

Phase III Randomized Study of Postradiotherapy Chemotherapy with Combination α -Difluoromethylornithine-PCV versus PCV for Anaplastic Gliomas¹

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ABSTRACT

Purpose: In the current study, we sought to determine whether the addition of DFMO (α -difluoromethyl ornithine; eflornithine), an inhibitor of ornithine decarboxylase, to a nitrosourea-based therapy procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, vincristine (PCV) would be more effective as a postirradiation adjuvant therapy for anaplastic gliomas (AG) than PCV alone.

Patients and Methods: After conventional radiation therapy, 249 AG patients were randomized to receive either DFMO-PCV (125 patients) or PCV alone (124 patients), with survival being the primary endpoint and progression-free survival being an important secondary endpoint. The starting dosage of DFMO was 3 grams/m² p.o. q. 8 h for 14 days before and 4 weeks after 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; PCV was administered as described previously (1). Clinical and radiological (gadolinium-enhanced magnetic resonance imaging) 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 hematological and other adverse effects were at 2-week intervals.

Results: In the DFMO-PCV arm, there were 114 evaluable patients with 78.1% anaplastic astrocytoma (AA), 3.5% anaplastic oligoastrocytoma (AOA), 14% anaplastic oligodendroglioma (AO), and 4.4% other malignant gliomas. These histological groupings were comparable with those of the 114 patients in the PCV arm: (a) 69.3% AA; (b) 7% AOA; (c) 21.1% AO; and (d) 2.6% malignant gliomas. Although improved survival estimates for the DFMO-PCV treatment group persisted over the course of the study, analysis of survival differences over the entire follow-up period did not yield significance ($P = 0.11$). However, careful analysis of the corresponding hazard and hazard ratio functions indicated that the real treatment difference was limited to the first 24 months of follow-up ($P = 0.02$). The median progression-free survival for the two treatment groups, as measured from postradiotherapy registration, was 71.1 months for the DFMO-PCV arm and 37.5 months for the PCV-only arm. Median survival, measured from registration, was 75.8 and 61.1 months, respectively, for the DFMO-PCV and PCV arms. The treatment effect persisted when the AA histology was separated from AO and AOA histologies. This effect persisted even after adjusting for the covariates of age, Karnofsky performance status, and extent of surgery. There was a statistically significant increase in grade 3 adverse events for diarrhea and anemia associated with DFMO-PCV. Grade 3 or 4 adverse events of nausea, ototoxicity, and thrombocytopenia were not significantly increased among groups.

Conclusions: The addition of DFMO to the nitrosourea-based PCV regimen in this Phase III study demonstrated a sustained benefit in survival probabilities for AG patients but not in the corresponding hazard rates. Survival analysis from registration found a DFMO-PCV median survival of 6.3 years (49 of 114 events), whereas that for PCV alone was 5.1 years (55 of 114 events). The hazard function demonstrated a difference over the first 2 years of study (hazard ratio 0.53, $P = 0.02$) but not after 2 years (hazard ratio 1.06, $P = 0.84$), supporting the conclusion that DFMO adds to the survival advantage of PCV chemotherapy for AG patients by direct temporal interaction with PCV.

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INTRODUCTION

In a previous publication (1), we reported the results from our randomized Phase III study of DFMO³ with PCV versus

³ The abbreviations used are: DFMO, α -difluoromethyl ornithine (eflornithine); CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; PCV, procarbazine, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, vincristine; MDACC, M. D. Anderson Cancer Center; AG, anaplastic glioma; AA,

PCV alone for the glioblastoma multiforme strata of the study, which demonstrated no benefit from the addition of DFMO. This study shows that DFMO-PCV is, however, superior to PCV for treating AGs.

DFMO, an irreversible inhibitor of ODC, can block polyamine accumulation, significantly reducing cell proliferation rates in various cell systems. The intracellular accumulation of polyamine polycations contributes to the conformational stabilization of DNA (2). DFMO as a single agent has infrequently shown activity against established human tumors in clinical trials (3, 4). In combination with nitrosourea alkylating agents, however, DFMO increased antitumor activity in various tumors studied in cell culture (5–11) and animal tumor models (12, 13). The molecular pharmacodynamics underlying this synergism has not been well established (6, 7, 10, 14, 15). Many of the preclinical DFMO combination studies with BCNU were obtained using the 9L gliosarcoma cell line in culture and rats with intracerebral 9L tumors (16–19).

In the clinical setting, we demonstrated previously encouraging results in Phase I and II studies of single agent DFMO (20), DFMO with mitoguanine (21), and DFMO with BCNU (22). In the DFMO-BCNU study, of 21 evaluable supratentorial AG patients, 10% experienced a partial response, and 48% had disease stabilization with a median response duration of 164 weeks for these 12 patients. The fact that responding patients had more durable responses than expected from the DFMO-BCNU combination led to the current study. Because we had shown previously that PCV was superior to BCNU for adjuvant chemotherapy of malignant gliomas, we elected to initiate a Phase III randomized trial with PCV instead of BCNU (23).

