factors in bevacizumab-naive versus bevacizumab-failure patients (categorical: Fisher's exact test; continuous: Kruskal–Wallis test) and performed a multivariate analysis using a forward stepwise Cox proportional hazards regression model with entry and exit criteria of $\alpha = 0.20$ (Supplementary material, Appendix S4). Bevacizumab-failure patients had a higher number of previous progressions/chemotherapy regimens ($P < .0001$) and lower KPS ($P = .04$) than bevacizumab-naive patients. The median time from glioblastoma diagnosis was 21 months in bevacizumab-failure versus 13 months for bevacizumab-naive patients ($P = .18$). Multivariate stepwise analysis suggested that the driving variable for decreased PFS was number of progressions/salvage chemotherapies ($P = .02$), favored over other candidate variables, including bevacizumab exposure. Multivariate analysis of OS showed that number of progressions/salvage chemotherapies ($P = .05$) and KPS ($P = .05$), but not prior bevacizumab exposure, were the driving variables for differences in survival.

There were no differences in outcome according to previous history of progression on temozolomide (PFS: $P = .58$; OS: $P = .18$) or according to *MGMT* promoter methylation status (PFS: $P = .76$; OS: $P = .14$), although analysis is limited by the small number of patients in whom this could be determined accurately (Table 1).

Grade III Recurrent Malignant Glioma Exploratory Cohort

Among the 10 patients with grade III MG, PFS6 was 30% (95% CI: 1.6–58.4); median PFS was 3 months. Median OS was 18 months; 1-year OS was 60% (95%

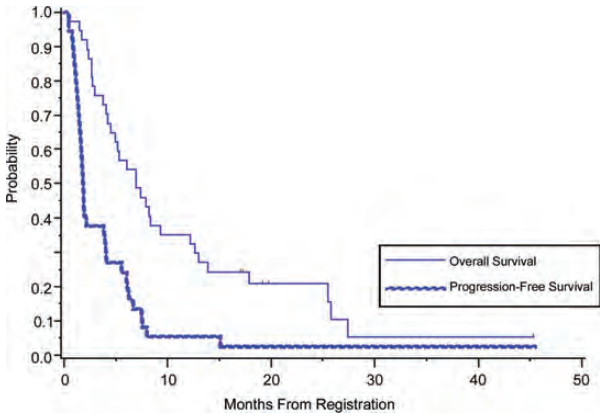

Fig. 1. OS and PFS (glioblastoma cohort, $N = 37$). PFS6: 19% (95% CI: 6–32); median PFS: 2 months; 1-year OS: 35%; median OS: 7 months.

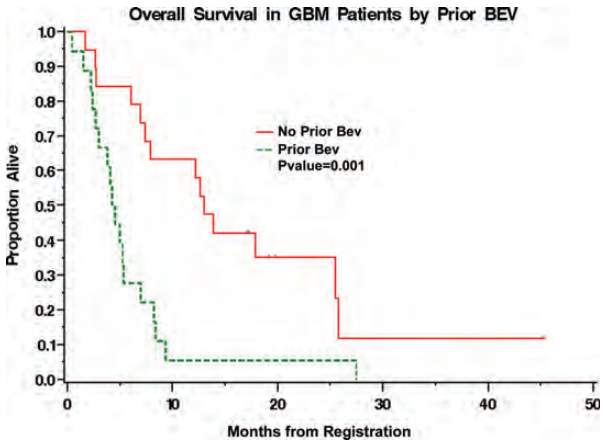

Fig. 2. Glioblastoma cohort ($N = 37$): OS according to previous history of bevacizumab (BEV) treatment. Patients with previous BEV failure: median OS: 4.3 mo, 6-mo OS: 28% (95% CI: 7–48); patients without BEV failure: median OS: 13 mo; 6-month OS: 84% (95% CI: 68–100); HR = 3.2; $P = .001$.

