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ORIGINAL REPORT

Phase II Trial of Temozolomide Plus O^6-Benzylguanine in Adults With Recurrent, Temozolomide-Resistant Malignant Glioma

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ABSTRACT

Purpose
This phase II trial was designed to define the role of O^6-benzylguanine (O^6-BG) in restoring temozolomide sensitivity in patients with recurrent or progressive, temozolomide-resistant malignant glioma and to evaluate the safety of administering O^6-BG in combination with temozolomide.

Patients and Methods
Patients were accrued into two independent strata on the basis of histology: glioblastoma multiforme (GBM) and anaplastic glioma. Both temozolomide and O^6-BG were administered on day 1 of a 28-day treatment cycle. Patients were administered a 1-hour O^6-BG infusion at a dose of 120 mg/m^2 followed immediately by a 48-hour infusion at a dose of 30 mg/m^2/d. Temozolomide was administered orally within 60 minutes of the end of the 1-hour O^6-BG infusion at a dose of 472 mg/m^2. The primary end point was objective response rate. Secondary end points included progression-free survival, overall survival, and safety.

Results
Sixty-six of 67 patients who enrolled were treated with temozolomide and O^6-BG. One of 34 patients (3%) with GBM (95% CI, 0.1% to 15%) and five of 32 assessable patients (16%) with anaplastic glioma (95% CI, 5% to 33%) were responders. The most commonly reported adverse events were grade 4 hematologic events experienced in 48% of the patients.

Conclusion
O^6-BG when added to a 1-day dosing regimen of temozolomide was able to restore temozolomide sensitivity in patients with temozolomide-resistant anaplastic glioma, but there seemed to be no significant restoration of temozolomide sensitivity in patients with temozolomide-resistant GBM.

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INTRODUCTION

Despite recent advances in therapeutic regimens, the diagnosis of malignant glioma still carries a poor prognosis. Although alkylators such as temozolomide and polifeprosan 20 with carmustine implant (Gliadel, Guilford Pharmaceuticals-MGI Pharma, Bloomington, MN) are US Food and Drug Administration–approved for treatment of malignant glioma, their ability to prolong survival is short-lived. Thus, innovative therapeutic agents and strategies are imperative.

One novel approach to fighting this deadly disease is to target the mechanisms of resistance to chemotherapy. Because O^6-alkylguanine-DNA alkyltransferase (AGT) has been shown to be a major factor in the resistance of tumor cells to alkylating agents such as carmustine and temozolomide,[1-3] perhaps targeting this DNA repair protein can help restore chemotherapy activity.

One such agent that can irreversibly inactivate AGT is a low molecular weight substrate, O^6-benzylguanine (O^6-BG). In vitro[1,4,5] and in vivo[6,7] studies using tumor cell lines and subcutaneous brain tumor xenografts demonstrate that O^6-BG increases the therapeutic effectiveness of temozolomide.

These studies suggest that combining O^6-BG with temozolomide may circumvent resistance and restore chemotherapy sensitivity. However, before one can combine these two agents, one must answer the following two important questions: what dosing schedule of O^6-BG is necessary to completely suppress AGT activity in brain tumors

for at least 48 hours, and what is the maximum-tolerated dose (MTD) of temozolomide in a 1-day dosing regimen that can be combined with O^6-BG?

Prior research endeavors have answered both of these questions. Weingart et al[8] answered this first question in a phase I trial of polifeprosan 20 with carmustine implant plus continuous infusion of intravenous O^6-BG in adults with recurrent malignant glioma by establishing the dose of O^6-BG to be 120 mg/m^2 for 1 hour followed by a continuous infusion of 30 mg/m^2/d for 2 days. Quinn et al[9] answered the second question in a phase I trial of temozolomide plus O^6-BG in adults with recurrent malignant glioma by establishing the MTD of temozolomide to be 472 mg/m^2 when administered as a single dose in conjunction with the above dosing schedule of O^6-BG.

The objectives of this phase II trial were twofold: to define the role of O^6-BG in restoring temozolomide sensitivity in patients with recurrent or progressive, temozolomide-resistant malignant glioma and to evaluate the safety of administering O^6-BG in combination with temozolomide.

