Phase I Trial of Single-Dose Temozolomide and Continuous Administration of O^6-Benzylguanine in Children with Brain Tumors: a Pediatric Brain Tumor Consortium Report

Alberto Broniscer,[1] Sridharan Gururangan,[6] Tobey J. MacDonald,[7] Stewart Goldman,[8] Roger J. Packer,[7] Clinton F. Stewart,[2] Dana Wallace,[3] Mary K. Danks,[4] Henry S. Friedman,[6] Tina Y. Poussaint,[9] Larry E. Kun,[5] James M. Boyett,[3] and Amar Gajjar[1] for the Pediatric Brain Tumor Consortium

Abstract

Purpose: To estimate the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) of escalating doses of temozolomide combined with O^6-benzylguanine in patients ≤21 years with recurrent brain tumors.

Experimental Design: Treatment strata consisted of patients who had previously received no or local radiotherapy (Str1) and patients who had undergone craniospinal radiotherapy or myeloablative chemotherapy (Str2). One-hour i.v. administration of O^6-benzylguanine at 120 mg/m^2 was followed by 48-h continuous infusion at 30 mg/m^2/day. Single-dose temozolomide at five dosage levels (267, 355, 472, 628, and 835 mg/m^2) was given at least 6 h after completion of O^6-benzylguanine bolus. Treatment was repeated after recovery from toxicities at least 4 weeks apart for a maximum of 12 courses. Dose escalation followed the modified continual reassessment method. Pharmacokinetic analyses of temozolomide and 5-triazeno imidazole carboxamide (MTIC) were done in 28 patients.

Results: A total of 44 and 26 eligible patients were enrolled on Str1 and Str2, respectively. Median age at study entry in each stratum was 8.6 and 11.3 years, respectively. Predominant diagnoses were high-grade/brainstem glioma in Str1 and medulloblastoma in Str2. Whereas the estimated MTDs of temozolomide for Str1 and Str2 were 562 and 407 mg/m^2, respectively, the doses recommended for phase II investigations are 472 and 355 mg/m^2, respectively. DLTs were predominantly neutropenia and thrombocytopenia. Three patients with gliomas experienced centrally confirmed partial responses to therapy. Four patients completed all planned therapy. Temozolomide and MTIC exposures were statistically associated with temozolomide dosage.

Conclusions: The current schedule of temozolomide and O^6-benzylguanine is safe and showed modest activity against recurrent brain tumors in children.

Authors' Affiliations: Departments of [1]Oncology, [2]Pharmaceutical Sciences, [3]Biostatistics, [4]Molecular Pharmacology, and [5]Radiological Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee; [6]Department of Pediatrics, The Preston Robert Tisch Brain Tumor Center at Duke, Duke University Medical Center, Durham, North Carolina; [7]Department of Neurology, Children's National Medical Center, Washington, District of Columbia; [8]Children's Memorial Hospital, Chicago, Illinois; and [9]Department of Radiology, Children's Hospital of Boston, Boston, Massachusetts

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Requests for reprints: Alberto Broniscer, Department of Oncology, St. Jude Children's Research Hospital, 332 North Lauderdale, Mail Stop 260, Memphis, TN 38105. Phone: 901-495-4925; Fax: 901-521-9005; E-mail: alberto.broniscer@stjude.org.

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Temozolomide has been used in the treatment of children with brain tumors, particularly those with high-grade gliomas, because of its activity against xenografts derived from pediatric patients (1, 2), its favorable penetration into the central nervous system (3, 4), and the promising results in the treatment of adults with high-grade glioma (5–7). Two pediatric phase I studies that used the 5-day schedule of temozolomide have established its maximum tolerated dose (MTD; refs. 8, 9). Unlike adult clinical trials (5–7), phase II studies of temozolomide showed only modest activity in children with newly diagnosed or recurrent high-grade and diffuse brainstem glioma (10–13).

Two main mechanisms account for the resistance of tumor cells to temozolomide: the repair enzyme methylguanine methyltransferase (MGMT) and the mismatch repair (MMR) system (14–16). Cytotoxicity of temozolomide is initiated by the methylation of the O^6 position of guanine that causes mispairing of O^6-methylguanine with thymine. The futile repair of this base mismatch by the MMR system generates single- and double-strand DNA breaks that activate cell death. MGMT prevents this process by transferring methylating groups

from the O^6 position of guanine to one of its internal cysteine residues.

