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Phase II Trial of Gliadel® plus O^6-Benzylguanine (O^6-BG) in Adults with Recurrent Glioblastoma Multiforme

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Abstract

Purpose—This phase II trial was designed to: define the efficacy of Gliadel® wafers in combination with an infusion of O^6-BG that suppresses tumor O^6-alkylguanine-DNA alkyltransferase (AGT) levels in patients with recurrent GBM for 5 days; and evaluate the safety of this combination therapy.

Patients and Methods—This was a phase II, open-label, single center trial. Upon gross total resection of the tumor, up to 8 Gliadel® wafers were implanted. Bolus infusion of O^6-BG was administered at 120 mg/m^2 over 1 hour on days 1, 3, and 5, along with a continuous infusion at 30 mg/m^2/day. The primary endpoints were 6-month overall survival (OS) and safety, and the secondary endpoints were 1-year, 2-year, and median OS.

Results—Fifty-two patients were accrued. The 6-month OS was 82% (95% CI, 72% to 93%). The 1-year and 2-year OS rates were 47% (95% CI, 35% and 63%) and 10% (95% CI, 3% to 32%), respectively. The median OS was 50.3 weeks (95% CI, 36.1 to 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^6-BG. Although systemically administered O^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^6-BG results in increased efficacy of Gliadel® wafers without added toxicity.

Keywords

Gliadel®; O^6-benzylguanine; glioblastoma multiforme

INTRODUCTION

While novel therapeutic regimens in recent years have enhanced survival for patients with malignant glioma, overall prognosis remains poor. Glioblastoma multiforme (GBM) is by far

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STATEMENT OF CLINICAL RELEVANCE This study reports on the efficacy and safety of a therapeutic regimen consisting of Gliadel® and O^6-Benzylguanine in the treatment of patients with recurrent glioblastoma multiforme.
Malignant glioma is the most common primary brain tumor, with poor survival despite standard therapies. Gliadel® is one of only two FDA-approved chemotherapeutic agents for the treatment of brain tumors, and novel regimens are needed to enhance efficacy for both newly diagnosed patients and patients at recurrence.

the most common histological subtype of glioma, with a two-year survival of only 8.7% (1). Recurrence of disease after or during therapy is the norm for malignant glioma (MG), with the majority of recurrences being local.(2) Carmustine wafer (polifeprosan 20 with carmustine implant (Gliadel® Wafer, [MGI Pharma, Inc., Bloomington, MN]), 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 MG patients.(3,4)

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

Unfortunately, when both O^6-BG and carmustine are administered systemically, O^6-BG not only enhances the activity of carmustine but it also enhances the hematopoietic toxicity of carmustine. Our phase I trial established the maximum tolerated dose of carmustine to be 40 mg/m^2 when combined with a dose of O^6-BG (1-hour IV bolus of 120 mg/m^2) sufficient to deplete AGT in gliomas 18 hours after administration.(7,8) This trial demonstrated the need for a marked reduction in the dose of carmustine compared with the usual dose of 200 mg/m^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^6-BG may be the underlying factor in the failure of this drug combination to cause frank tumor regressions in our phase II trial.

In an attempt to circumvent the enhanced hematopoietic toxicity of systemically administered O^6-BG and carmustine, Weingart et al performed a phase I trial where carmustine was administered locally as Gliadel® wafers in combination with systemically administered O^6-BG.(10) This clinical trial established the O^6-BG dose, required to completely deplete tumor AGT for 48 hours as 120 mg/m^2 over 1 hour followed by a 48-hour continuous infusion of 30 mg/m^2/d. This clinical trial also noted no added toxicity when systemically administered O^6-BG was combined with locally administered Gliadel® wafers.

The primary objectives of the current study were to 1) define the efficacy of Gliadel® wafers in combination with an infusion of O^6-BG that suppresses tumor AGT levels in patients with recurrent GBM for 5 days, and 2) evaluate the safety of this combination therapy.