PATIENTS AND METHODS

Patients

Eligible patients had a histologically confirmed diagnosis of progressive or recurrent glioblastoma multiforme (GBM) or anaplastic glioma (anaplastic astrocytoma [AA], anaplastic oligodendroglioma [AO], or anaplastic mixed AA and AO). These tumors must have shown resistance to temozolomide, defined as a $\geq$ 25% increase in tumor growth on contrast-enhanced magnetic resonance imaging (MRI) or computed tomography (CT) within 8 weeks of the last dose of temozolomide. Furthermore, all patients who experienced treatment failure with temozolomide as their most recent treatment were immediately enrolled onto the trial using temozolomide plus O^6-BG. Patients were $\geq$ 18 years old and required to have a Karnofsky performance score of $\geq$ 60%. An interval of at least 2 weeks since prior surgical resection (if conducted) or 4 weeks since prior chemotherapy (6 weeks for a nitrosourea-based regimen) had to have elapsed for the patient to be enrolled onto the clinical trial. All patients had evidence of residual tumor on postoperative MRI before starting temozolomide plus O^6-BG. Additional enrollment criteria included adequate pretreatment bone marrow function (hematocrit $>$ 29%, total granulocyte count $>$ 1,500 cells/μL, platelets $>$ 100,000 cells/μL), renal function (serum creatinine $<$ 1.5 mg/dL, serum urea nitrogen $<$ 25 mg/dL), and hepatic function (serum AST and bilirubin $<$ 1.5$\times$ upper limit of normal). For patients receiving corticosteroids, a stable dose for 1 week before entry onto the study was required, if clinically possible, with no escalation of dose above entry dose level. Patients of reproductive potential were required to take effective contraceptive measures for the duration of the study. The following patients were excluded from the study: pregnant women, potentially fertile women or men who were not using effective contraception method, and patients taking immunosuppressive agents other than corticosteroids. The protocol was reviewed and approved by the Duke University Health System institutional review board. Each patient signed an informed consent form.

Study Design and Treatment

This was a phase II, open-label, single-center trial with accrual goals defined within two separate strata: GBM and anaplastic glioma (AA or AO). Both temozolomide and O^6-BG were administered on day 1 of a 28-day treatment cycle. First, patients were treated with a 1-hour bolus infusion of O^6-BG at a dose of 120 mg/m^2 followed immediately by a 48-hour continuous infusion of O^6-BG at a dose of 30 mg/m^2/d. Next, temozolomide was administered orally, in a fasting state, at a dose of 472 mg/m^2 within 60 minutes of the end of the 1-hour administration of O^6-BG infusion.

Temozolomide was commercially available from Schering-Plough Research Institute (Kenilworth, NJ). O^6-BG was supplied by AOI Pharmaceuticals Inc (New York, NY).

Prophylactic antiemetics were permitted as needed. Neurologic stability was provided with the lowest corticosteroid dose when required. Colony-stimulating factors were permitted only for rescue from grade 4 neutropenia.

Surveillance and Follow-Up

The baseline examination included central review of tumor tissue, MRI (or CT if MRI was medically contraindicated), CBCs and blood chemistry tests, and a physical examination including a comprehensive neurologic examination. During therapy, weekly CBCs were obtained. Before subsequent cycles of chemotherapy, patients were required to repeat CBCs, blood chemistry tests, and a physical examination. In addition, after every two cycles of chemotherapy, patients were required to obtain repeat neuroimaging.

Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. Repeat cycles of chemotherapy were administered on schedule only if the patient met the following re-treatment criteria: total granulocyte count $>$ 1,500 cells/μL, platelets $>$ 100,000/μL, hematocrit $>$ 29 g/dL, AST $\leq$ 2.5$\times$ upper limit of normal, creatinine $<$ 1.5$\times$ upper limit of normal, and total bilirubin within normal limits; all other toxicities must have resolved to baseline or grade 1. Temozolomide dose was reduced by 25% in any patient with grade $\geq$ 3 nonhematologic or grade 4 hematologic toxicity or if re-treatment was delayed by longer than 2 weeks because of any grade toxicity. Patients who were delayed 3 weeks from treatment were removed from the trial unless there was evidence of tumor response.

