Phase 1 trial of irinotecan plus BCNU in patients with progressive or recurrent malignant glioma[1]

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Irinotecan is a topoisomerase I inhibitor previously shown to be active in the treatment of malignant glioma. We now report the results of a phase 1 trial of irinotecan plus BCNU, or 1,3-bis(2-chloroethyl)-1-nitrosourea, for patients with recurrent or progressive MG. Irinotecan dose escalation occurred independently within 2 strata: patients receiving enzyme-inducing antiepileptic drugs (EIAEDs) and patients not receiving EIAEDs. BCNU was administered at a dose of 100 mg/m^2 over 1 h every 6 weeks on the same day as the first irinotecan dose was administered. Irinotecan was administered intravenously over 90 min once weekly. Treatment cycles consisted of 4 weekly administrations of irinotecan followed by a 2-week rest with dose escalation in cohorts of 3 to 6 patients. Seventy-three patients were treated, including 49 patients who were on EIAEDs and 24 who were not on EIAEDs. The maximum tolerated dose for patients not on EIAEDs was 125 mg/m^2. The maximum tolerated dose for patients on EIAEDs was 225 mg/m^2. Dose-limiting toxicity was evenly distributed among the following organ systems: pulmonary, gastrointestinal, cardiovascular, neurologic, infectious, and hematologic, without a clear predominance of toxicity involving any one organ system. There was no evidence of increasing incidence of toxicity involving one organ system as irinotecan dose was escalated. On the basis of these results, we conclude that the recommended doses of irinotecan for a phase 2 clinical trial when given in combination with BCNU (100 mg/m^2) are 225 mg/m^2 for patients on EIAEDs and 125 mg/m^2 for patients not on EIAEDs. *Neuro-Oncology 6, 145–153, 2004 (Posted to Neuro-Oncology [serial online], Doc. 03-049, February 25, 2004. URL http://neuro-oncology.mc.duke.edu; 10.1215/S1152 8517 03 00049 8)*

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[3] Abbreviations used are as follows: AA, anaplastic astrocytoma; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine); CR, complete response; DLCO, diffusing capacity of the lung for carbon monoxide; DLT, dose-limiting toxicity; EIAED, enzyme-inducing antiepileptic drug; GBM, glioblastoma multiforme; MG, malignant glioma; MTD, maximum-tolerated dose; PD, progressive disease; PR, partial response; SD, stable disease.

Resistance to chemotherapy remains the central reason for the failure to cure patients with a diverse spectrum of malignancies. Malignant glioma (MG)[3] is a neoplasm with particularly dismal attributes in that virtually all tumors display marked de novo or

acquired drug resistance and ultimate lethal growth. Conventional treatment with surgery, radiotherapy, and alkylnitrosourea-based chemotherapy cures a minority of patients with anaplastic astrocytoma (AA) and no patients with glioblastoma multiforme (GBM) (Fine, 1994; Levin et al., 1985; Shapiro, 1986). Although BCNU, a contraction of the chemical name 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine), remains the community standard of care because of a modest increase in median survival (Chang et al., 1983; Green et al., 1983), resistance to this alkylnitrosourea invariably occurs, and the patient dies.

Combination chemotherapy is a treatment strategy designed to produce therapeutic effects that are more favorable than those of a single drug, while minimizing normal organ toxicity and thwarting the emergence of drug-resistant tumor cells (Rideout and Chou, 1991). An optimal combination involves drugs that are less than additive in producing host organ toxicity, but more than additive in producing an antitumor effect.

Irinotecan and BCNU are ideal candidates for combination chemotherapy because they exert their antitumor effects through interactions with different targets and have different organ toxicities. Irinotecan is a topoisomerase I inhibitor that stabilizes the covalent bond between topoisomerase I and DNA, a bond formed during synthesis of new DNA, thereby inhibiting the DNA re-ligation and ultimately leading to cell death. The dose-limiting toxicity (DLT) of irinotecan is diarrhea (Slichenmyer et al., 1993). BCNU produces its antitumor effect by covalently binding an alkyl group to a cellular molecule to form an adduct that cross-links DNA, which leads ultimately to cell death (Hall and Tilby, 1992). The DLT of BCNU is myelosuppression (Colvin and Chabner, 1990).

