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Phase 1 Trial of Temozolomide (TMZ) plus Irinotecan (CPT-11) plus O⁶-Benzylguanine (O⁶-BG) in Adults with Recurrent Malignant Glioma

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Abstract

Background—This is a phase I clinical trial conducted with patients who had recurrent or progressive malignant glioma (MG). The trial was designed to determine the maximum tolerated dose (MTD) and toxicity of irinotecan (CPT-11) when administered with temozolomide (TMZ) and O⁶-benzylguanine (O⁶-BG).

Methods—All three drugs, CPT-11, TMZ and O⁶-BG, were administered on day 1 of a 21-day treatment. First, patients were treated with a 1-hour bolus infusion of O⁶-BG at a dose of 120 mg/m² followed immediately by a 48-hour continuous infusion of O⁶-BG at a dose of 30 mg/m²/d. Second, within 60 minutes of the end of the 1-hour bolus infusion of O⁶-BG, TMZ was administered orally at a dose of 355 mg/m². Third, one hour after TMZ administration CPT-11 was infused over 90 minutes. Patients were accrued to one of two strata based on CYP3A1- and CYP3A4-inducing antiepileptic drug (EIAED) use; dose escalation was conducted independently within these strata.

Results—Fifty-five patients were enrolled. In both strata, the dose-limiting toxicities (DLTs) were hematologic and included grade 4 neutropenia, febrile neutropenia, leukopenia, and/or thrombocytopenia. For Stratum 1 (EIAEDs), when TMZ was administered at 355 mg/m², the MTD of CPT-11 was determined to be 120 mg/m². In contrast, for Stratum 2 (no EIAEDs), when TMZ was administered at 200 mg/m², the MTD of CPT-11 was determined to be 80 mg/m².

Conclusion—This study provides the foundation for a phase II trial of O⁶-BG in combination with CPT-11 and TMZ in MG.

Keywords

temozolomide; irinotecan; O⁶-benzylguanine; recurrent; malignant glioma

INTRODUCTION

Survival following the diagnosis of malignant glioma remains poor, despite recent advances in therapy. Although alkylators such as temozolomide (TMZ) and polifeprosan 20 with carmustine implant (Gliadel; Guilford Pharmaceuticals, Baltimore, MD) are FDA approved for treatment of malignant glioma (MG), their ability to prolong survival is short-lived. Thus, innovative therapeutic agents and strategies are imperative.

Few would argue with the reasoning that because cytotoxic drugs when used as single agents rarely have a major therapeutic impact combination treatment should be developed. Thus, the rationale for the combination of TMZ, CPT-11, and O⁶-BG is based on five factors. First, TMZ and CPT-11 each have demonstrated clinical activity when administered separately to patients with MG. In a multicenter, randomized Phase III trial, the addition of TMZ to radiotherapy for newly diagnosed glioblastoma resulted in a statistically significant survival benefit 1. These results were supported by earlier studies showing that TMZ was active in patients with recurrent MG 2-3. Although CPT-11 has shown some activity in MG, the magnitude of this activity remains unclear. In phase II MG trials, the response rates for CPT-11 have ranged from 2.2 - 44% 4-8. The variability in response rates can be explained by the variability in the following: 1) population (pediatrics vs. adults), 2) interpatient pharmacokinetics (dependent and independent of EIAED), 3) extent of prior therapy (newly diagnosed vs. recurrent), and 3) dosing regimen (every three weeks vs. weekly for the first 4 of 6 weeks).

Second, TMZ and CPT-11 are ideal candidates for combination chemotherapy because they exert their antitumor effects through interactions with different targets and have different organ toxicities. CPT-11 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 religation and ultimately leading to cell death 9. The DLT of CPT-11 is diarrhea 10-11. TMZ is an alkylating agent that transfers a methyl group to three sites, N⁷-guanine, N³-adenine, and O⁶-guanine 12. The toxic lesion is felt to be the O⁶-guanine adduct, which leads to a lethal cycle of DNA mismatch repair if the adduct is not removed by the DNA repair protein, O(6)-alkylguanine-DNA alkyltransferase (AGT). The DLT of TMZ is myelosuppression 13.

