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A Phase II Study of O6-Benzylguanine and Temozolomide in Pediatric Patients with Recurrent or Progressive High Grade Gliomas and Brainstem Gliomas: A Pediatric Brain Tumor Consortium Study

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Abstract

Purpose—To estimate the sustained (≥8 weeks) objective response rate in pediatric patients with recurrent or progressive high-grade gliomas (HGG, Stratum A) or brainstem gliomas (BSG, Stratum B) treated with the combination of O6-benzylguanine (O6BG) and temozolomide® (TMZ).

Patients and Methods—Patients received O6BG 120 mg/m^2/d IV followed by TMZ 75 mg/m^2/d orally daily for 5 consecutive days of each 28-day course. The target objective response rate to consider the combination active was 17%. A two-stage design was employed.

Results—Forty-three patients were enrolled; 41 were evaluable for response, including 25 patients with HGG and 16 patients with BSG. The combination of O6BG and TMZ was tolerable, and the primary toxicities were myelosuppression and gastrointestinal symptoms. One sustained (≥8 weeks) partial response was observed in the HGG cohort; no sustained objective responses were observed in the BSG cohort. Long-term (≥6 courses) stable disease (SD) was observed in 4 patients in Stratum A and 1 patient in Stratum B. Of the 5 patients with objective response or long-term SD, 3 underwent central review with 2 reclassified as low-grade gliomas.

Conclusions—The combination of O6BG and TMZ did not achieve the target response rate for activity in pediatric patients with recurrent or progressive HGG and BSG.

Keywords

glioma; pediatric; resistance; alkylating agent; brainstem glioma; AGT; MGMT

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Introduction

Temozolomide® (TMZ) is an alkylating agent that is active in a number of preclinical tumor models, including childhood CNS tumor xenografts.[1] It is approved for the treatment of refractory anaplastic astrocytoma, and has become standard treatment for adults with newly diagnosed glioblastoma administered concurrent with and following irradiation.[2] Despite the clinical activity observed in adults with high-grade gliomas,[3-7] pediatric clinical trials have not demonstrated significant improvement in outcome in children with high-grade gliomas (HGG) or brainstem (BSG) treated with TMZ. Response rates compiled from published studies are 11% in HGG and 4% in BSG,[8-10] and a recent Children's Oncology Group study, (ACNS0126), showed no improvement in median 1 year event free survival when temozolomide was administered with radiation therapy.[11, 12] The lack of efficacy in childhood gliomas has been attributed, at least in part, to overexpression of DNA repair proteins, particularly O^6-methylguanine-DNA methyltransferase (MGMT).[13, 14]

TMZ is an orally bioavailable prodrug that is rapidly absorbed and spontaneously converted to the reactive intermediate 5-(3-methyl-1-triazeno) imidazole-4-carboxamide (MTIC) at physiologic pH.[15, 16] MTIC methylates the N^7 and O^6-positions of guanine, and the N^3 position of adenine. Although the O^6-methylguanine adducts represent less than 10% of the methylation events, these lesions are primarily responsible for TMZ-mediated cytotoxicity by triggering the mismatch repair pathway and apoptosis.[17, 18] MGMT is a ubiquitous DNA repair protein overexpressed in some tumors and associated with drug resistance.[19, 20] It is a single turnover protein that specifically recognizes and removes the methyl adduct from the O^6-position of guanine, thus restoring DNA but inactivating itself in the process.[21] *MGMT* promoter methylation, which leads to lower MGMT protein expression, is associated with better clinical outcomes in adults and children with high-grade gliomas.[14, 17, 22]

O^6-benzylguanine (O6BG) is a modulating agent that inactivates and depletes MGMT by serving as a substrate and transferring its benzyl group to the active site of MGMT.[23] In preclinical studies, O6BG potentiates TMZ antitumor effects.[24] In clinical studies, the combination of O6BG and TMZ in pediatric patients is tolerable, albeit at substantially reduced doses of TMZ.[25, 26] The maximum tolerated dose of TMZ when given with a biologically active dose of O6BG for 5 days is 75 mg/m^2/d.[25] We hypothesized that the efficacy of TMZ would be enhanced when given with O6BG to children with high-grade and brainstem gliomas.

