

Phase I analysis of BCNU-impregnated biodegradable polymer wafers followed by systemic interferon alfa-2b in adults with recurrent glioblastoma multiforme

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Abstract *Introduction* Carmustine (BCNU)-impregnated biodegradable polymer wafers have been shown to prolong survival in patients with recurrent malignant glioma. Interferon alfa-2b (IFN α_{2b}) has demonstrated antitumor activity against a number of cancers, but its use in glioma has been limited. The use of these agents in combination is appealing because the mode of antitumor activity likely differs. This is a report of a phase I dose-finding study for IFN α_{2b} in combination with surgery and BCNU wafers in patients with recurrent glioblastoma. *Method* Patients with progressive malignant glioma that was confirmed intraoperatively to be glioblastoma were treated with surgical resection and implantation of 8 BCNU wafers. A week later, IFN α_{2b} was initiated three times a week at a dose of 3 MU/m², which was escalated in increments of 3 MU/m². The treatment cycle encompassed 8 weeks. Toxicity was monitored by clinical and laboratory testing. Correlative studies of methylguanine methyltransferase (MGMT) expression and gene expression array analysis were carried

out. *Results* Ten patients were enrolled, and 9 patients had evaluable data. Dose-limiting toxicity in the form of fatigue occurred at 9 MU/m². Two complete imaging responses were observed at the 3 MU/m² dose. MGMT expression and gene expression arrays did not correlate with toxicity or response. *Conclusions* Multimodal therapy with surgery, BCNU wafers, and IFN α_{2b} appears to be a feasible and safe treatment strategy. The maximum tolerated dose of IFN α_{2b} was determined to be 6 MU/m². Analysis of MGMT expression and gene expression was feasible.

Keywords Gene expression array · Gliadel wafer · Glioblastoma · Interferon alfa · Methylguanine methyltransferase

Introduction

Currently, there is no reliable curative therapy for glioblastoma multiforme (GBM), although useful palliation has been reported in selected applications of surgery, radiation, cytotoxic chemotherapy (both systemic and localized), and biologic therapy [1–4]. The inability of these interventions to obtain long-term control is likely due in part to one or more tumoral mechanisms that resist or rapidly repair the therapy effect. For example, O⁶-methylguanine-DNA methyltransferase (MGMT) is a DNA repair enzyme that is believed to confer resistance to alkylating agents [5], and nuclear factor-kappa B appears to inhibit apoptosis, a principle mechanism of action for many antitumor agents [6].

One possible method of addressing tumor resistance is the concurrent use of therapies whose proposed mechanisms of tumor killing are different. Carmustine (BCNU)-impregnated biodegradable polymer wafers have been

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demonstrated to improve survival when used in selected cases of recurrent malignant brain tumors and in newly diagnosed malignant gliomas [7–9]. The cytotoxic mechanism of BCNU is well known and understood in the therapy of brain tumors [10, 11]. Interferon alfa has been studied in newly diagnosed and recurrent malignant gliomas. In the newly diagnosed setting, it has been of limited value [12], but some responses have been observed in the recurrent setting [13, 14]. Low-dose interferon alfa has been shown to be efficacious in other cancer site settings, with evidence indicating antitumor activity via antiangiogenesis, and enhancement of immune response to tumors [15–17]. Studies have examined both high- and low-dose regimens of interferon alfa as an antineoplastic agent, with the low-dose regimen presenting the advantage of less toxicity [18, 19].

This study was developed to assess the toxicity and maximum tolerated dose of interferon alfa-2b (IFN α_{2b}) following surgery and BCNU wafer implantation in patients with progressive malignant glioma proven to be glioblastoma at the time of surgery.