PATIENTS AND METHODS

Patient Population

Eligible patients had a histologically confirmed diagnosis of recurrent GBM (including gliosarcoma) that was shown by contrast-enhanced MRI to have a unilateral, single focus of measurable CNS neoplasm that was supratentorial and measured at least 1.0 cm in diameter. Patients were ≥18 years old and had a Karnofsky performance score of ≥60%. An interval of at least 2 weeks since prior surgical resection (if conducted) or 4 weeks since prior chemotherapy (6 weeks for a nitrosourea-based regimen) had to have elapsed for the patient to be enrolled 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 SGOT <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 weeks. 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 immunosuppressive agents other than corticosteroids. Others excluded were patients who were not neurologically stable for 2 weeks prior to study entry, patients who were poor medical risks because of non-malignant systemic disease, as well as those with acute infection treated with intravenous 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 AIDS-related illness.

Study Design and Treatment

This was a phase II, open-label, single center trial. Upon gross total resection of the tumor and confirmation of GBM on intraoperative frozen section, each patient received up to 8 carmustine wafers.

Carmustine wafers were commercially available (Gliadel® Wafers, Guilford Pharmaceuticals, Baltimore, MD). 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 upon the size of the tumor resection cavity. The aim was to cover the entire surface of the resection cavity with 8 or fewer wafers with slight overlapping of wafers permitted. The number of wafers implanted was recorded.

Within 6 hours of completion of surgery, a bolus infusion of O^6-BG was administered at 120 mg/m^2 over 1 hour on day 1 and repeated every 48 hours on days 3 and 5. A continuous infusion of O^6-BG at 30 mg/m^2/day was administered immediately following the initial O^6-BG bolus on day 1 and continued until immediately prior to the last bolus injection. Continuous O^6-BG was sometimes interrupted for administration of the O^6-BG bolus. O^6-BG was supplied by AOI Pharmaceuticals, Inc. (New York, NY).

Surveillance and Follow-up

The baseline examination included central review of tumor tissue, MRI or CT (if MRI was medically contraindicated), complete blood counts and blood chemistry tests, and a physical examination including a comprehensive neurologic examination. Every 8 weeks, patients were required to repeat complete blood counts and blood chemistry tests, neuro-imaging, 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 O^6-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 months after initiation of protocol treatment.

The 6-month 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 months with the combination of Gliadel® wafers and O^6-BG, there would be no interest in further developing this treatment regimen. However, if at least 70% of the patients survived for 6 months, there would be genuine interest in pursuing this treatment regimen. The study was therefore designed to differentiate between 6-month survival rates of 50% and 70%. Statistically, the hypothesis to be tested was as follows: H_0: $p < 0.50$ vs H_1: $p > 0.70$, where p is the proportion of patients remaining alive at 6 months.

Fifty patients were to be accrued to this study. If 31 or more patients survived for at least 6 months, 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 endpoints included 1-year OS, 2-year 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 O^6-BG. A second patient received Gliadel® wafers and O^6-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% CI, 72% to 93%). The 1-year OS and 2-year OS were 47% (95% CI, 35% to 63%) and 10% (95% CI, 3% to 32%), respectively. The median OS was 50.3 weeks (95% CI, 36.1 to 69.4 weeks). Overall survival 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 (CNS) 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 7 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 FDA 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 demonstrates 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 *O*^{6}-alkylguanine lesions introduced by alkylating agents such as carmustine and temozolomide.(5)

Depletion of AGT activity by the selective inhibitor O^6-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 O^6-BG is their potential for doserelated 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 O^6-BG and carmustine led to a reduction in the maximum tolerated dose of systemic carmustine from 200 mg/m^2 to 40 mg/m2 because of markedly enhanced myelosuppression.(8)

In an attempt to circumvent this enhanced hematopoietic toxicity of systemically administered O^6-BG and carmustine, a phase I trial was performed where Gliadel® wafers were administered locally in combination with systemically administered O^6-BG.(10) This clinical trial established the O^6-BG dose required to completely deplete tumor AGT for 48 hours to be 120 mg/m^2 over 1 hour followed by a continuous infusion of 30 mg/m^2/d. This clinical trial noted no added toxicity when systemically administered O^6-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 O^6-BG that would ensure suppression of AGT activity for 5 days. Our results demonstrate that this infusion regimen of O^6-BG can improve the efficacy of Gliadel® wafers.

