Phase I trial of temozolomide plus O⁶-benzylguanine 5-day regimen with recurrent malignant glioma

Jennifer A. Quinn, Sara Xiaoyin Jiang, David A. Reardon, Annick Desjardins, James J. Vredenburgh, Jeremy N. Rich, Sridharan Gururangan, Allan H. Friedman, Darell D. Bigner, John H. Sampson, Roger E. McLendon, James E. Herndon, Jr., Amy Walker, and Henry S. Friedman

Departments of Surgery (J.A.Q., S.X.J., D.A.R., A.D., J.J.V., J.N.R., S.G., A.H.F., D.D.B., J.H.S., A.W., H.S.F.), Pathology (D.D.B., R.E.M.), and Biostatistics and Bioinformatics (J.E.H.), Duke University Medical Center, Durham, NC, USA

This phase I clinical trial conducted with patients who had recurrent or progressive malignant glioma (MG) was designed to determine the maximum tolerated dose (MTD) and toxicity of three different 5-day dosing regimens of temozolomide (TMZ) in combination with O⁶-benzylguanine (O⁶-BG). Both TMZ and O⁶-BG were administered on days 1–5 of a 28-day treatment cycle. A bolus infusion of O⁶-BG was administered at 120 mg/m² over 1 h on days 1, 3, and 5, along with a continuous infusion of O⁶-BG at 30 mg/m²/day. TMZ was administered at the end of the first bolus infusion of O⁶-BG and then every 24 h for 5 days during the continuous infusion of O⁶-BG. Patients were accrued to one of three 5-day dosing regimens of TMZ. Twenty-nine patients were enrolled into this study. The dose-limiting toxicities (DLTs) were grade 4 neutropenia, leukopenia, and thrombocytopenia. The MTD for TMZ for the three different 5-day dosing schedules was determined as follows: schedule 1, 200 mg/m² on day 1 and 50 mg/m²/day on days 2–5; schedule 2, 50 mg/m²/day on days 1–5; and schedule 3, 50 mg/m²/day on days 1–5 while receiving pegfilgrastim. Thus, the 5-day TMZ dosing schedule that

maximized the total dose of TMZ when combined with O⁶-BG was schedule 1. This study provides the foundation for a phase II trial of O⁶-BG in combination with a 5-day dosing schedule of TMZ in TMZ-resistant MG. *Neuro-Oncology* 11, 556–561, 2009 (Posted to *Neuro-Oncology* [serial online], Doc. D08-00202, March 16, 2009. URL <http://neuro-oncology.dukejournals.org>; DOI: 10.1215/15228517-2009-007)

Keywords: malignant glioma, O⁶-benzylguanine, phase I, recurrent, temozolomide

Gliomas are the most common type of primary malignant brain tumor, with poor survival despite conventional therapeutic regimens. The overall median survival from diagnosis for patients with malignant glioma (MG) is 48.2 weeks.¹ Currently, the only two chemotherapeutics approved by the FDA for the treatment of MG, temozolomide (TMZ) and carmustine, both exert their therapeutic effect through alkylation. Unfortunately, the majority of patients with MG treated with alkylators, including TMZ, demonstrate de novo or acquired resistance, with subsequent tumor progression. Thus, targeting these mechanisms of resistance may maximize potential therapeutic benefit of these alkylating agents.

A major mechanism for resistance to alkylating chemotherapeutics is mediated in part through a DNA repair protein, O⁶-alkylguanine-DNA alkyltransferase

Received July 29, 2008; accepted January 21, 2009.

Address correspondence to Jennifer A. Quinn, Dept. of Medicine, Division of Neurology, Duke University Medical Center, 047 Baker House, Box 3624, Durham, NC 27710, USA (quinn008@mc.duke.edu).

(AGT).²⁻⁴ AGT removes chlorethylation or methylation damage from the O⁶ position of DNA guanines before cell injury and cell death. O⁶-benzylguanine (O⁶-BG) is a nonreversible inhibitor of the enzyme AGT and has been found to enhance cytotoxicity of alkylating chemotherapies, such as TMZ, *in vitro*^{2,5,6} and *in vivo* xenografts.^{7,8} Thus, circumventing resistance and restoring chemotherapy sensitivity by combining O⁶-BG with TMZ may be a reasonable therapeutic strategy.

