Phase II Study of Protracted Daily Temozolomide for Low-Grade Gliomas in Adults

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Abstract

Purpose: Resistance to temozolomide chemotherapy is partly mediated by O^6-methylguanine-DNA methlytransferase (MGMT). Protracted treatment with temozolomide potentially overcomes MGMT resistance and improves outcome. We conducted a phase II study of protracted daily temozolomide in adults with low-grade gliomas.

Experimental Design: Patients with newly diagnosed oligodendroglioma or oligoastrocytoma with a MIB-1 index of >5% or recurrent low-grade gliomas received temozolomide (75 mg/m^2/day in 11-week cycles of 7 weeks on/4 weeks off). Treatment continued for a total of six cycles or until tumor progression or unacceptable toxicity. Primary end point was best overall response rate; secondary end points were progression-free survival, overall survival, and toxicity. We correlated response with MGMT promoter methylation and chromosome 1p/19q deletion status.

Results: Forty-four patients were treated (14 female, 30 male) with a median follow-up of 39.4 months. Median age was 43 years (range, 20-68 years) and median Karnofsky performance status was 90 (range, 70-100). The regimen was well tolerated. No patients had a complete response (0%), 9 had partial response (20%), 33 had stable disease (75%), and 2 had progressive disease (5%). A total of 21 patients eventually progressed with an overall median progression-free survival of 38 months. Patients with methylated MGMT promoter had a longer overall survival (P = 0.008). Deletion of either 1p or 19q chromosomes also predicted longer overall survival (hazard ratio, 0.17; 95% confidence interval, 0.03-0.93; log-rank P = 0.02).

Conclusions: A protracted course of daily temozolomide is a well-tolerated regimen and seems to produce effective tumor control. This compares favorably with historical data on the standard 5-day temozolomide regimen.

Low-grade gliomas constitute approximately 10% to 20% of primary brain tumors in adults (1). WHO grade I tumors such as pilocytic astrocytomas are often localized and can be surgically cured. WHO grade II tumors (diffuse infiltrating low-grade glioma) have a highly variable prognosis with curative resection being rare. The majority of low-grade gliomas are astrocytomas, although in recent years oligodendrogliomas and mixed oligoastroctyomas have been diagnosed with increasing frequency. Although these tumors are slow-growing, they usually lead to death by infiltration of brain and/or transformation into higher-grade tumors (WHO grade III-IV). The median survival times for patients with low-grade glioma is between 4.7 and 9.8 years, with range of up to 13 years for certain subtypes (2, 3).

The optimal management of progressive low-grade glioma is controversial because of a lack of data from prospective randomized trials. The standard treatment for low-grade glioma is surgical resection and radiotherapy. Most often radiotherapy is offered to patients with symptomatic and/or progressive disease. Several randomized trials have failed to show a survival difference in either early versus delayed radiation or with higher doses of radiation (4–7). The role of chemotherapy in low-grade glioma has been evaluated in several phase II trials. There is increasing evidence that conventional chemotherapeutic agents used for malignant gliomas, such as combination of procarbazine, lomustine, and vincristine (8–12), and temozolomide (13–18), are active in low-grade glioma in adults. There is also a

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Received 4/6/08; revised 7/22/08; accepted 9/2/08.

Grant support: Schering-Plough Corporation.

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Note: This work is original and preliminary data have been presented previously at 2007 Society for Neuro-Oncology meeting. These data have not been published in any journal. All authors have seen and approved the manuscript and declare no conflict of interests, except PW and DS, who have served on Schering Plough advisory boards.

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doi:10.1158/1078-0432.CCR-08-0888

Translational Relevance

The optimal management of progressive low-grade gliomas is controversial because of a lack of data from prospective randomized trials. The standard treatment for low-grade glioma is surgical resection and radiotherapy. There is increasing evidence that conventional chemotherapeutic agents used for malignant gliomas, such as temozolomide, are active in low-grade glioma. We report the first use of a protracted temozolomide regimen (75 mg/m^2/day in 11-week cycles of 7 weeks on/4 weeks off) in patients with low-grade glioma. This regimen was well tolerated and had significant activity comparing favorably with historical data on the standard 5-day temozolomide regimen. Tumor O^6-methylguanine-DNA methlytransferase (MGMT) promoter methylation and 1p/19q chromosome deletion status correlated with overall survival. An increase in progression-free survival among patients with tumors with unmethylated MGMT promoters relative to studies using standard temozolomide dosing suggests that this regimen may potentially overcome MGMT-mediated temozolomide resistance. This protracted temozolomide regimen is a feasible treatment option for patients with low-grade glioma.

suggestion that low-dose chemotherapy is effective in children (19). The standard procarbazine, lomustine, and vincristine regimen is associated with considerable toxicity limiting its use, and new regimens are being investigated (20). Patients with certain tumor genotypes, characterized by 1p and 19q deletion or methylation of the O^6-methylguanine-DNA methlytransferase (MGMT) promoter, may respond better to chemotherapy (1, 21–23). Currently chemotherapy is primarily used for recurrent disease. The role of chemotherapy in the initial management of low-grade glioma is controversial, and there are several large ongoing or planned studies to address this. The Eastern Cooperative Oncology Group and the North Central Cancer Treatment Group plan a phase III trial (E3F05) comparing radiation alone with combined chemoradiation with temozolomide for progressive/symptomatic low-grade glioma. In another phase III trial, the European Organization for Research and Treatment of Cancer (EORTC 22033/26033) and the National Cancer Institute of Canada (NCIC CE5) are comparing temozolomide chemotherapy alone versus radiation as first-line treatment for low-grade glioma.

