

Phase III Randomized Study of Postradiotherapy Chemotherapy with α -Difluoromethylornithine-Procarbazine, *N*-(2-Chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea, Vincristine (DFMO-PCV) Versus PCV for Glioblastoma Multiforme¹

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ABSTRACT

Although the efficacy of the nitrosourea-based combination chemotherapy procarbazine, *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea, and vincristine (PCV) has been previously demonstrated in the setting of anaplastic/intermediate-grade gliomas, the benefit for glioblastoma patients remains unproven. In the current study, we sought to determine whether the addition of α -difluoromethylornithine (eflornithine), an inhibitor of ornithine decarboxylase, which has shown encouraging results in the setting of recurrent glioma patients, to a nitrosourea-based therapy (PCV) would constitute a more effective adjuvant therapy in the treatment of glioblastoma multiforme patients in the post-radiation therapy setting.

Following conventional radiation therapy, 272 glioblastoma (GBM) patients were randomized to receive either α -difluoromethylornithine-PCV (DFMO-PCV; 134 patients) or PCV alone (138 patients), with survival and time to tumor progression being the primary endpoints. The starting dosage of DFMO was 3.0 g/m² p.o. q8h for 14 days before and after

treatment with *N*-(2-chloroethyl)-*N*-cyclohexyl-*N*-nitrosourea; PCV was administered as previously described¹. Clinical and radiological (Gadolinium-enhanced MRI) follow-ups were nominally at the end of each 6 or 8 week cycle (PCV at 6 weeks; DFMO-PCV at 8 weeks). Laboratory evaluations for hematologic and other adverse effects were at 2 week intervals.

There was no difference in median survival or median time-to-tumor progression between the two treatment groups, as measured from day of commencement of postradiotherapy chemotherapy [MS (months): DFMO-PCV, 10.5; PCV, 11.1; MTP (months): DFMO-PCV, 4.6; PCV, 4.4]. Overall survival, as measured from time of tumor diagnosis at first surgery, was 13.3 and 14.2 months at the median and 6.2 and 8.7% at 5 years, respectively, for the DFMO-PCV and PCV arms. The treatment effect was unchanged after adjustment for age, performance status (KPS), extent of surgery, and other factors using the multivariate Cox proportional hazard model. Adverse effects associated with DFMO consisted of gastrointestinal (diarrhea nausea/vomiting), cytopenias, and minimal ototoxicity (limited to tinnitus) at the dose range tested.

The addition of DFMO to the nitrosourea-based PCV regimen in this phase III study demonstrated no additional benefit in glioblastoma patients, underscoring the resistance of glioblastoma multiforme tumors to alkylating agents. For patients with anaplastic (intermediate grade) gliomas, in which the previously demonstrated benefit of post-radiation chemotherapy is more substantial, the evaluation of DFMO-PCV vs. PCV is still ongoing and hopefully will yield more encouraging results.

INTRODUCTION

Laboratory research accumulated in recent years demonstrated that polyamines influence many cellular processes that are associated with cellular growth. Blockade of polyamine accumulation by administration of inhibitors, such as DFMO (eflornithine)⁴, can result in a significant reduction in cell

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⁴ The abbreviations used are: DFMO, α -difluoromethylornithine (eflornithine); CCNU, *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea; PCV, procarbazine, CCNU, vincristine; GBM, glioblastoma multiforme; ODC, ornithine decarboxylase; MTP, median-time(s)-to-tumor progression; MS, median survival; KPS, Karnofsky performance status; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea, MDACC, M. D. Anderson Cancer Center; CI, confidence interval; MRI, magnetic resonance imaging; CT, computed tomography; NCI, National Cancer Institute; CR, complete response; PR, partial response; MR, minor response.

proliferation rates in a variety of cell systems (1–4). In cultured cells of normal and neoplastic origin, DFMO reduces ODC activity and significantly reduces cell proliferation. Specifically, DFMO has been shown to inhibit the growth of rat hepatoma cells, mouse mammary EMT 6 sarcoma cells, mouse L1210 leukemia cells, HCT cells, and human prostate adenoma cells in tissue culture (1, 2, 4, 5).

As single agents, polyamine inhibitors such as DFMO have only infrequently shown activity against human tumors in a clinical setting. In combination with other drugs that interfere with polyamine metabolism, such as methylglyoxal bis-guanylhydrazone (6), or drugs that interfere with DNA function (7–10), evidence implicates a synergistic interaction of DFMO against tumor cells.