To develop such a therapeutic strategy, we must first determine the most efficacious and least toxic dosing regimen of TMZ and O⁶-BG when administered together. Although *in vitro* studies suggest that a 5-day dosing schedule of TMZ when combined with the O⁶-BG may be more efficacious than a 1-day dosing schedule, *in vivo* studies have been far less convincing. An *in vitro* study by Wedge et al.⁹ found the potentiation of TMZ cytotoxicity by O⁶-BG to increase linearly on 5 consecutive days. However, when Wedge et al.¹⁰ explored the effect of single versus multiple administration of O⁶-BG/TMZ combination in a human melanoma xenograft model, the results were not as convincingly supportive of the 5-day regimen over the 1-day regimen. Although Wedge et al.¹⁰ concluded that prolonged administration of the combination can lead to an increase in the therapeutic index of TMZ, no direct comparison of the 5-day to the 1-day regimen of TMZ plus O⁶-BG was made to support this conclusion. In fact, the efficacy and toxicity of these two regimens looked remarkably similar in the mice that received a single dose of TMZ at 200 mg/kg pretreated with 35 mg/kg O⁶-BG and the mice that received a total dose of 200 mg/kg of TMZ over 5 days pretreated each day with 35 mg/kg O⁶-BG. However, the major motivation for evaluating a 5-day versus a 1-day schedule of TMZ in combination with O⁶-BG was that the initial phase I trial of TMZ showed activity only in patients treated with the 5-day regimen,¹¹ which led to this regimen being used in the subsequent phase II trials of TMZ.

We first explored the 1-day dosing schedule of TMZ in combination with O⁶-BG before exploring the 5-day dosing schedule of TMZ. Thus, we performed a phase I clinical trial in patients with MG to determine the maximum tolerated dose (MTD) of single-dose TMZ in combination with O⁶-BG.¹² The MTD was 472 mg/m² when TMZ was combined with O⁶-BG, a finding confirmed by Broniscer et al.¹³ In our phase II trial, we determined that O⁶-BG when added to a 1-day dosing regimen of TMZ was able to restore TMZ sensitivity in patients with TMZ-resistant anaplastic glioma, but no significant restoration of TMZ sensitivity was seen in patients with TMZ-resistant glioblastoma multiforme (GBM).¹⁴

Given the limited response in GBM on the 1-day dosing regimen of TMZ and O⁶-BG, we decided to explore the 5-day dosing regimen of TMZ in combination with O⁶-BG. However, before a 5-day dosing regimen of TMZ could be investigated, further clarification of the optimum dosing regimen of O⁶-BG in combination with the 5-day dosing regimen of TMZ was needed.

In our phase I and II trials utilizing the 1-day dosing regimen of TMZ,^{12,14} we chose an O⁶-BG dose of

120 mg/m² over 1 h followed by a continuous infusion of 30 mg/m²/day for 48 h. This O⁶-BG dosing regimen was considered to be optimal for two reasons. First, a preclinical study by Hirose et al.¹⁵ found that the maximal TMZ sensitization in gliomas through prolonged AGT depletion with continuous O⁶-BG exposure was achieved 2–3 days after TMZ exposure. Second, Weingart et al.¹⁶ showed in a phase I trial that this dosing regimen suppressed tumor AGT for at least 48 h. Given the results in both of these studies, we considered the optimum O⁶-BG regimen for a 5-day dosing schedule of TMZ to be one where tumor AGT suppression occurred with certainty for at least 5 days, with 7 days perhaps being preferable. Thus, in order to guarantee tumor AGT suppression for at least 5 days, we chose to repeat the O⁶-BG regimen used in our phase I and II trial on days 1, 3, and 5.