Temozolomide has become the standard chemotherapeutic regimen for high-grade gliomas. There is increasing evidence of its efficacy in low-grade glioma at standard dosing regimens (5 days every 28 days; refs. 13–18, 22–24). Because resistance to temozolomide chemotherapy may be mediated in part by the presence of MGMT and because protracted daily treatment may potentially deplete MGMT (25, 26) and increases dose-density (27–30), we conducted a phase II study to evaluate the therapeutic efficacy of protracted temozolomide regimen in adults with low-grade glioma with respect to response, survival, toxicity, and biomarkers of response.

Patients and Methods

Objectives. The primary objective of this study was to determine the radiographic and overall best response rate to oral administration of protracted daily temozolomide in patients with low-grade glioma. Secondary objectives were progression-free survival (PFS), overall survival (OS), safety, and toxicity of this regimen. In addition, we sought to correlate response with methylation status of the MGMT promoter (31), loss-of-heterozygosity of chromosomes 1p and 19q, MIB-1 index, and histopathologic subtypes.

Patient eligibility. The Institutional Review Boards of the Dana-Farber/Harvard Cancer Center and the University of Virginia approved this protocol. Eligibility criteria included patients with histologically confirmed, newly diagnosed low-grade (WHO grade II) oligodendroglioma or oligoastrocytoma with a MIB-1 index of >5% or recurrent low-grade glioma (oligodendroglioma, astrocytoma, or oligoastrocytoma); measurable disease on magnetic resonance imaging; and for recurrent low-grade glioma, unequivocal evidence of tumor progression on magnetic resonance imaging and on a stable dose of steroids for at least 5 d. Additional eligibility criteria included age ≥18 y, life expectancy >12 wk, Karnofsky performance status ≥70, and adequate hematologic function.

Treatment regimen. Patients were treated initially with temozolomide given at 75 mg/m^2/d (rounded to nearest 5 mg) for 49 consecutive d of each cycle, followed by 28 d off between cycles (1 cycle, 77 d). Therapy was continued until evidence of progression or unacceptable toxicity for a maximum of six cycles. All patients received sulphamethoxazole-trimethoprim for pneumocystis pneumonitis prophylaxis.

Dose modifications and patient follow-up. Patients were closely monitored throughout therapy for drug-related toxicity, and all adverse events were recorded and graded according to the NCI-CTC v2.0. Physical and neurologic examinations were done every visit. Hematologic testing was done every 2 wk. Study drug was held for the remainder of the cycle if the patient experienced a drug-related grade 3 or 4 thrombocytopenia or neutropenia or grade ≥3 nonhematological

Table 1. Characteristics of the study population (44 patients)

Patient characteristics	Patients, *n* (%)
Median age in y	43
Age range in y	20-68
Male:female	30:14
Median KPS	90
Prior treatments	
Surgeries (1, 2, or ≥3)	30 (68%;), 12 (27%), 2 (5%)
Radiation	12 (27%)
Chemotherapy	0%
Anticonvulsants	
EIAED	20 (45%)
Non-EIAED	15 (34%)
None	9 (21%)
Steriods at baseline	2 (5%)
Pathology*	
Oligodendroglioma	
Newly diagnosed	15 (32%)
Recurrent	11 (25%)
Oligoastrocytoma	
Newly diagnosed	3 (9%)
Recurrent	9 (20%)
Astrocytoma (Recurrent)	6 (14%)

Abbreviations: KPS, Karnofsky performance status; EIAED, enzyme-inducing antiepileptic drug.
*Pre-protocol pathologic diagnosis.

Table 2. Adverse events related to treatment regimen (N = 44)

	Grade 2	Grade 3	Grade 4
	Number	Number	Number
Anemia	0	0	0
Ataxia	0	0	0
Constipation	3	0	0
Fatigue	8	0	0
Hepatotoxicity	1	1	0
Hyperglycemia	1	0	0
Infection*	1	0	0
Leukopenia	16	5	0
Libido	1	0	0
Lymphopenia	10	6	0
Nausea/Vomiting	3	1	0
Neutropenia	11	3	1
Petechiae	0	1	0
Thrombocytopenia	2	3	2 †
Thrombosis	0	0	1
Weight Loss	1	0	0

NOTE: Hematologic toxicity was monitored with weekly complete blood counts during first 7 wk and on every visit.
*In setting of neutropenia.
† 1 patient with immune thrombocytopenic purpura.