Methods

Patients

To be eligible for enrollment in this study, patients needed to be age ≥ 18 years, have a prior diagnosis of supratentorial glioma (i.e., astrocytoma, oligodendroglioma, mixed glioma, anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic mixed glioma, or glioblastoma multiforme), and have radiographic evidence of disease progression, a Karnofsky Performance Status (KPS) score of >50 , and an estimated life expectancy of 12 weeks or more. Imaging progression was defined as a 25% or more increase in enhancing disease as determined by neuroradiologic assessment since the baseline of the participant's most recent treatment regimen. The progression must have been deemed appropriate for surgical debulking and implantation of BCNU wafers. Required laboratory parameters were white blood cell count $>2,000/\text{mm}^3$, hemoglobin >10 g/dL, and platelets $>75,000/\text{mm}^3$, adequate renal function (creatinine <1.7 mg or blood urea nitrogen [BUN] <40 mg/dL), adequate hepatic function (bilirubin <2.0 mg/dL, aspartate aminotransferase [AST] and alanine aminotransferase [ALT] $<4\times$ the upper limit of lab normal range, prothrombin time [PT] and partial thromboplastin time [PTT] within 1.5x the upper limit of lab normal range). Cardiac and pulmonary function needed to be clinically consistent with the ability to tolerate a craniotomy.

Participants were excluded if they were pregnant, and participants were required to agree to use effective

contraception from the day prior to surgery through 30 days after completion of IFN α_{2b} therapy. Participants were ineligible if they had completed radiation therapy less than 3 months prior to the initiation of the proposed BCNU wafer implantation, if less than 3 weeks had elapsed since their last non-nitrosourea chemotherapy, or if less than 6 weeks had elapsed since their last nitrosourea chemotherapy. There was no limit on the number of prior chemotherapeutic regimens, but BCNU wafer therapy could not have been previously used. Anatomic exclusions included tumors that crossed the midline, multifocal tumors, and posterior fossa or brain stem tumors. Also excluded were those participants with concurrent severe medical illness or psychiatric illness that precluded surgical candidacy. The study was approved by the Emory University Institutional Review Board, and participants provided written informed consent prior to enrollment.

Treatment plan

Treatment was initiated by image-guided craniotomy for tumor debulking and implantation of 8 BCNU wafers. At the time of surgery, frozen section analysis was undertaken. Interpretation confirming high-grade glioma with necrosis was required to proceed with BCNU wafer implantation (Gliadel[®] Wafer, MGI PHARMA, Inc., Baltimore, MD), and a final diagnosis of GBM was required prior to initiation of IFN α_{2b} . One week after surgery, IFN α_{2b} (Intron[®] A, Schering Corporation, Kenilworth, NJ) was initiated by subcutaneous injection three times a week. This was carried on for an 8-week cycle followed by clinical and magnetic resonance imaging (MRI) reassessment. For those with stable disease or response and no dose-limiting toxicity, an additional 8-week cycle was carried out. Therapy thereafter was dependent on patient response and physician judgment. Medical support with corticosteroids, anticonvulsants, and other agents was utilized as per standard medical practice.

Dosing with IFN α_{2b} was begun by treating three patient groups at 3 MU/m² three times a week, and then increasing the dose by 3 MU/m² for each successive dose level. The initial dosage was chosen based on prior experience and literature evidence. These data indicated that 3 MU/m² would be below previously documented toxic doses with the goal of reaching an eventual maximum tolerated dose that would yield approximately 1 serious toxicity in every three individuals treated [13, 20]. If no dose limiting toxicities were encountered in the first three patients, the study was designed to move to the next higher dose level. If a dose-limiting toxicity was encountered in 1 of the first 3 patients, the group was expanded to six patients. If a second dose-limiting toxicity was encountered, the prior dose level would be defined as the maximum tolerated dose. The

study was designed to proceed to the next dosage level if six patients received a given dose with only one individual having dose-limiting toxicity. The interval for treatment assessment prior to moving to the next higher dosage level was 6 weeks after completion of the first cycle of IFN α_{2b} by the last person in the cohort.