Because no one knows the optimum dosing regimen of TMZ when combined with O⁶-BG, we chose to investigate three different 5-day regimens. Schedule 1 escalated the TMZ dose on days 2–5 but used a fixed dose of TMZ at 200 mg/kg on day 1. The reason for this uncustomary dosing regimen was our desire to incorporate the fact that one patient who participated in our prior phase I trial¹² sustained a complete response while receiving the 1-day dosing regimen of TMZ at 200 mg/m² along with O⁶-BG. This patient remained without tumor progression for 6 years and 4 months despite initially failing TMZ. Unlike schedule 1, schedule 2 escalated equal doses of TMZ for all 5 days. Schedule 3, while replicating the dosing schedule of schedule 2, incorporated pegfilgrastim to determine whether the MTD could be escalated beyond the limits found in schedule 2.

We now report a phase I clinical trial conducted in patients with recurrent or progressive MG. The primary objectives of the trial were to determine the safety and MTD of three different 5-day dosing regimens of TMZ in combination with O⁶-BG. The secondary objective was to provide a preliminary assessment of response.

Patients and Methods

Patient Population

Eligible patients had a histologically confirmed diagnosis of progressive or recurrent MG. They must have had measurable disease on contrast-enhanced MRI or CT scan (for patients when MRI was medically contraindicated) performed within 2 weeks of study drug administration. Patients were >18 years old and required to have a Karnofsky performance scale (KPS) score of >70%. An interval of at least 2 weeks since prior surgical resection, 4 weeks since prior chemotherapy (6 weeks for a nitrosourea-based regimen), or 12 weeks from prior radiotherapy had to have elapsed for the patient to be enrolled into the clinical trial. Additional enrollment criteria included adequate pretreatment bone marrow function (total granulocyte count >2,000 cells/ μ l, platelets >125,000 cells/ μ l), renal function (serum creatinine <1.5 upper limit of normal), and hepatic function (serum glu-

tamic oxalacetic transaminase and bilirubin ≤ 2.5 times upper limit of normal). Patients of reproductive potential were required to take effective contraceptive measures for the duration of the study and for 2 months after completing therapy. All patients were informed of the investigational nature of the study and were required to sign an informed consent form as approved by the Duke University Health System Institutional Review Board.

The following patients were excluded from the study: pregnant or breast-feeding women, potentially fertile women or men who were not using an effective contraception method, patients treated in the past with the combination of TMZ plus O⁶-BG, patients with active infection requiring intravenous antibiotics, patients with a concurrently active primary malignancy, patients with known human immunodeficiency virus infection, and patients who received investigational drugs ≤ 2 weeks prior to starting on the study drug or who had not recovered from side effects of such therapy.

Study Design and Treatment

This was a phase I, open-label, single-center trial with accrual goals defined for three different dosing schedules of TMZ (Table 1). Schedule 1 consisted of a fixed dose of TMZ on day 1 at 200 mg/m² and an initial dose of 25 mg/m²/day on days 2–5. The dose on days 2–5 was escalated for each new dose level by 25 mg/m²/day to determine the MTD. Schedule 2 consisted of the same dose of temozolomide on days 1–5. The starting dose level was 25 mg/m²/day higher than the dose found to be the MTD on days 2–5 for schedule 1. This dose was planned to be escalated by 25 mg/m²/day in subsequent cycles to determine the MTD. Schedule 3 started with a dose level 25 mg/m²/day higher than the MTD from schedule 2, and patients received pegfilgrastim between days 7 and 14 of each cycle. Dose escalation was planned to proceed by 25 mg/m²/day for each dose level to determine the MTD.

Both TMZ and O⁶-BG were administered on days 1–5 of a 28-day treatment cycle. A 1-h bolus infusion of O⁶-BG was administered at 120 mg/m² on day 1 and every 48 h on days 3 and 5. A continuous infusion of O⁶-BG at 30 mg/m²/day was administered immediately following the initial bolus infusion of O⁶-BG on day 1 and continued until immediately prior to the last bolus infusion. TMZ was administered orally, within 60 min of the end of the first bolus infusion of O⁶-BG (120 mg/m²) and then every 24 h for 5 days during the continuous infusion of O⁶-BG (30 mg/m²/day).