toxicity. The temozolomide dose was reduced for next cycle (level -1: 55 mg/m^2/day; -2: 45 mg/m^2/day). If absolute neutrophil count <1,000/mm^3 or platelets <50,000/mm^3, then patient dose was reduced 1 level; otherwise, patient dose was unchanged for the subsequent cycle. Doses reduced for temozolomide-related toxicity were not reescalated. A new 11-wk cycle could begin when there was adequate hematologic, symptomatic toxicity and any nonhematologic, symptomatic toxicities were ≤grade 1. If temozolomide could not be administered on the scheduled day of dosing, the complete blood count was repeated weekly for up to 3 wk until absolute neutrophil count ≥1,500/mm^3 and platelet count ≥100,000/mm^3. If the counts remained below these values at 3 wk, the patient was removed from trial for toxicity.

Imaging and response assessment. Magnetic resonance imaging of the brain was done every cycle. Axial fluid attenuated inversion recovery and axial and coronal T1 pregadolinium and postgadolinium images were obtained and used for this study. Because most low-grade gliomas are nonenhancing, the area of abnormal fluid attenuated inversion recovery was used to assess response even if enhancing tumor was present. Responses were determined using this modified Macdonald criteria (32) in which the sum of the products of perpendicular diameters of all measurable fluid attenuated inversion recovery lesions was measured. Complete response was defined as disappearance of all measurable disease. Partial response was defined as ≥50% decrease in the sum of products of perpendicular diameters of all measurable lesions compared with the baseline. Progression was defined as a 25% increase in the sum of products of all measurable lesions over the smallest sum observed. Stable disease applied to those who did not qualify for complete response, partial response, or progression.

Biomarker evaluation. Fluorescence *in situ* hybridization analysis to detect chromosomal deletion of 1p and 19q was done by the BWH Clinical Cytogenetics Laboratory using Abbott Laboratories Inc./Vysis probe sets (32-231004) on dissociated whole nuclei isolated from 50-μm paraffin sections of blocks containing >80% tumor cell nuclei. We obtained DNA from paraffin-embedded sections for a retrospective analysis of MGMT promoter methylation status. DNA methylation status of the MGMT gene was done by OncoMethylome Sciences, Inc.

Statistical methods. Patients with at least one follow-up assessment after treatment initiation were scored for best overall response rate and disease control rate (DCR). Best overall response rate was defined as complete response plus partial response, and DCR was defined as best overall response rate plus stable disease. The trial was designed to have 90% power for a DCR of ≥50% versus a null DCR of ≤30% with a one-sided significance level of 0.1 using a two-stage design with continuation if at least 8 of the first 22 participants achieved a favorable DCR. The protocol specified that temozolomide would be considered a promising therapy if ≥18 patients of 44 achieved a favorable DCR. Favorable response rate was calculated as a simple proportion among evaluable participants with exact confidence bounds. PFS and OS were described using Kaplan-Meier product-limit estimates and Greenwood's variance formula. Nonparametric confidence bounds are reported as infinite if high censoring rates precluded observation of a specific, finite bound. The differences in estimated PFS and OS curves between groups defined by MGMT promoter methylation, 1p and 19q deletions, pathology, MIB1 index, and prior