This clinical trial shows significant improvement in 6-month OS when compared to 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 O^6-BG showed a 6-month OS of 82% (95% CI, 72% to 93%). The 1-year OS and 2-year OS were 47% (95% CI, 35% and 63%) and 10% (95% CI, 3% and 32%), respectively. The median OS was 50.3 weeks (95% CI, 36.1 to 69.4 weeks).

In this phase II trial, as well as in the phase I clinical trial utilizing Gliadel® wafers and O^6-BG in the treatment of malignant glioma,(10) Gliadel® wafers combined with O^6-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 reveals 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 O^6-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 O^6-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 O^6-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 O^6-BG regimen was far more rigorous, with 120 mg/m^2 infusions given on days 1, 3, and 5 and 30 mg/m^2/day continuous infusion whereas Weingart et al (10) only gave the day 1 dose of 120 mg/m^2 followed by 30 mg/m^2/day by continuous infusion for 2-14 days.

The data from this trial supports proceeding with clinical trials designed to test the hypothesis that O^6-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 O^6-BG dosing regimen would maximal therapeutic benefit be realized? Second, would sequential combination of Gliadel® wafers and O^6-BG followed by effective systemic chemotherapy have the greatest impact on overall survival by treating both the focal and diffuse nature of this disease?

In this clinical trial, tumor AGT activity was suppressed for at least 5-6 days since AGT remains undetectable for at least 18 hours after the 120 mg/m^2 bolus.(7) One could argue that since carmustine is released over several weeks from Gliadel® wafers, tumor AGT activity should to be suppressed by O^6-BG during this entire period to maximize potential therapeutic benefit. However the vast majority of the BCNU is released within the first 5-7 days following wafer placement.(27,28) Examination of the pharmacokinetics of the Weingart study detailed above (10) for the prolonged continuous infusion of O^6-BG raised the possibility that a higher dose of O^6-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 O^6-BG dosing regimen used in our study and proved in prior studies to deplete tumor AGT for 48 hours should be repeated 7 times to ensure prolonged tumor AGT depletion for a total of 14 days. Lastly, another alternative O^6-BG dosing regimen used in a clinical trial in pediatric tumors utilizes a daily 1-hour infusion O^6-BG at 120 mg/m^2 without a continuous infusion. Although research shows depletion of AGT at 18 hours after this 1-hour infusion of O^6-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 O^6-BG followed by effective systemic chemotherapy would have the greatest impact on overall survival. In developing clinical trials where Gliadel® wafers and O^6-BG are sequentially followed by effective systemic chemotherapy, overall survival can remain a valid endpoint 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.

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REFERENCES

1. CBTRUS. Statistical Report: Primary Brain Tumors in the United States 1997-2001. 2004.
2. Hochberg FH, Pruitt AA, Beck DO, DeBrun G, Davis K. The rationale and methodology for intra-arterial chemotherapy with BCNU as treatment for glioblastoma. J Neurosurg 1985;63:876–80. [PubMed: 2997415]
3. Westphal M, Hilt DC, Bortey E, et al. A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. Neuro Oncol 2003;5:79–88. [PubMed: 12672279]
4. Brem H, Piantadosi S, Burger PC, et al. Placebo-controlled trial of safety and efficacy of intraoperative controlled delivery by biodegradable polymers of chemotherapy for recurrent gliomas. The Polymer-brain Tumor Treatment Group. Lancet 1995;345:1008–12. [PubMed: 7723496]
5. 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. [PubMed: 7659775]
6. Dolan ME, Pegg AE. O6-benzylguanine and its role in chemotherapy. Clin Cancer Res 1997;3:837–47. [PubMed: 9815757]
7. Friedman HS, Kokkinakis DM, Pluda J, et al. Phase I trial of O6-benzylguanine for patients undergoing surgery for malignant glioma. J Clin Oncol 1998;16:3570–5. [PubMed: 9817277]
8. Friedman HS, Pluda J, Quinn JA, et al. Phase I Trial of Carmustine Plus O6-Benzylguanine for Patients With Recurrent or Progressive Malignant Glioma. J Clin Oncol 2000;18:3522–8. [PubMed: 11032594]
9. Quinn JA, Pluda J, Dolan ME, et al. Phase II Trial of Carmustine Plus O6-Benzylguanine for Patients With Nitrosourea-Resistant Recurrent or Progressive Malignant Glioma. J Clin Oncol 2002;20:2277–83. [PubMed: 11980998]
10. 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. [PubMed: 17264335]
11. Belanich M, Pastor M, Randall T, et al. Retrospective Study of the Correlation between the DNA Repair Protein Alkyltransferase and Survival of Brain Tumor Patients Treated with Carmustine. Cancer Res 1996;56:783–8. [PubMed: 8631014]
12. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-Repair Gene MGMT and the Clinical Response of Gliomas to Alkylating Agents. N Engl J Med 2000;343:1350–4. [PubMed: 11070098]
13. Friedman HS, McLendon RE, Kerby T, et al. DNA mismatch repair and O6-alkylguanine-DNA alkyltransferase analysis and response to Temodal in newly diagnosed malignant glioma. J Clin Oncol 1998;16:3851–7. [PubMed: 9850030]
14. Hegi ME, Diserens A-C, Godard S, et al. Clinical Trial Substantiates the Predictive Value of O-6-Methylguanine-DNA Methyltransferase Promoter Methylation in Glioblastoma Patients Treated with Temozolomide. Clin Cancer Res 2004;10:1871–4. [PubMed: 15041700]
15. Hotta T, Saito Y, Fujita H, et al. O6-alkylguanine-DNA alkyltransferase activity of human malignant glioma and its clinical implications. J Neurooncol 1994;21:135–40. [PubMed: 7861189]
16. Jaeckle KA, Eyre HJ, Townsend JJ, et al. Correlation of tumor O6 methylguanine-DNA methyltransferase levels with survival of malignant astrocytoma patients treated with bis-