PATIENTS AND METHODS

Patient Eligibility

This was a multi-institutional study of patients from The University of Texas MDACC, the Community Cancer Oncology Program, and the University of California at San Francisco. To be eligible for this study, patients had to fulfill the following criteria: (a) histologically confirmed diagnosis of AA, AOA, AO, or AG not otherwise specified (24, 25); (b) completed external beam radiation therapy with no previous chemotherapy except hydroxyurea during radiation therapy; (c) commencement of study treatment within 4 weeks of completing radiation therapy; (d) ≥ 16 years of age; (e) Karnofsky performance status ≥ 70 with a life expectancy of ≥ 8 weeks; (f) normal liver function (serum glutamic pyruvic transaminase and alkaline phosphatase levels less than or equal to two times normal values and total bilirubin ≤ 1.5 mg/dl); (g) normal hemogram, including ANC of $\geq 1,500/\text{mm}^3$ and platelets $\geq 125,000/\text{mm}^3$; and (h) lack of active infection, pregnancy (adequate contraception re-

quired), any disease that would obscure toxicity or dangerously alter drug metabolism, and serious intercurrent illness. All patients signed an Institutional Review Board-approved informed consent form.

Treatment

Eflornithine (DL- α -difluoromethyl-2, 5-diaminopentanoic acid hydrochloride, monohydrate; α -difluoromethyl ornithine; DFMO; RM 71,782) was prepared as a premixed 500-ml solution containing DFMO at a concentration of 200 mg/ml in 5% ethanol. The solution is stable when stored in a cool environment ($<85^\circ\text{C}$) out of direct light. The drug was made available by the Division of Cancer Treatment, NCI (National Service Center 337250) through a gift from Merrell Dow Pharmaceuticals, Inc.

After irradiation, patients were randomized to receive one of the two following treatment schedules:

Arm A: DFMO (Eflornithine) in Combination with PCV

DFMO, 3 grams/m² p.o. q. 8 h, days 1–14

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. (maximum 2 mg), days 22 and 43.

DFMO, 3 grams/m² p.o. q. 8 h, days 29–42

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

Arm B: PCV Alone

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. (maximum 2 mg), days 8 and 29

The cycle was repeated at 6-week intervals, for a total of seven cycles (26).

Evaluation for Toxicity and Dose Adjustments

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

Evaluation During Study. This analysis used 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 ANC fell below $750/\text{mm}^3$ and/or platelets fell below $50,000/\text{mm}^3$, counts were obtained more frequently until they rose above these levels. Serum creatinine, alkaline phosphatase, bilirubin, and

anaplastic astrocytoma; ODC, ornithine decarboxylase; PFS, progression-free survival; ANC, absolute neutrophil count; SURV, survival from registration; AE, adverse event; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea; SURV-D, survival from diagnosis at time of first surgery; AO, anaplastic oligodendroglioma; CI, confidence interval; CT, computed tomography; MRI, magnetic resonance imaging; AOA, anaplastic oligoastrocytoma; NCI, National Cancer Institute; MS, median survival.

alanine aminotransferase levels were assessed before each cycle of chemotherapy.

All toxicities encountered during the study were graded (0–4) using the NCI Common Toxicity Criteria and recorded before each course of therapy. Dose adjustments for life-threatening toxicities were required 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 ANC nadirs and platelet nadirs 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%.

DFMO dose modification was primarily for ototoxicity greater than grade 2 (tinnitus) and required that the DFMO dose be decreased by 25% for grades 3 or 4 ototoxicity; DFMO was not to be withheld, because ototoxicity was demonstrated earlier to be dose and schedule dependent and typically resolved after either dose reduction or a drug holiday. Audiometric testing was performed at the physician's discretion for patients who, from history and/or exam, demonstrated evidence of hearing loss.

Determination of Response

Patient and imaging parameters were evaluated on the basis of changes occurring since the last examination and scans using the methodology described by MacDonald *et al.* (27). Although 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 was composed of ≥ 12 scans at levels from the cranial base to the convexity to encompass the intracranial contents. Imaging studies were evaluated for parameters described previously (28, 29): (a) tumor size; (b) degree of contrast enhancement; (c) surrounding edema; and (d) ventricular size and compression.

A complete response was defined as an MRI (or CT scan) with no visible tumor, provided that the patient had no increase in glucocorticoid dose since the last evaluation period. A partial response was defined as less than a complete response but a $>50\%$ reduction in the product of the two largest tumor diameters, provided that the patient had no increase in glucocorticoid dose since the last evaluation period. A minor response was defined as an unequivocal reduction in tumor size that either was a $<50\%$ reduction in the product of two 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 an increase in tumor size by $>25\%$, provided that the dose of glucocorticoids had not been decreased since the last 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

PFS was measured from the first day of registration until progression was documented, at which time, the patient was removed from the protocol; if appropriate, patients were offered other treatments. Survival (SURV), measured from registration until death, was estimated using the Kaplan-Meier product limit method separately for each treatment group, and the groups were compared using a likelihood ratio test. SURV-D, measured from date of first diagnosis at surgery until death, was estimated using the Kaplan-Meier product limit method separately for each treatment group. The Cox proportional hazards regression model was used to adjust for potentially confounding study factors (30). All statistical tests were 2-sided tests.