O^6-benzylguanine (O^6-BG), a pseudosubstrate of MGMT, depletes this enzyme by transferring the benzyl group to its active cysteine site, which leads to MGMT degradation. Preclinical studies with cells lines derived from brain tumors, including those from pediatric patients, showed enhanced temozolomide activity when combined with O^6-BG, except for those cell lines with no or low baseline MGMT activity (15, 17, 18). The contribution of O^6-BG to the cytotoxicity of temozolomide in cell lines was variable and not dependent on their content of MGMT (15, 18). The addition of O^6-BG also increased the cytotoxic effects of temozolomide in an intracranial xenograft derived from medulloblastoma with high MGMT activity (1). The same combination produced inconsistent responses in three s.c. xenografts derived from glioblastoma with low MGMT activity (19–21).

Three clinical trials have shown that MGMT can be effectively depleted from tumor samples obtained at the time of surgery in adult patients with high-grade glioma following bolus ($n = 2$) or bolus and continuous administration of O^6-BG ($n = 1$; refs. 22–24). In the latter study, Quinn et al. (24) observed MGMT depletion in 12 of 13 tumor samples obtained after 1-h bolus of O^6-BG at 120 mg/m^2 followed by 48-h continuous administration at 30 mg/m^2/day.

Because of the successful depletion of MGMT after bolus and continuous administration of O^6-BG, we conducted this phase I multicenter clinical trial combining escalating, single-dose temozolomide and the same schedule of O^6-BG described above.

Materials and Methods

Eligibility. Patients ≤21 years of age with recurrent or refractory brain tumors were eligible for this study. Histologic confirmation at diagnosis or at the time of recurrence/progression was required for all patients except those with brainstem tumors. Other inclusion criteria consisted of (*a*) performance score ≥60 and life expectancy >8 weeks; (*b*) stable neurologic deficits and stable doses of dexamethasone for ≥1 week; (*c*) no more than two previous therapy regimens; (*d*) interval from previous chemotherapy >3 weeks (6 weeks if nitrosourea was used) and >6 months from myeloablative chemotherapy; (*e*) interval from previous craniospinal radiotherapy (dose ≥18 Gy), local radiotherapy, and local radiotherapy for symptomatic metastatic sites ≥3 months, ≥4 weeks, and ≥2 weeks, respectively; (*f*) no growth factors for >2 weeks; (*g*) adequate organ function, particularly hematopoietic (absolute neutrophil count [ANC] >1,000/μL, platelet count >100,000/μL [transfusion independent], hemoglobin >8 g/dL), renal (serum creatinine ≤1.5 times upper levels of institutional normal or glomerular filtration rate >70 mL/min/1.73 m^2), and hepatic [serum bilirubin ≤ upper limit of normal for age, alanine aminotransferase and aspartate aminotransferase (AST) <2.5 times upper levels of institutional normal]. Exclusion criteria consisted of pregnant and lactating patients, unwillingness to use acceptable forms of contraception when applicable, use of other anticancer or experimental therapy, presence of uncontrolled infections, and previous hypersensitivity to dacarbazine, temozolomide, or polyethylene glycol. Patients who had previously received temozolomide were eligible for this study if they had not received this medication for ≥3 months and had not experienced any grade 3/4 nonhematologic toxicity.

The institutional review board of each Pediatric Brain Tumor Consortium–participating institution approved the protocol before initial patient enrollment, and continuing approval was maintained throughout the study. Written informed consent for participation was obtained from patients or their legal guardians, and assents were obtained when appropriate.

Study design and treatment plan. Patients were stratified into two strata according to previous therapy: stratum 1 for patients who received only local or no radiotherapy and stratum 2 for patients who underwent craniospinal radiotherapy at doses >18 Gy or myeloablative chemotherapy.

O^6-BG was supplied by the Division of Cancer Treatment and Diagnosis, Cancer Therapy Evaluation Program, National Cancer Institute, and temozolomide (5-, 20-, 100-, and 250-mg capsules) was obtained through commercial sources.