Dose-limiting toxicity

Dose-limiting toxicity was defined as the emergence of a grade 3 or 4 nonhematologic systemic toxicity (National Cancer Institute Common Toxicity Criteria 2.0 [21]), except nausea and vomiting not treated with sufficient antiemetics. Hematologic criteria for dose-limiting toxicity were platelet count $<25,000/\text{m}^3$, and febrile neutropenia (oral temperature $>38.5^\circ\text{C}$ and absolute neutrophil count $<500/\text{mm}^3$). Grade 4 neurologic toxicity was set as the criteria for removal from study, but neurologic deficits that were present prior to initiation of therapy were not included. Seizure activity, deep venous thrombosis, and pulmonary emboli were not included as dose-limiting toxicities. Patients were removed from the study if treatment was delayed for Grade 3 and 4 hematologic and nonhematologic toxicities whose medical management would be complicated by resumption of IFN α_{2b} treatment.

Data safety monitoring was conducted by a 4-member committee after completion of the first cycle of IFN α_{2b} by the first patient, prior to each dose escalation, and at the point when maximum tolerated dose was declared. Removal from the study was based on development of treatment-related dose-limiting toxicity, development of a non-treatment-related medical circumstance precluding study continuation, refusal to continue therapy, or evidence of disease progression. Participants were removed if a non-treatment-related event precluded ongoing treatment for 14 or more days. Disease progression was defined as at least one of the following: $>25\%$ increase in tumor size as determined by MRI; a neurologic decline that was not explained by treatment, metabolic causes, or drug toxicity; a $<25\%$ increase in tumor volume and a worsening of neurologic status on a stable or increasing dose of steroids; or MRI evidence of a new lesion. At the time of progression, further treatment was provided to each patient dependent up the nature of their progression, KPS scores, and prior therapeutic history.

Study assessments

During the first cycle of IFN α_{2b} administration, patients underwent routine general and neurologic exam and complete blood count with differential, platelet count, BUN/creatinine, AST, ALT, alkaline phosphatase, and total bilirubin assessment every 2 weeks. During subsequent cycles, assessments were every 2–4 weeks, depending on

the patient's status. Imaging assessment by MRI or computerized axial tomography was obtained within 48 h of surgery to serve as a treatment baseline, within 7 days of completion of the first cycle of IFN α_{2b} , and then within 7 days prior to and within 7 days after the completion of each subsequent cycle.

Though assessment of toxicity was the focus of this study, response also was assessed by imaging and clinical status using modified McDonald criteria [22]. This required patients to have discontinued corticosteroid therapy for 4 weeks or more in cases of complete response and the need for stable or decreasing corticosteroid dosage over at least 4 weeks in cases of partial response. Response data were assessed independently by the Data Safety Monitoring Committee.

MGMT expression and microarray gene expression profiling

To evaluate the feasibility of assessing surrogate markers of efficacy and toxicity, tumor tissue was analyzed for MGMT and screened using gene expression profiling.

The presence of MGMT was assessed immunohistochemically in specimens from tumor resections. Formalin-fixed, paraffin-embedded brain tumor tissues were cut at a thickness of 5 μm . The slides were deparaffinized in two xylene baths for 5 min each, and rehydrated in an alcohol series (100%, 85%, and 70%). Staining for MGMT was carried out using the Catalyzed Signal Amplification System (DAKO, Cat#K1500) following the manufacturer's instructions. Following incubation to block endogenous biotin and nonspecific proteins, the slides were incubated with a primary mouse anti-MGMT antibody (O⁶-methylguanine-DNA methyltransferase clone mT3.1 [5 $\mu\text{g}/\text{mL}$]; Chemicon International, Temecula, CA), a biotinylated linking antibody, and then streptavidin peroxidase. The slides were counterstained with Mayer's hematoxylin. Primary mouse IgG1 antibody was used as the negative control in each run and endothelial staining was used as an internal positive control. The immunoreactivity was quantified by counting staining tumor cell nuclei over 1,000 cells and expressed as percentage of the positive cells.

