Phase II Study of Neoadjuvant 1, 3-Bis (2-Chloroethyl)-1-Nitrosourea and Temozolomide for Newly Diagnosed Anaplastic Glioma

A North American Brain Tumor Consortium Trial

Susan M. Chang, M.D.[1]
Michael D. Prados, M.D.[1]
W. K. Alfred Yung, M.D.[2]
Howard Fine, M.D.[3]
Larry Junck, M.D.[4]
Harry Greenberg, M.D.[4]
H. Ian Robins, M.D., Ph.D.[5]
Minesh Mehta, M.D.[5]
Karen L. Fink, M.D., Ph.D.[6]
Kurt A. Jaeckle, M.D.[7]
John Kuhn, M.D.[8]
Kenneth Hess, Ph.D.[9]
Clifford Schold, M.D.[10]

[1] Department of Neurological Surgery, Neuro-Oncology Service, University of California at San Francisco, San Francisco, California.

[2] Department of Neuro-Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[3] Neuro-Oncology Branch, National Cancer Institute, Bethesda, Maryland.

[4] Department of Neurology, University of Michigan, Ann Arbor, Michigan.

[5] Department of Radiotherapy, University of Wisconsin at Madison, Madison, Wisconsin.

[6] Department of Neurology, University of Texas Southwestern Medical Center, Dallas, Texas.

[7] Department of Neurology, Mayo Clinic, Jacksonville, Florida.

[8] Department of Pharmacology, The University of Texas at San Antonio, San Antonio, Texas.

[9] Department of Biostatistics and Applied Mathematics, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[10] Office of Clinical Research, University of Pittsburgh, Pittsburgh, Pennsylvania.

BACKGROUND. Temozolomide (TMZ) and 1, 3-bis (2-chloroethyl)-1-nitrosourea (BCNU) are reported to be active agents in anaplastic glioma (AG). TMZ has also been shown to deplete alkyltransferase, a DNA repair enzyme that contributes to nitrosourea resistance. The objective of the current study was to determine the efficacy and toxicity profile of a combination of these agents before radiotherapy in newly diagnosed AG.

METHODS. Eligibility criteria included histologically confirmed newly diagnosed AG with measurable enhancing disease, a Karnofsky performance score (KPS) ≥ 60, normal pulmonary function, and normal laboratory parameters. In addition, informed consent was obtained from all patients. BCNU given at a dose of 150 mg/m^2 intravenously was followed after 2 hours by TMZ given at a dose of 550 mg/m^2 orally on Day 1 of a 42-day cycle to a maximum of 4 cycles, unless there was tumor progression or unacceptable toxicity.

RESULTS. Forty-one eligible patients were accrued. Their median age was 40 years. Seventy-six percent of patients had a KPS of 90–100. The histology was 81% anaplastic astrocytoma, 12% anaplastic oligodendroglioma, and 7% mixed tumors. Twenty-two percent of patients did not complete 4 cycles because of toxicity, mainly hematologic. Forty-six percent of patients experienced Grade 3 or 4 (according to National Cancer Institute Common Toxicity Criteria) thrombocytopenia. Twenty percent had Grade 4 granulocytopenia. Two patients died while receiving therapy, 1 of progressive disease and the other of *Pneumocystis carinii* pneumonia. The complete and partial response rates were 2% and 27% respectively. An additional 54% of patients had stable disease. Seventeen percent developed progressive disease (10% after the first cycle and 7% after the second cycle).

CONCLUSIONS. This neoadjuvant strategy was associated with significant myelosuppression and a modest response rate in patients with newly diagnosed AG. ***Cancer* 2004;100:1712–6.** © *2004 American Cancer Society.*

KEYWORDS: anaplastic glioma, 1, 3-bis (2-chloroethyl)-1-nitrosourea (BCNU), temozolomide, neoadjuvant strategy.