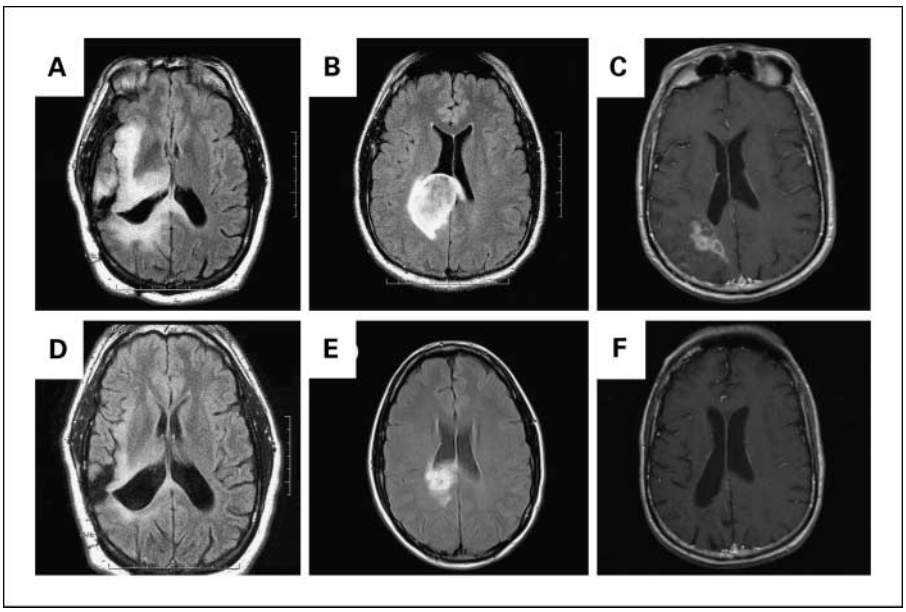

Fig. 1. Magnetic resonance imaging responses of low-grade glioma. *A, D,* FLAIR sequences of 42-year-old with recurrent oligoastrocytoma before (*A*) and after six cycles of temozolomide (*D*). FLAIR, fluid attenuated inversion recovery. *B, E*, FLAIR sequences of 36-year-old with recurrent low-grade astrocytoma, before (*B*) and after six cycles of therapy (*E*). *C, F,* T1-weighted postcontrast sequences of a 56-year-old diagnosed with low-grade oligoastrocytoma in 1990, treated with radiation and then recurring in 2002 with enhancement (*C*). Biopsy of the enhancing lesion showed low-grade glioma and after treatment with six cycles had a near complete response of the enhancing disease (*F*).

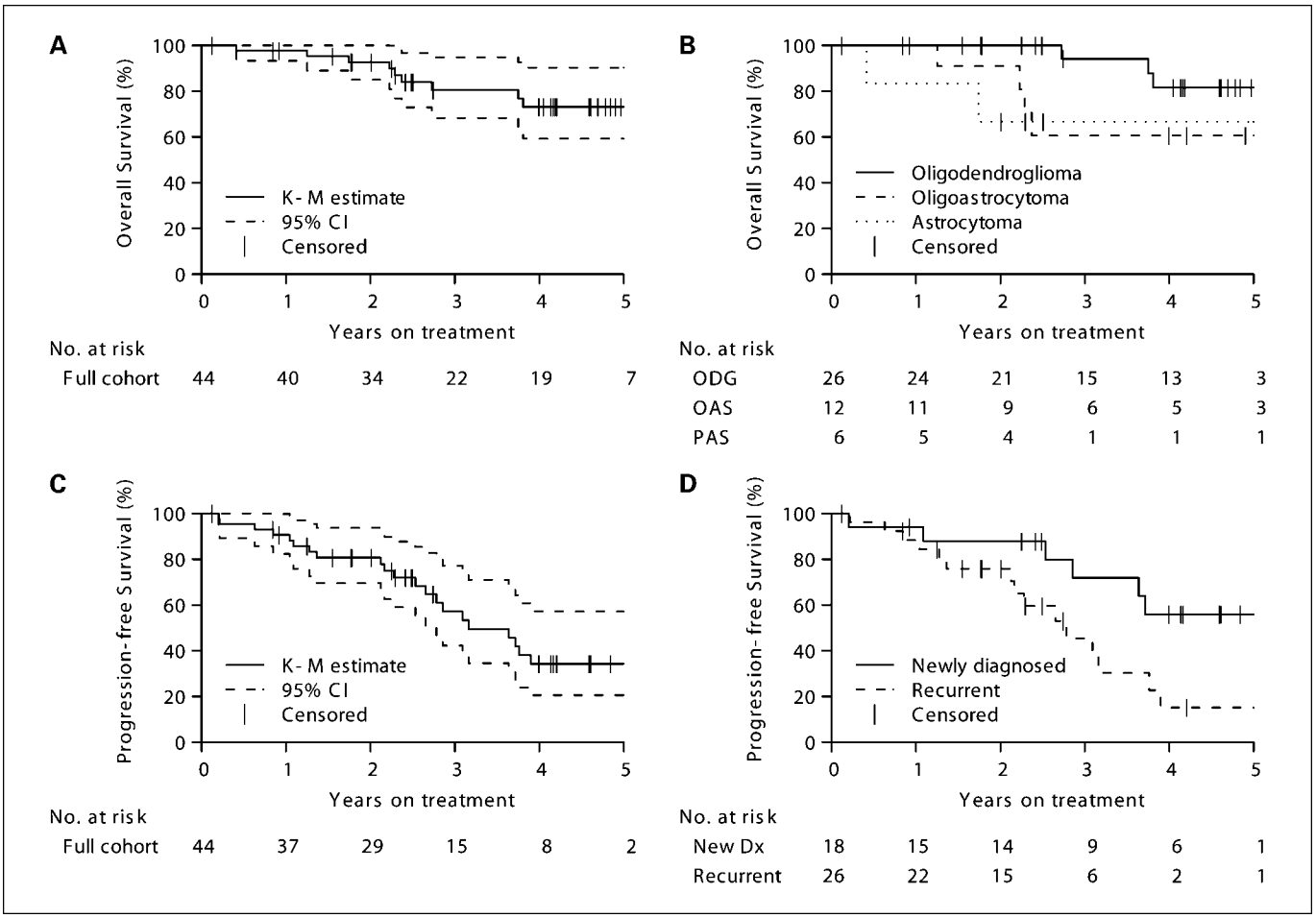

Fig. 2. Kaplan-Meier OS and PFS estimates. OS for all patients (*A*, $n = 44$) and subsets of tumor types (*B*, ODG versus PAS; HR, 0.14; 95% CI 0.03-0.71; $P = 0.018$). PFS for all patients (*C*) and subsets of newly diagnosed versus recurrent tumors (*D*, HR, 0.35; 95% CI, 0.13-0.91; $P = 0.03$). Tumor subtype did not significantly affect PFS. Bottom line below figures lists the number of patients at risk at each year. ODG, oligodendroglioma; OAS, oligoastrocytoma; PAS, pure astrocytoma.