Patients and Methods

Eligibility

Patients ≤21 years of age with recurrent or progressive HGG or BSG after prior therapy were eligible. Patients must have received standard therapy, including radiation therapy prior to failure. Eligible patients were required to have bidimensionally measurable disease on radiographic studies; no more than two recurrences/progression; a Karnofsky or Lansky performance score ≥60; recovered from the toxic effects (to grade < 2 using CTCAE v.3.0) of prior therapy; received their last dose of known myelosuppressive chemotherapy or biologic therapy ≥3 weeks prior to registration (≥6 weeks for nitrosourea), and nonmyelosuppressive agent ≥7 days prior to registration; received their last fraction of radiation ≥12 weeks prior to registration; adequate hematologic function (ANC>1500/mm^3, Hb >8 gm/dl, ALC ≥500/mm^3, platelets ≥100,000/mm^3); an age-adjusted normal serum creatinine or a creatinine clearance >60 mL/min/1.73m^2; SGOT and SGPT up to 2.5 times the upper limit of normal; and total bilirubin up to 1.5 times the upper limit of normal. Patients previously treated with TMZ were eligible provided they did not have severe (grade ≥3) toxicity associated with prior use. The institutional review boards of each PBTC

institution approved the protocol before initial patient enrollment; continuing approval was maintained throughout the study. All patients or their legal guardians (for patients <18 years) gave written informed consent, and assent was obtained as appropriate at the time of enrollment.

Study Design

The primary endpoint of this study was estimation of the confirmed, sustained (≥8 weeks) objective response rate (complete response + partial response) in children with HGG and BSG treated with the combination of O6BG and TMZ. Patients were stratified at enrollment by tumor type (Stratum A: HGG; Stratum B: BSG). This trial used a two-stage Gehan-type design, with each stratum accruing 16 patients in the first stage and expanded to 25 patients in the second stage if ≥1 evaluable patient demonstrated a sustained objective response. A response rate of 17% in either stratum was considered to be of sufficient activity to warrant further evaluation of O6BG and TMZ in that disease. Because there is a 44% probability of observing no responses in a cohort of 16 patients if the true response rate is 5%, the study was to be considered non-informative if stopped after the first stage.

Drug Administration

O6BG was supplied by the Clinical Trials Evaluation Program (CTEP) of the Division of Cancer Treatment, National Cancer Institute. TMZ® (Schering Plough Corp., New Jersey) was purchased commercially. All patients received ondansetron or granisetron prior to and during the 5-day course of therapy. O6BG 120 mg/m^2 was administered as a 60-minute intravenous infusion daily for 5 days every 28 days. TMZ capsules (75 mg/m^2) were administered orally 30 minutes after the completion of each O6BG infusion. Patients received subsequent courses every 28 days if ANC >1500/mm^3 and platelets >100,000/mm^3. Patients who did not experience grade ≥ 2 toxicity had the TMZ dose-escalated to 100 mg/m^2/d on subsequent courses. Hematologic toxicity was presumed to be related to TMZ and patients with significant toxicity (ANC <500/mm^3 for ≥ 3 days, platelet count <50,000/mm^3, or myelosuppression requiring treatment delay of ≥ 14 days duration) had the TMZ dose reduced to 55 mg/m^2/d for subsequent cycles. The TMZ dose was also reduced for patients with any grade 4 toxicity or dose-related intolerable grade 3 toxicity at least possibly related to the combination of O6BG and TMZ. The dose of O6BG remained at 120 mg/m^2/dose for all patients. Adverse events were graded according to version 3.0 of the National Cancer Institute Common Toxicity Criteria (http://ctep/info/nih/gov). Patients were treated on study for a maximum of 12 courses or until one of the off-study criteria were met.

Evaluation of Response

Any patient who completed at least two courses of the combination of O6BG and TMZ were considered evaluable for the primary endpoint. Patients who completed fewer than two courses of therapy were considered evaluable if they experienced disease progression or died from any cause after receiving any O6BG and TMZ. Standard MR imaging including T1, T2, FLAIR and post-gadolinium diethylene triamine pentaacetic acid (DTPA) T1-weighted imaging of the brain was obtained prior to therapy, prior to cycle 3, and at alternate cycles thereafter. Echoplanar MR diffusion imaging and T2* gradient echo perfusion imaging were done. Single voxel MR Point Resolved Spectroscopy (PRESS) was obtained in the region of solid tumor enhancement prior to therapy and on follow-up examinations.

The definition of response was based on maximal two-dimensional measurements of tumor obtained on MRI and confirmed 8 weeks after initial documentation of an objective response. (Table 1) Blinded central pathology review of patients in Stratum A was

retrospectively performed to confirm the diagnosis and determine the relationship of drug sensitivity to MGMT expression.