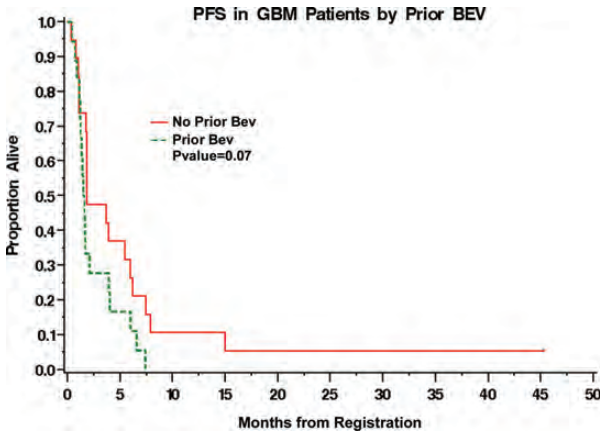

Fig. 3. Glioblastoma cohort ($N = 37$): PFS according to previous bevacizumab (BEV) treatment. Patients with previous BEV failure: PFS6: 11.1% (95% CI: 0.0–25.6), median PFS: 1.5 mo; patients without bevacizumab failure: PFS6: 26.3% (95% CI: 6.5–46.1), median PFS: 1.8 mo; HR = 1.9; $P = .07$.

CI: 30–90). One patient achieved PR, 4 achieved SD, and 5 achieved PD.

Toxicity

Treatment was well tolerated; no patient discontinued treatment as a result of toxicity, and there were no toxic deaths. Lymphopenia was the most frequent toxicity (grade 3 = 10 patients, grade 4 = 1), but no lymphopenia-related infection was reported. The only other grade 4 toxicity was thrombocytopenia ($n = 1$). Grade 3 toxicities consisted of increased alanine aminotransferase ($n = 2$), fatigue (2), hyponatremia (2), hypokalemia (1), pulmonary embolism (1), neuropathy (1), and infection (1).

Mutational Analysis

In an exploratory analysis, we evaluated FFPE tissue from 38 patients (28 glioblastomas and 10 grade III tumors) for the presence of 76 mutations previously described in gliomas using mass spectrometry genotyping/iPLEX assay (Sequenom [Supplementary material, Appendix S1]). A total of 9 different mutations was found in 13 patients (Table 2), including 8 (29%) glioblastomas and 5 (50%) grade III MGs. Mutations were found in *EGFR* ($N = 10$), *IDH1* ($N = 5$), and *ERBB2* ($N = 1$). All *IDH1* mutations were found in grade III ($N = 3$) or secondary glioblastomas ($N = 2$) and none in recurrent primary glioblastomas. *EGFR* extracellular-domain mutations were found in both glioblastomas ($N = 7$) and grade III gliomas ($N = 3$). None of the analyzed mutations involving phosphatidylinositol 3-kinase (PI3K), BRAF, or KRAS was found. Table 2 summarizes the mutations found, along with patient characteristics and treatment outcomes.

Discussion

This study was designed to test the efficacy of metronomic temozolomide in the typical population enrolled in clinical trials for recurrent glioblastoma, irrespective of prior treatments. The primary efficacy endpoint (based on pre–bevacizumab-era historical controls) was met, with 19% PFS6, despite the inclusion of a heavily pretreated population, including nearly half of the patients with a history of bevacizumab failure. This regimen was therefore deemed active, warranting further investigation.

Enrollment to this trial coincided with the rise of bevacizumab for glioblastoma salvage therapy in the United States. Although this confounded the interpretation of our results in comparison with historical controls, it also provided the unique opportunity to evaluate the same cytotoxic regimen in patients who were bevacizumab naive or had failed bevacizumab. Results in bevacizumab-naive glioblastoma seemed particularly favorable (PFS6: 26%, median OS: 13 mo) and comparable to other active salvage treatments; activity was also observed in grade III tumors. Given the excellent toxicity profile and ease of drug administration, this regimen could potentially constitute a viable alternative to bevacizumab and nitrosoureas as second-line treatment for glioblastoma and a suitable platform for combination with targeted agents. Conversely, bevacizumab-failure patients achieved a much shorter PFS6 of 11% and median OS of 4.3 months, highlighting the need for alternative therapies in this population.