Recent studies with human glioma xenograft D-54 MG have shown that irinotecan given with BCNU produces striking antitumor activity, with a greater than additive effect at all doses tested (Coggins et al., 1998). Other recent studies elucidate the optimal regimen (Castellino et al., 2000) and the likely mechanism of this enhancement (Pourquier et al., 2000; Sekikawa et al., 2000). These studies strongly suggest that maximal enhancement of antitumor activity without an increase in toxicity is seen when BCNU and irinotecan are started on the same day. Taken together, these studies suggest that O^6-alkylation with temozolomide or BCNU is required to enhance the antitumor activity of irinotecan.

We now report a phase 1 trial of irinotecan plus BCNU with patients who have recurrent or progressive malignant glioma that is designed (1) to determine the maximum tolerated dose (MTD) of irinotecan when administered with a standard dose of BCNU and (2) to define the toxicity of this regimen.

Patients and Methods

Protocol Objectives

The objectives of the study were as follows: to define the MTD of irinotecan when administered following BCNU (100 mg/m^2), to characterize any toxicity associated with the combination of irinotecan and BCNU, and to note antitumor activity.

Patient Eligibility Criteria

For entry into the study, patients were required to have a histologically confirmed primary malignant glioma (AA, GBM, or gliosarcoma) with evidence of recurrence or progression, measurable on contrast-enhancing MRI, or on CT when MRI was medically contraindicated. Patients were eligible if they were 18 years of age or older with a Karnofsky performance status $\geq$60% at study entry. An interval of at least 3 weeks since prior surgical resection, and 6 weeks since prior radiotherapy or chemotherapy, must have elapsed for the patient to be enrolled into the clinical trial unless there was unequivocal evidence of tumor progression. Additional enrollment criteria included adequate pretreatment bone marrow, renal, hepatic, and pulmonary function (hematocrit concentration >29%, absolute neutrophil count >1500 cells/μl, platelet count >125,000 cells/μl, serum creatinine level <1.5 mg/dl, blood urea nitrogen <25 mg/dl, serum aspartate aminotransferase and bilirubin level <1.5 times the upper limit of normal, and diffusing capacity of the lung for carbon monoxide [DLCO] $\geq$60% after correction for low hemoglobin). For patients on corticosteroids, a stable dose for 1 week before entry was required. Women of reproductive potential were required to take contraceptive measures for the duration of the therapy. All patients were informed of the investigational nature of the study and were required to sign informed consent forms approved by the Duke University Medical Center Institutional Review Board.

Exclusion criteria included the following: (1) pregnancy, (2) co-medication that might interfere with the study results, for example, immunosuppressive agents other than corticosteroids, and (3) prior failure of irinotecan or BCNU.

Treatment Design

Recently published research (Friedman et al., 1999) revealed a significant drug-drug interaction between irinotecan and enzyme-inducing antiepileptic drugs (EIAEDs) (phenytoin, carbamazepine, and phenobarbital) leading to a 2-fold higher irinotecan clearance and lower systemic levels of irinotecan and its major metabolites, SN-38 and SN-38G. Therefore, patients were accrued independently into 2 separate strata beginning with the accrual of the thirty-second patient. The first stratum consisted of patients not receiving phenytoin, carbamazepine, or phenobarbital. The second consisted of patients receiving one or more of these antiepileptic drugs.

Cohorts of 3 to 6 patients were treated with BCNU at a dose of 100 mg/m^2, followed approximately 1 h later by irinotecan at an initial dose of 20 mg/m^2. Additional cohorts of 3 to 6 patients were treated with escalating doses of irinotecan until DLT was observed. The first 3 assessable patients at a dose level must have been followed for 6 weeks following the initial dose of irinotecan/

BCNU without experiencing DLT prior to entry of patients at the next dose level.

BCNU was commercially available and administered intravenously in 0.9% saline over 1 h. BCNU was administered every 6 weeks on the same day as the first irinotecan dose. Irinotecan was commercially available and administered intravenously in D5W (dextrose 5% in water) over 90 min starting 1 h after the completion of the BCNU infusion. A treatment cycle consisted of 4 weekly administrations of irinotecan followed by a 2-week rest.