To better understand the rate at which death or disease progression occurred in patients in each treatment arm over time, we evaluated the hazard rates. These hazard rates represent sensitive measures of the instantaneous event rate among the event-free cohorts (*e.g.*, instantaneous death rates among the surviving patients). To assess whether DFMO-PCV and PCV-alone patients have different event time distributions, we compared their respective hazard functions by estimating the ratios of the hazard functions. Hazard functions were estimated from the data using a fixed bandwidth kernel approach incorporating boundary kernels (31). Because the magnitude of the hazard ratio indicates the difference in death rates, we monitored how the ratio changed over time to assess if the relative death rate between the groups was changing over time (32). CIs for the hazard function and hazard ratio function estimates were computed using bootstrap resampling.

The MDACC served as the central registration site. All patients' scans and pathology slides were reviewed at MDACC. Registration and randomization were accomplished via a computer-generated randomization program. Patients were stratified only by histology (glioblastoma multiforme *versus* AG). The two strata had different accrual goals and constitute two separate studies. For AG patients, the trial was powered to detect a MS of 54.5 months as distinct from 36.2 months based on a one-sided log-rank test with $\alpha = 0.05$ and a power of 80%. The target sample size in the original 1989 protocol for the AG patient group was 303, based on an accrual rate of 1.5 patients/week and a 2-year postaccrual follow-up period (33). The required number of deaths in the original one-sided testing was 152; with two-sided testing, it would have been 194 with an O'Brien-Fleming interim analysis (34). Thus, a planned interim analysis based on the two-sided α calculations would have taken place after ~ 97 deaths. We conducted our analysis at 114 deaths. Because of slow accrual (0.6 patients/week), new design parameters for the AG patients were established in 1996. These were needed to yield the number of deaths necessary to obtain the specified power for α to determine the specified inference. This adjustment led to a maximum accrual of 249 patients, a maximum accrual period of 7.7 years, and a maximum study duration of 10.4 years.

RESULTS

Patient Characteristics. A total of 249 patients were randomized to receive either DFMO-PCV (125) or PCV alone (124). Patients were accrued between September 23, 1992 and

Table 1 Patient characteristics of the 242 patients analyzed in this study

	All patients				Evaluable patients ^a			
	PCV/DFMO		PCV		PCV/DFMO		PCV	
Patients	121		121		114		114	
Median age (range)	41.8		42.6		41.6 (19–73)		42.3 (18–76)	
Male:female	72:49		67:54		68:46		62:51	
Mean KPS ^b	90.6		89.3		91.0		90.1	
Histology	All registered				Allowed histologies			
AA	95	79%	86	71.1%	89	78.1%	79	69.3%
AOA	4	3.2%	8	6.6%	4	3.5%	8	7.0%
AO	17	13.7%	24	19.8%	16	14.0%	24	21.1%
Anaplastic glioma not otherwise specified (MG)	5	4.0%	3	2.5%	5	4.4%	3	2.6%
Surgery								
Biopsy	44	34%	37	31%	39	34%	34	30%
Subtotal resection	50	42%	52	43%	48	42%	48	42%
Gross total resection	27	24%	32	26%	27	24%	32	28%
Radiation therapy		Gy		Gy		Gy		Gy
Mean dose, Gy ^c	119	59.4	121	58.6	112	59.5	113	58.9
Radiosurgery boost, Gy	2	72.2	1	69.8	2	72.2	1	69.8
Hydroxyurea with RT					11		15	

^a Evaluable patients with correct histology (AA, AOA, AO, MG) and evidence that they received ≥ 2 weeks of prescribed therapy.

^b KPS, Karnofsky performance status.

^c Dose excludes patients who received a radiosurgery boost.

Table 2 Toxicity associated with treatment in patients receiving ≥ 14 days of treatment even if removed from response evaluation for incorrect diagnosis

Patients with AEs refers to the worst AE experienced by a patient in a specific category. Total AE will include multiple AEs for a given patient. Toxicity grade 4 not cited when no events are observed.

Toxicity	Toxicity grade	DFMO-PCV ($n = 117$) ^a		PCV ($n = 115$)	
		Patients with AE (%)	Total AE	Patients with AE (%)	Total AE
Anemia	3	9 (7.7) ^b	17	2 (1.7)	3
	4	1 (0.9)	1	0	0
Diarrhea	3	8 (6.8) ^c	11	0	0
	4	16 (13.7)	20	11 (9.6)	13
Granulocytopenia	3	42 (35.9)	84	42 (36.5)	68
	4	16 (13.7)	20	11 (9.6)	13
Leukopenia	3	39 (33.3)	101	36 (31.3)	64
	4	7 (6.0)	8	3 (2.6)	3
Nausea/vomiting	3	18 (15.4)	36	13 (11.3)	16
	4	1 (0.9)	1	2 (1.7)	3
Ototoxicity	3	2 (1.7)	2	0	0
Skin	3	8 (6.8)	11	7 (6.1)	8
Thrombocytopenia	3	31 (26.5)	50	20 (17.4)	29
	4	8 (6.8)	12	5 (4.3)	6
Miscellaneous	3	18 (15.4)	3	14 (12.2)	18
	4	1 (0.9)	4	2 (1.7)	3

^a n = patients that are evaluable for toxicity and exceeds those evaluable for response.

^b Test of proportion $P = 0.068$.

^c Test of proportion $P = 0.013$.