Several phase II trials testing intensified temozolomide schedules have been conducted (Table 3), but the optimal regimen remains to be defined. A direct comparison across trials is difficult, given the varying inclusion criteria and study settings, but overall it appears that signs of activity were observed irrespective of the regimen used. A regimen of comparable efficacy used temozolomide 150 mg/m^2 given 7/14 days; toxicities were more frequent, likely reflecting the 150% greater cumulative dose of this agent in comparison with our regimen.[13] It must also be noted that an intensified adjuvant temozolomide schedule (75–100 mg/m^2 × 21 d q 4 wk) failed to improve survival in a recent phase III trial in newly diagnosed glioblastoma,[28] although those results may not be applicable to recurrent disease, given potential differences in chemosensitivity and patient selection. Dedicated randomized trials are thus needed to define the role and optimal metronomic temozolomide schedule in recurrent glioblastoma. Interestingly, results of trials combining daily temozolomide and bevacizumab[29,30] (Tables 3 and 4) were disappointing and comparable or inferior to our single-agent results. This raises the intriguing question of whether bevacizumab could decrease the activity of temozolomide when given at low doses, perhaps by decreasing drug penetration.[31–34]

To our knowledge, this is the first prospective study reporting outcomes in bevacizumab-failure patients in whom bevacizumab was discontinued. Because of fears of a rebound effect, bevacizumab is often continued despite progression on the drug.[23–27,35] Three small prospective studies in bevacizumab-failure patients used continued bevacizumab with different cytotoxic agents and found a median OS of 3–6 months (Table 4).[9,36,37] The most favorable results were observed with combined bevacizumab, carboplatin, and irinotecan (PFS6: 16%; median OS: 6 mo),[37] but 48% of patients in that study had failed bevacizumab as part of first-line treatment, whereas the other studies included patients failing salvage bevacizumab; moreover, that same drug combination was not active in bevacizumab-naive patients.[38] Therefore, outcomes of continued bevacizumab regimens were poor and not superior to our results, suggesting that it is justifiable to discontinue bevacizumab after bevacizumab failure[39,40] and that conducting clinical trials in that setting is feasible. Interestingly, our multivariate analysis suggested that the poor outcomes in bevacizumab failures seemed to be at least partially explained by poor KPS and greater number of progressions (Supplementary material, Appendix S4), although the possibility that bevacizumab exposure may induce a more aggressive, invasive phenotype[41,42] cannot be entirely ruled out.

Table 2. Mutations found in the patients enrolled in the trial with corresponding patient characteristics and clinical outcomes ($N = 38$)

Pt	Path	MGMT	Time from Diagnosis (mo)	Prior BEV	Response	PFS (mo)	OS (mo)
EGFR_P596L							
1	AA	Unmethylated	0.6	No	PD	1.63	7.33
2	GBM	Invalid	30	Yes	NE	0.37	0.37
3	GBM	Invalid	22	No	SD	7.4	17.2
4[a]	AO	Invalid	1.6	No	PD	1.83	8.9
5	GBM	Invalid	55	No	PD	1.9	19.8
EGFR_C620Y							
6	GBM	Unmethylated	17	Yes	SD	5.93	8.2
EGFR_G598V							
7	GBM	Unmethylated	31	Yes	PD	1.5	2.4
EGFR_I91L							
8	GBM	Unmethylated	17	Yes	NE	1.2	2.6
EGFR_T263P							
9	AA	Methylated	26	No	SD	8.03	15.57
EGFR_V651M							
10[a]	GBM (secondary)	Methylated	22	No	PR	5.47	12.63
IDH1_R132L_H							
11	AO	Methylated	163	No	SD	1.77	3.13
4[a]	AO	Invalid	1.6	No	PD	1.83	8.9
12	AO	Invalid	25	No	SD	6.9	21.1
10[a]	GBM (secondary)	Methylated	22	No	PR	5.47	12.63
IDH1_R132C_G_S							
13	GBM (secondary)	Unmethylated	10	No	NE	0.4	1.7
ERBB2_L49H							
10[a]	GBM (secondary)	Methylated	22	No	PR	5.47	12.63

