Phase II Trial of Gliadel plus O\textsuperscript{6}-Benzylguanine in Adults with Recurrent Glioblastoma Multiforme


Abstract

**Purpose:** This phase II trial was designed to define the efficacy of Gliadel wafers in combination with an infusion of O\textsuperscript{6}-benzylguanine (O\textsuperscript{6}-BG) that suppresses tumor O\textsuperscript{6}-alkylguanine-DNA alkyltransferase (AGT) levels in patients with recurrent glioblastoma multiforme for 5 days and to evaluate the safety of this combination therapy.

**Experimental Design:** This was a phase II, open-label, single center trial. On gross total resection of the tumor, up to eight Gliadel wafers were implanted. Bolus infusion of O\textsuperscript{6}-BG was administered at 120 mg/m\textsuperscript{2} over 1 hour on days 1, 3, and 5, along with a continuous infusion at 30 mg/m\textsuperscript{2}/d. The primary end points were 6-month overall survival (OS) and safety, and the secondary end points were 1-year, 2-year, and median OS.

**Results:** Fifty-two patients were accrued. The 6-month OS was 82\% [95\% confidence interval (95\% CI), 72-93\%]. The 1- and 2-year OS rates were 47\% (95\% CI, 35-63\%) and 10\% (95\% CI, 3-32\%), respectively. The median OS was 53 weeks (95\% CI, 36.1-69.4 weeks). Treatment-related toxicity with this drug combination included grade 3 hydrocephalus (9.6\%), grade 3 cerebrospinal fluid (CSF) leak (19.2\%), and grade 3 CSF/brain infection (13.4\%).

**Conclusion:** The efficacy of implanted Gliadel wafers may be improved with the addition of O\textsuperscript{6}-BG. Although systemically administered O\textsuperscript{6}-BG can be coadministered with Gliadel wafers safely, it may increase the risk of hydrocephalus, CSF leak, and CSF/brain infection. Future trials are required to verify that inhibition of tumor AGT levels by O\textsuperscript{6}-BG results in increased efficacy of Gliadel wafers without added toxicity.

Although novel therapeutic regimens in recent years have enhanced survival for patients with malignant glioma, overall prognosis remains poor. Glioblastoma multiforme (GBM) is by far the most common histologic subtype of glioma, with a 2-year survival of only 8.7\% (1). Recurrence of disease after or during therapy is the norm for malignant glioma, with the majority of recurrences being local (2). Carmustine wafer [polifeprosan 20 with carmustine implant (Gliadel wafer, MGI Pharma, Inc.)], which delivers chemotherapy as carmustine directly to the tumor cavity, has been shown in two phase III trials to improve survival both in newly diagnosed and in recurrent malignant glioma patients (3, 4).

However, a major mechanism of resistance to alkylating agents such as carmustine and temozolomide is O\textsuperscript{6}-alkylguanine-DNA alkyltransferase (AGT), a DNA repair protein known to remove and repair O\textsuperscript{6}-alkylguanine lesions introduced by alkylating agents (5). The substrate O\textsuperscript{6}-benzylguanine (O\textsuperscript{6}-BG) inactivates AGT, and because this protein requires de novo synthesis for replenishment, O\textsuperscript{6}-BG thus effectively enhances carmustine activity both in vitro and in vivo (6).

Unfortunately, when both O\textsuperscript{6}-BG and carmustine are administered systemically, O\textsuperscript{6}-BG enhances not only the activity of carmustine but also the hematopoietic toxicity of carmustine. Our phase I trial established the maximum tolerated dose of carmustine to be 40 mg/m\textsuperscript{2} when combined with a dose of O\textsuperscript{6}-BG (1-hour i.v. bolus of 120 mg/m\textsuperscript{2}) sufficient to deplete AGT in gliomas 18 hours after administration (7, 8). This trial showed the need for a marked reduction in the dose of carmustine compared with the usual dose of 200 mg/m\textsuperscript{2} when carmustine is used alone. The myelosuppression seen in the phase I trial was also seen in the phase II trial (9). This profound reduction of carmustine when given in combination with O\textsuperscript{6}-BG may be the underlying factor in the failure of this drug combination to cause frank tumor regressions in our phase II trial.
The primary objectives of the current study were to (1) define the efficacy of Gliadel wafers in combination with an infusion of O6-BG that suppresses tumor AGT levels in patients with recurrent GBM for 5 days and (2) evaluate the safety of this combination therapy.