Supported by National Institutes of Health (NIH) Grants CA62399 and CA62422 (Susan M. Chang and Michael D. Prados), CA62412 (W.K.A. Yung), CA62407 (Howard Fine), CA62421 (H. Ian Robins and Minesh Mehta), CA62455 (Karen L. Fink), CA62405 (Clifford Schold), and CA62426 (John Kuhn) and by the following General Clinical Research Center grants: M01-RR00079 (Susan M. Chang and Michael D. Prados), M01-RR00042 (Larry Junck and Harry Greenberg), M01-RR03186 (H. Ian Robins and Minesh Mehta), M01-RR00633 (Karen L. Fink), and M01-RR00056 (Clifford Schold).

The authors thank Sharon Reynolds, Department of Neurological Surgery, University of California at San Francisco, for editorial support.

Address for reprints: Susan M. Chang, M.D., Neuro-Oncology Service, University of California-San Francisco, 400 Parnassus Avenue, A808, San Francisco, CA 94143; Fax: (415) 353 2167; E-mail: changs@neurosurg.ucsf.edu

Received October 31, 2003; revision received December 12, 2003; accepted January 27, 2004.

© 2004 American Cancer Society
DOI 10.1002/cncr.20157
Published online 8 March 2004 in Wiley InterScience (www.interscience.wiley.com).

In the current multimodality approach to the management of malignant gliomas, maximum, safe resection is the initial treatment goal and radiotherapy remains the single most effective adjuvant treatment.[1] The role of chemotherapy in the management of malignant astrocytomas remains controversial. To our knowledge, no single agents or drug combinations studied have been shown to be more effective than the nitrosoureas. Single-agent 1, 3-bis (2-chloroethyl)-1-nitrosourea (BCNU) has been reported to have a similar efficacy compared with combination chemotherapy.[2] A recent metaanalysis by the Glioma Meta-analysis Trialists Group demonstrated a survival advantage to adjuvant chemotherapy (mainly nitrosoureas) for patients with malignant glioma, with a 15% relative decrease in the risk of death.[3]

Temozolomide (TMZ), an imidazotetrazinone, has shown antitumor activity in both glioma cell lines and in vivo models.[4–7] A response rate of 35%, with progression-free survival rates of 46% at 6 months and 24% at 12 months, were reported in clinical studies of patients with anaplastic astrocytoma (AA) at first recurrence.[8] Nausea, emesis, and minimal significant myelosuppression were some of the recorded toxicities. Based on these results, there is interest in evaluating the role of neoadjuvant TMZ in patients with malignant glioma.

The rationale for the combination of these agents is that they may have synergistic activity due to TMZ's depletion of O6 alkylguanine-DNA alkyltransferase (AGT), the DNA repair enzyme that is believed to contribute to BCNU resistance. This was demonstrated in preclinical models in which schedule dependency was observed not only for potential synergism but also for toxicity.[6] A Phase I study of the combination of BCNU and TMZ was completed by the North American Brain Tumor Consortium (NABTC).[9] In that study, patients were assigned randomly to two schedules of drug administration. In 1 arm, BCNU was administered first followed by TMZ 2 hours later. In the second arm, TMZ was given first and BCNU was given 4 hours later. The combination of TMZ followed by BCNU resulted in less hematologic toxicity than in the TMZ-first arm. No other significant nonhematologic toxicities were observed during the first cycle. Pulmonary toxicity occurred in subsequent cycles. The maximum tolerated doses (MTD) were 150 mg/m^2 of BCNU given intravenously followed by 550 mg/m^2 of TMZ given 2 hours later. This is the treatment plan used for the current study.

The primary objective of the current study was to assess the response rate of the combination of BCNU and TMZ administered neoadjuvantly to patients with anaplastic glioma (AG). Because the Phase I NABTC study focused on the toxicity for the first cycle of the combination of agents administered, another important objective of our Phase II study was to evaluate the potential cumulative toxicity of repeated cycles of the combination of these agents further in a larger number of patients.

MATERIALS AND METHODS

Patient Eligibility

Patients were eligible if they were age $\geq$ 18 years with a newly diagnosed, previously untreated, histopathologically confirmed, and centrally reviewed AG. Central pathology review was performed by Dr. Richard Davis at the University of California at San Francisco (UCSF). Measurable disease defined as contrast-enhancing bidimensional disease was mandated. Other eligibility criteria were a Karnofsky performance score (KPS) $\geq$ 60, as well as normal hematologic, renal, and hepatic function. Pulmonary function as measured by the carbon monoxide diffusing capacity (DLCO) was required to be $\geq$ 80%. Patients were not permitted to be pregnant or nursing and all patients (both men and women) agreed to practice birth control during the study. All patients or their surrogates signed an institutional review board-approved consent form.