radiation therapy were tested by the log-rank test. Hazard ratios (HR) were estimated by Cox regression. Bias in the sample of participants with assessments of MGMT methylation or 1p or 19q deletions were tested by *t*-test and Fisher's exact test comparing those with versus those without each type of assessment. The reported log-rank and Cox regression *P* values were confirmed using permutation tests with 5,000 repetitions.

Results

Population characteristics. Forty-five patients were enrolled with low-grade gliomas. One patient was lost to follow-up prior to initiating treatment and is excluded from all summaries. Of the 44 evaluable participants (Table 1), median age at baseline was 43 years (range, 20-68 years), 30 were men, 26 had pure oligodendroglioma (11 recurrent), 6 had pure astrocytoma (all recurrent), and 12 had mixed oligoastrocytoma (9 recurrent). Median baseline Karnofsky performance status was 90 (range, 70-100). Twelve patients (27%) had prior radiation and none had prior chemotherapies. Only two patients were on steroids at baseline, both of whom progressed during treatment; one died within 5 months and the other was lost to follow-up.

Toxicity of regimen. The regimen was generally well tolerated. There were no opportunistic infections or treatment-related deaths. Six patients discontinued therapy secondary to symptoms possibly or probably related to the treatment protocol. Three patients came off treatment during the first cycle for unacceptable toxicities; one for persistent grade 3 thrombocytopenia, one for grade 2 nausea, and one for worsening of preexisting depression and agitation which was felt to be unrelated to temozolomide. The other three discontinued therapy during cycles 3 and 4, one due to immune thrombocytopenic purpura, one due to grade IV leucopenia/neutropenia, and one due to lymphopenia and sinus tachycardia. Grade II to V toxicities associated with treatment are listed in Table 2. These toxicities generally resolved with treatment break. Fatigue was common but mild. Many patients did not require antiemetics for nausea and was easily controlled in those with nausea. Only one thrombotic event was noted in this study.

Response and survival. Of the 44 evaluable patients, the median treatment cycles was 4.7 (12.5 months; range, 0.2-6.0) and median follow-up time was 39.4 months (range, 1.5-71.8 months). No patient had a complete response (0%), 9 had a partial response (20%), 33 had stable disease (75%), and 2 had progressive disease (5%); giving a favorable overall response rate of 20% [95% confidence interval (CI), 10-35%] and a DCR of 95% (95% CI, 85-99%). Patients with partial

Table 3. Cytogenetics and MGMT status of tumors (N = 44)

Tumor characteristics	Cases, *n*
Cytogenetics	
1p	
Deleted	21
Intact	13
Not done*	10
19q	
Deleted	19
Intact	12
Not done*	13
MGMT status	
Methylated	12
Unmethylated	8
Uninformative	6
Not done	18

*Not done due to histologic diagnosis of pure astrocytoma and oligodendroglial tumors which were technically uninformative.

responses responded at a median of 3.5 cycles (Fig. 1). Of the two patients who progressed during cycle 1, one was found to have gliomatosis cerebri on reanalysis. By November 2007, a total of 21 (48%) patients had progressed and 10 (23%) patients had died. Partial responses by tumor subtypes were 3 of 26 in pure oligodendroglioma, 2 of 6 in pure astrocytoma, and 4 of 12 in mixed oligoastrocytoma (P = 0.19).

From enrollment, the median OS was >72 months (95% C, 67 to inf) and the estimated 1-, 3-, and 5-year OS rates were 98%, 81%, and 73% respectively (Fig. 2A). OS differed significantly by tumor type (log-rank P = 0.031; Fig. 2B); patients with oligodendroglioma lived longer than those with pure astrocytoma (HR, 0.14; 95% CI, 0.03-0.71; P = 0.018); OS of patients with mixed oligoastrocytoma did not differ significantly (HR, 0.43; 95% CI, 0.09-1.95; P = 0.27). OS of patients with newly diagnosed tumors was substantially longer than those with recurrent tumors although the difference was not significant (HR, 0.30; 95% CI, 0.06-1.41; P = 0.11). It is possible, however, that the small sample size may have contributed to the failure to detect a difference between these groups of patients.

From enrollment, overall median PFS was 38 months (95% CI, 33 to inf) and the 1-, 3-, and 5-year PFS rates were 91%, 57%, and 34% respectively (Fig. 2C). PFS was significantly greater among patients with newly diagnosed versus recurrent tumor (HR, 0.35; 95% CI, 0.13-0.91; P = 0.03; Fig. 2D). PFS did not differ significantly by tumor type (log-rank P = 0.23).