With respect to malignant gliomas, the combination of DFMO with the BCNU nitrosourea has shown consistent potentiation of 9L cell kill and increased survival in rats with intracerebral 9L tumors (2, 6–9). In the patient setting, Prados *et al.* (11) had demonstrated encouraging results in a Phase I-II study of DFMO-BCNU in the treatment of patients with recurrent gliomas. Of the 21 evaluable supratentorial anaplastic glioma patients, 2 (10%) experienced a PR and 10 (48%) had disease stabilization with therapy. The MS for these 12 patients was 119 weeks (measured from the initiation of chemotherapy), which was far superior to the MS of 56 weeks for the entire anaplastic glioma group of 21 patients.

The encouraging results with the DFMO-BCNU combination in the setting of recurrent anaplastic gliomas formed the basis for the current Phase III study, in which patients with newly diagnosed high-grade malignant gliomas received DFMO together with a nitrosourea combination in the postradiotherapy setting. Because the three-drug regimen PCV has been demonstrated to be more effective against anaplastic astrocytomas than the nitrosourea BCNU alone (12), the study randomized between DFMO-PCV *versus* PCV alone for two histological strata: anaplastic astrocytoma and GBM. This report presents data related only to the GBM stratum of the randomized study.

PATIENTS AND METHODS

Patient Eligibility

This was a multi-institutional study of patients from The University of Texas M. D. Anderson Cancer Center, the Metro-Minnesota Community Cancer Oncology Programs (CCOP), and the University of California at San Francisco. To be eligible for registration in this study, patients had to fulfill the following criteria: (a) histologically confirmed diagnosis of GBM or gliosarcoma; (b) completed external beam radiation therapy with no prior chemotherapy except hydroxyurea during radiation therapy; (c) commencement of study treatment within 4 weeks of completing radiation therapy; (d) ≤ 16 years of age; (e) KPS, ≥ 70 with a life expectancy of at least 8 weeks; (f) normal liver function (serum glutamic pyruvic transaminase, alkaline phosphatase levels ≤ 2 times normal values and total bilirubin ≤ 1.5 mg/dl); (g) normal hemogram including absolute neutrophil count of $\geq 1500/\text{mm}^3$ and platelets, $\geq 125,000/\text{mm}^3$; and (h) lack of active infection, pregnancy (adequate contraception required), any disease that would obscure toxicity or dangerously alter drug metabolism, and serious intercurrent illness. All of the

patients signed an Institutional Review Board-approved informed consent form.

The MDACC served as the central registration site. All of the patients' scans and pathology slides were reviewed at MDACC before registration and randomization via a computer-generated randomization program. Patients were stratified only by histology (GBM *versus* anaplastic glioma). The two strata had different accrual goals and they constitute two separate studies. For GBM, the trial was powered to detect a MS of 75 weeks as different from 50 weeks based on a 1-sided log-rank test with $\alpha = 5\%$ and $\beta = 20\%$. The target sample size in the original protocol for the GBM patient group was 237, based on an accrual rate of 1.5 per week and a 26-week post accrual follow-up period. The final accrual for the GBM study presented in this report was 272, because the target size was increased because of slow accrual.

Treatment

Eflornithine⁵ was prepared as a premixed 500-ml solution containing DFMO at a concentration of 200 mg/ml in 5% ethanol. The solution was stored in a cool environment ($< 85^\circ\text{C}$) out of direct light, under which conditions it is stable. The drug was made available by the Division of Cancer Treatment, NCI (NSC 337250) through a gift from Merrell Dow Pharmaceuticals, Inc.

Before initiating the randomized study, we conducted a Phase I evaluation of escalating DFMO doses of 2, 2.5, and 3 g/m²/day based on the DFMO dosage used in the BCNU-DFMO and DFMO studies (11, 13). There were four to seven patients in each treatment cohort. Dose-limiting diarrhea and combined myelotoxicity with PCV determined the dosage used in this study. Ototoxicity was not observed to be a dose-limiting toxicity in our Phase I study. After randomization, patients received one of the two following treatment schedules:

Arm A: DFMO (eflornithine) in combination with PCV

DFMO, 3.0 g/m² p.o. every 8 h, days 1–14

PCV beginning day 15

CCNU, 110 mg/m² p.o., day 15

Procarbazine, 60 mg/m²/day p.o., days 22–35

Vincristine, 1.4 mg/m² i.v. (max, 2.0 mg), day 22 and day 43

DFMO, 3.0 g/m² p.o. every 8 h, days 29–42

The cycle was repeated at 8-week intervals, for a total of seven cycles.