Dose Escalation and Statistical Consideration

Succeeding dose levels of irinotecan were as follows: 20, 40, 60, 80, 100, 125, 150, 175, 200, 225, 250, 275, and 300 mg/m^2. A modified classical "3+3" dose escalation design was employed in this study, which permitted up to 3 additional patients to be accrued at a given dose level as long as none of the first 3 patients enrolled at that dose level experienced a DLT. The dose level was escalated in successive cohorts of 3 patients as long as no DLT was observed. If 1 instance of DLT was observed among the initial 3 assessable patients treated at a dose level, an additional 3 patients had to be treated at that dose level with no further DLT in order for dose escalation to proceed. If 2 instances of DLT were observed at a dose level, the MTD was determined to be surpassed, and a total of 6 patients were treated at the previous level to ensure its tolerability. The MTD was therefore the highest dose causing DLT in no more than 1 of 6 patients at that dose level. Any patient who had stable or responding disease who developed DLT could continue to be treated at the next lowest dose level, provided the patient's toxicity resolved to grade 1 or lower and no more than 2 weeks were required for recovery. However, the patient was removed from study if DLT occurred on the lower dose.

Dose-limiting toxicity was defined as grade 3 or greater nonhematopoietic toxicity or grade 4 hematologic toxicity. A DLCO $\leq$60% was considered a DLT. Furthermore, failure to recover from any non-DLT to no greater than grade 1 toxicity within 2 weeks of the end of the cycle (i.e., 8 weeks from drug administration) was considered a DLT.

Toxicity Evaluation

Toxicity was graded according to the National Cancer Institute's Common Toxicity Criteria Version 2.0 (NCI, 1999). Complete blood counts were obtained weekly from patients, and each patient underwent a physical examination, pulmonary function studies, measure of blood urea nitrogen/creatinine, liver function studies, and serum electrolyte measurements prior to initiating each cycle of therapy.

Response Evaluation

Determination of overall response was based on radiographic changes in tumor size as revealed by CT or MRI, and clinical criteria including steroid requirement and neurologic examination. Complete response (CR) was defined as the complete disappearance of all enhancing or nonenhancing tumor from baseline on consecutive scans at least 6 weeks apart while the patient was not receiving corticosteroids and was neurologically stable or improved. Partial response (PR) was defined as $\geq$50% reduction from baseline in the size (measured as the product of the largest perpendicular diameters) of enhancing or nonenhancing tumor maintained for at least 12 weeks, use of a stable or reduced corticosteroid dose, and stable or improved neurologic exam. Progressive disease (PD) was defined as >25% increase in size of enhancing or nonenhancing tumor, or any new tumor on MRI scan after 6 weeks of therapy, or neurological worsening of the patient without a documented non-neurologic etiology while on a stable or increased corticosteroid dose. Stable disease (SD) was defined as any other clinical status not meeting the criteria for CR, PR, and PD that was observable for at least 12 weeks.

Results

Patient Data

A total of 73 patients (45 males and 28 females) were enrolled onto the study (Table 1). Their demographic data are summarized in Table 2. At study entry, median age was 48 years (range, 20–75 years). Consistent with the epidemiology of CNS neoplasms in adults, the majority of patients (73%) had GBM, whereas cases of AA were less frequent (22%), and anaplastic oligodendroglioma (4%) and anaplastic mixed glioma (1%) were rare. Upon study entry, all patients had progressive tumor following initial therapy with resection, radiotherapy, or chemotherapy. All but 1 patient received prior radiotherapy, and all but 7 patients underwent initial tumor resection. Thirty-one patients received between 1 and 3 prior chemotherapy regimens. In addition to radiotherapy, 3 patients received liquid brachytherapy with radioisotope-labeled monoclonal antibodies.