Third, TMZ given in combination with CPT-11 displays enhanced antitumor activity compared with the two agents used alone. Houghton et al 14 first discovered that in glioblastoma xenografts the activity of the combination of CPT-11 and TMZ was significantly greater than the activity of either agent administered alone. Patel et al 15 confirmed this synergistic activity and went on to show that this activity was schedule dependent. When TMZ preceded CPT-11, a substantial increase in antitumor activity was seen compared with the response to the agents used alone. However, when CPT-11 preceded TMZ, a dramatic decrease in this enhanced activity was seen. The mechanism of this enhanced activity was elucidated by Pourquier et al 16 when he demonstrated in vitro and in N-methyl-N'-nitrosoguanidine-treated Chinese hamster ovary cells that O⁶ alkylation of guanine induces topoisomerase I-DNA covalent complexes, thereby decreasing the topoisomerase I-mediated DNA religation.

Fourth, AGT seems to be a major mechanism of resistance to TMZ. An inverse relationship between AGT levels and response was seen in patients with MG treated with preradiation TMZ 17. Furthermore, Hegi et al 18 have shown a relationship between inactivation of the AGT gene by promoter methylation and survival in patients with newly diagnosed glioblastoma multiforme (GBM) treated with surgery, radiotherapy and TMZ. No clinical trials have been performed to elucidate the relationship between AGT levels and CPT-11

efficacy in MG patients, despite marginal preclinical studies purporting a relationship between AGT and CPT-11 19–21. Accordingly no CPT-11 dose modifications based on AGT were performed. These studies suggest that the efficacy of TMZ but not necessarily CPT-11 could be enhanced by depletion of tumor AGT by O⁶-BG.

Fifth, the addition of O⁶-BG to the combination of TMZ and CPT-11 dramatically increased the growth delay of AGT-positive D-456 MG xenografts compared with the growth delay in these xenografts following administration of either agent alone or in combination 22. These five factors suggest that the combination of TMZ, O⁶-BG, and CPT-11 may be a highly effective clinical intervention.

We now report a phase I clinical trial in patients with recurrent or progressive MG. This trial was designed to determine the maximum tolerated dose (MTD) and DLT of CPT-11 when administered following TMZ and O⁶-BG. The secondary objectives were to further define the toxicity of this regimen and to evaluate for antitumor activity.

MATERIALS AND METHODS

Patients

Eligible patients had histologically confirmed diagnosis of progressive or recurrent primary MG (glioblastoma multiforme, gliosarcoma, anaplastic astrocytoma, anaplastic oligodendroglioma, and anaplastic mixed oligoastrocytoma). Patients were ≥18 years old and required to have Karnofsky performance score ≥60%. Patients must have had measurable disease on contrast-enhanced MRI. An interval of at least 2 weeks since prior surgical resection or 6 weeks since prior chemotherapy or radiotherapy had to have elapsed for the patient to be enrolled into the clinical trial. Additional enrollment criteria included adequate pretreatment bone marrow function (hemoglobin ≥ 10 g/dl, total granulocyte count ≥ 1,500 cells/μl, platelet count ≥ 100,000 cells/μl), renal function (BUN and serum creatinine < 1.5 times upper limit of normal [ULN]), and hepatic function (total serum bilirubin < 1.5 times ULN, SGOT and SGPT < 2.5 times ULN, and alkaline phosphatase < 2 times ULN). Patients of reproductive potential were required to take effective contraceptive measures for the duration of the study. The protocol was reviewed and approved by the Duke University Health System Institutional Review Board. Each patient signed an informed-consent form.

The following patients were excluded from the study: patients who had previously failed CPT-11, nursing or pregnant women, potentially fertile women or men who were not using an effective contraception method, patients with previous active malignancy treated in the past year, and patients taking immunosuppressive agents other than corticosteroids, patients with active infection requiring intravenous antibiotics, patients with known human immunodeficiency virus infection, or those experiencing frequent vomiting or a medical condition that could interfere with oral medication intake.

Study Design and Treatment

This was a phase I, open-label, single-center trial with accrual goals defined within two separate strata. Previous studies confirmed that the metabolism of CPT-11 is significantly enhanced by concurrent use of CYP3A1- and CYP3A4-inducing antiepileptic drugs (EIAEDs) including phenytoin, carbamazepine, phenobarbital, oxcarbazepine, and primidone (4, 6, 8). Therefore, patients were accrued independently into each stratum: Stratum 1, patients receiving EIAEDs, and Stratum 2, patients not receiving EIAEDs.