OS and PFS were lower among patients with a history of radiation therapy (n = 12), presumably reflecting a therapeutic

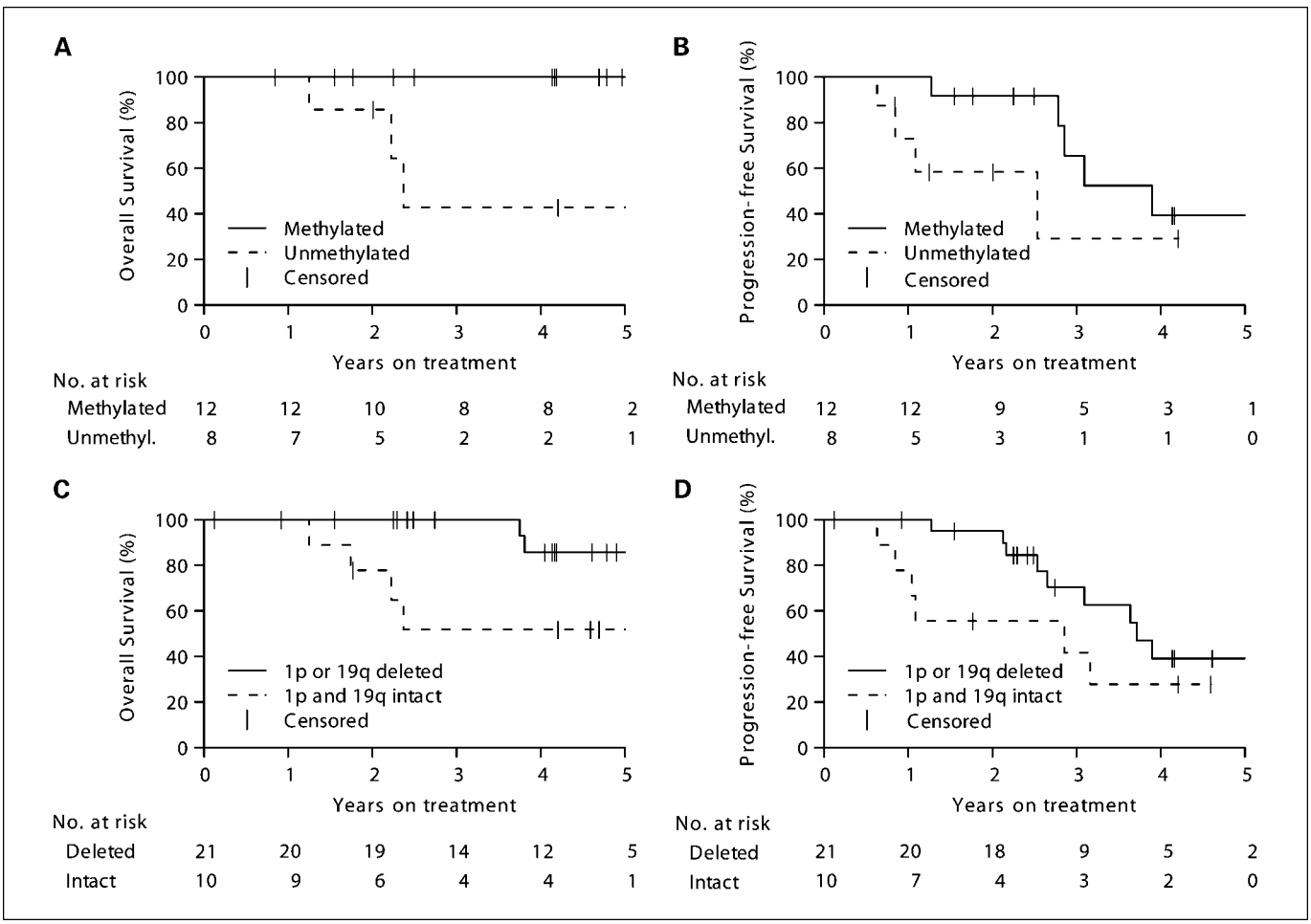

Fig. 3. MGMT methylation and 1p and 19q deletion status and OS and PFS. OS for subset of patients with MGMT status (*A*, n = 20; log-rank P = 0.008) and 1p or 19q deletion (*C*, n = 31; log-rank P = 0.02). PFS for subset of patients with MGMT status (*B*) and subsets 1p/19q deletion (*D*).

bias in response to more refractory disease. OS does not differ as a function of previous radiation therapy when risk was adjusted for baseline Karnofsky performance status score. Prior radiation continued to be associated with lower PFS even after adjustment for baseline Karnofsky performance status score.