Treatment Plan

The dose schedule consisted of an intavenous dose of BCNU of 150 mg/m^2 followed 2 hours later by an oral dose of TMZ of 550 mg/m^2 on Day 1 every 42 days for a maximum of 4 cycles. Radiotherapy was subsequently adminstered at the discretion of the treating physician but was not mandated in the protocol. Dose reductions were specified for toxicity. Weekly complete blood counts and differentials were obtained during therapy. A full chemistry panel evaluating hepatic and renal function was obtained before every course of chemotherapy. In addition, after every two cycles, lung function was assessed by measuring the DLCO. Clinical evaluations, including steroid requirements and neuroimaging, were obtained before each course of chemotherapy. Evaluation criteria were based on the standard criteria of Macdonald et al.[10] A complete response (CR) was defined as complete resolution of all enhancing disease and a partial response (PR) was defined as a reduction of $>$ 50% in lesion size using bidimensional measurements. Disease progression (DP) indicated an increase in lesion size of $>$ 25%. A magnetic resonance imaging scan was the imaging study of choice, but a computed tomography scan was allowed. The protocol required that each patient be followed with the same imaging modality throughout the trial. Treatment was continued for a total of four cycles unless there was evidence of tumor progres-

TABLE 1
Patient Characteristics (n = 41)

Median age (range)	40 (18–71) yrs
Karnofsky performance score (%)	
90–100	76
70–80	19
60	5
Male (%)	54
White (%)	98
Histology and extent of resection (%)	
Anaplastic astrocytoma	81
Biopsy only	52
STR	36
GTR	12
Anaplastic oligodendroglioma	12
Biopsy only	0
STR	80
GTR	20
Anaplastic mixed	7
Biopsy only	0
STR	67
GTR	33

STR: Subtotal resection, GTR: macroscopic (gross) total resection.

sion, toxicity precluding continuation of therapy, or patient refusal. Toxicities were graded using the National Cancer Institute Common Toxicity Criteria.[11]

The primary end point was a CR or PR to the chemotherapy regimen. Response was measured after the maximum number of cycles was completed by the patient. Central review of the responses was mandated and was performed by the Department of Neuroradiology at UCSF. A 1-stage design with estimation of a response rate of 40% was the goal. With enrollment of 45 patients, the 95% confidence interval (95% CI) on the true response would be 26–56% or approximately $\pm$ 15%.

RESULTS

A total of 50 patients were enrolled in the study. After central review of pathology, 7 patients were found to have histology other than AG (6 patients had glioblastoma multiforme and 1 patient had Grade 2 oligoastrocytoma). Two other patients were not eligible, one because the DLCO requirement was not met and one because measurable disease was not documented.

The characteristics of the 41 eligible patients are shown in Table 1. Their median age was 40 years and the majority of patients had a KPS of $\geq$ 70. AA was the predominant histology. The extent of resection based on histology is presented in Table 1.

A summary of the disposition of all the patients treated is shown in Table 2. Twenty-four patients (59%) completed 4 cycles of therapy as planned. Of these 24 patients, 10 received full doses for all cycles,

TABLE 2
Disposition of Patients

Disposition of patients	No.	(%)
No. completed four cycles therapy	24	(59)
No. progressed/dead of PD before four cycles[a]	7[a]	(17)
No. off because of toxicity/death due to toxicity	9[b]	(22)
No. refused therapy (after two cycles)	1	(2)

PD: progressive disease.

[a] Six patients progressed and one died.

[b] Eight patients experienced toxicity and discontinued therapy and one patient died. The death was secondary to *Pneumocystis carinii* pneumonia.

TABLE 3
Toxicity Profiles

Toxicity	Grade (%)[a,b]				
	1	2	3	4	5
Anemia	44	12	7	0	0
Granulocytopenia	12	15	1	20	0
Infection	0	0	0	2	2
Thrombocytopenia	34	2	24	22	0
Pulmonary function	2	2	0	0	0
Nausea	20	20	5	0	0
Emesis	5	2	7	0	0
Fatigue	34	15	2	0	0

[a] Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria.