All three drugs, TMZ, O⁶-BG, and CPT-11, were administered on day 1 of a 21-day treatment cycle until unacceptable toxicity or tumor progression occurred. First, patients

were treated with a 1-hour bolus infusion of O⁶-BG at a dose of 120 mg/m² followed immediately by a 48-hour continuous infusion of O⁶-BG at a dose of 30 mg/m²/d, based on the desire to prevent regeneration of AGT and removal of O⁶-methyladducts before futile rounds of DNA mismatch repair lead to cell death 23. CPT-11 is not affected by AGT and no timing considerations with O⁶-BG were necessary for this agent. Second, within 60 minutes of the end of the 1-hour bolus infusion of O⁶-BG, TMZ was administered orally at a dose of 355 mg/m², which represents a one level reduction from the single dose MTD of TMZ when given in combination with O⁶-BG 23. Third, one hour after TMZ administration, CPT-11 was infused over 90 minutes.

Dose escalation for CPT-11 for Stratum 1 was as follows: 60, 90, 120, 150, 180, 210, 240, and 270 mg/m². Dose escalation for CPT-11 for Stratum 2 was as follows: 40, 60, 80, 100, 125, 150, 175, and 200 mg/m². If the first dose level of CPT-11 exceeded the MTD, the dose of TMZ for the next cohort of patients was reduced to 267 mg/m². If this dose of TMZ plus the first dose level of CPT-11 still exceeded the MTD, the TMZ dose was reduced for the next cohort of patients to 200 mg/m². If this dose of TMZ (200 mg/m²) in combination with the first dose level of CPT-11 exceeded the MTD, the study was to be closed.

O⁶-BG was supplied by AOI Pharmaceuticals, Inc. (New York, NY). TMZ was commercially available from Schering-Plough Research Institute (Kenilworth, NJ). CPT-11 was commercially available from Pfizer Pharmaceuticals (New York, NY).

Surveillance and Follow-up

The baseline examination included central review of tumor tissue, MRI or CT (if MRI was medically contraindicated), complete blood counts and blood chemistry tests, and a physical examination including a comprehensive neurologic examination. During therapy, complete blood counts and blood chemistry tests were obtained weekly. Prior to subsequent cycles of chemotherapy, patients were required to repeat the complete blood count and blood chemistry tests and the physical and neurologic examination. Additionally, after every 2 cycles of chemotherapy, patients were required to obtain repeat neuro-imaging.

Toxicity was graded according to the National Cancer Institute's Common Toxicity Criteria version 3.0. Repeat cycles of chemotherapy were administered on schedule only if the patient met the following retreatment criteria: total granulocyte count > 1000 cells/ μ l, platelets > 100,000/ μ l, hemoglobin > 10 g/dl, SGOT $\leq$ 2.5 times upper limit of normal, creatinine < 1.5 times upper limit of normal, and total bilirubin within normal limits, and all other toxicities must have resolved to baseline or grade 1. The CPT-11 dose was reduced by 20% in any patient with grade $\geq$ 3 non-hematologic toxicity that was felt to be probably or definitely related to the administration of CPT-11.

Objective assessments of overall response were based on tumor assessment from MRI scans (CT if MRI was medically contraindicated) interpreted in the light of corticosteroid use, as suggested by Macdonald et al 24 with appropriate support from the neurologic examination. Thus, complete response (CR) was disappearance of all enhancing tumor on consecutive MRI scans at least one month apart and no corticosteroid use except for physiologic doses. Partial response (PR) was $\geq$ 50% reduction in contrast enhancement for lesions on consecutive MRI scans at least one month apart, and at least stable corticosteroid use, with stable or improved neurologic condition. Progressive disease (PD) was $\geq$ 25% increase in contrast enhancement of any lesion or any new enhancing tumor on MRI scans, and stable or increased corticosteroid use, with or without neurologic progression. Stable disease (SD) was all other situations.

Statistical Analysis

In establishing the optimal CPT-11 dose when combined with TMZ and O⁶-BG, a modified classic “3 + 3” dose escalation design was employed, which permitted up to three additional patients to be accrued at a given dose level as long as none of the first three patients enrolled at that dose level experienced a DLT. The dose level was escalated in successive cohorts of three patients as long as no DLT was observed. If one instance of DLT was observed among the initial three assessable patients at any dose level, an additional three patients had to be treated at that dose level with no further DLT in order for dose escalation to proceed. If two instances of DLT were observed at a dose level, the MTD was determined to be surpassed, and a total of six patients were treated at the previous level to ensure its tolerability. The MTD was therefore the highest dose level at which DLT was experienced by no more than 1 of 6 patients. 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.