Abbreviations: Pt, patient; Path, most recent pathology prior to enrollment; BEV, bevacizumab.
[a]Patients with >1 mutation found.

Table 3. Phase II trials of metronomic temozolomide schedules for glioblastoma

Reference	*N*	TMZ Daily Dose, Schedule	ORR (%)	PFS6 (%)	Median OS (mo)
Khan[11]	27	75 mg/m^2, 42/70 d	14	27	8
Brandes[12]	33	75 mg/m^2, 21/28 d	9	30	10
Wick[13]	64	150 mg/m^2; 7/14 d	15	44	9
Perry[10]		50 mg/m^2, 28/28 d			
	33	*Early progression on adjuvant TMZ*	3	27	NA
	27	*Progression on extended TMZ*	0	7	NA
	28	*Rechallenge*	11	36	NA
Kong[15]	38	40–50 m^2, 28/28 d	5	32	13
Abacioglu[14]	16	100 mg/m^2, 21/28 d	7	25	7
Verhoeff[30]	15	50 mg/m^2, 28/28 d + bevacizumab 10 mg/kg q 21 d	NA	7	4
Desjardins[29]	32	50 mg/m^2, 28/28 d + bevacizumab 10 mg/kg q 14 d	28	19	6
This study	37	50 mg/m^2, 28/28 d			
		All patients	11	19	7
		Bevacizumab failures	0	11	4
		Bevacizumab naive	20	26	13

Abbreviations: TMZ, temozolomide; ORR, objective response rate (complete + partial responses); NA, not available.

Finally, we performed a Sequenom-based tissue analysis in order to characterize the frequency of glioma-associated mutations[43] within patients who enroll in clinical trials for recurrent disease. A total of 13 (38%) patients displayed one or more mutations involving the *EGFR*, *IDH1*, and *ERBB2* genes, but overall the

Table 4. Outcomes of clinical trials conducted in recurrent glioblastoma patients with history of bevacizumab (BEV) failure

Reference	Regimen	*N*	Patients who had Failed BEV as Part of First-Line Treatment	PFS6 (%)	Median OS (mo)	ORR
Reardon[36]	BEV + daily TMZ 50 mg/m^2	10	0	0	3	0
	BEV + VP-16 50 mg/m^2 (3 wk on/1 wk off)	13	15%	8	4.7	0
	All pts	23	15%	4	4.1	0
Reardon[37]	BEV + carboplatin + CPT-11	25	48%	16	5.8	0
Kreisl[9]	BEV + CPT-11	19	0	≤5[a]	NA	0
This study	Daily TMZ 50 mg/m^2, no BEV	18	0	11	4.3	0

Abbreviations: ORR, objective response rate (complete + partial responses); TMZ, temozolomide; VP-16, etoposide; NA, not available.
[a]Only 1 patient received >3 cycles.