O^6-BG was administered i.v. as a 1-h bolus of 120 mg/m^2 followed by 48-h continuous infusion at 30 mg/m^2/day. Single-dose temozolomide was administered p.o. at least 6 h after completion of O^6-BG bolus. Six dosage levels of temozolomide were planned for each treatment stratum: 267, 355, 472, 628, 835, and 1,111 mg/m^2. Treatment was repeated at least 4 weeks apart once patients recovered from toxicities for a total of 12 courses. Criteria to initiate subsequent courses of chemotherapy consisted of ANC ≥1,000/μL, platelets ≥75,000/μL (transfusion independent), hemoglobin ≥8 g/dL (transfusion independent), AST ≤2.5 times upper limit of institutional normal, creatinine and bilirubin ≤1.5 times upper limit of institutional normal, and recovery of all other toxicities to grade ≤1. The dose of chemotherapy was calculated based on the body surface area before each course.

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. The dose-limiting toxicity (DLT) evaluation period was defined as the first 4 weeks from the start of therapy. DLT was considered as any of the following toxicities attributable to temozolomide and/or O^6-BG: (*a*) grade 4 neutropenia >7 days; (*b*) grade 4 anemia; (*c*) grade 4 thrombocytopenia; (*d*) need of ≥2 platelet transfusions within 7 days; (*e*) delay of ≥14 days of planned interval between courses because of hematologic toxicity; (*f*) any grade 3/4 nonhematologic toxicity except for grade 3 fever or infection and grade 3 nausea and vomiting unless patient was receiving optimal anti-emetic therapy; (*g*) failure to recover from any non-DLT to ≤grade 1 within 6 weeks from temozolomide administration.

In each stratum, the modified continual reassessment method (CRM; ref. 25) was used to estimate the dose of temozolomide where 25% of patients would be expected to experience DLT (CRM-estimated MTD). Prior probabilities of DLT for the six protocol-prescribed dosage levels were based on the adult experience with the same strategy (24). In each stratum, treatment started at the first dosage level, and at least two patients were treated at each dosage level; a third patient could be treated at the same dosage level if toxicity information was pending for the first two patients. The CRM was continuously updated, and decisions about dose escalation were made as toxicity information became known for each patient. Patients were treated at the dosage level closest to the CRM-estimated MTD without skipping any levels at which fewer than two patients had been evaluated. Dose escalation was completed when at least six evaluable patients had been treated at the protocol-prescribed dosage level closest to the CRM-estimated MTD, which was defined as the dose-finding MTD.

A logistic regression model was used to investigate the relationship between age and dosage of temozolomide and the occurrence of DLT. Ordinary least-squares regression was used to analyze the association between temozolomide dose and the area under concentration-time curve (AUC) of temozolomide and 5-triazeno-imidazole carboxamide (MTIC). The dose of temozolomide was evaluated as a continuous variable in the regression models. Visual inspection of the scatter plots and residuals were done to validate the model assumptions.

Imaging evaluation was obtained before beginning of therapy and after every other course of treatment thereafter. Complete and partial responses were defined as complete disappearance, and ≥50% shrinkage of tumor as measured by bi-dimensional measurements, respectively, accompanied by stable or improved neurologic findings for at least 6 weeks on a stable or decreasing dose of corticosteroids.

Progressive disease was defined as worsening neurologic findings attributed to tumor progression and/or increase of >25% in bi-dimensional tumor measurements and/or need of increasing doses of corticosteroid to maintain stable neurologic exam. Stable disease consisted of all other situations not defined above. Objective responses to therapy were confirmed by central radiographic review.

Supportive care. Central venous access was recommended for all patients treated on this study. Irradiated, leukocyte-depleted blood products were used to maintain platelet count >20,000 to 30,000/mm^3 and hematocrit >20% to 25%. All patients received *Pneumocystis cariini* prophylaxis beginning with the first course of therapy. Whereas ondansetron was recommended as the anti-emetic of choice during treatment, the use of corticosteroids as anti-emetics was contra-indicated. Episodes of fever and neutropenia were managed according to institutional guidelines.