Seventy-three patients were registered in the study at 1 of 11 dose levels of irinotecan. Following initiation of this study, it was demonstrated that EIAEDs affect irinotecan pharmacokinetics (Friedman et al., 1999). Therefore, the protocol was amended to stratify patients into 2 strata: stratum 1, composed of patients not receiving phenytoin, carbamazepine, or phenobarbital; and stratum 2, composed of patients receiving either phenytoin, carbamazepine, or phenobarbital. Stratification by EIAED use began at an irinotecan dose of 175 mg/m^2, and accrual into stratum 1 occurred in a retrospective manner, whereas accrual into stratum 2 occurred in a prospective manner. Since the MTD was higher for patients treated on stratum 2, 49 patients were enrolled into this stratum, whereas 24 patients were enrolled into stratum 1.

The MTD was reached at an irinotecan dose of 125 mg/m^2 for patients allocated to stratum 1. The MTD was reached at an irinotecan dose of 225 mg/m^2 for patients allocated to stratum 2.

Table 1. Patient profile and evaluation stratified by AED use and irinotecan dose

Patient	Diagnosis	Age (years)	Prior Therapy	Irinotecan Dose (mg/m^2)	Cycles	Best Response	Toxicities	
							Non-Dose-Limiting	Dose Limiting
No EIAED Use								
1	GBM	64	Resection, XRT	20	1	PD		
3	GBM	51	Resection, XRT	20	1	PD		
4	GBM	52	Resection, XRT	20	4	SD		
5	GBM	70	Resection, XRT, Etoposide	20	1	PD		
9	AA	64	Resection, XRT, Tamoxifen	40	5	SD		
11	GBM	49	Resection, XRT	40	1	PD		
13	GBM	63	Resection, XRT	60	1	PD		
18	GBM	44	Resection, XRT, PCV	60	5	SD		
20	GBM	57	Resection, XRT	80	4	SD		
21	GBM	61	Resection, XRT	80	4	SD		
26	GBM	39	XRT	100	11	SD		
32	GBM	38	Resection, XRT, Gliadel, Carbo, PCV	125	2	PD		
51	AA	31	Resection, XRT	125	2	PD		
54	GBM	65	Resection, XRT, Temo	125	1	PD*		
62	GBM	48	Resection, XRT, Temo, Thalidomide	125	1	PD		ANC 4, Vasovagal 3
63	GBM	48	Resection, XRT	125	3	SD		
67	GBM	58	Resection, XRT	125	3	SD		
68	GBM	47	Resection, XRT	125	1	PD		
36	GBM	43	Resection, XRT	150	2	PD		↓ DLCO 3, ANC 4
37	GBM	70	Resection, XRT, Gliadel	150	3	SD		
43	GBM	40	Resection, XRT, Gliadel	175	2	SD	PE 4 + DVT 4**	
44	GBM	40	Resection, XRT	175	1	PD		
46	GBM	71	Resection, XRT	175	1	PD*		Infection with neutropenia 3, diarrhea 3
48	GBM	54	XRT	150	1	PD		ANC 4, Hgb 4, Infection 4
EIAED Use								
2	GBM	42	Resection, XRT, Paclitaxol	20	2	PD		
6	GBM	46	Resection, XRT, Temo	20	1	PD		
7	GBM	74	Resection, XRT	20	2	PD		
8	AA	49	XRT	40	5	PR		
10	GBM	52	Resection, XRT	40	1	SD		Pulmonary fibrosis 5
12	GBM	66	Resection, XRT	40	1	PD	Pulmonary infiltrates 1	
14	AA	44	Resection, XRT	60	3	SD		
15	AA	40	Resection, XRT	60	1	PD		
16	AO	50	Resection	60	7	SD		
17	AO	56	Resection, XRT	60	9	SD		
19	AA	55	Resection, XRT	80	1	PD*	Plt 3	Infection 3
22	GBM	55	Resection, XRT	80	3	SD		
23	GBM	46	Resection, XRT	80	6	SD		
24	AA	42	XRT, Temo, Tamoxifen	100	1	PR		Infection 5
25	GBM	20	Resection, XRT, Temo	100	1	PD		
27	GBM	47	Resection, XRT	100	3	SD		
28	GBM	48	Resection, XRT, Gliadel, Temo	100	2	PD		
29	GBM	67	Resection, XRT	100	1	PD		
30	GBM	53	Resection, XRT	125	2	PD		
31	AA	64	XRT, PCV	125	2	PD		Febrile neutropenia 3
33	AOA	28	XRT, Temo, Topotecan	125	4	SD		