Materials and Methods

**Patient population.** Eligible patients had a histologically confirmed diagnosis of recurrent GBM (including gliosarcoma) that was shown by contrast-enhanced magnetic resonance imaging (MRI) to have a unilateral, single focus of measurable central nervous system neoplasm that was supratentorial and measured at least 1.0 cm in diameter. Patients were ≥18 y old and had a Karnofsky performance score of ≥60%. An interval of at least 2 wk since prior surgical resection (if conducted) or 4 wk since prior chemotherapy (6 wk for a nitrosourea-based regimen) had to have elapsed for the patient to be enrolled into the clinical trial. The number or type of prior chemotherapy treatments or failures did not limit eligibility, except prior treatment with Gliadel wafers, which excluded enrollment. Additional enrollment criteria included adequate pretreatment bone marrow function (hematocrit >29%, total granulocyte count >1,000 cells/μL, platelets >100,000 cells/μL), renal function (BUN and serum creatinine <1.5 times upper limit of laboratory normal), and hepatic function (serum aspartate aminotransferase <3 times upper limit of normal and bilirubin <2 times upper limit of normal). Patients were required to have recovered from any effects of major surgery and have a life expectancy of greater than 12 wk. Patients of reproductive potential were required to take effective contraceptive measures for the duration of the study. All patients were informed of the investigational nature of the study and were required to provide signed informed consent as approved by the institutional review board.

The following patients were excluded from the study: pregnant women, potentially fertile women or men who were not using an effective contraception method, and patients taking immuno suppressive agents other than corticosteroids. Others excluded were patients who were not neurologically stable for 2 wk before study entry, patients who were poor medical risks because of nonmalignant systemic disease, as well as those with acute infection treated with i.v. antibiotics, patients with frequent vomiting or medical condition that could interfere with oral medication intake (e.g., partial bowel obstruction), patients with a history of another primary malignancy that was currently clinically significant or currently required active intervention, and known HIV positivity or acquired immunodeficiency syndrome–related illness.

**Study design and treatment.** This was a phase II, open-label, single center trial. On gross total resection of the tumor and confirmation of GBM on intraoperative frozen section, each patient received up to eight carmustine wafers. Carmustine wafers were commercially available (Gliadel wafers, Guilford Pharmaceuticals). Each wafer contained 7.7 mg of carmustine, for a total carmustine dose of up to 61.6 mg. The actual number of wafers received by each patient was dependent on the size of the tumor resection cavity. The aim was to cover the entire surface of the resection cavity with eight or fewer wafers with slight overlapping of wafers permitted. The number of wafers implanted was recorded.

Within 6 h of completion of surgery, a bolus infusion of O6-BG was administered at 120 mg/m² over 1 h on day 1 and repeated every 48 h on days 3 and 5. A continuous infusion of O6-BG at 30 mg/m²/d was administered immediately following the initial O6-BG bolus on day 1 and continued until immediately before the last bolus injection. Continuous O6-BG was sometimes interrupted for administration of the O6-BG bolus. O6-BG was supplied by Aol Pharmaceuticals, Inc.

**Surveillance and follow-up.** The baseline examination included central review of tumor tissue, MRI or computed tomography (if MRI was medically contraindicated), complete blood counts and blood chemistry tests, and a physical examination including a comprehensive neurologic examination. Every 8 wk, patients were required to repeat complete blood counts and blood chemistry tests, neuroimaging, and a physical examination. Toxicity was graded according to the National Cancer Institute’s Common Toxicity Criteria version 3.0. Patients were eligible to receive systemic chemotherapy if there was evidence of tumor progression or recurrence.

**Statistical analysis.** The primary objective of this phase II study was to determine whether the administration of O6-BG with Gliadel wafers to patients with recurrent GBM is a treatment regimen worthy of further investigation in a randomized clinical trial. The basis for making this determination was to be the proportion of patients who survived at least 6 mo after initiation of protocol treatment.

The 6-mo overall survival (OS) of patients with recurrent GBM treated in a 1995 clinical trial with Gliadel wafers alone was 56% (4). Thus, if no more than 50% of the patients survived 6 mo with the current regimen of Gliadel wafers and O6-BG, there would be no interest in further developing this treatment regimen. However, if at least 70% of the patients survived for 6 mo, there would be genuine interest in pursuing this treatment regimen. The study was therefore designed to differentiate between 6-mo survival rates of 50% and 70%. Statistically, the hypothesis to be tested was as follows: H₀: p < 0.50 versus H₁: p > 0.70, where p is the proportion of patients remaining alive at 6 mo.

Fifty patients were to be accrued to this study. If 31 or more patients survived for at least 6 mo, further investigation of the treatment regimen was warranted. Otherwise, further development of the treatment regimen was not to be considered without modification of the treatment regimen. The type I error and type II error were 0.066 and 0.085, respectively.

Kaplan-Meier curves were used to graphically display the distribution of survival time, where survival is defined as the time between initiation of treatment and death. The survival time was censored for patients alive at last follow-up. Intention-to-treat population was used for all analyses. Secondary end points included 1-y OS, 2-y OS, and median survival.