Pharmacokinetic studies. In patients consenting to pharmacokinetic studies, 3 mL of blood were collected in a lithium heparin tube before and at 0.25, 0.5, 1, 2.5, 4, and 6 h after temozolomide administration to assess the disposition of temozolomide and its active metabolite MTIC. After processing, plasma levels of temozolomide and MTIC were measured by isocratic high-performance liquid chromatography (26). Two milliliters of blood were collected before and at 26 ($\pm$3) and 32 ($\pm$3) h after the start of O^6-BG infusion to measure the plasma levels of O^6-BG and its active metabolite O^6-benzyl-8-oxoguanine (8-oxoBG). Samples were centrifuged within 30 to 60 min after collection at 1,000 $\times$ *g* for 10 min and frozen at -80°C until analysis. Plasma levels of O^6-BG and 8-oxoBG were measured by isocratic high-performance liquid chromatography as previously described (27).

Temozolomide and MTIC plasma concentration-time data were modeled using a posteriori (MAP) Bayesian estimation as implemented in ADAPT II (28). The prior parameter estimates were derived from a pediatric population (29). A first-order absorption, one-compartment linear model that included first-order MTIC formation and elimination was used to simultaneously describe temozolomide and MTIC disposition. The AUC values for temozolomide and MTIC were calculated from the model parameters. These estimates then allowed for calculation of temozolomide systemic clearance. A noncompartmental approach was used to assess O^6-BG systemic clearance from its steady-state plasma concentrations. Inter- and intrapatient variability of pharmacokinetic parameters was calculated when deemed appropriate.

Biological studies. DNA was extracted from formalin-fixed, paraffin-embedded blocks (n = 16) or fresh-frozen tumor samples (n = 1) and peripheral blood lymphocytes using a Stratagene DNA extraction kit. Analysis of microsatellite instability (MSI) was conducted by examining 17 separate loci (D1S102, D2S123, D2S390, BAT26, D4S174, D4S392, BAT25, D6S253, D7S460, D10S212, D11S935, D14S48, D14S49, D15S118, D17S250, D18S53, and D19S246), including four of five loci recommended by the National Cancer Institute workshop for differences in number of single- or double-nucleotide repeats between constitutional and tumor DNA (30).

PCR amplification and subsequent capillary electrophoresis was used to analyze MSI. The PCR reaction mix was composed of 9 μL of True Allele PCR Premix (Applied Biosystems), 1 μL of DNA, and primer sets labeled with fluorescent phosphoramidite at a final concentration of 50 pmol/L. Supplementary Table S1 provides details about the primers used. PCR was done using an MJ Research PTC-200 Peltier Thermal Cycler. Initial denaturing at 95°C for 12 min was followed by 10 cycles at 94°C for 15 s, 55°C for 45 s, and 72°C for 1 min. Final reaction consisted of 20 cycles at 89°C for 15 s, 55°C for 15 s, and 72°C for 30 s, followed by extension at 72°C extension for 10 min. The ROX400HD size standard was used in all samples as an internal ladder to align data from different capillaries. One microliter of fluorescent-labeled PCR products, size standard, and Ultrapure Formamide (Invitrogen) were added into each well of the sample plate. PCR products were denatured at 95°C for 5 min and then cooled in ice for 2 min to avoid re-annealing of the complementary strands. PCR products were separated by capillary electrophoresis using ABI 3730XL DNA Analyzer. GeneMapper 4.0 software (Applied Biosystems) was used to analyze the data.

Results

Seventy-two patients were enrolled in this study between October 2002 and June 2005. Two patients were ineligible because of previous treatment with >2 chemotherapy regimens (n = 1) and need of increasing doses of corticosteroids within 1 week of study entry (n = 1). Table 1 provides the clinical characteristics and information about previous therapy for all eligible patients.

A total of 17 patients was not assessable for the MTD estimate. Six patients were prescribed an incorrect dose of O^6-BG (15 mg/m^2/day for 48 h) before the study was amended to the correct dose of this medication. Eleven patients were not assessable because of use of hematopoietic growth factor (n = 1), early death before start of therapy (n = 1), or because they did not complete the DLT evaluation period for reasons unrelated to toxicity (n = 5) or did not receive the intended

Table 1. Clinical characteristics and previous treatment for all eligible patients