June 11, 1999, and data analyses were based on data collected through September 1, 2002. Pathology slides for all patients registered to the study were reviewed by the referee pathologist (J.M.B.) using criteria published by Burger *et al.* (24), as well as those of the referee pathologist (25). Six patients had a diagnosis that did not fit the protocol for this study, and 1 patient was injured after registration and received no treatment. 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 was composed of patients with slightly more AA (78 *versus* 69%) and a slightly higher proportion of male patients (60 *versus* 55%) compared with the group that received PCV alone (Table 1). The treatment effect was unchanged after adjustment for these differences using the multivariate Cox proportional hazards regression model.

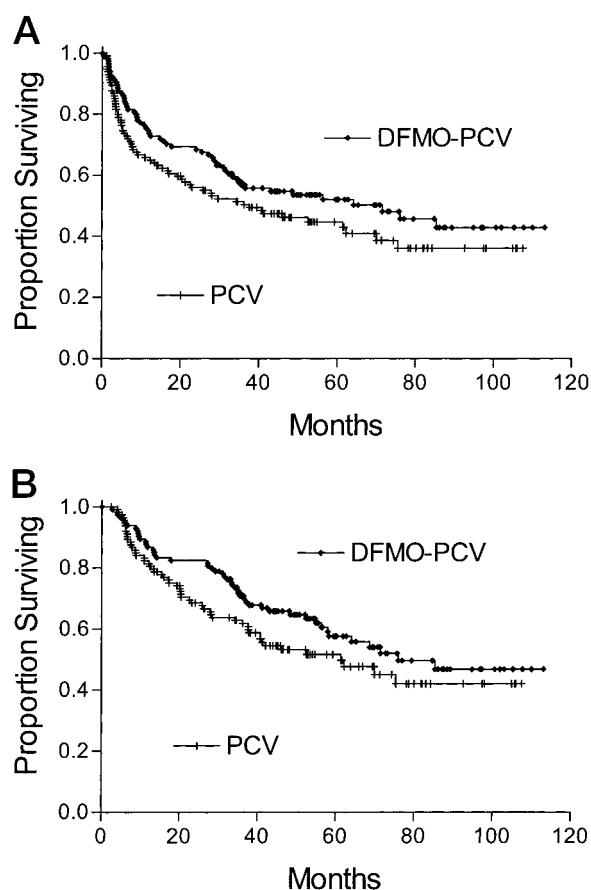


Fig. 1 Kaplan-Meier PFS (A) and survival (SURV; B) estimates from start of postradiotherapy chemotherapy for the 228 evaluable patients who had correct histological diagnoses and received >2 weeks of protocol therapy.

Twenty-one patients (8.4%, 21 of 249), 11 patients in the PCV-DFMO arm and 10 in the PCV arm, were not evaluable for response. Six patients were not evaluated because of an inappropriate histological diagnosis for inclusion in the study, 5 refused treatment after randomization, 4 refused because of treatment side effects, 1 had an accident and received no treatment, 2 did not receive treatment because of medical problems, 2 refused without completing DFMO because of clinical deterioration, and 1 was lost to follow-up within weeks of randomization.

Toxicity. In general, both treatment arms were well tolerated. Table 2 lists grades 3 and 4 AEs. Of the toxicities cited in Table 2, only diarrhea and anemia were statistically more prevalent in DFMO-PCV versus PCV patients. Two patients (1.7%) in the DFMO-PCV group were recorded as having experienced grade 3 ototoxicity (sensorineural hearing loss). Diarrhea was an AE that was restricted to the DFMO-PCV group, and 8 patients (6.8%, 8 of 117) had grade 3 diarrhea. Only 1 patient had a 25% dose reduction of DFMO because of diarrhea. Anemia was seen in 10 (8.5%, 10 of 117) of the DFMO-PCV patients and in only 2 PCV patients (1.7%, 2 of 115). Other myelotoxicities were nearly equal among the two treatment arms.

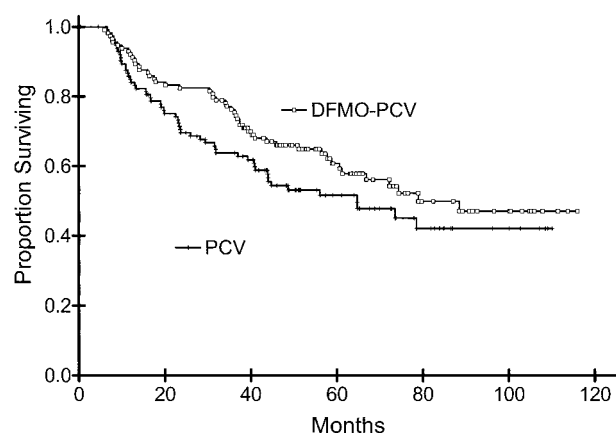


Fig. 2 Kaplan-Meier survival estimates from first diagnosis for patients treated with DFMO-PCV versus PCV. The Kaplan-Meier representations are for the 228 evaluable patients who had correct histological diagnoses and received >2 weeks of protocol therapy. The MS for the DFMO-PCV group (49 of 114 events) was 78.6 months, whereas the MS for the patients treated with PCV alone (55 of 114 events) was 64.4 months. Hazard ratio 0.74 (95% CI = 0.5, 1.08) with $P = 0.12$.