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,[10] with appropriate support from the neurologic examination. This drug combination was administered for a maximum of 16 months or until unacceptable toxicity or tumor progression occurred.

Statistical Analysis

The primary end point was objective response rate (ORR) defined as the percentage of patients with complete response (CR) or partial response (PR) according to the modified Macdonald criteria. The secondary end points were safety, 6-month, and median progression-free survival (PFS), and 6-month and median overall survival (OS).

On the basis of a two-stage Simon minimax design[11] with an α level of 10% and 90% power, 32 patients (18 patients in stage 1 of the study and an additional 14 patients in stage 2) within each stratum were required to test the null hypothesis that the true ORR was $\leq$ 5% versus the alternative hypothesis that the true ORR was $\geq$ 20%. At least one objective response within each stratum was needed in stage 1 to allow expansion of the trial to stage 2. At the end of the study, at least four objective responses within each stratum were needed to reject the null hypothesis.

Efficacy and safety analyses included all patients who received at least one dose of temozolomide and O^6-BG. The number and proportion of patients who achieved an objective response (CR or PR) was summarized, along with the corresponding exact two-sided 95% CI, calculated by a method derived from the binomial distribution. PFS, defined as the time between initiation of treatment and the first occurrence of disease progression, disease, or death, and OS, defined as the time between initiation of treatment and death, were summarized by using the Kaplan-Meier method.[12] Estimates for the median event time and 6-month rates were generated within the Kaplan-Meier framework.

RESULTS

Patient Characteristics

Sixty-seven patients were enrolled. Sixty-six patients received at least one dose of temozolomide and O^6-BG. One patient with AA did not receive any protocol treatment because of withdrawal of consent and is excluded from all summaries. Patient demographics and baseline disease characteristics of the 66 patients who received protocol treatment are listed in Table 1. Central pathologic review confirmed that 34 patients had GBM, 28 patients had AA, and four patients had

Table 1. Patient Demographics and Clinical Characteristics

Characteristic	No. of Patients (N = 66)	%
Age, years		
Median	51	
Range	21-69	
Sex		
Male	44	67
Female	22	33
Karnofsky performance score, %		
< 70	3	4
70-80	33	51
90-100	30	45
No. of progressions		
Median	2	
Range	1-5	
Time from diagnosis, weeks		
Median	60	
Range	12-702	
Histological diagnosis		
AA	28	42
AO	4	6
GBM	34	52

Abbreviations: AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; GBM, glioblastoma multiforme.

AO. The median age was 51 years, and the majority of patients were male. Fifty-five percent of patients had a Karnofsky performance score of ≤ 80. All patients had progressive or recurrent disease after prior therapy with temozolomide, and the majority of patients (59%) had at least two prior progressions. The median time from diagnosis to the start of therapy with temozolomide plus O^6-BG was 60 weeks (range, 12 to 72 weeks).

Response

The overall ORR for all histologies was 9% (95% CI, 3% to 19%). One (3%) of 34 patients with GBM (95% CI, 0.1% to 15%) and five (16%) of 32 patients with anaplastic glioma (95% CI, 5% to 33%) were responders to the combination of temozolomide and O^6-BG. Table 2 details the response in each histology subgroup. One patient died as a result of a perforated cecum 2 days after treatment with this drug combination. Five patients with anaplastic glioma who had a response completed at least a full year of therapy. Of these five patients, disease progression did not occur in three patients for 131, 93, and 89 weeks, respectively, and the other two patients remain disease-free 185 and 164 weeks from enrollment, respectively. Of interest, one patient with an anaplastic glioma who completed a year of therapy remained progression free for 143 weeks but never realized a response by the modified Macdonald criteria. However, this patient's positron emission tomography scan was hypometabolic on completion of a full year of therapy.