	Stratum 1 (n = 44) n (%)	Stratum 2 (n = 26) n (%)
Age at diagnosis (y)		
Median	6	6.7
Range	0.2-17.9	0.6-15.5
Age at study entry (y)		
Median	8.6	11.3
Range	0.4-20.2	2.4-18.6
Gender		
Male	23 (52)	20 (77)
Female	21 (48)	6 (23)
Race		
Caucasian	23 (52)	20 (77)
African-American	11 (25)	1 (4)
Other	10 (23)	5 (19)
Histologic diagnoses		
Ependymoma	14 (32)	4 (15)
Cellular	6 (14)	2 (7.5)
Anaplastic	8 (18)	2 (7.5)
High-grade glioma	13 (30)	3 (12)
Glioblastoma	6 (14)	2 (8)
AA	2 (5)	0 (0)
Others	5 (11)	1 (4)
Brainstem glioma	9 (20)	1 (4)
Low-grade glioma	7 (16)	2 (8)
JPA	4 (9)	1 (4)
Others	3 (7)	1 (4)
Medullo/PNET	1 (2)	16 (61.5)
Medullo	0 (0)	10 (38.5)
PNET	1 (2)	6 (23)
Performance score at study entry		
Median	90	85
Range	60-100	60-100
Previous therapy		
Radiotherapy	38 (86)	26 (100)
Chemotherapy	42 (95)	23 (88)
Temozolomide and/or nitrosourea	16 (36)	7 (27)

NOTE: Two patients with primary spinal cord glioblastoma were enrolled on stratum 1 of this study.
Abbreviations: AA, anaplastic astrocytoma; JPA, juvenile pilocytic astrocytoma; medullo, medulloblastoma; PNET, primitive neuroectodermal tumor.

Table 2. Attribution of toxicities by grade during the DLT evaluation period

Toxicity	Number of patients*	Stratum 1				Number of patients*	Stratum 2			
		Grade					Grade			
		1	2	3	4		1	2	3	4
Neutropenia	19	1	1	4	13	12	2	4	0	6
Anemia	17	7	4	5	1	8	2	4	1	1
Thrombocytopenia	15	3	2	8	2	7	1	1	5	0
Transfusion: platelets	7	—	—	5	2	3	—	—	3	0
Gastrointestinal	8	5	2	1	0	5	5	0	1	0
Constitutional symptoms	6	4	2	0	0	1	0	1	0	0
Infection/febrile neutropenia	4	0	0	4	0	1	0	0	1	0
Metabolic/laboratory	4	3	1	0	0	1	1	0	0	0
Hepatic	2	2	0	0	0	2	1	1	0	0
Pain	1	1	0	0	0	2	2	0	0	0

*Patients who did not experience toxicity were excluded.

dose of medications (n = 4). Eleven patients (five in stratum 1 and six in stratum 2) were treated at the respective MTD to better characterize the influence of age on the toxicity. Therefore, the MTD estimate was based on toxicities observed in 27 and 15 patients for strata 1 and 2, respectively.

Toxicities. Table 2 summarizes the toxicities attributed to therapy observed during the DLT evaluation period. Most grades 3 and 4 toxicities were hematologic, particularly neutropenia and thrombocytopenia. Table 3 provides details of neutropenia and thrombocytopenia according to temozolomide dosage level during the DLT evaluation period. In stratum 1, temozolomide dosage reached 835 mg/m^2 because only 1 of 15 patients treated at lower dosage levels experienced DLT. However, four of five patients experienced DLTs at 835 mg/m^2, and temozolomide dosage was reduced to 628 mg/m^2. Because five of seven additional patients treated at 628 mg/m^2 experienced DLTs, the CRM-estimated MTD and the dose-finding MTD for stratum 1 were 530 and 472 mg/m^2, respectively. After the MTD had been estimated in stratum 1, a central review identified one DLT at the 472-mg/m^2 dosage level, which did not fit the protocol definition, and an institutional audit declared one of the patients treated at the 835-mg/m^2 dosage level ineligible. Based on this new information, the revised CRM-estimated MTD and the dose-finding MTD in stratum 1 were changed to 562 and 628 mg/m^2, respectively. DLTs in stratum 1 were grade 4 neutropenia for >7 days (n = 5) at 628 mg/m^2; grade 4 thrombocytopenia (n = 2), neutropenia for >7 days (n = 1), anemia (n = 1), and need for >2 platelet transfusions within 7 days (n = 1) at 835 mg/m^2. In stratum 2, temozolomide dosage was escalated to 472 mg/m^2 because no DLTs were observed at lower dosage levels. However, four of six patients had DLTs at 472 mg/m^2. Therefore, the dosage was reduced, and three additional patients treated at the 355-mg/m^2 level did not experience DLT. The CRM-estimated MTD and the dose-finding MTD for stratum 2 were 407 and 355 mg/m^2, respectively. DLTs for stratum 2 consisted of grade 4 neutropenia for >7 days (n = 3) and anemia (n = 1) at 472 mg/m^2. The only significant nonhematologic toxicities observed during DLT evaluation period consisted of grade 3 vomiting in one patient in each stratum and grade 3 hypoxia in one patient in stratum 1, which was not attributed to temozolomide. Although patients who experienced DLT tended to be younger than those who did not experience DLT, there was no significant correlation between