For microarray gene expression profiling, fresh tumor samples were taken for immediate freezing during surgery. Briefly, total RNA from the frozen brain tumor tissues was prepared by RNeasy[®] Lipid Tissue Mini Kit (QIAGEN[®], Cat# 74804, Valencia, CA) according to the manufacturer's instructions. The following steps were performed at the Yerkes Microarray Core at Emory University (Atlanta, GA). Double-strand cDNA was synthesized from 5 μg of purified total RNA and then transcribed in vitro to form biotin-labeled cRNA with the Two-Cycle Target Labeling Kit (Affymetrix, Cat# 900494, Santa Clara, CA). The

cRNA was fragmented and hybridized for 16 h at 45°C to microarrays (GeneChip® Human Genome U133 plus 2.0 arrays, Affymetrix) representing approximately 39,000 human genes. The microarrays were washed and stained using the Affymetrix fluidics station, and then scanned using an Affymetrix GeneArray Scanner 3000. Data were analyzed with Affymetrix GeneSpring 7.2 software. The raw expression value derived from the scanner for the samples was imported into the software. The raw values were normalized with the default setting. Quality control was performed at the sample level and at the gene level by setting the filter flag at present or marginal. Further filtering steps were conducted to identify the genes that were the most different between the patient groups of interest.

Statistics

Data were summarized with descriptive statistics. Statistical analysis of gene microarray data was conducted using a simple analysis of variance with significance set at $P < 0.05$.

Results

Ten individuals were enrolled over a 17-month period beginning in December 2003. The Table 1 summarizes their baseline data. The final pathologic diagnosis on the tissue obtained at the time of surgery was GBM in all nine patients. Four patients had received one prior course of systemic

chemotherapy (temozolomide in all cases), four patients had received two prior courses of systemic chemotherapy (temozolomide and lomustine, temozolomide and celecoxib, temozolomide and SDX-102 [L-alanosine], and temozolomide and bortezomib, respectively), and one patient had received three prior courses of systemic chemotherapy (temozolomide, lomustine/vincristine/etoposide, isotretinoin).

Three patients were treated at the 3 MU/m² dose, followed by three patients at the 6 MU/m² dose, followed by four patients at the 9 MU/m² dose level. One patient in the 9 MU/m² group was removed from the study due to cardiac arrhythmia that was thought to be unrelated to IFN α_{2b} but which precluded additional active tumor treatment based on its debilitating effects. This patient was replaced by another individual treated at the same dosage. This left nine patients for evaluation. Four patients completed at least one cycle of IFN α_{2b} . All patients received eight BCNU-impregnated biodegradable wafers at resection. No patients were lost to follow-up.

IFN α_{2b} therapy

In the 3 MU/m² group, two patients completed more than one cycle of IFN α_{2b} and one individual stopped after 6 weeks of treatment. In the 6 MU/m² group, one patient completed more than one cycle of IFN α_{2b} , one patient withdrew consent after 3 weeks of treatment, and one patient experienced tumor progression after 5 weeks of IFN α_{2b} treatment. In the 9 MU/m² group, one patient completed one cycle of IFN α_{2b} and then experienced tumor progression. There was no increase in combined white blood cell counts during therapy for any of the patients. Two patients developed dose-limiting toxicity (Grade 3) consisting of fatigue; one after three doses of IFN α_{2b} and one after six doses. The fatigue was associated with a subjective decline in cognition and ability to carry out activities of daily living. Thus, dose-limiting toxicity was declared at 9 MU/m², and the 6 MU/m² dose was declared the maximum tolerated dose. The Fig. 1 summarizes toxicities determined to be possibly, probably, or definitely related to the study treatment.

Treatment response

By review of imaging, two individuals in the 3 MU/m² group demonstrated treatment response. The first individual demonstrated a partial response with a reduction of tumor by >50% after completion of the first cycle of IFN α_{2b} therapy. This response has remained durable with progression-free survival of 183 weeks after surgery. The second individual had a complete response after the second cycle of IFN α_{2b} therapy. This response has remained

Table 1 Patient characteristics ($N = 9$)

Age (years)	
Mean	45.5
Median	51
Range	35–55
Gender	
Men	4
Women	5
KPS ^a	
Median	70
Range	60–100
Original histologic diagnosis	
Astrocytoma	1
Anaplastic astrocytoma	2
Glioblastoma multiforme	6
Prior chemotherapy	
1 regimen	4
2 regimens	4
3 regimens	1