Arm B: PCV alone⁽¹⁾

CCNU, 110 mg/m² p.o., day 1

Procarbazine, 60 mg/m² p.o./day, days 8–21

Vincristine, 1.4 mg/m² i.v. (max, 2.0 mg), days 8 and 29

The cycle was repeated at 6-week intervals, for a total of seven cycles.

⁵ Eflornithine, DL- α -difluoromethyl-2,5-diaminopentanoic acid hydrochloride, monohydrate.

Evaluation for Toxicity and Dose Adjustments

Pretreatment Evaluation. Pretreatment evaluation consisted of a complete medical history, Karnofsky performance status, and general physical and neurological examinations. Contrast-enhanced MRI and/or CT scan of brain within 2 weeks prior to treatment (patients from Centers outside the MDACC had their scans reviewed at MDACC before study entry). Blood tests (complete blood count, differential, platelet count, creatinine, bilirubin, alkaline phosphatase, and alanine aminotransferase) the results of which conformed to entry guidelines (see "Patient Eligibility") were obtained within 1 week prior to study entry.

Evaluation during Study. Evaluation during study consisted of the same clinical, radiological, and laboratory exams as those listed for "Pretreatment Evaluation." A complete neurological examination and neuro-imaging (contrast-enhanced MRI or CT scan) were performed before each cycle of chemotherapy. Complete blood count, differential, and platelet counts were obtained every 2 weeks during PCV and PCV-DFMO courses. If the absolute neutrophil count fell below $750/\text{mm}^3$ and/or platelets fell below $50,000/\text{mm}^3$, then counts were obtained more frequently until counts rose above these levels. Serum creatinine, alkaline phosphatase, bilirubin, and alanine aminotransferase tests were performed before each cycle of chemotherapy. All of the toxicities encountered during the study were graded (0–4) according to the NCI Common Toxicity Criteria and were recorded before each course of therapy. Dose adjustments for life-threatening toxicities were to be reported immediately to the Study Chairman, the Institutional Review Board, and the NCI.

Dose Modification. Doses of procarbazine and CCNU were modified at the beginning of each new course according to the grade of absolute neutrophil count and platelet-nadir experienced during the previous course: (a) grade 0, increase by 25%; (b) grades 1 and 2, no change; (c) grade 3, decrease previous dose by 25%; and (d) grade 4, decrease initial dose by 50%.

Dose modification for DFMO was based on ototoxicity. For ototoxicity greater than grade 2 (tinnitus), the dose was to be decreased 25%; therapy was not to be withheld, inasmuch as ototoxicity was demonstrated earlier to be dose- and schedule-dependent and typically resolved after either a dose reduction or a drug holiday. Audiometric testing was performed at the physician's discretion for patients who, on history and/or examination, demonstrated evidence of hearing loss.

Determination of Response

Patient and imaging parameters were evaluated on the basis of changes since the last examination and scans, as previously described by MacDonald *et al.* (14). Whereas neurological performance was monitored by grading both symptoms and signs, determination of objective response was based on changes in the contrast-enhanced MRI or CT scans. Each imaging study comprised 12 or more scans at levels from the cranial base to the convexity to encompass the intracranial contents. Imaging studies were evaluated as per previously described parameters (14, 15): tumor size, degree of contrast enhancement, surrounding edema, and ventricular size and compression.

A CR was defined as a MRI (or CT) scan with no visible

tumor, provided that the patient had had no increase in glucocorticoid dose since the last evaluation period. A PR was defined as less than a CR but greater than a 50% reduction in the product of the two largest tumor diameters, provided that the patient had had no increase in glucocorticoid dose since the last evaluation period. A MR was defined as an unequivocal reduction in tumor size that either was less than a 50% reduction in the product of the two largest diameters or could not be measured. "Stable disease" was defined as <25% change in tumor size (with the patient receiving stable or decreasing doses of glucocorticoids). "Progressive disease" was defined as a >25% increase in tumor size, provided that the dose of glucocorticoids had not been decreased since the last evaluation period; if the glucocorticoid dose was concomitantly decreased, theoretically, the progressive disease designation had to be confirmed at the next evaluation period.