Kaplan-Meier estimates of distributions of PFS included all eligible patients who received at least one dose of O6BG and TMZ in each stratum. PFS was measured from the date of initial treatment to the earliest date of disease progression or death for patients who experienced disease progression, and to the date of last contact for patients who remained at risk for disease progression. Cox proportional hazards models were used to explore relationships between PFS and functional changes in tumor measured by MR perfusion/diffusion imaging, volumetrics (FLAIR or T2; enhanced volume), cyst formation, and MR spectroscopy studies. Because of the limited number of subjects and imaging studies, these analyses were considered exploratory.

Immunohistochemistry

Immunohistochemistry for the detection of methylguanine methyltransferase (MGMT) and Ki-67 expression was performed as previously described.[27, 28] For both assays, nuclei of 100 tumor cells were quantitated independently by two observers to determine the percentage of positive immunoreactive nuclei. The results were accepted if the comparison of the two independent quantitations differed by <5%. Cytoplasmic-only and granular nuclear reactivity were regarded as negative with both antigens.

Results

Patient Characteristics

Forty-three patients were enrolled between December 2005 and November 2007. Forty-one patients were evaluable for response, including twenty-five patients in Stratum A and sixteen patients in Stratum B. Two patients were ineligible and therefore not evaluable for response due to the lack of measurable disease in one and <12 weeks since completion of radiation therapy in one. Characteristics of evaluable patients are listed in Table 2.

Responses and progression-free survival

One partial response was observed in Stratum A during the first stage. This cohort was subsequently expanded to twenty-five patients, but no additional sustained objective responses were observed. No objective responses were observed in the 16 patients in Stratum B. The overall response rate was 4% for HGG (exact 95% confidence interval 0.1%-20%) and 0% for BSG cohorts. However, stable disease (≥ 6 courses) was observed in 4 patients (16%) in Stratum A and 1 patient (6%) in Stratum B. Six-month progression-free survival (PFS) was 16 ± 6.6% for Stratum A and 0% for Stratum B.

Volumetric analysis (FLAIR or T2; enhanced volume, cyst formation), and diffusion were evaluated on follow-up imaging; a limited number of perfusion and MR spectroscopy studies were performed. Analyses showed no significant change over time for volumetric studies, cyst formation, diffusion, or perfusion. The log of volume (FLAIR) at baseline was suggestive of an association with progression-free survival (PFS) in stratum A (p=0.033). Similarly, an association was suggested between the square root of the enhancing volume at baseline and PFS (p =.045). MRS variables, including choline, choline to N-acetylaspartase ratio (Cho:NAA), and change in lipid were not associated with PFS survival.

Central Pathology Review

Pathology material was available and evaluable for twenty patients in Stratum A. Material was not reviewed on five patients because pre-trial tumor was not available (n=1), there was insufficient material for slides (n=2) and slides could not be obtained by the treating site

(n=2). The material was reviewed independently for histologic diagnosis by three neuropathologists. Ki-67 labeling index and MGMT labeling index were scored by a single neuropathologist. Material was available for review on three of the five patients in Stratum A with either an objective response or long-term stable disease. Upon central review of these 3 cases, the diagnoses included ganglioglioma (n=1), anaplastic astrocytoma (n=1), and one case classified differently by each neuropathologist as pilocytic astrocytoma, ganglioglioma, or astrocytic glioma. The Ki-67 index was 1-3 and the MGMT labeling index was 0-<10 for those three patients. In comparison, for the nonresponders, the Ki-67 index was >3 in 14 of 16 patients (88%) and MGMT labeling index was ≥10 in 13 of 16 patients (81%).

Toxicity

Forty-one patients received a total of 133 courses (range 1-12; median 2) of O6BG and TMZ. The combination was well tolerated in this population. Toxicities at least possibly related to this combination are listed by grade in Table 3. As expected, myelosuppression was the most common toxicity with Grade 4 neutropenia occurring in 15% of courses. Thirteen patients were able to tolerate dose escalation of TMZ to 100 mg/m^2/d; ten patients required a dose reduction to 55 mg/m^2/d for myelosuppression.