RESULTS

Patient Data

A total of 55 patients with recurrent MG were enrolled at Duke University Medical Center between July 2003 and March 2005. Demographic and clinical characteristics of patients are detailed in Table 1.

Toxicity Evaluation

Table 2 summarizes the frequency and type of DLT observed at each dose level per stratum. For Stratum 1 (EIAEDs), DLT was not observed until the dose of CPT-11 was escalated to 120 mg/m². One patient experienced a DLT of grade 4 neutropenia at this dose. Once the CPT-11 dose was escalated to 150 mg/m², two patients experienced a DLT, one with grade 4 neutropenia and the other with grade 4 leukopenia. For Stratum 1 when TMZ was administered at 355 mg/m², the MTD of CPT-11 was determined to be 120 mg/m². In contrast, for Stratum 2 (no EIAEDs), three patients experienced DLTs at the initial CPT-11 dose of 40 mg/m². At this dose level, two patients experienced grade 4 neutropenia with grade 4 thrombocytopenia, and one patient experienced grade 4 neutropenia. Subsequently, the TMZ dose was de-escalated, first to 267 mg/m² and then ultimately to 200 mg/m² after two patients experienced grade 4 neutropenia at 267 mg/m². Once the dose of TMZ was established at 200 mg/m², the CPT-11 dose was escalated to 60 mg/m² and then to 80 mg/m² with one DLT of grade 4 neutropenia experienced at each dose level. The last dose escalation of CPT-11, to 100 mg/m², proved to be too toxic, with two DLT events of grade 4 febrile neutropenia and grade 4 neutropenia with grade 4 thrombocytopenia. For Stratum 2 when TMZ was administered at 200 mg/m², the MTD of CPT-11 was determined to be 80 mg/m².

Seven patients were not evaluable for DLTs (Table 2). Two patients received Neupogen (Amgen Manufacturing, Ltd, Thousand Oaks, CA) by home physician intervention for grade 3 neutropenia before the ANC nadir was reached. Another patient did not obtain labs during cycle one, although they went on to receive a total of 17 cycles without experiencing toxicities. Three patients died of disease progression, which was verified on MRI, two patients on day 11 and one patient on day 19 of their first cycle. On day 19 of the first cycle, one patient died suddenly after experiencing signs and symptoms suspicious for pulmonary embolism, which was not verified by autopsy.

Seven patients died while participating in this study. Three patients died of disease progression from cerebral herniation during their first cycle of therapy. The other 4 patients died of adverse events that were not dose-limiting. Two patients experienced signs and symptoms consistent with pulmonary embolism and died suddenly, one during their first cycle and one during their fourth cycle. One patient died of an intracranial hemorrhage during the second cycle of therapy, despite no evidence of thrombocytopenia or coagulopathy. One patient died during the seventh cycle of therapy after presenting to medical attention with pneumonia and sepsis with positive blood cultures for *Pseudomonas aeruginosa* accompanied by grade 4 leukopenia and grade 3 thrombocytopenia.

Response

There were 53 of the 55 patients evaluable for response by MRI. All had progressed at the time of this report. Although it was not a response study, there were no reports of any patients who had an objective response (>50% decrease in size of tumor). The median progression-free survival time measured by the Kaplan-Meier technique from the date of initiation of therapy was 6.4 weeks (95% CI, 6 to 7.71 weeks). Seventeen patients were progression free for 13 weeks or longer, and 2 patients (both with GBMs) were progression free for 6 months or longer. These results are difficult to compare to the literature since typically there are limited summaries of response rates in phase 1 studies. Nevertheless, a review of 6-month PFS in adults with recurrent grade 4 (9 weeks) and grade 3 glioma (13 weeks) have been reported 25. Unfortunately, it is impossible to meaningfully relate these results to those in our phase 1 trial.

DISCUSSION

The goal of this study was to define the MTD and DLT of CPT-11 when administered following TMZ and O⁶-BG once every three weeks to patients receiving and not receiving EIAEDs. In the current Phase I trial for Stratum 1 (EIAEDs), when TMZ was administered at 355 mg/m², the MTD of CPT-11 was determined to be 120 mg/m². In contrast, for Stratum 2 (no EIAEDs), when TMZ was administered at 200 mg/m², the MTD of CPT-11 was determined to be 80 mg/m². The DLTs were hematologic and included grade 4 neutropenia, leukopenia, and thrombocytopenia. The pharmacokinetics of CPT-11 in these 2 strata were not evaluated but the available literature is clear regarding the reduced levels of CPT-11 and SN-38 levels seen in patients receiving EIAEDs 6.