Table 3 Response designations for the 228 evaluable patients

Response	DFMO-PCV	PCV
Complete response	1.8%	0.9%
Partial response	7.0%	4.4%
Stable disease	81.6%	82.5%
Progressive disease	7.0%	10.5%
Not evaluable for response	2.6%	1.8%

Survival Statistics. All registered patients eligible for treatment ($n = 242$) were analyzed on an “intent-to-treat” basis. In addition, analyses were performed using evaluable patients from both treatment arms who actually received the prescribed treatment and were defined as those who actually received the prescribed chemotherapy for at least half of the first course. Kaplan-Meier analyses demonstrated differences in PFS (Fig. 1A) and SURV (Fig. 1B) from registration, although the differences were not statistically significant by global likelihood ratio tests ($P = 0.12$ – 0.13). Similarly, Kaplan-Meier analysis demonstrated differences in SURV-D (Fig. 2) without statistical significance ($P = 0.12$). Table 3 shows the responses observed for evaluable patients by treatment arm. Most patients had stable disease (82%), with complete and partial responses accounting for 8.8% in the DFMO-PCV arm and 5.3% in the PCV arm. There was, however, no statistical difference in response rates for the two treatment arms.

Table 4 summarizes median PFS, median SURV, P_s for intent to treat, and evaluable patients for the entire AG group and AA subgroup of patients. In all cases, the DFMO-PCV arm appears to be clinically superior to the PCV arm, but the difference is not statistically significant by global likelihood ratio tests. Interestingly, the difference between PFS for the two arms was ~34 months for the evaluable patients, but the differences diminished in the survival groups to 15–25 months, probably in response to the effectiveness of secondary salvage

Table 4 Collated median PFS and SURV for evaluable and ITT^a patients treated with DFMO-PCV compared with PCV

	Evaluable DFMO- PCV	Evaluable PCV	<i>P</i>	ITT DFMO- PCV	ITT PCV	<i>P</i>
AG PFS	71.1 mos	37.5 mos	0.13	56.2 mos	34.1 mos	0.10
AA PFS	56.2 mos	22.2 mos	0.18	42.9 mos	17.0 mos	0.15
AG SURV	75.8 mos	61.1 mos	0.12	71.2 mos	52.4 mos	0.11
AA SURV	71.2 mos	46.0 mos	0.12	63.9 mos	41.1 mos	0.11

^a ITT, intent to treat.

therapies instituted at the time of tumor progression. For evaluable AG patients, median SURV was 75.8 months for DFMO-PCV and 61.1 months for PCV patients. The comparable SURV-D was 78.6 months for DFMO-PCV and 64.4 months for PCV patients. For the AA strata, median SURV was 71.2 months for DFMO-PCV versus 46 months for the PCV arm ($P = 0.12$ by likelihood ratio test).

The recognized covariates were similar in both arms (Table 1). In addition, the multivariate Cox proportional hazards regression model demonstrates commonly recognized relationships for evaluable AG patients; survival is positively affected by younger age, higher performance status, and extent of surgery and is better for AO than for AA patients (Table 5). Too few patients received hydroxyurea to determine its impact on survival.

To better understand why Kaplan-Meier survival curves for the DFMO-PCV versus PCV-alone group that appear consistently different over a decade are not statistically significant by the global likelihood ratio test, we analyzed the data using hazard ratio functions. Because the magnitude of the hazard ratio indicates the difference in event rates, we compared the two treatments, DFMO-PCV and PCV-alone, by computing a hazard ratio as the hazard rate for patients with PCV-alone treatment divided by the hazard rate for patients with DFMO-PCV treatment. Thus, a hazard ratio of 1 would indicate that the hazard rates are equivalent and that there is no treatment effect; a hazard ratio between 0 and 1 would indicate that the hazard rate is lower in patients with DFMO-PCV treatment and that it is better than PCV alone; a hazard ratio > 1 indicates that the hazard rate is higher with DFMO-PCV treatment, and the treatment is inferior to PCV alone. The Cox proportional hazards regression model was used to estimate an average global hazard ratio. The Cox proportional hazards model assumes that the hazard ratio is constant over the follow-up period; however, when this assumption is violated, the model still provides an estimate of the average hazard ratio, but this estimate may not apply to any particular part (time) of follow-up. One method to estimate the hazard ratio when it varies in the follow-up period is to partition the follow-up period into intervals to estimate the hazard ratios separately for each interval. Another method is to compute the individual hazard functions and take their ratio. In this study, we have used these two methods to indicate how the hazard ratio is changing over time.

With these explanations in mind, let us reconsider the analysis (e.g., likelihood ratio test) that, by including data over the entire time period, assumes that the treatment effect (as measured by the hazard ratio) is constant over this entire period.