individual frequency of tested mutations was low. The oncogenic role of some of the identified extracellular-domain *EGFR* mutations has been validated in functional assays,[44,45] and testing EGFR inhibitors in those patients could be of interest. However, none of the 33 mutations involving the PI3K complex was found, suggesting that a very large number of recurrent patients would have to be screened in order to study a PI3K inhibitor in a trial restricted to PI3K mutated tumors. In addition, the wide variability in outcome observed in patients with *IDH1* mutations was unexpected. These mutations have been reported to confer a better prognosis,[46] but the outcome of these patients in this trial was variable (OS: 3–21 mo), likely influenced by pretreatment characteristics such as time from initial diagnosis and previous therapies (Table 2). This suggests that even in trials for molecularly selected tumors, further patient selection and stratification based on pretreatment clinical characteristics may be necessary, which will add to the feasibility challenges that such trials will face. Of note, Sequenom-based methodology requires that point mutations be chosen and prespecified; other panels have been proposed,[47,48] including mutations that are different from the ones we tested. The adoption of next-generation sequencing may overcome this limitation in the future.

A few other limitations apply to this study. Our multivariate analysis was performed post hoc and was based on a relatively small number of patients. We used Macdonald criteria for assessment of response, which allows for a comparison with historical controls; criteria of the Revised Assessment of Neuro-Oncology[49] have since been proposed and may define progression earlier. The median time between diagnosis and enrollment was unusually long (21 mo), although the longest times were observed among bevacizumab-failure patients, who fared poorly; therefore, selection bias does not seem to explain the activity observed.

In summary, we provide further evidence that daily-dose temozolomide is a safe and active regimen in recurrent glioblastoma. We also demonstrate the differences in outcomes between bevacizumab-naive and bevacizumab-failure patients in the prospective setting and provide historical controls that can be used for sample-size calculation and clinical trial interpretation in bevacizumab-failure patients. Our findings support the development of separate trials or stratification according to bevacizumab exposure but also highlight the prognostic importance of other variables, such as number of previous recurrences and KPS, for that purpose. Importantly, our results in bevacizumab failures were comparable to trials of continuation of bevacizumab after progression, suggesting that one should not fear discontinuing bevacizumab for the purposes of pursuing alternative treatments or clinical trials.

Supplementary Material

Supplementary material is available at *Neuro-Oncology Journal* online (http://neuro-oncology.oxfordjournals.org/).

Acknowledgments

Presented in part at the American Society of Clinical Oncology (ASCO) annual meeting 2010 and Society for Neuro-Oncology (SNO) annual meeting 2010.

Conflict of interest statement. L. E. A. is currently an employee of Hoffmann–La Roche. M.K. has received honoraria from Roche. A. O. has received compensation for consulting from Roche. A. B. L. has received compensation for consulting from Merck/Schering-Plough, BMS, Campus Bio, Cephalon, Eisai, Genentech, GSK, Imclone, Sigma Tau, and Abbott and reports research funding from Merck/Schering-Plough, Genentech, Keryx, Sigma Tau, Astra Zeneca, Medimmune, and Boehringer Ingelheim.

Funding

This study was an investigator-initiated clinical trial funded by the Memorial Sloan-Kettering Cancer Center Department of Neurology Research and Development Fund. Schering-Plough/Merck provided drug, partial financial support, and analysis of *MGMT* promoter methylation status.