[b] Percentages represent toxicities observed over all cycles received.

4 required a dose reduction of 1 level, and 10 required a dose reduction of 2 levels. Twenty-two percent of patients did not complete 4 cycles because of toxicity, mainly hematologic. Forty-six percent of patients experienced Grade 3 or 4 thrombocytopenia. Twenty percent had Grade 4 granulocytopenia. Two patients died while receiving therapy, one of DP and the other of *Pneumocystis carinii* pneumonia. Seventeen percent developed DP (10% after the first cycle and 7% after the second). For patients who were not able to complete therapy because of toxicity, 7% stopped after the first cycle, 13% after the second, and 2% after the third. One patient (2%) refused therapy after the second cycle. Table 3 shows the toxicity profiles of the patients (percentages represent toxicities observed over all cycles received). Myelosuppression was the most common and most severe toxicity observed.

Response data are shown in Table 4. Ten patients were not evaluable for response because of toxicity, DP, or patient withdrawal. Twelve of the 41 patients (29%) achieved an objective response (a CR or PR) with a 95% binomial CI of 16–46%. Of the PRs observed, eight patients had AA histology, two had mixed glioma histology, and one had anaplastic oligodendro-

TABLE 4
Response Results

Characteristics	CR (%)	PR (%)	SD (%)	PD (%)
All patients (n = 41)	1 (2)	11 (27)	22 (54)	7 (17)
Patients evaluable for response (n = 31)	1 (3)	8 (26)	15 (48)	7 (23)

CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

glioma histology. For the fully evaluable patients (n = 31), 3% achieved a CR (AA histology) and 26% achieved a PR. Another 48% had stable disease and 23% had DP.

DISCUSSION

To our knowledge, effective chemotherapeutic options for patients with malignant glioma are limited. The most commonly used agents are the nitrosoureas and TMZ. For patients with recurrent AA, objective response rates of 14% and 35% have been reported for the nitrosoureas and TMZ, respectively.[8,9,12] Preclinical data have demonstrated a synergistic effect for the combination of BCNU and TMZ[6] and Phase I trials have defined the MTD for the combination. In the NABTC Phase I trial,[9] the sequence of BCNU followed by TMZ was selected based on higher doses of the agents administered with this regimen, compared with TMZ administered before BCNU. These agents have overlapping toxicities and the dose-limiting toxicities encountered for the first cycle of the combination were hematologic. Pulmonary toxicity also occurred after subsequent cycles.[9] We chose a single-dose schedule of TMZ based on the NABTC Phase I trial. Although multiday dosing schedules for this drug combination were being evaluated, cumulative toxicity was our primary concern. Other clinical trials have since reported other dose schedules for the combination of BCNU/TMZ.

The primary objective of the current study was to evaluate the role of this combination of agents in the neoadjuvant setting in patients with newly diagnosed AG. The advantages of selecting this patient population are the lack of confounding effects secondary to radiotherapy and the ability to determine whether there is a synergistic effect between BCNU and TMZ. A secondary objective was to further characterize the toxicity profile of the combination.

Several studies published to date have addressed the neoadjuvant use of chemotherapy for malignant glioma.[13–16] In these studies, the majority of patients had glioblastoma multiforme histology, so it is difficult to assess the response rates specifically for the smaller samples of patients with AA. Delay or omission of radiotherapy, especially in children, has been the major goal of neoadjuvant chemotherapy. There is also a theoretic rationale for administering chemotherapeutic agents before radiotherapy to allow the maximum distribution of the agents that may otherwise be compromised by the alteration of blood vessels by radiotherapy. This strategy is safe once frequent assessment of response is incorporated in the design of the study and radiotherapy is used at the first indication of clinical or radiographic DP. To our knowledge, no comparative study has been performed to date evaluating this strategy in newly diagnosed patients with malignant glioma. Although there are some reports regarding this strategy, many of them include patients with Grade 4 tumors (glioblastoma multiforme).[17–20] Therefore, it is difficult to compare our results with the results of those studies.