Table 3. Grades of neutropenia and thrombocytopenia during DLT evaluation period according to dosage level and treatment stratum

Temozolomide dosage level (mg/m$_2$)	Number of patients	Grade of neutropenia						Grade of thrombocytopenia					
		0	1	2	3	4	DLT	0	1	2	3	4	DLT
Stratum 1													
267	3	3	0	0	0	0	0	3	0	0	0	0	0
355	3	2	0	0	1	0	0	3	0	0	0	0	0
472	7	2	0	1	3	1	0	3	3	0	1	0	1 (0)
628	10	1	1	0	0	8	5	3	0	1	6	0	0
835	5 (4)	0	0	0	0	5 (4)	2 (1)	0	0	1	1	3 (2)	3 (2)*
Stratum 2													
267	3	2	0	1	0	0	0	3	0	0	0	0	0
355	6	1	1	3	0	1	0	4	1	1	0	0	0
472	6	0	1	0	0	5	3	1	0	0	5	0	0

NOTE: Numbers in parenthesis in stratum 1 reflect the results after exclusion of one DLT at the 472-mg/m^2 dosage level that was not confirmed on central review and after the exclusion of one patient retrospectively declared ineligible at the 835 mg/m^2 dosage level.
*Another patient required >2 platelet transfusions within 7 d, which was considered a dose-limiting toxicity.

Table 4. Pharmacokinetic parameters of temozolomide and MTIC

	Dosage level				
	267 mg/m² (*n* = 5)	355 mg/m² (*n* = 4)	472 mg/m² (*n* = 11)	628 mg/m² (*n* = 5)	835 mg/m² (*n* = 3)
TMZ C_{max} (μg/mL)	6.6 (2.7-19.5)	17.8 (13.2-19.6)	11.6 (6.3-26.5)	13.4 (9.5-18.8)	14.1 (5.2-22.3)
TMZ T_{max} (h)	1.2 (0.2-1.5)	1.1 (0.5-1.2)	1.7 (0.2-2.4)	1.5 (1.2-9)	1.2 (1-2.7)
CL_{TMZ} (L/h/m²)	11.0 (4.3-28.3)	5.2 (4.5-8)	9.6 (5.1-15.9)	9.2 (2.5-11.2)	11.4 (11.1-12)
TMZ $AUC_{0\rightarrow\infty}$ (μg·mL·h)	29.4 (17-62.3)	68.0 (58.7-78.9)	49.4 (29.6-91.9)	68 (56-174.8)	72.7 (69.5-72.7)
MTIC $AUC_{0\rightarrow\infty}$ (μg·mL·h)	0.9 (0.5-4.1)	2.25 (2.1-3.6)	2.7 (2-5.4)	4.4 (3.4-6.7)	6 (0.9-7)

NOTE: *n*, number of patients. Results are shown as median values and range in parenthesis.
Abbreviations: TMZ, temozolomide; C_{max}, maximal concentration; T_{max}, time for maximal concentration; CL, apparent oral clearance; $AUC_{0\rightarrow\infty}$, area under concentration-time curve from zero to infinity.

age and occurrence of DLT among 27 assessable patients in stratum 1 ($P = 0.34$).

Pharmacokinetic studies. Of 43 patients where consent was obtained for pharmacokinetic studies, analyses of temozolomide and MTIC and of O^6-BG and 8-oxoBG were done in 28 (65%) and 19 (44%) patients, respectively. Pharmacokinetic studies were not done in the remaining patients because of logistical problems (e.g., lack of venous access or technical issues related to sample processing).