Toxicity prevalence was summarized by type and maximum grade experienced according to the National Cancer Institute’s Common Toxicity Criteria version 3.0.
Results

**Patient characteristics.** Fifty-two patients were enrolled between May 2004 and February 2007. Two patients who were enrolled did not meet eligibility criteria. One patient was found to have a second lesion in the contralateral cerebral hemisphere on postoperative MRI. This patient underwent resection and received Gliadel wafers but did not receive O6-BG. A second patient received Gliadel wafers and O6-BG despite difficulty in confirming the original histology of GBM as the on-study histology. Although on prior resection the patient’s histology was GBM, histology for enrollment on this trial was found to be grade II astrocytoma despite the presence of necrosis. Demographic and baseline characteristics are presented in Table 1.

**Overall survival.** The 6-month OS was 82% [95% confidence interval (95% CI), 72-93%]. The 1-year OS and 2-year OS were 47% (95% CI, 35-63%) and 10% (95% CI, 3-32%), respectively. The median OS was 50.3 weeks (95% CI, 36.1-69.4 weeks). OS is presented as a Kaplan-Meier curve in Fig. 1.

**Toxicity.** There were no treatment-related deaths nor were there treatment-related grade 4 adverse events. Treatment-related adverse events are summarized in Table 2. The most common treatment-related adverse events included grade 3 cerebrospinal fluid (CSF) leak (19.2%), grade 3 brain and/or CSF infection (13.4), and grade 3 hydrocephalus (9.6%). Grade 3 hyponatremia (3.8%) and grade 2 superficial wound infection (3.8%) were far less common. Other treatment-related adverse events included grade 4 fever, grade 3 epidural hygroma, grade 3 epidural hematoma, and grade 3 central nervous system hemorrhage, all occurring in 1.9% of patients.

These adverse events were found to be highly interrelated. Five of the six patients who developed hydrocephalus developed a CSF leak. All seven patients with a CSF/brain infection also had a CSF leak. Both patients, one with an epidural hemorrhage and one with a subdural hygroma, developed hydrocephalus, but only the patient with the epidural hemorrhage went on to develop a CSF leak and a CSF infection.

**Discussion**

Gliadel wafers are approved by the Food and Drug Administration for the treatment of patients with newly diagnosed, high-grade malignant glioma as an adjunct to surgery and radiation. Gliadel wafers are also indicated to treat recurrent GBM in addition to surgery. The approval was based on clinical trial results showing the median survival of patients with high-grade malignant gliomas increased to 13.9 months from 11.6 months (10), and the median survival of patients with recurrent GBM increased to 6.4 months from 4.6 months (4).

This modest improvement in survival, especially among recurrent GBM patients treated with Gliadel wafers, reflects the difficulty in treating a disease that shows not only local but diffuse brain involvement and not only *de novo* but acquired chemotherapy resistance. Both of these dichotomies need to be addressed for further treatment progress to be realized.

Unfortunately, either *de novo* or acquired resistance to alkylators occurs in the majority of patients with malignant glioma. A major factor in the resistance of tumor cells to alkylating agents is AGT activity (11–17). AGT is a DNA repair protein known to remove and repair O6-alkylguanine lesions introduced by alkylating agents such as carmustine and temozolomide (5).

Depletion of AGT activity by the selective inhibitor O6-BG enhances the cytotoxicity of chloroethylators and methylators (18–23). However, the main limitation in the clinical use of systemic alkylating agents in combination with O6-BG is their potential for dose-related acute toxicity to the hematopoietic system. The enhanced bone marrow toxicity seen in animal studies (24–26) was substantiated in a phase I clinical trial when systemically administered O6-BG and carmustine led to a reduction in the maximum tolerated dose of systemic carmustine from 200 mg/m² to 40 mg/m² because of markedly enhanced myelosuppression (8).

In an attempt to circumvent this enhanced hematopoietic toxicity of systemically administered O6-BG and carmustine, a phase I trial was done where Gliadel wafers were administered

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**Table 1. Patient characteristics**

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<td>Prior nitrosourea</td>
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Abbreviations: LGA, low-grade astrocytoma; GS, gliosarcoma.
locally in combination with systemically administered O6-BG (10). This clinical trial established the O6-BG dose required to completely deplete tumor AGT for 48 hours to be 120 mg/m2 over 1 hour followed by a continuous infusion of 30 mg/m2/d. This clinical trial noted an added toxicity when systemically administered O6-BG was combined with locally administered Gliadel wafers.

Building on this prior study, we investigated the efficacy and toxicity of Gliadel wafers when combined with a dosing schedule of O6-BG that would ensure suppression of AGT activity for 5 days. Our results show that this infusion regimen of O6-BG can improve the efficacy of Gliadel wafers.