Table 1. Continued

Patient	Diagnosis	Age (years)	Prior Therapy	Irinotecan Dose (mg/m^2)	Cycles	Best Response	Toxicities	
							Non Dose Limiting	Dose Limiting
EIAED Use								
34	GBM	44	Resection, XRT	125	2	PD		
35	GBM	75	Resection, XRT	125	1	PD		
38	GBM	50	Resection, XRT, MAb	150	1	PD		
39	GBM	46	Resection, XRT	150	1	PD		
40	GBM	51	Resection, XRT, Temo	150	6	SD		
41	GBM	47	XRT	150	8	SD		
42	GBM	38	Resection, XRT, Temo	175	4	SD		
45	GBM	37	Resection, XRT	175	1	PD		
47	AA	37	Resection, XRT	175	3	SD		
49	GBM	35	Resection, XRT	200	3	SD		
50	GBM	55	Resection, XRT, CCNU, MAb, Tamoxifen, Etoposide	200	1	PD		
52	AA	37	Resection, XRT, Temo	200	1	PD		
53	GBM	45	Resection, XRT, CCNU, Temo	200	1	PD		
55	AA	31	Resection, XRT, Temo	200	1	PD	Infection 2	N/V 3
56	GBM	58	Resection, XRT, CCNU, Temo	225	1	PD*	NA	NA
57	GBM	45	Resection, XRT, Gliadel	225	1	PD		
58	AA	53	Resection, XRT, CCNU, Temo	225	2	PD		
59	GBM	75	Resection, XRT, Thiotepa, Carbo	225	1	PD	Plt 3	
60	GBM	36	Resection, XRT	225	5	PR		
61	AA	23	Resection, XRT, Gliadel, Temo	225	1	PD		
72	GBM	35	Resection, XRT	225	1	PD*	NA	NA
73	AO	41	Resection, XRT, Temo, MAb	225	7	PR		
64	AA	41	Resection, XRT, CCNU, Temo	250	1	SD		N/V 3, Diarrhea 3
65	AA	34	XRT, Temo	250	1	PD*	Cerebral herniation 5*	
66	AA	41	Resection, XRT	250	1	PD		
69	GBM	49	Resection, XRT	250	3	PR		
70	GBM	52	Resection, XRT, Temo	250	1	NA		Cardiopulmonary arrest 5
71	GBM	53	Resection, XRT	250	9	SD		

*Clinical evidence of tumor progression but MRI not performed.

**Adverse event attributed to tumorigenesis or tumor progression but not attributed to drug.

Abbreviations and symbol used: AA, anaplastic astrocytoma; AED, anti-epileptic drug; ANC, absolute neutrophil count; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; Carbo, carboplatin; CCNU, lomustine; DLCO, diffusing capacity of the lung for carbon monoxide; DVT, deep vein thrombosis; EIAED, enzyme-inducing antiepileptic drug; GBM, glioblastoma multiforme; MAb, monoclonal antibody; MI, myocardial infarction; NA, nonassessable; N/V, nausea and vomiting; PCV, procarbazine, lomustine, vincristine; PD, progressive disease; PE, pulmonary embolism; Plt, platelets; PR, partial response; SD, stable disease; Temo, temozolomide; XRT, radiation therapy; ↓, decreased.

Toxicity Evaluation

Of the 73 patients who enrolled onto the study, 71 were assessable for toxicity (Table 3). Two patients could not be completely evaluated for toxicity because they failed to return for follow-up after their first cycle.

Twenty-two adverse events were observed in 15 patients, 18 of which were instances of DLT (Table 4). There was no evidence of an increasing incidence of toxicity involving one organ system as the irinotecan dose was escalated. The observed toxicity was evenly distributed among the following organ systems: pulmonary, gastrointestinal, cardiovascular, neurologic, infectious, and hematologic, without a clear predominance of toxicity involving any one organ system.