Criteria for Removing Patients from the Study

Patients were removed from the study for the following reasons: (a) progressive disease as defined previously after a minimum of one course (6–8 weeks, depending on the chemotherapy regimen); (b) development of unacceptable toxicity even at reduced doses of chemotherapy; (c) patient refusal to continue therapy; and (d) patient noncompliance with protocol requirements.

Statistics

Time-to-progression was measured from the 1st day of treatment until progression was documented, at which time the patient was removed from the protocol; if appropriate, patients were offered other treatments. Survival, measured from the 1st day of treatment with postradiotherapy chemotherapy until death, was estimated using the Kaplan-Meier product limit method separately for each treatment group, and the groups were compared using a log-rank test. Overall survival, measured from the date of first diagnosis at surgery until death, was estimated using the Kaplan-Meier product-limit method separately for each treatment group. The Cox regression model was used to identify patient characteristics that were of possible prognostic significance.

RESULTS

Patient Characteristics. A total of 272 patients were randomized to receive either DFMO-PCV (134) or PCV alone (138). Patients were accrued between September 23, 1992, and February 6, 1998, and data analyses were based on data collected through November 30, 1999. Table 1 summarizes patient characteristics in the two treatment groups. Although randomization yielded a balance with respect to most patient characteristics, the DFMO-PCV group consisted of patients with a slightly higher median age (52.7 years compared with 49.6) and a higher proportion of female patients (38 versus 26%) compared with the group that received PCV alone (Table 2). The treatment effect was unchanged after adjustment for these differences using the multivariate Cox proportional hazard model.

Twenty-eight (10%) patients were still alive at last contact with a median follow-up of 4 years. Twenty-three patients were listed as "inevaluable" (primarily because they refused therapy

Table 1 Patient characteristics of the 272 patients entered into study

Characteristic	DFMO-PCV arm	PCV arm
Number	134	138
Female patients	51 (38%)	36 (26%)
Median age (range), yr	52.7 (20–75)	49.6 (18–75)
Karnofsky performance score		
100	16 (12%)	28 (20%)
90	73 (54%)	68 (49%)
80	38 (28%)	29 (21%)
70	7 (5%)	13 (9%)
Median radiation dose (range), Gy	60 (40.4–72)	60 (50–70.4)
Diagnosis to registration (range), weeks	12.9 (8.1–21.0)	12.4 (7.0–18.0)
Registered and followed at MDACC	50 (37%)	38 (28%)
Extent of surgery		
Biopsy	20 (15%)	23 (17%)
Subtotal resection	63 (47%)	67 (49%)
Gross total resection	51 (38%)	48 (35%)

after randomization), but a review of data revealed that results were unaffected by their exclusion. Forty were registered later than 4 weeks after radiation completion; of these, 3 were registered later than 6 weeks after radiation completion (6.8, 8.0, and 10.5 weeks). Two patients were registered before completion of radiation therapy.

Toxicity. In general, both of the treatment arms were well tolerated. Table 3 lists the adverse effects that were above grade 2. Although DFMO in higher doses has been associated with sensorineural hearing deficits, no patients in the DFMO-PCV group was recorded to have experienced ototoxicity worse than grade 2 (defined as tinnitus). Diarrhea was an adverse effect that was restricted to the DFMO-PCV group. In one case, the diarrhea was that of grade 4, with ≥ 10 stools/day and a need for parenteral support.

With respect to bone marrow suppression, leukopenia was the most frequently encountered hematological reaction, being approximately 17% in both patient groups. Interestingly, whereas thrombocytopenia was more frequent among the DFMO-PCV group, granulocytopenia was more frequent in the PCV limb (Table 3). One patient on the PCV treatment arm experienced a grade 4 elevation in bilirubin, which resolved within 3 days of onset, and one patient receiving DFMO-PCV experienced dehydration leading to renal failure.

Response. Kaplan-Meier analyses demonstrated no difference in survival (Fig. 1) or progression-free survival (Fig. 2) between the two treatment groups: MS with DFMO-PCV treatment, 10.5 months; MS with only PCV treatment, 11.1 months; MTP for the respective groups were 4.6 and 4.4 months. Univariate hazard ratio for overall survival (DFMO-PCV versus PCV alone) was 1.0 (95% CI, 0.9–1.1; $P = 0.55$), which did not change after adjustment for covariates. Univariate hazard ratio for progression-free survival was 1.0 (95% CI 0.9, 1.1; $P = 0.68$), which did not change after adjustment. Overall survival, as measured from time of tumor diagnosis at first surgery, was 13.3 and 14.2 months at the median and, at 5-year, 6.2 and 8.7%, respectively, for the DFMO-PCV and PCV arms.