Tables 4 and 5 summarize the pharmacokinetic parameters of temozolomide and MTIC, and O^6-BG and 8-oxoBG at various dosage levels during course 1, respectively. There was a statistical association between the actual dose of temozolomide (in mg/m²) and the $AUC_{0\rightarrow\infty}$ of temozolomide ($P = 0.035$) and MTIC ($P = 0.0015$; Supplementary Figs. S1 and S2). No obvious differences were noted in O^6-BG pharmacokinetic parameters among patients treated with different temozolomide dosages; however, the number of assessable patients for each dosage level was limited.

Response to therapy. Three patients with gliomas had an objective response to therapy that were confirmed by central radiographic review, and five patients experienced disease stabilization for at least 6 months (Table 6). Four patients completed all planned therapy.

Biological studies. Supplementary Table S2 shows details about the analysis of MSI. MSI in at least one locus was identified in the tumor of 10 of 17 patients analyzed.

Discussion

We used the modified CRM study design to estimate the MTD of single-dose temozolomide combined with the current schedule of O^6-BG in children with recurrent brain tumors based on their previous therapy. For patients in stratum 1, the CRM design was particularly helpful in re-estimating the MTD after one patient at the 835-mg/m² dosage level was retrospectively declared ineligible and another DLT at the 472-mg/m² dosage level was found not to meet study criteria upon central review. Although the revised CRM-estimated MTD and the dose-finding MTD in stratum 1 were 562 and 628 mg/m², respectively, half of our patients treated at the 628-mg/m² dosage level experienced DLTs (Table 3). Therefore, we declared 472 mg/m² as the recommended dose for phase II investigation in this setting, which is identical to the MTD reached in adults with recurrent high-grade glioma who received the same therapy (24). Of note, whereas there was a substantial variation in dose-finding MTD before and after central review, the CRM-estimated MTD varied by 32 mg/m² only. The corresponding recommended phase II dose for patients in stratum 2 was 355 mg/m². Although our patients were stratified based on previous need of very intensive treatment, even our patients in stratum 1 were heavily pretreated (Table 1).

Only one other pediatric clinical trial that used a different regimen of temozolomide and O^6-BG has been published thus

Table 5. Pharmacokinetic parameters of *O*⁶-benzylguanine and *O*⁶-benzyl-8-oxoguanine

	Dosage level				
	267 mg/m² (*n* = 5)	355 mg/m² (*n* = 2)*	472 mg/m² (*n* = 7)	628 mg/m² (*n* = 2)	835 mg/m² (*n* = 3)
O^6-BG C_{SS} (ng/mL)	31.8 (23.3-40.2)*	20.1 †	24.9 (14.5-81.4)	37.3 (19.4-55.2)	20.2 (14.2-29.9)
8-oxoBG C_{ss} (ng/mL)	730.5 (689-772)*	747 †	346 (258-612)	289 (99-478)	153 (88-238)
$CL_{O^6\text{-BG}}$ (L/h/m²)	42.5 (31.1-53.8)*	62.3 †	50.2 (15.4-86.4)	43.5 (22.6-64.4)	62.0 (41.8-87.9)

NOTE: *n*, number of patients. Results are shown as median values and range in parenthesis.
Abbreviations: CL, apparent oral clearance; C_{ss}, plasma concentration at steady state; O^6-BG, O^6-benzylguanine; 8-oxoBG, O^6-benzyl-8-oxoguanine.
*Three of these patients received O^6-BG at 15 mg/m²/d at this dosage level. Their median O^6-BG C_{ss}, 8-oxoBG C_{ss}, and CLO^6_{BG} values were 22.6 ng/mL (16.5-43.3), 588 ng/mL (66-1079), and 27.7 L/h/m² (14.5-37.8), respectively.
†One of these patients received O^6-BG at 15 mg/m²/d at this dosage level. The O^6-BG C_{ss}, 8-oxoBG C_{ss}, and CLO^6_{BG} values were 55.5 ng/mL, 83.7 ng/mL, and 11.3 L/h/m², respectively.