Four patients experienced the following adverse events involving the pulmonary system: grade 1 pulmonary infiltrate; grade 3 decrease in DLCO; grade 4 pulmonary embolism, along with bilateral deep vein thrombosis; and grade 5 pulmonary fibrosis confirmed by autopsy. Adverse events involving the gastrointestinal system were all grade 3 and consisted of diarrhea experienced by 3 patients and nausea and vomiting experienced by 2 patients. One patient died of a cardiopulmonary arrest preceded by a seizure within 24 h after the discovery of a large atrial thrombus downstream from an indwelling central venous

Table 2. Demographic characteristics

Characteristics	Number of Patients	%
Total number of patients	73	
Age, years		
Median	48	
Range	20–75	
Gender		
Male	45	62
Female	28	38
Histology		
Glioblastoma multiforme	53	73
Anaplastic astrocytoma	16	22
Anaplastic oligodendroglioma	3	4
Anaplastic oligoastrocytoma	1	1
Prior therapy		
Radiotherapy	72	99
Chemotherapy	31	42
Monoclonal antibody	3	4
Resection	66	90

catheter. Neurologic adverse events involved 1 patient with a grade 3 vasovagal episode and another patient who died after exhibiting symptoms consistent with cerebral herniation 1 day after infusion of his first dose of BCNU/irinotecan. Six patients had evidence of an infectious process: 2 patients with grade 3 infection with neutropenia, 1 patient with grade 3 febrile neutropenia, and 3 patients with infection without neutropenia (grade 2, grade 3, and grade 5). An autopsy performed on the patient who died of infection without neutropenia revealed pulmonary and kidney abscesses, valvular vegetations, acute respiratory distress syndrome, diffuse intravascular coagulation, and myocardial infarction. Hematopoietic adverse events involved 2 patients with grade 4 neutropenia and 2 patients with grade 3 thrombocytopenia.

In patients who had a grade 3 or greater nonhematopoietic toxicity or grade 4 hematologic toxicity, the toxicity for all but 2 patients was thought to be possibly drug related. Tumorigenesis and tumor progression were thought to be the major contributing factors in the patient who experienced a grade 4 pulmonary embolism, along with bilateral deep vein thrombosis, and in the patient who died after exhibiting symptoms consistent with cerebral herniation.

Antitumor Activity

Sixty-six patients were assessable for antitumor activity (Table 5). Disease was not reassessed in 7 patients. Six of the 7 patients experienced neurologic deterioration, with cerebral herniation occurring in 1 patient. One of the 7 patients died of a pulmonary embolism after the discovery of bilateral deep vein thrombosis.

Patients remained on study for a median period of 2 cycles; the range was 1 to 11 cycles. The median time to tumor progression was 2 cycles. Forty-two patients (58%) demonstrated PD as their best response. Disease control (SD + PR + CR) was seen in 30 patients (40%), with 25 patients (35%) demonstrating SD and 5 patients (7%) demonstrating a PR as their best response. Twenty-four (33%) patients completed ⩾3 cycles of therapy. Furthermore, 8 patients (11%) completed 6 or more cycles of treatment, and 1 patient completed 11 cycles of treatment. No CRs were seen.

Table 3. Toxicity observed in patients, stratified by EIAED use

	Irinotecan Dose (mg/m^2)	Number of Patients	Toxicities (number of patients) Not Dose Limiting	Dose Limiting	Nonassessable
No EIAEDs	20	4	0	0	0
	40	2	0	0	0
	60	2	0	0	0
	80	2	0	0	0
	100	1	0	0	0
	125	7	0	1	0
	150	3	0	2	0
	175	3	0	2	0
EIAEDs	20	3	0	0	0
	40	3	1	1	0
	60	4	0	0	0
	80	3	1	1	0
	100	5	0	1	0
	125	5	0	1	0
	150	4	0	0	0
	175	3	0	0	0
	200	5	1	1	0
	225	8	1	0	2
	250	6	0	3	0

Abbreviation: EIAED, enzyme-inducing anti-epileptic drug.