The rationale for using a frequency of once every three weeks in the dosing regimen for TMZ, CPT-11, and O⁶-BG is based on several factors. First, of the two competing dosing regimens of CPT-11 (once every three weeks vs. weekly for the first 4 of 6 weeks), neither has proven to be more efficacious nor less toxic than the other. In prior Phase I and II MG trials utilizing CPT-11, the variability in population, interpatient pharmacokinetics, dosing regimen, and extent of prior therapy makes drawing any conclusions as to a preferred CPT-11 dosing regimen difficult, if not impossible.

Second, the schedule-dependent enhancement of antitumor activity seen in preclinical studies made it necessary to administer TMZ prior to but within 5 days of CPT-11. This requirement persuaded us to choose the once-every-three-weeks dosing regimen because we had no prior experience in administering TMZ in combination with O⁶-BG on a weekly basis. Since our prior phase I and II trials of TMZ plus O⁶-BG utilized a 28-day cycle length for drug administration, a modification in cycle length from 28 to 21 days was thought to be safe, along with, as detailed in the Methods Section, a concomitant dose reduction of TMZ from 472 mg/m² to 355 mg/m². In our present study, it is unclear whether the reduction in cycle length of TMZ plus O⁶-BG from 28 to 21 days or the addition of CPT-11 necessitated the eventual dose reduction in TMZ to 200 mg/m² in those who did not require EIAEDs.

Despite this TMZ dose reduction, 12 patients who received no EIAEDs showed stable disease for 4-18 cycles.

The current study describes a Phase I trial combining CPT-11 plus TMZ plus O⁶-BG and demonstrates that these drugs can be safely administered together in a once-every-three-weeks dosing regimen, with and without EIAED. This was a phase I trial and the primary endpoint was not response. Nevertheless, the lack of any responses was disheartening, and it is possible that the final MTDs represent sub-therapeutic doses or that the timing of administered doses does not maximize therapeutic benefit. These questions can only be asked in a future Phase II trial using this combination of drugs.

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Table 1

Demographic and clinical characteristics of patients

Characteristic	Value (percent)	
	EIAED	Non-EIAED
Total N	22	33
Age, Years		
Median	48	53
Range	32-66	21-75
Sex, male	13 (59%)	19 (58%)
Race, white	21 (95%)	33 (100%)
KPS		
Median	80	80
Range	70-90	70-100
Histology		
GBM	21 (95%)	31 (94%)
Anaplastic Oligodendroglioma	1 (4.5%)	
Anaplastic Astrocytoma		2 (6%)
Prior therapy		
Radiotherapy	22 (100%)	33 (100%)
Chemotherapy	22 (100%)	33 (100%)
Temozolomide Failure	19 (86%)	30 (90.9%)
Resection	17 (77%)	30 (90.9%)
No. of Prior Chemotherapies		
1	11 (50%)	19 (58%)
2	10 (45%)	8 (24%)
>2	1 (4.5%)	6 (18%)

Table 2

Dose-Limiting Toxicities

Stratum	TMZ Dose	CPT-11 Dose	No. Patients Treated	No. Patients Evaluable*	No. Patients DLT	Type DLT
Stratum 1 EIAED N = 23	355	60	6	6	0	---
		90	3	3	0	---
		120	6	6	1	Gr. 4 neutropenia
		150	7	6 ^a	2	Gr. 4 neutropenia Gr. 4 leukopenia
Stratum 2 Not On EIAED N = 33	355	40	8	6 ^{b,c}	3	Gr. 4 neutropenia + thrombocytopenia × 2 Gr. 4 neutropenia
	267	40	5	4 ^d	2	Gr. 4 neutropenia × 2
	200	40	4	3 ^d	0	---
	200	60	7	6 ^e	1	Gr. 4 neutropenia
	200	80	7	6 ^f	1	Gr. 4 neutropenia
	200	100	2	2	2	Gr. 4 febrile neutropenia Gr. 4 neutropenia + thrombocytopenia

* Some patients were not evaluable for the following reasons.

^a One patient died of disease progression (PD) on day 11 of cycle 1.

^b One patient died of PD on day 19 of cycle 1.

^c One patient died after experiencing signs and symptoms suspicious for pulmonary embolism on day 19 of cycle 1.

^d One patient received Neupogen (Amgen) for grade 3 neutropenia before nadir was reached during cycle 1.

^e One patient had no labs during cycle 1.

^f One patient died of PD on day 11 of cycle 1.