Table 6. Radiologic responses to therapy according to diagnosis and dosage level of temozolomide

	Age at study entry/gender	Diagnosis	Previous use of nitrosourea or temozolomide	Dosage level of temozolomide (mg/m^2)	Best radiologic response	Number of courses completed
1	11.9/F	Malignant glioma	Yes	267	SD	12
2	8.1/F	Medulloblastoma	Yes	355	SD	5
3	12.3/F	Pineoblastoma	Yes	355	SD	11
4	12.5/F	Medulloblastoma	No	355	SD	5
5	16.9/M	Malignant glioma	No	355	PR	4
6	16.2/M	Ependymoma	No	472	SD	6
7	12/F	Anaplastic astrocytoma	Yes	472	SD	11
8	4/F	Pilocytic astrocytoma	No	472	PR	12
9	17.8/M	Glioblastoma multiforme	Yes	628	SD	5
10	2.1/F	Anaplastic ependymoma	No	628	SD	12
11	0.4/M	Anaplastic ependymoma	No	628	PR	12

Abbreviations: SD, stable disease; PR, partial response.

far (31). In that phase I study, patients with recurrent solid tumors received a 5-day schedule of temozolomide, which was administered within 30 min of completion of a 1-h bolus of O^6-BG. A total of 32 of 41 patients enrolled on that study had primary brain tumors, and the remainder had sarcomas. The MTD of temozolomide administered over 5 days following a bolus dose of 120 mg/m^2 of O^6-BG was 75 mg/m^2/day. The cumulative dose of temozolomide in that study was very similar to that obtained for patients in stratum 2 of the current study. One of the main limitations of the previous pediatric study was the lack of patient stratification based on previous intensive myelosuppressive therapy. Whereas only 7 of 32 assessable children had previously received craniospinal radiotherapy, four of those seven patients experienced DLTs.

The toxicity observed in our patients resembled that already described in children treated with the 5-day regimen of temozolomide alone or combined with O^6-BG with a predominance of myelosuppression, particularly neutropenia and thrombocytopenia (8–13, 31). No unexpected side effects attributable to this treatment combination were observed among our patients.

Our analysis showed that the pharmacokinetic parameters after single-dose temozolomide are mostly similar to those observed in patients receiving the traditional 5-day schedule of this medication (8, 29). Similar to previous pediatric studies, we found a statistical association between temozolomide exposure and dosage (8, 32). In addition, we also showed that the exposure to MTIC increased with increasing dosage of temozolomide.

A total of 11 (20%) of 54 assessable patients who were treated at different dosage levels of temozolomide in the current study benefited from therapy (Table 5), compared with 9 (28%) of 32 in the previous pediatric study (31). We cannot draw conclusions about the antitumor activity of the current treatment schedule versus the 5-day regimen of temozolomide and O^6-BG because multiple variables influenced the response to treatment. For example, whereas one-third of our patients had been previously exposed to temozolomide or nitrosourea, only one patient in the previous pediatric study had received temozolomide (31). In addition, thus far, both regimens have been tested in children only within phase I studies.

Within the context of a phase I study, we analyzed the influence of drug pharmacokinetics and limited tumor biology on the response to therapy. Although no relationship between temozolomide and MTIC pharmacokinetic parameters and response to therapy was observed (data not shown), this analysis was limited by the small number of assessable patients. MSI, which is defined as a change in length in repetitive nucleotide sequences present within tumor but not normal tissue DNA, is a marker of a disrupted MMR system (33, 34). We did extensive analysis of MSI in a subset of our patients because an intact MMR system is necessary for the cytotoxic effects of temozolomide (5, 16). However, we could not draw any conclusions about the influence of MSI status on the response to the current treatment strategy because of the limited number of assessable patients. Four of six patients with medulloblastoma harbored MSI on locus D17S250 in the long arm of chromosome 17. Likewise, the small number of patients with medulloblastoma in the current study limited our ability to draw any conclusions about this finding. MSI had previously been evaluated in low- and high-grade glioma in children (35, 36), but to our knowledge, no information is available about its occurrence in ependymoma or medulloblastoma, the two most common histologic tumor types in the current study. We were unable to evaluate MGMT expression or the methylation status of the respective gene promoter in the current study. Although several studies have shown an association between MGMT overexpression or lack of methylation of the respective gene promoter with worse response to temozolomide therapy (5, 37, 38), a recent study showed that there is not yet a proven standardized MGMT assay to predict response to temozolomide (39).

Combining temozolomide and O^6-BG in the current schedule is feasible and has shown activity across different histologic types of recurrent/progressive pediatric brain tumors. Additional ongoing clinical trials both in children and adults are exploring the role of optimizing this drug combination strategy in the treatment of patients with brain tumors.

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