Table 4. Nonhematologic and hematologic toxicity

Adverse Event	Grade (number of patients) 1	2	3	4	5	Total patients with toxicity No.	%
Nonhematologic toxicity							
Pulmonary							
Fibrosis					1	1	1.4
Embolism				1		1	1.4
Decreased DLCO			1			1	1.4
Infiltrates	1					1	1.4
Gastrointestinal							
Diarrhea			3			3	4.1
Nausea/vomiting			2			2	2.7
Cardiovascular							
Cardiopulmonary arrest					1	1	1.4
Neurologic							
Vasovagal episode			1			1	1.4
Cerebral herniation					1	1	1.4
Infection/febrile neutropenia infection							
With neutropenia			2			2	2.7
Without neutropenia		1	1		1	3	4.1
Febrile neutropenia			1			1	1.4
Hematologic toxicity							
Neutropenia				2		2	2.7
Leukocytopenia						0	0
Thrombocytopenia			2			2	2.7
Anemia						0	0

Abbreviation: DLCO, diffusing capacity of the lung for carbon monoxide

Discussion

New treatment strategies, including gene therapy, cancer vaccines, and antiangiogenesis agents, are expected to play a more prominent role in the future treatment of human malignancies, including MG. However, until that time, it is likely that chemotherapy will remain the major intervention for those patients whose tumors cannot be cured with surgery and radiotherapy. Therefore, in the interim, combination chemotherapy is one treatment strategy designed to produce therapeutic effects that are more favorable than those of a single drug, yet minimize normal organ toxicity and prevent emergence of drug-resistant tumor cells.

Irinotecan and BCNU are ideal candidates for combination chemotherapy because they exert their antitumor effects through interactions with different targets, and they produce toxicities affecting different organs. BCNU, an alkylating agent, produces its antitumor effect by covalently binding an alkyl group to a cellular molecule to form an adduct and ultimately a cross-link (Hall and Tilby, 1992). Irinotecan, a topoisomerase I inhibitor, has a target different from that of BCNU. Irinotecan stabilizes the intermediate that is formed by the covalent bond between topoisomerase I and DNA, thereby allowing the topoisomerase to cleave the DNA, but inhibit re-ligation. Single-strand breaks are irreversibly converted to double-strand breaks through interaction with the replication machinery, and the cell is killed. The symptoms of the organ toxicity caused by irinotecan and by BCNU also differ. The DLT of irinotecan is diarrhea (Slichenmyer et al., 1993), whereas the DLT of BCNU is myelosuppression (Colvin and Chabner, 1990).

In addition to the less than additive toxicity to organs that is produced by this combination chemotherapy, there is preclinical evidence that BCNU may enhance the antitumor activity of irinotecan. Recent studies with human glioma xenograft D-54 MG have shown that when irinotecan is given in combination with BCNU, antitumor activity is striking, with a greater than additive effect at all doses tested (Coggins et al., 1998). Moreover, the increase in activity was schedule dependent (Castellino et al., 2000), with the greatest enhancement of activity seen when BCNU was given on day 1 and irinotecan was given on days 1 to 5 and 8 to 12. Delay of irinotecan to day 3 or 5 or delay of BCNU to day 8 substantially reduced the enhanced activity. These results suggest that the presence of a BCNU-induced adduct or a cross-link before administration of irinotecan is critical for enhanced activity. To resolve the question of whether a monoadduct or a cross-link was the critical lesion responsible for the enhanced antitumor activity, temozolomide was given in combination with irinotecan in the treatment of an MG-derived xenograft in athymic nude mice. This combination produced a greater than additive increase in activity compared with the 2 agents used alone. This increase in activity was schedule dependent, with the greatest enhancement of activity seen when

Table 5. Response to treatment, stratified by EIAED use

	Irinotecan Dose (mg/m^2)	Number of Patients	Number of Cycles		Response			
			Median	Range	PD	SD	PR	NA
No EIAEDs	20	4	1	1–4	3	1	0	0
	40	2	3	1–5	1	1	0	0
	60	2	3	1–5	1	1	0	0
	80	2	4	4	0	2	0	0
	100	1	11	11	0	1	0	0
	125	7	2	1–3	4	2	0	0
	150	3	2	1–3	1	2	0	0
	175	3	1	1–2	2	1	0	0
EIAEDs	20	3	2	1–2	3	0	0	0
	40	3	1	1–5	1	1	1	0
	60	4	5	1–9	1	3	0	0
	80	3	3	1–6	1	2	0	0
	100	5	1	1–3	3	1	1	0
	125	5	2	1–4	4	1	0	0
	150	4	3.5	1–8	2	2	0	0
	175	3	3	1–4	1	2	0	0
	200	5	1	1–3	4	1	0	0
	225	8	1	1–7	6	0	2	2
	250	6	1	1–9	2	2	1	1