Discussion

TMZ administered after O6BG on a daily for 5-day schedule repeated every 28 days resulted in an overall sustained objective response rate of 4% for HGG and 0% for BSG, which did not meet an activity level of interest in either stratum. If one considers patients with long-term stable disease (≥6 courses), as responders, then the response rate was 20% for the high-grade glioma cohort, although two of these patients were subsequently reclassified on central review as low-grade gliomas, and 6-month PFS remained low at 16%. In phase II trials in adults with recurrent glioblastoma treated with temozolomide alone, objective response rates were 8-19%, and up to 45% of patients had stable disease. [4, 29] In the Phase II trial of TMZ plus O6BG in adults with TMZ-resistant glioma, objective response rates based on MacDonald criteria were 3% for patients with glioblastoma multiforme and 16% for patients with anaplastic gliomas, but the median progression-free survival was less than 8 weeks.[30] This is in agreement with our study in that the addition of O6BG to TMZ did not appear to significantly improve outcome. A Phase II study of O6BG administered with BCNU to patients with nitrosourea-resistant recurrent and progressive malignant glioma also did not show any significant benefit.[31]

Prior studies have demonstrated both significant potentiation of alkylating agents *in vitro* when administered with O6BG[23, 24] and a correlation between MGMT expression and outcome for patients with high grade gliomas treated with TMZ.[32, 33] Although the numbers are small and MGMT was not measured close to treatment on this study, we noted that the three patients with an objective response or long term stable disease for whom tissue was available all had a lower MGMT labeling index than the majority of the nonresponders.

The lack of potentiation of TMZ activity when administered with O6BG may be due to a number of factors. TMZ activity is known to be dose and schedule-dependent[1, 16, 34] and the TMZ dose of 75 mg/m^2/d for 5 days used in this study may not be optimal. TMZ has good CSF penetration with a CSF:plasma ratio of 33%.[35] Increasing age and body surface area are associated with increased TMZ clearance and C_{max} and decreasing TMZ and MTIC AUC, [36] so a higher exposure would be expected in children compared to adults. MGMT expression within the tumor may not be effectively depleted: O6BG and its metabolites do not penetrate well across the intact blood:brain barrier,[37] although studies in adults showed that 100 mg/m^2 O6BG effectively reduced MGMT levels to <10 fmol/mg protein in brain tumors.[38] Furthermore, although we studied MGMT activity in tumors from tissue obtained

at diagnosis, MGMT activity in patients' tumors at the time of treatment on this study is unknown. Radiation therapy and glucocorticoids can increase MGMT expression[21] and therefore samples obtained at diagnosis may not be representative of the MGMT status at the start of protocol therapy. Alternative mechanisms of resistance may also exist, including a defective mismatch repair system.[39]

Several approaches may be used to try to enhance the activity of O6BG with TMZ. Because TMZ dosing is limited by enhanced myelosuppression, protection of the marrow may allow intensified TMZ dosing, and *MGMT* gene transduction into hematopoietic progenitor cells has been proposed.[21] Alternate TMZ scheduling, such as low-dose, continuous daily dosing may provide another approach to promote efficacy.

An important aspect of this study was the central pathology review of patients in Stratum A. Of the 5 patients in Stratum A that demonstrated an objective response or long-term stable disease, HGG could be confirmed in only 1 case as two cases were reclassified as low-grade gliomas and material was not available in the other two cases. This emphasizes the need for central pathology review, particularly in small trials estimating efficacy of an agent in a particular disease cohort.

In summary, this study of O6BG and TMZ, administered on a daily x 5 schedule every 28 days to children with high-grade gliomas and brainstem gliomas, did not appear to significantly improve the activity of temozolomide alone. Further strategies to optimize TMZ dosing need to be evaluated.