References

1. 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.
2. Chang SM, Lamborn KR, Kuhn JG, et al. Neurooncology clinical trial design for targeted therapies: lessons learned from the North American Brain Tumor Consortium. *Neuro Oncol*. 2008;10:631–642.
3. Yung WK, Albright RE, Olson J, et al. A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. *Br J Cancer*. 2000;83:588–593.
4. Wong ET, Hess KR, Gleason MJ, et al. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol*. 1999;17:2572–2578.
5. Lamborn KR, Yung WK, Chang SM, et al. Progression-free survival: an important end point in evaluating therapy for recurrent high-grade gliomas. *Neuro Oncol*. 2008;10:162–170.
6. Ballman KV, Buckner JC, Brown PD, et al. 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 Oncol*. 2007;9:29–38.
7. Vredenburgh JJ, Desjardins A, Herndon JE, II, et al. Phase II trial of bevacizumab and irinotecan in recurrent malignant gliomas. *Clin Cancer Res*. 2007;13:1253–1259.
8. Friedman HS, Prados MD, Wen PY, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol*. 2009;27:4733–4740.
9. Kreisl TN, Kim L, Moore K, et al. 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.
10. Perry JR, Belanger K, Mason WP, et al. Phase II trial of continuous dose-intense temozolomide in recurrent malignant glioma: RESCUE study. *J Clin Oncol*. 2010;28:2051–2057.
11. Khan RB, Raizer JJ, Malkin MG, Bazylewicz KA, Abrey LE. A phase II study of extended low-dose temozolomide in recurrent malignant gliomas. *Neuro Oncol*. 2002;4:39–43.
12. Brandes AA, Tosoni A, Cavallo G, et al. Temozolomide 3 weeks on and 1 week off as first-line therapy for recurrent glioblastoma: phase II study from Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO). *Br J Cancer*. 2006;95:1155–1160.
13. Wick A, Felsberg J, Steinbach JP, et al. Efficacy and tolerability of temozolomide in an alternating weekly regimen in patients with recurrent glioma. *J Clin Oncol*. 2007;25:3357–3361.
14. Abacioglu U, Caglar HB, Yumuk PF, Akgun Z, Atasoy BM, Sengoz M. Efficacy of protracted dose-dense temozolomide in patients with recurrent high-grade glioma. *J Neurooncol*. 2011;103:585–593.
15. Kong DS, Lee JI, Kim JH, et al. Phase II trial of low-dose continuous (metronomic) treatment of temozolomide for recurrent glioblastoma. *Neuro Oncol*. 2010;12:289–296.
16. Tolcher AW, Gerson SL, Denis L, et al. Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules. *Br J Cancer*. 2003;88:1004–1011.
17. Kurzen H, Schmitt S, Naher H, Mohler T. Inhibition of angiogenesis by non-toxic doses of temozolomide. *Anticancer Drugs*. 2003;14:515–522.
18. Kim JT, Kim JS, Ko KW, et al. Metronomic treatment of temozolomide inhibits tumor cell growth through reduction of angiogenesis and augmentation of apoptosis in orthotopic models of gliomas. *Oncol Rep*. 2006;16:33–39.
19. Franceschi E, Omuro AM, Lassman AB, Demopoulos A, Nolan C, Abrey LE. Salvage temozolomide for prior temozolomide responders. *Cancer*. 2005;104:2473–2476.
20. Omuro AM, Faivre S, Raymond E. Lessons learned in the development of targeted therapy for malignant gliomas. *Mol Cancer Ther*. 2007;6:1909–1919.
21. 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.
22. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005;352:997–1003.
23. Iwamoto FM, Abrey LE, Beal K, et al. Patterns of relapse and prognosis after bevacizumab failure in recurrent glioblastoma. *Neurology*. 2009;73:1200–1206.
24. Lu-Emerson C, Norden AD, Drappatz J, et al. Retrospective study of dasatinib for recurrent glioblastoma after bevacizumab failure. *J Neurooncol*. 2011;104:287–291.
25. Norden AD, Young GS, Setayesh K, et al. Bevacizumab for recurrent malignant gliomas: efficacy, toxicity, and patterns of recurrence. *Neurology*. 2008;70:779–787.
26. Quant EC, Norden AD, Drappatz J, et al. Role of a second chemotherapy in recurrent malignant glioma patients who progress on bevacizumab. *Neuro Oncol*. 2009;11:550–555.
27. Torcuator RG, Thind R, Patel M, et al. The role of salvage reirradiation for malignant gliomas that progress on bevacizumab. *J Neurooncol*. 2010;97:401–407.
28. Gilbert MR, Wang M, Aldape KD, et al. RTOG 0525: a randomized phase III trial comparing standard adjuvant temozolomide (TMZ) with a dose-dense (dd) schedule in newly diagnosed glioblastoma (GBM). *ASCO Meeting Abstracts*. 2011;29:2006.
29. Desjardins A, Reardon DA, Coan A, et al. Bevacizumab and daily temozolomide for recurrent glioblastoma. *Cancer*. 2011;118:1302–1312.
30. Verhoeff JJ, Lavini C, van Linde ME, et al. Bevacizumab and dose-intense temozolomide in recurrent high-grade glioma. *Ann Oncol*. 2010;21:1723–1727.
31. 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.
32. Thompson EM, Frenkel EP, Neuwelt EA. The paradoxical effect of bevacizumab in the therapy of malignant gliomas. *Neurology*. 2011;76:87–93.
33. Zhou Q, Guo P, Gallo JM. Impact of angiogenesis inhibition by sunitinib on tumor distribution of temozolomide. *Clin Cancer Res*. 2008;14:1540–1549.
34. Ma J, Pulfer S, Li S, Chu J, Reed K, Gallo JM. Pharmacodynamic-mediated reduction of temozolomide tumor concentrations by the angiogenesis inhibitor TNP-470. *Cancer Res*. 2001;61:5491–5498.
35. Zuniga RM, Torcuator R, Jain R, et al. Rebound tumour progression after the cessation of bevacizumab therapy in patients with recurrent high-grade glioma. *J Neurooncol*. 2010;99:237–242.
36. Reardon DA, Desjardins A, Peters K, et al. Phase II study of metronomic chemotherapy with bevacizumab for recurrent glioblastoma after progression on bevacizumab therapy. *J Neurooncol*. 2011;103:371–379.
37. Reardon DA, Desjardins A, Peters KB, et al. Phase 2 study of carboplatin, irinotecan, and bevacizumab for recurrent glioblastoma after progression on bevacizumab therapy. *Cancer*. 2011;117:5351–5358.