Table 5 Multiple covariate model utilizing treatment, extent of surgery, age, KPS, and histologic diagnosis for evaluable patients

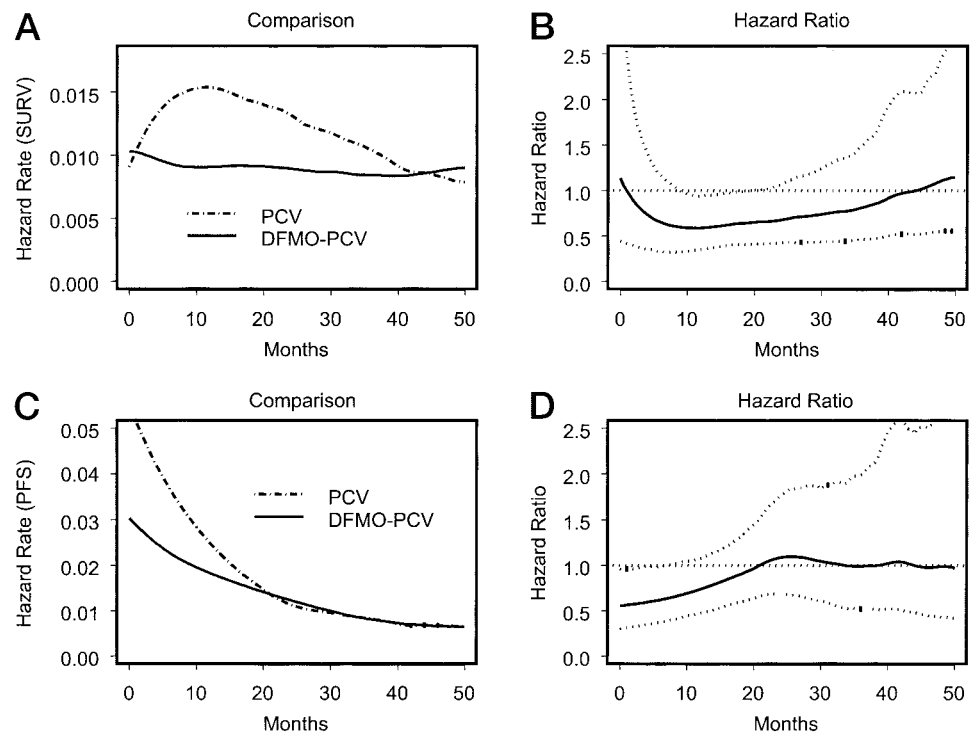
Variables	Survival hazard ratio (95% CI)	PFS hazard ratio (95% CI)
DFMO-PCV vs. PCV	0.74 (0.50, 1.10) $P = 0.13$	0.72 (0.50, 1.03) $P = 0.073$
Age, 20-year increase	2.27 (1.57, 3.28) $P < 0.0001$	1.94 (1.41, 2.66) $P < 0.0001$
KPS, 10-point increase	0.61 (0.47, 0.79) $P = 0.0002$	0.73 (0.57, 0.93) $P = 0.011$
Extent of surgery		
GTR vs. BX ^a	0.48 (0.28, 0.84) $P = 0.0074$	0.55 (0.33, 0.92) $P = 0.020$
STR vs. BX	0.65 (0.42, 1.00) $P = 0.048$	0.75 (0.50, 1.12) $P = 0.017$
AA vs. Non-AA	1.76 (1.09, 2.86) $P = 0.016$	1.72 (1.11, 2.66) $P = 0.011$

^a GTR, gross total resection; STR, subtotal resection.

Yet from Fig. 3, we have to question this assumption. Fig. 3 depicts the comparative hazard rates for the two treatment arms for SURV (Fig. 3A) and PFS (Fig. 3C) patients. From Figs. 3, B and D, it is apparent that the hazard ratio is not constant over time, and thus, the global analysis that assumes that the hazard function is constant over time is clearly invalid. The corresponding hazard functions converge at ~ 42 months for SURV patients (Fig. 3B) and at ~ 24 months for PFS (Fig. 3D) patients. On the basis of the observation that the hazard ratio for PFS is < 1 until ~ 24 months, we portioned the hazard functions into two mutually exclusive intervals and performed analyses separately in two time periods, before and after 24 months. For evaluable PFS patients, when we partitioned the follow-up period at 24 months and censored all observations beyond 24 months (32), the overall average crude hazard ratio for SURV in the first 24 months was 0.53 with $P = 0.02$ (0.55 with $P = 0.033$ after adjustment). When AA patients were analyzed separately, these relationships persisted ($P = 0.021$), whereas for non-AA patients, the relationships persisted but were not significant ($P = 0.27$). For analysis corresponding to the second period, we excluded all patients dying or censored during the first 24 months (leaving 166 patients for analysis). After 24 months, the average hazard ratio was 1.07 with $P = 0.83$. A similar relationship was seen for PFS, where the adjusted hazard ratio for AG patients was 0.63 with $P = 0.033$; for the AA subgroup, however, the hazard ratio was 0.67 with $P = 0.099$. These findings imply that the survival advantage for AG patients with a 1-year treatment of DFMO-PCV is limited to the first 2 years after treatment compared with patients treated with PCV alone.

Another way of viewing data from the first 24 months of the study is to use events charting (35). Fig. 4A depicts the time from registration to death (event) or censoring before 24 months for SURV patients; a similar charting of PFS data is shown in Fig. 4B. These plots demonstrate the impact of DFMO on PCV chemotherapy during the first 24 months of treatment by a reduction in the number of events in the DFMO-PCV arm compared with the PCV alone arm. DFMO-PCV patients had 32% fewer events (death or progression) at 24 months for PFS and 53% fewer events (death) at 24 months for SURV.