TMZ was commercially available as Temodar from Schering-Plough Research Institute (Kenilworth, NJ, USA). Pegfilgrastim was commercially available as Neulasta from Amgen (Thousand Oaks, CA, USA). O⁶-BG was supplied by AOI Pharmaceuticals, Inc. (New York, NY, USA).

Surveillance and Follow-up

The baseline examination included central review of tumor tissue, MRI or CT (if MRI was medically contraindicated), complete blood counts (CBCs) and blood chemistry tests, and a physical examination, including a comprehensive neurologic examination. During therapy, weekly CBCs were obtained. Prior to subsequent cycles of chemotherapy, patients were required to repeat CBC and blood chemistry tests, a physical examination, and neuroimaging.

Toxicity was graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0 (http://ctep.cancer.gov/reporting/ctc_v30.html). Dose-limiting toxicity (DLT) must have occurred during the first cycle of therapy and was defined as the following: grade >4 neutropenia for >4 days, grade >3 febrile neutropenia (absolute neutrophil count $<1 \times 10^9/L$, fever $>38.5^\circ C$), grade >4 thrombocytopenia, grade >3 nonhematologic toxicity that is related to study drug (except grade >3 diarrhea without maximal intensive loperamide support, or grade >3 nausea or vomiting without maximal antiemetic therapy). Any adverse event causing >14 days of interruption to therapy (ie, a delay beyond day 42) would be considered a DLT, and the patient would be taken off study. The TMZ dose was reduced by 25 mg/m²/day if the patient developed a DLT. Patients who experienced recurrence of a DLT were taken off study.

Repeat cycles of chemotherapy were administered on schedule only if the patient met the following retreatment criteria: total granulocyte count $>1,000$ cells/ μL ; platelets $>100,000/\mu L$; serum glutamic oxalacetic transaminase, total bilirubin, and creatinine ≤ 2.5 times upper limit of normal; and all other toxicities resolved to baseline or grade 1.

Objective assessments of overall response were based on tumor assessment from MRI scans (CT if MRI was medically contraindicated) interpreted in light of corticosteroid use, as suggested by Macdonald et al.,¹⁷ with appropriate support from the neurologic examination.

This drug combination was administered for a maximum of 12 months or until unacceptable toxicity or

Table 1. Temozolomide plus O⁶-BG schedules

Schedule	Temozolomide	O ⁶ -BG
1	Day 1: 200 mg/m ² Days 2–5: escalation from 25 mg/m ² /day	One-hour 120 mg/m ² bolus on days 1, 3, 5
2	Days 1–5: escalation from 75 mg/m ² /day	Continuous 30 mg/m ² /day infusion on days 1–5
3	Days 1–5: escalation from 75 mg/m ² /day with pegfilgrastim between days 7 and 14	

tumor progression occurred. If the patient demonstrated ongoing radiographic and/or clinical improvement after 12 months of therapy, then the patient could continue therapy until there was evidence that maximal benefit had been achieved.

Statistical Analysis

In establishing the optimal TMZ dose when it was combined with 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, 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 that caused DLT in no more than one of six patients at that dose level. Any patient who had stable or responding disease that developed a 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 Characteristics

Between September 23, 2005, and April 10, 2006, a total of 29 patients with recurrent or progressive MG (WHO grades III and IV) were enrolled into the study at one of three dosing schedules for TMZ. Demographic and clinical characteristics of patients are detailed in Table 2.