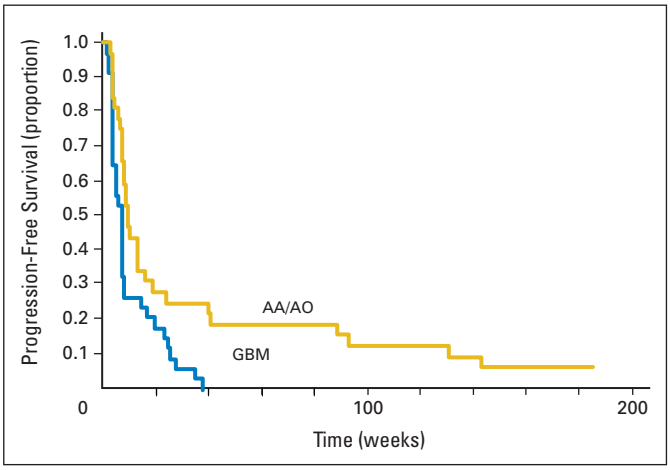

Fig 1. Kaplan-Meier estimates of progression-free survival according to histology. AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; GBM, glioblastoma multiforme.

PFS

The 6-month PFS was 17% overall (95% CI, 9% to 27%), 9% for GBM (95% CI, 2% to 21%), and 25% for anaplastic glioma (95% CI, 12% to 41%). The median PFS was 7.9 weeks overall (95% CI, 7.6 to 9.6 weeks), 7.5 weeks for GBM (95% CI, 4.2 to 7.9 weeks), and 9.6 weeks for anaplastic glioma (95% CI, 7.9 to 16.1 weeks). There was a significant statistical difference between the two histologies ($P = .0049$). The PFS data are illustrated by the Kaplan-Meier curve in Figure 1.

OS

The 6-month OS was 47% (95% CI, 35% to 58%), 26% for GBM (95% CI, 13% to 42%), and 69% for anaplastic glioma (95% CI, 50% to 82%). The median OS was 24.7 weeks (95% CI, 20.9 to 31.0 weeks), 19.4 weeks for GBM (95% CI, 13.7 to 24.3 weeks), and 33 weeks for anaplastic glioma (95% CI, 28.0 to 49.6 weeks). There was a significant statistical difference between the two histologies ($P = .0004$). The OS data are presented as a Kaplan-Meier curve in Figure 2.

Table 2. Overall Survival, Progression-Free Survival, and Objective Response Rate According to Histology

	Overall		GBM		AA/AO	
Variable	No. of Patients	95% CI	No. of Patients	95% CI	No. of Patients	95% CI
Overall survival						
Median, weeks*	24.7	20.9 to 31.0	19.4	13.7 to 24.3	33	28 to 49.6
At 6 months, %	47	35 to 58	26	13 to 42	69	50 to 82
Progression-free survival						
Median, weeks*	7.9	7.6 to 9.6	7.5	4.2 to 7.9	9.6	7.9 to 16.1
At 6 months, %	17	9 to 27	9	2 to 21	25	12 to 41
Objective response rate,* %	9	3 to 19	3	0.1 to 15	16	5 to 33

Abbreviations: AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; GBM, glioblastoma multiforme.
*There was a statistically significant difference between the two histologies.

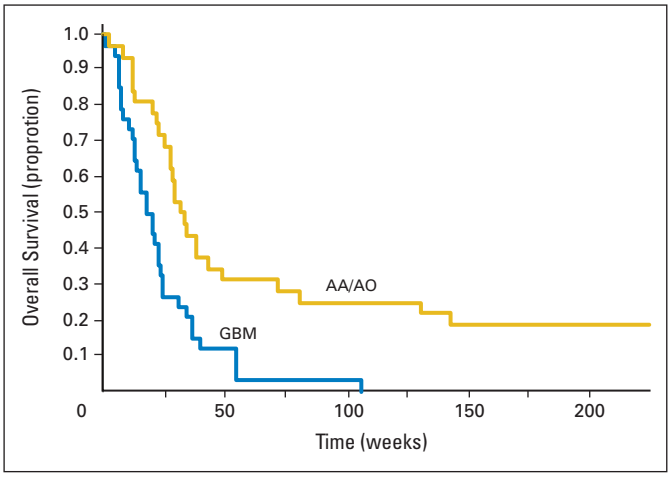

Fig 2. Kaplan-Meier estimates of overall survival according to histology. AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; GBM, glioblastoma multiforme.