Fig. 3 Hazard function estimates with bootstrap 95% CI using evaluable patients are summarized as 2×2 plot matrices for SURV (A and B) and PFS (C and D). Included are the comparative hazard rates for the two treatment groups and hazard ratios (B and D) of these two hazard functions with 95% CI (dotted lines). In B, the hazard ratio is <1 until ~ 42 months, whereas in D, it is <1 until ~ 24 months.



Lastly, we considered whether the patient data will change sufficiently to justify later analyses. At this time, the average length of follow-up for the 124 patients whose follow-ups continue was 264 weeks, with $\sim 75\%$ of these patients having a follow-up > 2 years. Additional follow-up is unlikely to change the findings of this study, because the events will be occurring during the second period of follow-up, in which there is no treatment advantage (*i.e.*, deaths are occurring at the same rate in both treatment arms). Furthermore, it would require a prohibitively long time to accumulate the number of deaths required under the original plan. Although the average hazard rate of the observed data is ~ 0.003 deaths/person-week of follow-up, the rate declines over time and is ~ 0.0025 at 200 weeks. To reach 194 deaths (see study design in "Patients and Methods"), we would need ~ 90 more deaths. A death rate of 0.0025 would thus require ~ 615 additional person-years of follow-up, equating to an average of ~ 5 years of additional follow-up for each person. Waiting 5 years to observe deaths would not change the established finding that a significant treatment benefit for PCV combined with DFMO exists primarily in the first 24 months of follow-up.

DISCUSSION

Despite encouraging results from preclinical and Phase I/II clinical studies supporting a potential synergism between DFMO and a nitrosourea, adding DFMO to the nitrosourea PCV combination provided no significant improvement in survival over PCV alone for patients with glioblastoma multiforme (1). The current randomized cooperative trial, however, for the first time demonstrated a survival advantage for patients with AG who were treated with the nitrosourea combination of PCV and

DFMO. On the basis of Kaplan-Meier representations, the survival advantage was observed nearly throughout the 10-year trial and was reflected in actual survival gains of 1–2 years for patients taking DFMO-PCV compared with those taking PCV alone. Using hazard functions, PFS for DFMO-PCV was found to be superior to PCV alone only during the first 24 months after registration; after 24 months, the hazard rates were similar.

One criticism of the study may be that median SURV and SURV-D for patients treated under this protocol appear somewhat better than the published literature on PCV trials. Table 6 summarizes survival data from several studies that used PCV therapies for AG during the past 20 years. The fact that results from our PCV alone arm are better than results from other series begs the question why. One explanation is that unlike earlier studies, only limited field irradiation was used. Another explanation could be that patients in the current study were randomized and registered after completing irradiation. By protocol, however, patients were not excluded if their tumors grew during radiation therapy. Theoretically, one would expect the survival rate to be higher if failing patients are not offered access to the protocol because they represent more aggressive tumor phenotypes. However, patients whose tumors grew during irradiation represent a small cohort of AG patients. Of 174 consecutive patients irradiated at MDACC, only 23 (13%) demonstrated progression at the time of their first postirradiation MRI.⁴ Thus, although the possibility that some eligible patients were not enrolled in the study because of tumor progression during irra-

⁴ W. K. A. Yung, personal communication, 2002.

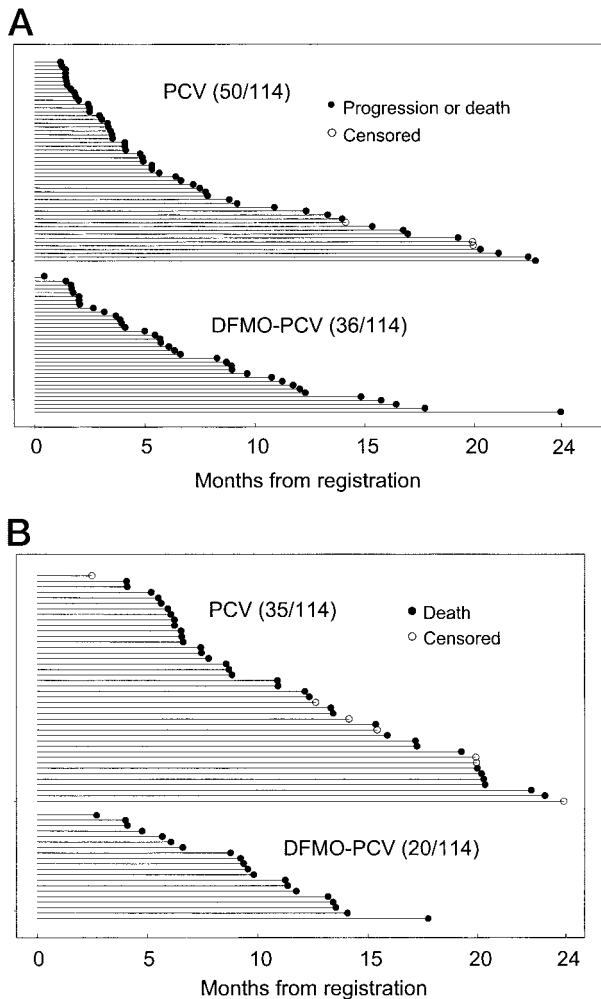


Fig. 4 Event charts of observations based on the 228 evaluable patients for the initial 24 months of study after postirradiation registration for those PFS (A) and SURV (B) patients. The values in parentheses are events/evaluable patients. For DFMO-PCV patients, there were 28% fewer events at 24 months for PFS patients and 43% fewer events at 24 months for SURV patients.