Although there was no significant difference in the two treatment groups with respect to overall survival from first

surgery, survival from first postradiotherapy chemotherapy, and progression-free survival, further analysis did demonstrate a significant interaction between treatment and age ($P = 0.028$), such that the survival hazard ratio for treatment (DFMO-PCV versus PCV alone) was 0.5 (95% CI, 0.2–1.1) for patients 40 or younger; for patients over 40, the hazard ratio was 1.3 (95% CI, 0.9–1.7).

For both groups combined (total of 272 patients), 225 progressed (189 while on treatment), and 47 were alive and progression-free after a median follow-up of 11 months. As shown in Table 2, a Cox model with four prognostic factors (age, KPS, extent of resection, side of tumor) accounted for 30% (value of R^2) variation in survival.

In each of the two treatment groups, there were 13 responders. The DFMO-PCV group had 2 CR, 5 PR, and 6 MR. The group treated with PCV alone had no CR, but had 6 PR and 7 MR (see “Determination of Response” in “Patients and Methods” section for response criteria). Twenty-three patients were listed as inevaluable (14 in the DFMO-PCV group, 9 in the PCV-alone group), but a review of the data revealed that results were unaffected by their exclusion.

DISCUSSION

One of the features that characterize rapidly growing cells is their intracellular accumulation of polyamines, whose polycationic nature contributes to the conformational stabilization of DNA (16). Because ODC is the key regulatory enzyme in the biosynthesis of the polyamine precursor putrescine (17), the induction of ODC in proliferating cells has been suggested to be a marker of malignant tumors (18), including gliomas (19, 20). Whereas ODC activity is highly elevated in malignant glioma, the normal adult brain has relatively low ODC activity (21, 22). Combined with its important role in DNA metabolism, the tumor-selective nature of ODC elevation identified this regulatory enzyme as a logical target for anti-glioma therapeutics.

We as well as other groups have previously reported extensive *in vitro* (7, 9, 23–28) and *in vivo* results from animal studies (8, 29–31) that demonstrated the anti-glioma effects of DFMO, an irreversible inhibitor of ODC (32). In addition, these preclinical studies had also shown that DFMO potentiates the cytotoxicity of the nitrosourea BCNU (8, 28). As a result, a Phase I-II study was subsequently performed to evaluate DFMO in combination with BCNU in recurrent malignant glioma patients (11). Whereas the DFMO-BCNU combination demonstrated only minimal activity in patients with recurrent GBM, results with patients with recurrent anaplastic gliomas were more impressive (PR, 10%; stable disease, 48%) with what appeared to be an unexpected cohort of long-term survivors. As such, the data supported a need for a prospective randomized study comparing the DFMO-BCNU versus BCNU alone, at least in the anaplastic glioma population.

Because we had previously shown that the nitrosourea-based combination of PCV to be superior to BCNU alone in patients with anaplastic gliomas (12), we felt obligated to conduct a Phase III study that randomized patients to receive either DFMO-PCV or PCV alone. In this multi-institutional study, patients afflicted with anaplastic glioma or GBM were accrued