Toxicity

Table 3 summarizes the adverse events (AEs). The most commonly reported AEs were grade 4 hematologic events experienced in 48% of the patients, for whom a 25% temozolomide dose reduction was required for subsequent dose administrations. The most frequent hematologic AEs were grade 4 neutropenia (47%), followed by grade 4 thrombocytopenia (12%). One patient died as a result of a perforated cecum 2 days after initiation of treatment with this drug combination. This event was unlikely to be drug related, given that before enrollment she complained of abdominal pain. One patient was not assessable for toxicity because the patient never received this drug combination.

DISCUSSION

Temozolomide and polifeprosan 20 with carmustine implant are both US Food and Drug Administration–approved drugs because of their ability to prolong survival in patients diagnosed with malignant glioma. Unfortunately, all patients with this disease will eventually experience treatment failure with these agents.

Resistance to both temozolomide and carmustine is mediated in part through the DNA repair protein AGT. Three clinical trials[13-15] reported an inverse relationship between AGT levels and survival for patients with malignant glioma treated with carmustine. Esteller et al[16] also confirmed this relationship, albeit using methylation of the AGT promoter gene in lieu of quantization of AGT levels. Likewise, this same inverse relationship between AGT levels and response was seen in patients with malignant glioma treated with preradiation temozolomide.[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 GBM treated with surgery, radiotherapy, and temozolomide. These studies suggest that the efficacy of temozolomide and carmustine could be enhanced by depletion of tumor AGT.

Depletion of tumor AGT activity by a selective inhibitor, O^6-BG, enhances the cytotoxicity of chloroethylators and methylators including temozolomide and carmustine.[1,4,7,17,19-21] However, the main limitation in systemic administration of alkylating agents in combination with O^6-BG is their potential for dose-related toxicity to the hematopoietic system. This was seen in animal studies[22-24] and confirmed in a phase I clinical trial,[25] where systemic administration of O^6-BG and carmustine markedly enhanced myelosuppression and reduced the carmustine MTD from 200 to 40 mg/m^2, representing an 80% dose reduction. This profound reduction in the carmustine dose was most likely the underlying factor in the failure of this drug combination to cause frank tumor regression in the phase II trial.[26]

Given that temozolomide is inherently less toxic than carmustine, particularly to hematopoietic cells, it was hypothesized that perhaps temozolomide in combination with O^6-BG would produce less myelosuppression than carmustine in combination with O^6-BG. Thus, a phase I clinical trial was performed in patients with malignant glioma to determine the MTD of single-dose temozolomide in combination with O^6-BG.[9] When combined with O^6-BG, the MTD of temozolomide was found to be 472 mg/m^2. The requirement for an 80% dose reduction seen with carmustine when combined with O^6-BG was seen to a lesser extent (50%) with temozolomide when combined with O^6-BG.

Thus, one of the aims of this study was to evaluate the safety of administering O^6-BG in combination with temozolomide, especially to the hematopoietic system. As expected, myelosuppression was the

Table 3. Adverse Events

	Grade 3		Grade 4		Grade 5	
Adverse Event	No. of Patients	%	No. of Patients	%	No. of Patients	%
Hematologic						
Anemia			1	1		
Lymphopenia			1	1		
Thrombocytopenia			8	12		
Neutropenia			31	47		
Neutropenia, febrile	1	1				
Nonhematologic						
Infection without neutropenia	2	3				
Seizure	4	6				
Thrombosis/embolism			2	3		
GI perforation					1	1

most commonly reported AE. Grade 4 hematologic events were experienced in 48% of the patients for whom a 25% temozolomide dose reduction was required for subsequent dose administrations. The most frequent hematologic AEs were grade 4 neutropenia (47%) followed by grade 4 thrombocytopenia (12%).