Biomarkers of response and survival. The results of MGMT promoter methylation status, and 1p and 19q deletion status are shown in Table 3. No variable tested (e.g. age, Karnofsky performance status, MIB-1 index, pathology, EIAED use, follow-up time, completed cycles, OS, or PFS) differed significantly in patients with versus without assessments of MGMT promoter methylation or 1p/19q deletion status ($P > 0.15$ for all). Among the 20 patients with evaluable MGMT status, OS was greater among patients whose tumors had methylated MGMT promoter (log-rank $P = 0.008$; Fig. 3A). The median OS was not estimable among patients whose tumors had methylated MGMT promoter (100% OS at time of analysis) and was 29 months (95% CI, 17 to inf) in patients whose tumors had unmethylated MGMT promoter. Among the 12 patients with methylated MGMT promoter and average follow-up of 47 months, no (0%) patient died. Among the 8 patients with unmethylated MGMT promoter and median follow-up of 30 months, 3 (38%) patients died ($P = 0.05$ by Fisher's exact test). Median PFS for patients with methylated tumors was 47 months versus 30 months for patients with unmethylated tumors (Fig. 3B).

Of 38 patients with oligodendroglioma or oligoastrocytoma, 1p and 19q status was determined in 34 (89%) and 31 (82%), respectively. The test was not done in the patients with astrocytomas. Deletion of either 1p or 19q predicted longer OS (HR, 0.17; 95% CI, 0.03-0.93; log-rank $P = 0.02$; Fig. 3C). Deletion of either 1p or 19q on its own or loss of both seemed to be protective but not significantly associated with OS likely due to the small sample size (Table 4). The median OS was not estimable for either group (fewer than half died before censoring; lower 95% confidence bound >72 months for patients with single or codeletion and 27 months for patients with both loci intact). Among the 21 patients with single or codeletions (18 codeleted) with an average follow-up of 45 months, 2 (10%) patients died. Among the 10 patients with both loci intact with an average follow-up of 39 months, 4 (40%) patients died. Median PFS for patients with single or codeletions was 45 months versus 34 months for patients with both loci intact (Fig. 3D). The data suggest cosegregation of MGMT and 1p and/or 19q deletion with an odds ratio of 16 (95% CI, 0.75-900; $P = 0.09$). There was no association between having a partial response and either MGMT status (odds ratio, 0.6; 95% CI, 0.04-10.6; $P = 1.0$) or with 1p and/or 19q deletion (odds ratio, 2.1; 95% CI, 0.17-116; $P = 1.0$) in this small sample. We could not test independently for MGMT methylation and 1p/19q deletion due to insufficient data ($n = 15$). Of 20 assessed for MGMT, 25% did not have 1p/19q determined; of 30 assessed for 1p/19q, 50% did not have MGMT determined.

MIB-1 index was <5% in 13 (30%), 5% to 10% in 17 (38%), and >10% in 15 (32%) patients, and the mean MIB-1 index was 7.3% (95% CI, 1.0-21%). MIB-1 index was not significantly associated with OS or PFS, possibly due to the small sample size.

Of the 21 patients who progressed, 13 (29%) patients underwent repeat surgery and most (12 of 13) pathologies showed progression to higher-grade glioma by report (reflecting in part surgical selection of patients whose pathology might have changed based on imaging features).

Discussion

This regimen using a protracted (7 weeks on/4 weeks off) course of temozolomide was a well-tolerated regimen. Five

Table 4. Univariate predictors of overall survival and progression-free survival

Predictor	Level	OS		PFS	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Best response	PR vs. SD	1.42 (0.29-7.07)	0.001	0.70 (0.20-2.42)	<0.001
	PD vs. SD	12.50 (2.28-68.5)		Inf-(NE,Inf)	
Age (y)	Per 5 y	0.94 (0.69-1.27)	0.87	0.84 (0.66-1.06)	0.12
AED use	EIAED vs. none	1.26 (0.24-6.48)	0.96	1.53 (0.49-4.83)	0.75
	non-EIAED vs. none	1.17 (0.19-7.09)		1.40 (0.39-5.01)	
Pathology	OAC vs. ODG	3.06 (0.68-13.8)	0.031	1.51 (0.57-4.00)	0.28
	PAS vs. ODG	7.18 (1.40-36.8)		2.68 (0.83-8.66)	
Recurrent	Yes vs. no	3.37 (0.71-16.0)	0.11	2.88 (1.10-7.58)	0.024
1p	Deleted vs. intact	0.24 (0.04-1.32)	0.075	0.51 (0.18-1.41)	0.20
19q	Deleted vs. intact	0.26 (0.05-1.44)	0.098	0.90 (0.32-2.56)	0.85
1p and 19q	Both deleted vs. either intact	0.32 (0.06-1.75)	0.17	0.74 (0.27-2.06)	0.57
1p or 19q	Either deleted vs. both intact	0.17 (0.03-0.93)	0.020	0.48 (0.17-1.36)	0.18
1p and 19q	Per deletion	0.43 (0.17-1.08)	0.061	0.74 (0.42-1.30)	0.31
MIB-1	5-10% vs. <5%	0.37 (0.08-1.67)	0.39	0.50 (0.18-1.38)	0.29
	>10% vs. <5%	0.51 (0.11-2.32)		0.42 (0.13-1.34)	
MGMT	Methylated vs. not	0.00 (0.00-NE)	0.008	0.36 (0.09-1.38)	0.15
RT	Yes vs. no	4.76 (1.33-17.0)	0.008	2.83 (1.18-6.80)	0.026