diation cannot be discounted, we expect that they will account for considerably <13% of patients and will not greatly impact the survival statistics. Interestingly, in the glioblastoma strata, survival in the PCV arm differed little from survival in historic PCV trials (1), even though the likelihood of progression during irradiation was considerably higher (40%).⁴

On the basis of outcome in this randomized study, however, the DFMO-PCV arm is superior to PCV alone and represents one of the clearest demonstrations of the clinical benefit of chemotherapy for the treatment of patients with AG. The conclusions drawn from analyses of the GBM and AG strata of this cooperative trial raise questions and possibilities for new treatment opportunities. Why does DFMO work for AG patients and not for GBM patients? Would the AG patients have done even better with DFMO treatment for an additional year after completing DFMO-PCV therapy?

We believe that the observed efficacy of DFMO is related

Table 6 Comparison of survival from diagnosis (current study) or pre-irradiation registration for anaplastic glioma patients treated with CCNU, procarbazine, and vincristine combinations

Treatment	n	Median survival, months
WBRT-HU-PCV (23) ^a	36	36.2
WBRT-mPCV (53)	31	34.2
BrdUrd-LFRT-PCV (54)	138	48
BrdUrd-LFRT-PCV (55, 56)	189	34.8–42.9
LFRT-PCV-current study	114	64.4
LFRT-DFMO-PCV-current study	114	78.6

^a WBRT, whole brain radiotherapy; LFRT, limited field radiation therapy; HU, hydroxyurea; mPCV, dose modified PCV; BrdUrd, bromodeoxyuridine.

in part to tumor ODC levels, because patients having tumors with relatively low levels of ODC appear to respond best to DFMO and DFMO-nitrosourea combinations. This conclusion is based on published observations showing that: (a) ODC levels are directly correlated with malignancy grade of glioma (36–40); (b) DFMO (+/-methylglyoxal bisguanylhydrazone) activity was not seen in patients with glioblastoma multiforme or medulloblastoma but was observed in patients with mid-grade AG (20, 21) who historically have lower ODC levels; and (c) for DFMO in combination with BCNU, activity was observed infrequently in glioblastoma multiforme patients at recurrence and was most obvious in patients with mid-grade AG (1, 22) who historically have lower ODC levels. Because ODC levels have been found to be directly related to malignancy grade for medulloblastoma (36, 37) and adenocarcinomas of the breast (41–47), lung (48), and colon (49, 50), we also anticipate that DFMO with a nitrosourea or nitrosourea combination would be more active against tumors that exhibit similar ODC relationships with malignant tumor grade, *i.e.*, patients with low ODC levels will respond better (a longer, more durable, response) than those with high ODC levels. It would be beneficial if this putative relationship between response to DFMO and tumor ODC levels could be demonstrated directly in prospective or retrospective studies rather than by inference, as is the case here.

Another treatment insight comes from the observation that the hazard function analyses show that the relative treatment effect of adding DFMO to PCV was manifest during the year of treatment but declined thereafter (being lost after 24 months when considering PFS and after 42 months when considering SURV). This implies that the major therapeutic gain from the addition of DFMO to PCV occurred in the first year of treatment and was sustained during the second year. This suggests that maintenance therapy during the second year, or possibly longer, might increase survival. An option would thus be to continue treatment with DFMO alone, which has shown activity as monotherapy for patients with AG (20, 21). The fact that the large differences between DFMO-PCV and PCV for PFS (33.6 months at median; Table 4) diminished >50% (14.7 months) when SURV was used may indicate the fact that AG tumors are sensitive to a variety of chemotherapy agents, and effective salvage therapies were used at progression (25, 51). This does not, however, detract from the important observation that the

addition of DFMO to a nitrosourea combination, such as PCV, improves survival for patients with AG.

Finally, this trial could provide insights that would be helpful when designing chemotherapy trials of newer drugs targeted to signaling pathways (inhibitors of farnesyltransferase and receptor tyrosine kinases and others) that will, in many cases, need to be combined with other agents to improve survival sufficiently so as to truly benefit cancer patients and gain regulatory approval. This may be more important for some cancers than for others, *e.g.*, patients with AG live considerably longer today than they did 30 years ago (52), even though AG is not readily cured. As a result, Phase III clinical trials could take in excess of 10–12 years to prove the benefit of chemotherapy combinations for regulatory approval based on current practice and guidelines. Obviously, this is a prohibitively long period of time for pharmaceutical company support given that AG accounts for ~0.5% of cancer cases and does not produce the same economic incentive that treating lung or breast cancer might engender. To improve opportunities for developing new and better treatments for patients with AG, it would be helpful to consider alternative strategies that place greater reliance on PFS and molecular surrogates of therapeutic relevance and benefit. We hope that this study provides a stimulus for the development of alternative controlled clinical trial approaches by both clinical investigators and pharmaceutical companies.

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