chloroethylnitrosourea: a Southwest Oncology Group study. J Clin Oncol 1998;16:3310–5. [PubMed: 9779706]

17. Schold SC Jr. Brent TP, von Hofe E, et al. O6-alkylguanine-DNA alkyltransferase and sensitivity to procarbazine in human brain-tumor xenografts. J Neurosurg 1989;70:573–7. [PubMed: 2926498]
18. Baer JC, Freeman AA, Newlands ES, Watson AJ, Rafferty JA, Margison GP. Depletion of O6-alkylguanine-DNA alkyltransferase correlates with potentiation of temozolomide and CCNU toxicity in human tumour cells. Br J Cancer 1993;67:1299–302. [PubMed: 8512814]
19. 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;2:735–41. [PubMed: 9816224]
20. 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;51:3367–72. [PubMed: 1647266]
21. Felker GM, Friedman HS, Dolan ME, Moschel RC, Schold C. Treatment of subcutaneous and intracranial brain tumor xenografts with O6-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Chemother Pharmacol 1993;32:471–6. [PubMed: 8258196]
22. Friedman HS, Dolan ME, Pegg AE, et al. Activity of temozolomide in the treatment of central nervous system tumor xenografts. Cancer Res 1995;55:2853–7. [PubMed: 7796412]
23. 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;74:1030–6. [PubMed: 8855970]
24. Page J, Giles H, Phillips W. Preclinical toxicology study of O6-benzylguanine (NSC-637037) and BCNU (carmustine, NSC0409962) in male and female Beagle dogs. Proc Am Assoc Cancer Res 1994:1952.1994
25. Rodman L, Giles H, Tomaszewski J. Preclinical toxicology study of O6-benzylguanine (NSC-637037) and 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC 409962) in mice. American Association for Cancer Res 1994:1954.1994
26. Rogers, T.; Rodman, L.; Tomaszewski, J. Preclinical toxicology and pharmacokinetic studies of O6-benzylguanine in mice and dogs. American Association for Cancer Res; 1994. p. 1953American Association for Cancer Res; 1994
27. Domb AJ, Rock M, Perkin C, Yipchuck G, Broxup B, Villemure JG. Excretion of a radiolabelled anticancer biodegradable polymeric implant from the rabbit brain. Biomaterials 1995;16:1069–72. [PubMed: 8519927]
28. Grossman SA, Reinhard C, Colvin OM, et al. The intracerebral distribution of BCNU delivered by surgically implanted biodegradable polymers. J Neurosurg 1992;76:640–7. [PubMed: 1545259]
29. Schold SC Jr. Kokkinakis DM, Chang SM, et al. O6-benzylguanine suppression of O6-alkylguanine-DNA alkyltransferase in anaplastic gliomas. Neuro Oncol 2004;6:28–32. [PubMed: 14769137]