Abbreviations: EIAEDs, enzyme-inducing anti-epileptic drugs; NA, nonassessable; PD, progressive disease; PR, partial response; SD, stable disease

temozolomide was given on day 1 and irinotecan was given on days 1 to 5 and 8 to 14. Delay of the start of irinotecan to day 3 or day 5 did not alter this enhanced activity. However, when irinotecan was administered first on day 1 followed by temozolomide on day 1, 3, or 5, the enhancement of activity was substantially reduced. These results strongly suggest that the critical lesion responsible for the enhanced antitumor activity is an adduct at the O^6-position of guanine and not a cross-link.

Recent work suggests a mechanism for this enhanced activity of irinotecan when administered after temozolomide or BCNU. Pourquier et al. (2000) demonstrated that O^6-alkylation of guanine induces topoisomerase I-DNA covalent complexes in vitro and in Chinese hamster ovary cells treated with *N*-methyl-*N*'-nitro-*N*-guanidine. This increase in topoisomerase I cleavage complexes would be expected to increase cellular sensitivity to topoisomerase I inhibitors, including irinotecan.

Together, this work suggests that O^6-alkylation with temozolomide or BCNU is the mechanism responsible for enhanced antitumor activity when these agents are administered before irinotecan. Given the potential for a favorable therapeutic index, this study explored the MTD and toxicities of BCNU in combination with irinotecan. In this phase 1 trial of BCNU (100 mg/m^2) plus irinotecan, the MTD of irinotecan was shown to be 225 mg/m^2 for patients receiving EIAEDs and 125 mg/m^2 for patients not receiving these drugs. The observed toxicity was evenly distributed among the pulmonary, gastrointestinal, cardiovascular, neurologic, infectious, and hematologic systems, without a clear predominance of toxicity involving any one organ system. Further, we detected no evidence of increasing incidence of toxicity involving one organ system as the irinotecan dose was escalated.

Stratification by EIAED therapy was performed in this phase 1 trial after information on the drug-drug interaction between irinotecan and EIAEDs became available. The interaction between irinotecan and the EIAEDs phenytoin, carbamazepine, and phenobarbital was first noted by Friedman et al. while treating adults with recurrent or progressive MG with irinotecan in a phase 2 trial (Friedman et al., 1999). In this study, a 2-fold increase in irinotecan clearance and lower systemic levels of irinotecan, SN-38, and SN-38G were observed in patients receiving EIAEDs. Since publication of this phase 2 trial, other researchers have substantiated this interaction (Crews et al., 2002; Mathijssen et al., 2002), although the exact mechanism of this interaction awaits elucidation. Since our clinical trial showed an impressive difference in MTD for the 2 groups of patients stratified by EIAED use, future studies using irinotecan alone or in combination with other antineoplastic agents should stratify accordingly.

Despite the phase 1 dose escalation format of this study, encouraging evidence of antitumor activity was noted with the study regimen. Disease control was seen in 43 patients (60%), with 38 patients (52%) demonstrating SD and 5 patients (7%) demonstrating a PR as their best response. Twenty-four patients (33%) completed ⩾3 cycles of therapy. Furthermore, 8 patients (11%) completed ⩾6 cycles of treatment, and 1 patient completed 11 cycles of treatment.

In conclusion, irinotecan plus BCNU combination chemotherapy is well tolerated and has activity in

patients with progressive or recurrent malignant glioma. The current phase 1 trial of irinotecan plus BCNU has defined the therapeutic approach for a newly opened phase 2 trial of irinotecan plus BCNU. Studies of temozolomide in combination with irinotecan or topotecan, as well as studies that use O^6-benzylguanine to enhance the sensitivity of alkylators and methylators such as temozolomide and BCNU when used with irinotecan, are indicated.

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