Another aim of this study was to identify the role of O^6-BG in restoring temozolomide sensitivity in patients with recurrent or progressive temozolomide-resistant malignant glioma. Unfortunately, the lack of a clinical rationale to justify a biopsy at recurrence precluded quantitation of tumor AGT levels, which could have provided insights if other non-AGT mechanisms of resistance were operational. We determined that O^6-BG when added to a 1-day dosing regimen of temozolomide was able to restore temozolomide sensitivity in patients with temozolomide-resistant anaplastic glioma, but there seemed to be no significant restoration of temozolomide sensitivity in patients with temozolomide-resistant GBM.

Despite the encouraging response to temozolomide and O^6-BG in patients with anaplastic glioma, it is somewhat disappointing that a greater response and improvement in PFS and OS were not realized in GBM, especially if AGT was indeed the primary mechanism responsible for tumor resistance to temozolomide. Although O^6-BG in combination with temozolomide was able to reverse the resistance in a minority of patients with recurrent or progressive, temozolomide-resistant malignant glioma, it was unable to reverse this resistance in the majority of these patients. The explanation for the inability of this combination to reverse resistance may lie in the two following reasons.

First, the dose reduction in temozolomide necessary to avoid hematologic toxicity when combined with O^6-BG may be an underlying factor in the failure of this drug combination to restore sensitivity. As a 1-day dosing regimen, temozolomide required a 50% dose reduction when delivered with O^6-BG. Alternative routes of administration of either O^6-BG or alkylators have been investigated in hopes of reducing systemic toxicity while maximizing efficacy. Administration of O^6-BG locally by intracerebral infusion through an Ommaya reservoir with concomitant oral administration of temozolomide was attempted in one patient.[27] This therapy was well tolerated and caused tumor stabilization for four months, but its success at depleting AGT and thus prolonging survival above and beyond the effect of temozolomide alone is unknown at this time. The administration of carmustine locally in the form of polifeprosan 20 with carmustine implant in combination with systemic O^6-BG was performed in a phase I[8] and a phase II clinical trial.[28] Both of these trials support the idea that local administration of carmustine in the form of polifeprosan 20 with carmustine implant in combination with systemic O^6-BG may mitigate the systemic toxicities of alkylators with improved efficacy.[28]

Second, combining O^6-BG with a 5-day dosing regimen of temozolomide may be more effective than a 1-day dosing regimen, especially if the total dose of temozolomide can be increased. To optimize a 5-day dosing regimen of temozolomide in combination with O^6-BG, one could hypothesize that suppressing AGT beyond 48 hours for at least 5 days while administering temozolomide may improve efficacy. Because we know that the above O^6-BG regimen completely suppresses O^6-BG for at least 48 hours, perhaps repeating this exact regimen every 48 hours for a period of 5 to 7 days may optimize AGT suppression while administering a 5-day dosing regimen of temozolomide. Although other O^6-BG regimens have been used in combination with temozolomide[29] and polifeprosan 20 with carmustine implant,[8] there is no evidence to suggest that these dosing regimens are adequate to suppress AGT levels in brain tumors.

Although the current trial of O^6-BG when added to a 1-day dosing regimen of temozolomide was able to restore temozolomide sensitivity in patients with temozolomide-resistant anaplastic glioma, no significant restoration of temozolomide sensitivity was seen in patients with temozolomide-resistant GBM. The limited response seen in GBM propels the research in a direction that explores alternative dosing regimens of temozolomide and O^6-BG and alternative administration routes for chemotherapy. Thus, additional investigation in combining temozolomide and O^6-BG in a 5-day dosing schedule needs to be undertaken in a phase I trial. Also, additional exploration of the safety and efficacy of combining polifeprosan 20 with carmustine implant with O^6-BG should be pursued. In addition, the role of drug delivery needs to be addressed, given that it is possible that potentially higher interstitial pressure seen in GBMs compared with anaplastic gliomas may be limiting drug delivery. This question might be addressed in future studies by incorporation of dynamic contrast-enhanced MRIs to evaluate tumor blood flow and potentially drug delivery.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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