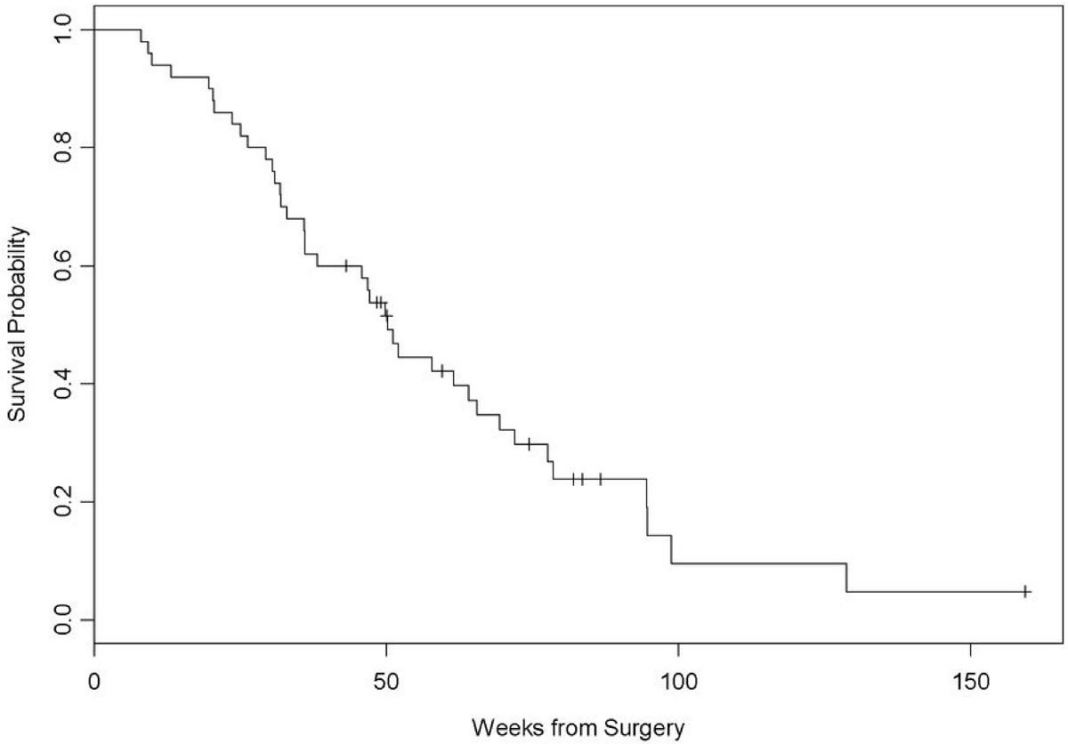

Fig 1.
Kaplan-Meier estimates of overall survival

Table 1

Patient Characteristics

Characteristic	No. of Patients	%
Total no. of Patients	52	
Age, years		
Median, 51.5		
Range, 25-69		
Sex		
Male	30	58
Female	22	42
Karnofsky performance status, %		
60	1	2
70	2	4
80	12	23
90	25	48
100	12	23
No. of progressions		
Median, 1		
Range, 1-3		
Time from diagnosis, weeks		
Median, 33.4		
Range, 12.4-232.9		
Histological diagnosis		
LGA	1	2
GS	3	6
GBM	48	92
Prior nitrosourea	18	35

LGA = low grade astrocytoma, GS = gliosarcoma, GBM = glioblastoma multiforme

Table 2

Adverse Events in 52 Patients

Adverse Event	No. of Patients	%
Hyponatremia (Gr3)	2	3.8
CSF Leak (Gr3)	10	19.2
Superficial Wound Infection (Gr2)	2	3.8
Brain/CSF Infection (Gr3)	7	13.4
Hydrocephalus (Gr3)	5	9.6
Fever (Gr3)	1	1.9
Epidural Hygroma (Gr3)	1	1.9
Epidural Hematoma (Gr3)	1	1.9
CNS Hemorrhage (Gr3)	1	1.9

Gr2, grade 2; Gr3, grade 3.