The combination of BCNU and TMZ at this dose schedule was found to have a modest effect with a CR or PR rate of 29% for fully evaluable patients. Another 48% of patients acheived stable disease whereas 23% progressed before completing the 4 cycles of therapy. This was well below the a priori stated 40% response rate that was the goal of the study. This relatively high percentage was used as a measure of "success" because of the expectation that these agents would work synergistically with a resulting high response rate. The difficulty in determining the benefit of the combination of these agents in this patient population, however, lies in the lack of historical controls to establish the response rate of the individual agents used neoadjuvantly in patients with AA. Compared with the results reported by Gilbert at al.,[21] this does not appear to be more advantageous than single-agent TMZ given on a 5/28-day standard regimen, which had a 34% objective response rate in 21 patients with newly diagnosed AA before radiotherapy. The combination of BCNU/TMZ was certainly also more toxic, which may have precluded the possibility of this combination having greater efficacy than TMZ alone in what would be considered a fairly good prognostic group of patients.

The toxicity profile for the current study was predominantly hematologic. As a result, 22% of patients did not complete the 4 cycles of therapy. The dose schedule for the current study was selected based on the findings of the Phase I study. However, it is important to note that the determination of dose-limiting toxicities and MTD in the Phase I study was based on the tolerance of the first cycle only. This is an inherent limitation in extrapolating how patients may tolerate extended cycles and emphasizes the importance of continued toxicity assessment in Phase II studies.[22,23] Although some information regarding the

feasibility of administering multiple cycles of therapy may be gained from Phase I studies, this is often limited because of the refractory nature of the disease and the likelihood of early progression.

There have been other studies of BCNU in combination with an agent that may inhibit AGT, an enzyme shown to be important for nitrosourea drug resistance.[24,25] When BCNU is used in combination with O6-benzylguanine, an inhibitor of AGT, the MTD of BCNU was found to be only 40 mg/m^2. This is in contrast to a dose of 200 mg/m^2 of BCNU when used as a single agent. The toxicity precluding dose escalation was myelosuppression, similar to the result in our study of the combination of TMZ with BCNU. The hypothesis is that AGT also is necessary in normal dividing cells to allow repair of the alkylation of the DNA by BCNU and that the inhibition of AGT by O6-benzylguanine is nonspecific in its effects. A similar mechanism may be present when TMZ and BCNU are used in combination, thereby limiting the combination strategy.

The neoadjuvant strategy used in the current study is associated with significant myelosuppression and a modest response rate in patients with newly diagnosed AG. The dose schedule used in the current trial is not sufficiently efficacious or well tolerated to merit further study. The use of alternative dosing schedules of TMZ after a fixed dose of BNCU in patients with AG will be required if this neoadjuvant strategy is to be evaluated further.