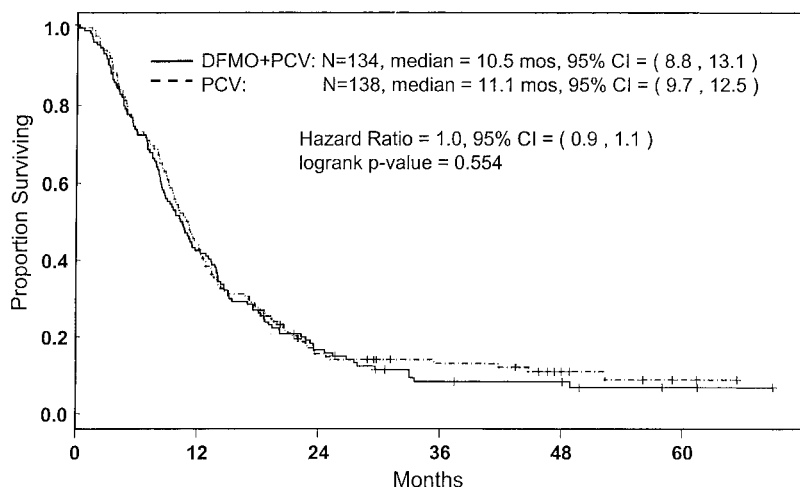
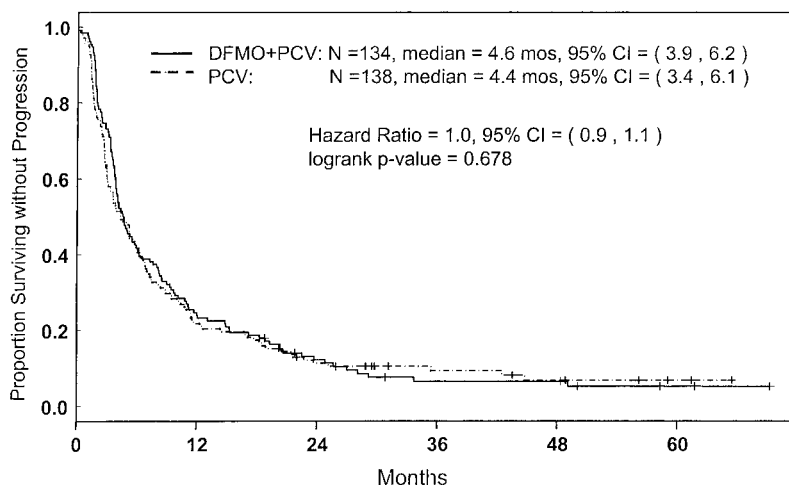


Fig. 1 Kaplan-Meier survival estimates from the start of postradiotherapy chemotherapy for patients treated with DFMO-PCV versus PCV. A total of 272 glioblastoma patients were randomized in the postradiotherapy setting to receive either DFMO-PCV or PCV alone. No difference was observed for survival. The MS for the DFMO-PCV group was 10.5 months (95% CI, 9.7–12.8), whereas the MS for the patients treated with PCV alone was 11.1 months (95% CI, 8.7–13.1).

Fig. 2 Kaplan-Meier estimates of progression-free survival from start of postradiotherapy chemotherapy. The curves show that the addition of DFMO to PCV was not associated with any extension of progression-free survival compared with the PCV-alone group. The MTP for the DFMO-PCV group was approximately 4.6 months and 4.4 months for the PCV group.



and stratified at entry by histology. The current report presents only the data pertinent to the GBM population.

Despite the encouraging preclinical and Phase I-II clinical studies supporting a synergism between DFMO and a nitrosourea, the addition of DFMO to PCV provided no significant extension in MS from postirradiation randomization (10.5 months; Fig. 1), overall MS from diagnostic surgery (13.3 months), or progression-free survival (MTP, 4.6 months; Fig. 2) compared with treatment with PCV alone. As expected, age and KPS at study entry were identified to be of potential prognostic significance (Table 2). Although overall and progression-free survival were unchanged after adjustment for differences between patient groups, further analysis of the data did suggest an apparent benefit of DFMO-PCV over PCV in younger patients; for patients 40 or younger, survival hazard ratio for treatment with DFMO-PCV versus PCV alone was 0.5 (95% CI, 0.2–1.1; $P = 0.028$), whereas for patients 40 or older, the hazard ratio was 1.3 (95% CI, 0.9–1.7). No explanation or judgment can be currently drawn from this observation.

Whether a higher dose of DFMO might have produced more encouraging results in this study is moot. Patients with recurrent anaplastic glioma in a short, Phase I study of PCV-DFMO before the randomized study began did not tolerate DFMO at the monotherapy dose of 3.6 g/m² (13). However, in contrast, DFMO was administered at a significantly lower dose (2.0 g/m² per dose) in our previous Phase I-II study assessing the DFMO-BCNU combination, in which the combination was superior in anaplastic glioma patients compared with historical controls treated with BCNU alone (11). Importantly, whereas the aforementioned studies of DFMO as a monotherapy or in combination with BCNU produced encouraging results, the antitumor activity of DFMO in these previous studies was readily apparent only with patients with anaplastic gliomas.