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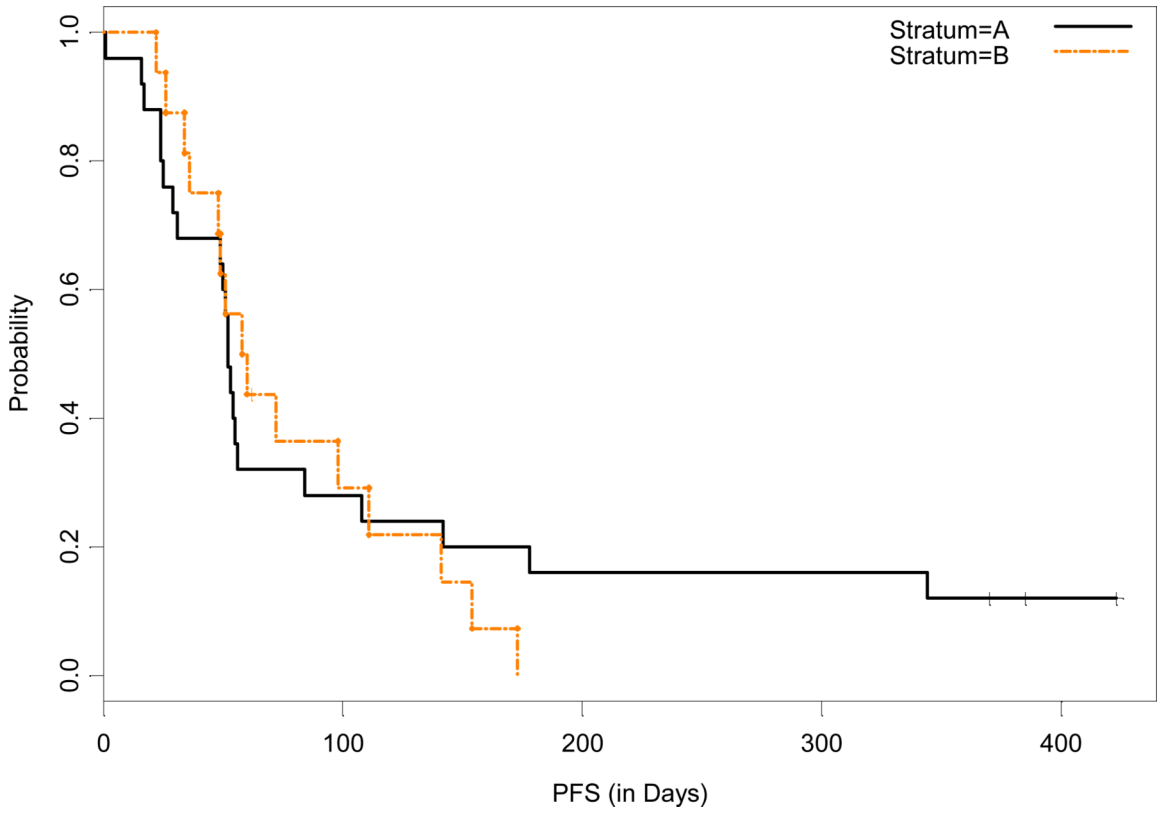

Figure 1. Progression-Free Survival

The median PFS for Stratum A was 52 days (95% CI on the median: 49-108 days) and 6-month PFS was 16% (95% CI: 6.52%-39.3%). For patients on Stratum B, median survival was 60 days (95% CI on the median: 49-154 days) and 6-month PFS was 0%.

Table 1

Definition of Response

Complete response (CR):	Complete disappearance of all enhancing tumor and mass effect, on a stable or decreasing dose of steroids, with stable/improving neurologic examination and maintained for at least 8 weeks.
Partial Response (PR):	≥50% reduction in tumor size based on maximal cross-sectional measurements, on a stable or decreasing dose of steroids, with stable/improving neurologic examination and maintained for at least 8 weeks.
Stable Disease (SD):	Neurologic examination is at least stable, steroid dose is not increased, and MR imaging does not meet the criteria for PR nor PD.)
Progressive Disease (PD):	Progressive neurologic abnormalities or appearance of new tumors or >25% increase in the sum of the product of the 2 longest perpendicular diameters.

Table 2

Patient Characteristics

	Stratum A	Stratum B
Number of evaluable patients:	25	16
Median (range) age [yr]:	14.4 (1.6-21.3)	6.9 (2.1-16.2)
Median (range) Performance Score:	90 (60-100)	85 (60-100)
Median (range) Prior # chemotherapy regimens:	1 (0-3)	1 (0-3)
Prior temozolomide:	19	5
Median (range) 2-dimensional tumor size (cm^2):	5.28 (.034-48.24)	14.74 (.0168-22.5)

Table 3

Toxicities attributable to O6BG and TMZ in 41 patients

TOXICITY	GRADE			
	1	2	3	4
Leukopenia	5	10	6	4
Neutropenia	-	4	5	13
Lymphopenia	4	6	10	1
Thrombocytopenia	16	3	7	1
Anemia	12	5	3	-
Nausea	9	1	-	-
Vomiting	5	-	1	-
Diarrhea	2	2	-	-
Constipation	1	-	-	-
Abdominal pain	1	-	-	-
Fatigue	3	-	-	-
Headache	-	-	1	-
Flushing	1	-	-	-
↑ AST/ALT	3/7	0/1	-	-
Infection	-	2	2	-
Urinary retention:	2	-	-	-
Alopecia:	2	1	-	-