This clinical trial shows significant improvement in 6-month OS when compared with the results found in a phase III randomized placebo-controlled clinical trial where patients with recurrent malignant glioma were treated with Gliadel wafers alone (4). GBM patients treated with Gliadel wafers alone showed a 6-month OS of 56%, 1-year OS of 20%, 2-year OS of 10%, and a median survival of 28 weeks. GBM patients treated with Gliadel wafers and O6-BG showed a 6-month OS of 82% (95% CI, 72%-93%). The 1-year OS and 2-year OS were 47% (95% CI, 35-63%) and 10% (95% CI, 3-32%), respectively. The median OS was 50.3 weeks (95% CI, 36.1-69.4 weeks).

In this phase II trial, as well as in the phase I clinical trial using Gliadel wafers and O6-BG in the treatment of malignant glioma (10), Gliadel wafers combined with O6-BG showed no systemic toxicity as is seen with systemically administered carmustine. However, the adverse events of hydrocephalus, CSF leak, and CSF/brain infection were seen at a higher frequency in our clinical trial than seen in the phase III trial where patients with recurrent malignant glioma were treated with Gliadel wafers alone or placebo (4). Our data reveal an interrelationship between these adverse events that suggests a phenomenological process that needs to be elucidated. Given this interrelationship, one suspects that Gliadel wafers combined with O6-BG may be causing a cascade of events to take place at a higher frequency than would otherwise occur with Gliadel wafers alone. Gliadel wafers combined with O6-BG may be triggering an inflammatory reaction that then produces either a communicating or a noncommunicating hydrocephalus followed by CSF leak from the craniotomy site and thus increases the risk of brain/CSF infection. Whether this cascade of events is truly at play and, if so, whether it is triggered by Gliadel wafers and O6-BG or carmustine wafers alone cannot be ascertained from this clinical trial but will have to be determined by subsequent clinical trials.

Although the toxicity seen by Weingart et al. (10) was much less, our O6-BG regimen was far more rigorous, with 120 mg/m2 infusions given on days 1, 3, and 5 and 30 mg/m2/d continuous infusion, whereas Weingart et al. (10) only gave the day 1 dose of 120 mg/m2 followed by 30 mg/m2/d by continuous infusion for 2 to 14 days.

The data from this trial support proceeding with clinical trials designed to test the hypothesis that O6-BG will increase the efficacy of Gliadel wafers and improve survival. However, in the design of the next clinical trial, two important questions should be addressed. First, how long should tumor AGT levels be suppressed and by which O6-BG dosing regimen would maximal therapeutic benefit be realized? Second, would sequential combination of Gliadel wafers and O6-BG followed by effective systemic chemotherapy have the greatest effect on OS by treating both the focal and diffuse nature of this disease?

In this clinical trial, tumor AGT activity was suppressed for at least 5 to 6 days because AGT remains undetectable for at least 18 hours after the 120 mg/m2 bolus (7). One could argue that because carmustine is released over several weeks from Gliadel wafers, tumor AGT activity should be suppressed by O6-BG during this entire period to maximize potential therapeutic benefit. However, the vast majority of the 1,3-bis(2-chloroethyl)-1-nitrosourea is released within the first 5 to 7 days following wafer placement (27, 28). Examination of the pharmacokinetics of the Weingart study detailed above (10) 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 and it is not clear how long tumor AGT levels remained suppressed. Perhaps the O6-BG dosing regimen used in our study and proved in prior studies to deplete tumor AGT for 48 hours should be repeated seven times to ensure prolonged tumor AGT depletion for a total of 14 days. Lastly, another alternative O6-BG dosing regimen used in a clinical trial in pediatric tumors uses a daily 1-hour infusion of O6-BG at 120 mg/m2 without a continuous infusion. Although research shows depletion of AGT at 18 hours after this 1-hour infusion of O6-BG (29), it is highly unlikely and has never been proven that this depletion is continuous from 18 to 24 hours, which makes this a less attractive alternative.

Given the recent success of treating malignant glioma with such treatment regimens as temozolomide, or CPT-11 and bevacizumab, and given the focal and diffuse parenchymal involvement of this disease, perhaps sequential combination of Gliadel wafers and O6-BG followed by effective systemic chemotherapy would have the greatest effect on OS. In developing clinical trials where Gliadel wafers and O6-BG are sequentially followed by effective systemic chemotherapy, OS can remain a valid end point without confounding the survival analysis. This is especially important in clinical trials using Gliadel wafers, where progression-free survival remains difficult to determine, given the difficulty of ascertaining disease progression based on a combination of clinical and MRI findings, and confounded by the inflammatory effects from Gliadel wafers, which accompany most focal therapy.

### Disclosure of Potential Conflicts of Interest

H.S. Friedman has received a commercial research grant from and is a consultant for Eisai, Inc.
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Jennifer A. Quinn, Sara Xiaoyin Jiang, James Carter, et al.


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