38. Reardon DA, Desjardins A, Peters KB, et al. Phase II study of carboplatin, irinotecan, and bevacizumab for bevacizumab naive, recurrent glioblastoma. *J Neurooncol*. 2012;107:155–164.
39. Kesselheim JC, Norden AD, Wen PY, Joffe S. Discontinuing bevacizumab in patients with glioblastoma: an ethical analysis. *Oncologist*. 2011;16:1435–1439.
40. Miles D, Harbeck N, Escudier B, et al. Disease course patterns after discontinuation of bevacizumab: pooled analysis of randomized phase III trials. *J Clin Oncol*. 2011;29:83–88.
41. de Groot JF, Fuller G, Kumar AJ, et al. Tumor invasion after treatment of glioblastoma with bevacizumab: radiographic and pathologic correlation in humans and mice. *Neuro Oncol*. 2010;12:233–242.
42. Ebos JM, Kerbel RS. Antiangiogenic therapy: impact on invasion, disease progression, and metastasis. *Nat Rev Clin Oncol*. 2011;8:210–221.
43. Verhaak RG, Hoadley KA, Purdom E, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. 2010;17:98–110.
44. Lee JC, Vivanco I, Beroukhim R, et al. Epidermal growth factor receptor activation in glioblastoma through novel missense mutations in the extracellular domain. *PLoS Med*. 2006;3:e485.
45. Idbaih A, Aimard J, Boisselier B, et al. Epidermal growth factor receptor extracellular domain mutations in primary glioblastoma. *Neuropathol Appl Neurobiol*. 2009;35:208–213.
46. Yan H, Parsons DW, Jin G, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med*. 2009;360:765–773.
47. Chi AS, Batchelor TT, Dias-Santagata D, et al. Prospective, high-throughput molecular profiling of human gliomas. *J Neurooncol*. 2012;110:89–98.
48. MacConaill LE, Campbell CD, Kehoe SM, et al. Profiling critical cancer gene mutations in clinical tumor samples. *PLoS One*. 2009;4:e7887.
49. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J Clin Oncol*. 2010;28:1963–1972.