NOTE: HR and 95% CI are from Cox regression. *P* values are from log-rank tests.
Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease; NE, not estimable; AED, antiepileptic drug; EIAED, enzyme-inducing antiepilepsy drug; OAC, oligoastrocytoma; ODG, oligodendroglioma; PAS, pure astrocytoma; MIB-1, MIB-1 labeling index; RT, radiation therapy.

patients (11%) had grade 3 or 4 thrombocytopenia and 4 (9%) had grade 3 or 4 neutropenia. Although 6 patients (14%) had grade 3 lymphopenia and 10 patients (23%) had grade 2 lymphopenia, no patient developed an opportunistic infection. This may be related in part to the longer breaks between treatment cycles with this regimen compared with other dose-dense regimens (29), and the use of prophylactic therapy for pneumocystis pneumonitis. Nausea and fatigue were generally mild.

This regimen seems to produce effective tumor control with a DCR of 95%, a 12-month PFS of 91%, a median PFS of 38 months, and a median OS of >72 months. This regimen compares favorably with the published studies of standard (5 days every 28 days) dosing of temozolomide, in which the median PFS ranged from 10 to 31 months (13–18, 22, 23). Two other recently published protracted temozolomide regimen of 3 weeks on/1 week off report a DCR of 84%, a 12-month PFS of 74%, and a median PFS that was not reached after a median follow-up of 19.8 months (28) and DCR of 87%, a 12-month PFS of 73%, and a median PFS of 21.8 months (30). Direct comparison of results is not possible due to differences in study designs, tumor histologies, extent of resection, genetics, and MGMT status.

We also found that MGMT methylation and 1p or 19q deletion status significantly correlated with OS and PFS consistent with prior studies on the prognostic value of these biomarkers (1, 16, 22, 23, 33–35). Analysis of 1p deletion alone and codeletion provided a positive trend, but the study's power is limited due to small sample size.

Furthermore, when comparing the patients with unmethylated tumors (who are less responsive to the standard temozolomide regimen) with patients with methylated tumors, we found that patients with unmethylated tumors also had longer OS and PFS compared with the patients who received standard temozolomide regimen. In this study, the patients with methylated tumors had a median PFS of 47 months and median OS was not reached, and the patients with unmethylated tumors had a median PFS of 30 months and a median OS of 29 months. This compares favorably with the study by Everhard et al. who reported a PFS of 29.5 months in patients with methylated tumors and a PFS of 6 months in patients with unmethylated tumors (23). The much larger increase in PFS among patients with unmethylated tumors raises the possibility that the protracted regimen used in this study might have overcome MGMT-mediated resistance to temozolomide.

There are several possible explanations for the responses seen with this prolonged temozolomide dosing regimen. Firstly, the 49-day schedule offers the advantage of at least a 2-fold increase in drug exposure when compared with the 5-day schedule. In addition to increased temozolomide dose density, the regimen is associated with decreased temozolomide-related toxicity, thus allowing patients to tolerate more chemotherapy. Secondly, the protracted temozolomide exposure may result in MGMT depletion resulting in improved sensitivity to temozolomide, especially in patients with unmethylated tumors (25, 27). MGMT is a DNA repair protein that stoichiometrically removes temozolomide-induced methylation DNA damage, thereby protecting cells from methylating chemotherapeutic agents (31). Low tumor levels of MGMT protein have been shown to increase the response rate to temozolomide. Although MGMT levels vary across and within tumors, each tumor cell has a defined quantity of MGMT protein. Thirdly, recent literature on metronomic dosing of chemotherapy also suggests a potential antiangiogenic effect due to cytotoxic effects on dividing tumor endothelial cells (36, 37).

The tumors of the patients who progressed (at least 29%) transformed into high-grade glioma during or after treatment. It is unclear if these patients were biologically different or if treatment selected for more aggressive phenotype. In recent studies of patients with glioblastoma treated with temozolomide, those tumors that progressed during temozolomide treatment had loss of the mismatch repair enzyme MSH6, which is required for temozolomide cytotoxicity (38, 39). Future studies will have to address this potential adverse effect of treatment and how to overcome this if borne out.

In conclusion, we report the first use of a protracted temozolomide regimen in low-grade glioma. This regimen was well tolerated and had significant activity comparing favorably with standard dosing of temozolomide. Tumor MGMT methylation and 1p/19q status correlated with OS. An increase in PFS among patients with tumors with unmethylated MGMT promoters relative to studies using standard temozolomide dosing suggests that this regimen may potentially overcome MGMT-mediated temozolomide resistance.

Disclosure of Potential Conflicts of Interest

P. Wen and D. Schiff have received commercial research support from Schering-Plough Corporation.

Acknowledgments

We gratefully acknowledge the support of the Par Fore the Cure, Sam Longo and Amos Wasgett Brain Tumor Clinical Research Funds.

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