GBMs are characteristically more resistant to conventional alkylating agents, a property that has been attributed to various genetic aberrations that distinguish GBM tumors from the lower-grade gliomas of the anaplastic or low-grade type (33). However, whether GBMs are distinct from the lower-grade histologies specifically with respect to their susceptibility to

Table 2 Multiple covariate model using treatment, extent of surgery, radiation dose, sex, age, Karnofsky performance score, side of tumor, treating institution, and time of diagnosis to registration and actual postradiotherapy chemotherapy

Variable	Contrast	Coefficient	SE	P
Treatment	DFMO-PCV vs. PCV	0.07	0.13	0.61
Surgery extent	GTR ^a vs. biopsy	-0.49	0.20	0.013
	STR vs. biopsy	-0.28	0.19	0.23
Radiation	↑ 1 cGy	-0.0000018	0.00019	0.92
Sex	Male vs. female	0.012	0.14	0.93
Age	↑ year	0.029	0.006	<0.001
Karnofsky score	↑ unit	-0.055	0.009	<0.001
Side of tumor	Left vs. right	-0.49	0.14	<0.001
Treatment center	MDACC vs. others	-0.032	0.14	0.83
Diagnosis to registration	↑ 1 week	-0.01	0.009	0.15

^a GTR, gross total resection; cGy, centi-Gray; STR, subtotal resection.

Table 3 Toxicity associated with treatment

Toxicity	Grade	Treatment group	
		DFMO-PCV n (%)	PCV n (%)
Anemia	3	8 (5.9)	6 (4.3)
	4	1 (0.7)	0
Granulocytopenia	3	12 (8.8)	21 (15)
	4	12 (8.8)	11 (7.9)
Leukopenia	3	21 (15.6)	24 (17.1)
	4	2 (1.5)	5 (3.6)
Thrombocytopenia	3	16 (11.8)	5 (3.6)
	4	17 (12.6)	3 (2.1)
Transaminase increase	3	1 (0.7)	2 (1.4)
	4	3 (2.2)	1 (bilirubin increase) (0.7)
Ototoxicity	3	0	0
	4	0	0
Nausea alone		3 (2.2)	4 (2.9)
Vomiting		7 (5.2)	5 (3.6)
Diarrhea		8 (5.9)	0
Fatigue		2 (1.5)	1 (0.7)
Headache		1 (0.7)	1 (0.7)
CNS ^a		2 (1.5)	1 (0.7)
Infection		2 (1.5)	2 (1.4)

^a CNS, central nervous system.

perturbations in polyamine metabolism is not known. *In vitro*, cell lines derived from human high-grade gliomas as well as rodent glioma and gliosarcomas models are sensitive to DFMO (7, 9, 23–28), although this sensitivity is not universal among all GBM cell lines (28). Nonetheless, *in vitro* studies that specifically compare the relative sensitivities of gliomas of different grades to ODC inhibition are lacking. In contrast, there have been *in vivo* studies with functional neuro-imaging techniques that indirectly measure ODC activity in patients, but these have not demonstrated any significant difference in the activity of this enzyme as a function of glioma grade (34).

The lack of an apparent potentiation between DFMO and PCV in the current study with GBM patients raises the question of whether DFMO would be more active if it were administered in combination with another class of drug or treatment modality. Compared with the extensive *in vitro* and preclinical data that supported the combinatory regimen of DFMO and nitrosourea, the data pertaining to DFMO combination with other agents is significantly less. In the rodent model, radiation,

when given concurrently with DFMO, led to an additive effect on the reduction polyamine levels, which correlated with an increase in the survival of rats orthotopically implanted with malignant glioma (35). DFMO in combination with *cis*-platinum has been mixed. Whereas synergism was observed between DFMO and *cis*-platinum in a rodent model (36), another study showed that the DFMO with *cis*-platinum was actually less cytotoxic than platinum alone (28). With respect to biological response modifiers, whereas *in vitro* results demonstrated the DFMO with IFN was additive in terms of antitumor effect (37), subsequent Phase II evaluation of this combination failed to confirm this result in the patient setting (38).

In conclusion, we have presented the results of a prospective, randomized Phase III study of 272 patients comparing the DFMO-PCV combination versus PCV alone in patients with GBM in the postradiotherapy setting. We observed no significant advantage of the addition of DFMO to the nitrosourea regimen in terms of the two primary end points, survival and progression-free survival (MTP). Whereas the results of this study of GBM patients are “negative,” (i.e., the addition of DFMO to PCV did not improve survival) they underscore the importance of controlled trials in the assessment of cancer therapeutics. Moreover, we are hopeful that the evaluation of DFMO-PCV versus PCV in anaplastic glioma patients may yield encouraging results when it is analyzed in early 2001.

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