Toxicity Evaluation

Of the 29 patients enrolled into the study, 28 were assessable for toxicity (Table 3). On schedule 1, at a TMZ dose of 200 mg/m² on day 1 and 25 mg/m²/day on days 2–5, all five patients were assessable for toxicity, none of whom experienced a DLT. On schedule 1 at a TMZ dose of 200 mg/m² on day 1 and 50 mg/m²/day on days 2–5, six of seven patients were assessable for toxicity. The patient that was not assessable for toxicity was lost to follow-up after study enrollment. One of the six patients experienced grade 4 neutropenia, which was dose limiting, and another patient experienced grade 3 thrombocytopenia, which was not dose limiting. On schedule 1 at a TMZ dose of 200 mg/m² on day 1 and 75 mg/m²/day on days 2–5, all five patients were assessable for toxicity. Two patients experienced a DLT: one patient developed grade 4 leukopenia with grade 4 thrombocytopenia, and another patient developed grade 4 neutropenia with grade 3 thrombocytopenia. Two patients developed grade 3 neutropenia, which was not dose limiting. On schedule 2 at a TMZ dose of 75 mg/m²/day on days 1–5, all six patients were assessable for toxicity. Two patients experienced a DLT: one patient developed grade 4 neutropenia with grade 4 thrombocytopenia, and another patient developed grade 4 neutropenia. One patient experienced grade 3 neutropenia, which was not dose limiting. On schedule 3 at a TMZ dose of 75 mg/m²/day on days 1–5 while receiving pegfilgrastim, all six patients were assessable for toxicity. Two patients experienced grade 4 neutropenia, which was dose limiting, and one patient experienced grade 3 neutropenia with grade 3 thrombocytopenia, which was not dose limiting. Thus, after the MTD was exceeded at a TMZ dose of 75 mg/m²/day for all three schedules, the MTD was established at 50 mg/m²/day. Note that this was inferred for schedules 2 and 3 since no patients on these schedules were treated at 50 mg/m²/day.

Response

All 29 patients who enrolled into the study were assessable for response. Seventeen patients showed disease progression after one cycle of chemotherapy. Three patients had stable disease for three cycles, and seven patients had stable disease for two cycles. Two patients

Table 2. Demographic and clinical characteristics of patients

Characteristic	Value
Total <i>n</i>	29
Age, years	
Median	56
Range	35–66
Sex, <i>n</i> (%) male	23 (79%)
Race, <i>n</i> (%) white	28 (97%)
KPS score, %	
Median	80
Range	70–95
Histology, <i>n</i> (%)	
Glioblastoma multiforme	28 (96.6%)
High-grade astrocytoma	1 (3.4%)
Prior therapy, <i>n</i> (%)	
Radiotherapy	29 (100%)
Chemotherapy	29 (100%)
Temozolomide	29 (100%)
Temozolomide failure	26 (89.7%)
Monoclonal antibody	1 (3.4%)
Resection	26 (89.7%)
No. prior chemotherapies, <i>n</i> (%)	
1	8 (28.6%)
2	13 (46.4%)
>3	8 (27.6%)

Table 3. Grade 3, 4, and 5 toxicities attributed to drug treatment

Schedule	Dose (mg/m ² /day)	Assessable/ Total Patients	DLT	Non-DLT
1	25	5/5	0	0
1	50	6/7	1 (grade 4 ANC)	1 (grade 3 PLT)
1	75	5/5	2 (1 grade 4 WBC plus grade 4 PLT; 1 grade 4 ANC plus grade 3 PLT)	2 (grade 3 ANC)
2	75	6/6	2 (1 grade 4 ANC plus grade 4 PLT; 1 grade 4 ANC)	1 (grade 3 ANC)
3	75	6/6	2 (grade 4 ANC)	1 (grade 3 ANC, grade 3 PLT)

Abbreviations: DLT, dose-limiting toxicity; ANC, absolute neutrophil count; WBC, white blood cell count; PLT, platelet count.

had a partial response. One patient who responded was enrolled in schedule 2 and received TMZ at 75 mg/m²/day on days 1–5 for all 12 cycles. Even though this patient originally failed TMZ, the patient demonstrated a sustained response to TMZ and O⁶-BG for 14 months. Another patient who responded was enrolled in schedule 3 and received TMZ at 75 mg/m²/day on days 1–5 with pegfilgrastim but required a dose reduction to 50 mg/m²/day on days 1–5 because of grade 4 neutropenia. Although this patient also originally failed TMZ, the patient demonstrated a sustained response to TMZ and O⁶-BG for seven cycles.