REFERENCES

1. Chang S, Theodosopoulos P, Sneed P. Multidisciplinary management of adult anaplastic astrocytomas. *Semin Radiat Oncol.* 2001;11:163–169.
2. Prados MD, Scott C, Sandler H, et al. A phase 3 randomized study of radiotherapy plus procarbazine, CCNU, and vincristine (PCV) with or without BUdR for the treatment of anaplastic astrocytoma: a preliminary report of RTOG 9404. *Int J Radiat Oncol Biol Phys.* 1999;45:1109–1115.
3. Chemotherapy for high-grade glioma. *Cochrane Database Syst Rev.* 2002CD003913.
4. Friedman HS, Dolan ME, Pegg AE, et al. Activity of temozolomide in the treatment of central nervous system tumor xenografts. *Cancer Res.* 1995;55:2853–2857.
5. O'Reilly SM, Newlands ES, Glaser MG, et al. Temozolomide: a new oral cytotoxic chemotherapeutic agent with promising activity against primary brain tumours. *Eur J Cancer.* 1993;29A:940–942.
6. Plowman J, Waud WR, Koutsoukos AD, Rubinstein LV, Moore TD, Grever MR. Preclinical antitumor activity of temozolomide in mice: efficacy against human brain tumor xenografts and synergism with 1,3-bis(2-chloroethyl)-1-nitrosourea. *Cancer Res.* 1994;54:3793–3799.
7. Stevens MF, Newlands ES. From triazines and triazenes to temozolomide. *Eur J Cancer.* 1993;29A:1045–1047.
8. Yung WK, Prados MD, Yaya-Tur R, et al. Multicenter phase II trial of temozolomide in patients with anaplastic astrocytoma or anaplastic oligoastrocytoma at first relapse. Temodal Brain Tumor Group. *J Clin Oncol.* 1999;17:2762–2771.
9. Schold SC Jr., Kuhn JG, Chang SM, et al. A phase I trial of 1,3-bis(2-chloroethyl)-1-nitrosourea plus temozolomide: a North American Brain Tumor Consortium study. *Neuro-oncol.* 2000;2:34–39.
10. Macdonald DR, Cascino TL, Schold SC Jr., Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol.* 1990;8:1277–1280.
11. (CTCAE) CTCfAEv. Available from URL: http://ctep.cancer.gov/reporting/ctc.html [accessed December 10, 2003].
12. Wong ET, Hess KR, Gleason MJ, et al. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials [abstract]. *J Clin Oncol.* 1999;17:2572.
13. Kirby S, Macdonald D, Fisher B, Gaspar L, Cairncross G. Pre-radiation chemotherapy for malignant glioma in adults. *Can J Neurol Sci.* 1996;23:123–127.
14. Friedman HS, McLendon RE, Kerby T, et al. DNA mismatch repair and O6-alkylguanine-DNA alkyltransferase analysis and response to Temodal in newly diagnosed malignant glioma. *J Clin Oncol.* 1998;16:3851–3857.
15. Recht L, Fram RJ, Strauss G, et al. Preirradiation chemotherapy of supratentorial malignant primary brain tumors with intracarotid cis-platinum (CDDP) and i.v. BCNU. A phase II trial. *Am J Clin Oncol.* 1990;13:125–131.
16. Vinolas N, Gil M, Verger E, et al. Pre-irradiation semi-intensive chemotherapy with carboplatin and cyclophosphamide in malignant glioma: a phase II study. *Anticancer Drugs.* 2002;13:163–167.
17. Grossman SA, Norris LK. Adjuvant and neoadjuvant treatment for primary brain tumors in adults. *Semin Oncol.* 1995; 22:530–539.
18. Grossman SA, Scheidler VR, Ahn H, et al. Complete and partial responses of newly diagnosed malignant astrocytomas following continuous infusion BCNU and cisplatin [abstract]. *Proc Am Soc Clin Oncol.* 1989;8:88.
19. Gilbert MR, Lundsford LO, Kondziolka D, et al. A phase II trial of continuous infusion chemotherapy, external beam radiotherapy and local boost radiotherapy for malignant gliomas [abstract]. *Proc Am Soc Clin Oncol.* 1993;12:176.
20. Grossman SA, O'Neill A, Grunnet M. Phase III study comparing 3 cycles of infusional BCNU/cisplatin followed by radiation with radiation and concurrent BCNU for patients with newly diagnosed supratentorial GBM: ECOG 2394-SWOG 4508 [abstract]. *Proc Am Soc Clin Oncol.* 2000;19:158.
21. Gilbert MR, Friedman HS, Kuttesch JF, et al. A phase II study of temozolomide in patients with newly diagnosed supratentorial malignant glioma before radiotherapy. *Neuro-oncol.* 2002;4:261–267.
22. Bryant J, Day R. Incorporating toxicity considerations into the design of two-stage phase II clinical trials. *Biometrics.* 1995;51:1372–1383.
23. Sargent DJ, Chan V, Goldberg RM. A three-outcome design for phase II clinical trials. *Control Clin Trials.* 2001;22:117–125.
24. Quinn JA, Pluda J, Dolan ME, et al. Phase II trial of carmustine plus O(6)-benzylguanine for patients with nitrosourea-resistant recurrent or progressive malignant glioma. *J Clin Oncol.* 2002;20:2277–2283.
25. Friedman HS, Pluda J, Quinn JA, et al. Phase I trial of carmustine plus O6-benzylguanine for patients with recurrent or progressive malignant glioma. *J Clin Oncol.* 2000;18: 3522–3528.