Discussion

The results of this trial demonstrate that when TMZ is combined with O⁶-BG, the MTD for TMZ for three different 5-day dosing schedules was as follows: schedule 1 at a dose of 200 mg/m² on day 1 and 50 mg/m²/day on days 2–5, schedule 2 at a dose of 50 mg/m²/day on days 1–5, and schedule 3 at a dose of 50 mg/m²/day on days 1–5 while receiving pegfilgrastim. Thus, the 5-day TMZ dosing schedule that maximizes the total dose of TMZ when combined with O⁶-BG is schedule 1, with TMZ administered at 200 mg/kg on day 1 and 50 mg/m²/day on days 2–5. DLT was limited to myelosuppression. Severe but reversible neutropenia, leukopenia, and thrombocytopenia were observed at TMZ doses of 50 and 75 mg/m². Despite prior TMZ failure, two patients demonstrated a sustained response for 7 and 14 months. Subsequent studies will need to address toleration of repeated cycles of the MTD, information not readily available in this trial.

Although other O⁶-BG regimens have been used in combination with TMZ and polifeprosan 20 with carmustine implant (Gliadel), there is no evidence to suggest that these dosing regimens are adequate to suppress AGT levels in brain tumors. In a phase I trial after inser-

tion of Gliadel, Weingart et al.¹⁶ attempted to deplete tumor AGT for up to 14 days using an O⁶-BG dosing regimen consisting of 120 mg/m² over 1 h followed by a continuous infusion of 30 mg/m²/day for up to 14 days. Unfortunately, examination of the pharmacokinetics for the prolonged continuous infusion of O⁶-BG raised the possibility that a higher dose of O⁶-BG will be needed in future studies to suppress tumor AGT activity for the full 2 weeks. Another alternative O⁶-BG dosing regimen used in a phase I trial after treatment with a 5-day dosing regimen of TMZ utilized a daily 1-h infusion O⁶-BG at 120 mg/m² without a continuous infusion.¹⁸ Although research shows depletion of AGT at 18 h after this 1-h infusion of O⁶-BG, it is highly unlikely and has never been proven that this depletion is continuous from 18 to 24 h, which makes this a less attractive alternative.

The phase I trial of O⁶-BG and TMZ reported here has defined the therapeutic approach for our ongoing phase II trial of TMZ plus O⁶-BG, which will enroll patients with recurrent TMZ-resistant MG. This will provide the evidence needed to determine whether TMZ plus O⁶-BG can restore TMZ sensitivity in patients for whom this agent has previously failed. Other strategies to overcome TMZ resistance mediated by AGT include a dose-dense TMZ schedule, an approach currently being evaluated by the Radiation Therapy Oncology Group and European Organisation for Research and Treatment of Cancer.^{19,20}

Acknowledgments

This study was supported by National Institute of Neurological Disorders and Stroke grant 5P50 NS20023-25, National Institutes of Health (NIH) Specialized Programs of Research Excellence grant 5P50 CA108786-4, and NIH merit award R37 CA 011898-38. S.X.J. was supported by NIH grant TL1 RR024126.

References

1. Laws ER, Parney IF, Huang W, et al. Survival following surgery and prognostic factors for recently diagnosed malignant glioma: data from the Glioma Outcomes Project. *J Neurosurg.* 2003;99:467–473.
2. Dolan ME, Mitchell RB, Mummert C, Moschel RC, Pegg AE. Effect of O⁶-benzylguanine analogues on sensitivity of human tumor cells to the cytotoxic effects of alkylating agents. *Cancer Res.* 1991;51:3367–3372.
3. Pegg AE, Dolan ME, Moschel RC. Structure, function, and inhibition of O⁶-alkylguanine-DNA alkyltransferase. *Prog Nucleic Acid Res Mol Biol.* 1995;51:167–223.
4. Hegi ME, Diserens A-C, Godard S, et al. Clinical trial substantiates the predictive value of O⁶-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. *Clin Cancer Res.* 2004;10:1871–1874.
5. Bobola MS, Tseng SH, Blank A, Berger MS, Silber JR. Role of O⁶-methylguanine-DNA methyltransferase in resistance of human brain tumor cell lines to the clinically relevant methylating agents temozolomide and streptozotocin. *Clin Cancer Res.* 1996;2:735–741.
6. Wedge SR, Porteous JK, Newlands ES. 3-Aminobenzamide and/or O⁶-benzylguanine evaluated as an adjuvant to temozolomide or BCNU treatment in cell lines of variable mismatch repair status and O⁶-alkylguanine-DNA alkyltransferase activity. *Br J Cancer.* 1996;74:1030–1036.
7. Friedman HS, Dolan ME, Pegg AE, et al. Activity of temozolomide in the treatment of central nervous system tumor xenografts. *Cancer Res.* 1995;55:2853–2857.
8. Wedge SR, Newlands ES. O⁶-benzylguanine enhances the sensitivity of a glioma xenograft with low O⁶-alkylguanine-DNA alkyltransferase activity to temozolomide and BCNU. *Br J Cancer.* 1996;73:1049–1052.
9. Wedge SR, Porteous JK, May BL, Newlands ES. Potentiation of temozolomide and BCNU cytotoxicity by O⁶(6)-benzylguanine: a comparative study in vitro. *Br J Cancer.* 1996;73:482–490.
10. Wedge SR, Porteous JK, Newlands ES. Effect of single and multiple administration of an O⁶-benzylguanine/temozolomide combination: an evaluation in a human melanoma xenograft model. *Cancer Chemother Pharmacol.* 1997;40:266–272.
11. Newlands ES, Blackledge GR, Slack JA, et al. Phase I trial of temozolomide (CCRG 81045: M&B 39831: NSC 362856). *Br J Cancer.* 1992;65:287–291.
12. Quinn JA, Desjardins A, Weingart J, et al. Phase I trial of temozolomide plus O⁶-benzylguanine for patients with recurrent or progressive malignant glioma. *J Clin Oncol.* 2005;23:7178–7187.
13. Broniscer A, Gururangan S, MacDonald TJ, et al. Phase I trial of single-dose temozolomide and continuous administration of O⁶-benzylguanine in children with brain tumors: a pediatric brain tumor consortium report. *Clin Cancer Res.* 2007;13:6712–6718.
14. Quinn JA, Desjardins A, Reardon D, et al. Phase II trial of temozolomide (Temodar) plus O⁶-benzylguanine (O⁶-BG) in the treatment of patients with Temodar-resistant malignant glioma [abstract]. *Neuro-Oncology.* 2004;6:380.
15. Hirose Y, Kreklau EL, Erickson LC, Berger MS, Pieper RO. Delayed repletion of O⁶-methylguanine-DNA methyltransferase resulting in failure to protect the human glioblastoma cell line SF767 from temozolomide-induced cytotoxicity. *J Neurosurg.* 2003;98:591–598.
16. Weingart J, Grossman SA, Carson KA, et al. Phase I trial of polifeprosan 20 with carmustine implant plus continuous infusion of intravenous O⁶-benzylguanine in adults with recurrent malignant glioma: new approaches to brain tumor therapy CNS consortium trial. *J Clin Oncol.* 2007;25:399–404.
17. Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol.* 1990;8:1277–1280.
18. Warren KE, Widemann BC, Fox E, et al. Phase I trial of O⁶-benzylguanine (O⁶BG) and temozolomide (TMZ) in pediatric patients with solid tumors [abstract]. *Neuro-Oncology.* 2003;5:360 (TP-07).
19. National Cancer Institute. Radiation Therapy and Temozolomide in Treating Patients with Newly Diagnosed Glioblastoma or Gliosarcoma. Available at www.clinicaltrials.gov/ct/gui/show/NCT00304031?order=1. Accessed July 1, 2008.
20. European Organisation for Research and Treatment of Cancer. Brain Tumour Group: Ongoing Trials. Available at <http://groups.eortc.be/brain/html.trials.html>. Accessed July 1, 2008.