^a KPS—Karnofsky Performance Status score

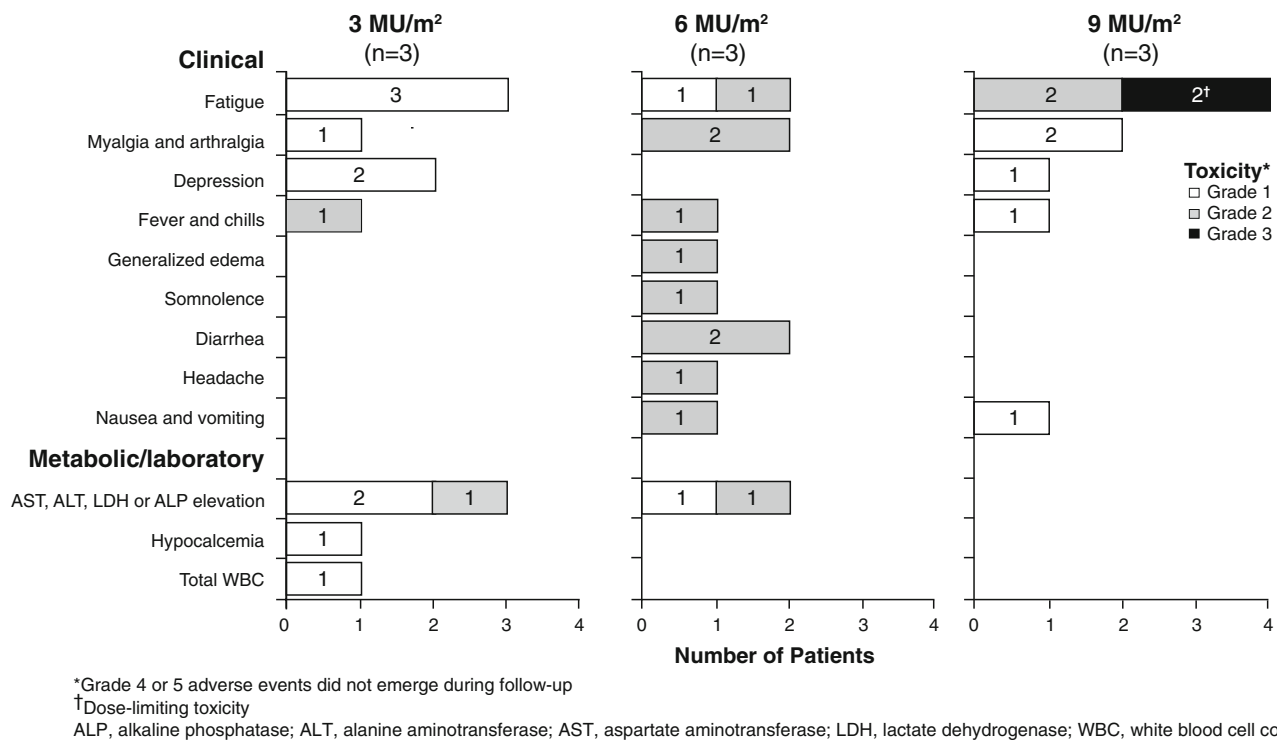


Fig. 1 Toxicities by IFN α_{2b} dose

lasting with progression-free survival of 167 weeks after surgery (each response calculated at the time of data analysis for this report). The two responders had an initial diagnosis of glioblastoma. The first individual had received radiation and concurrent temozolomide followed by 3 monthly cycles of temozolomide prior to progression. The therapy described in this study was used for the first imaging recurrence 7 months after initial diagnosis. The second responder had initial therapy with radiation alone 10.5 years before participation in this study. Treatment with ¹²⁵I brachytherapy was used at first recurrence 2 years after initial diagnosis. At second recurrence the patient began a monthly regimen of temozolomide. Four months later, the therapy described in this study was initiated. Median survival in the nine analyzed patients was 25.5 weeks and mean survival was 25.5 weeks. Six-month progression-free survival was 22% (2/9).

Correlative analyses

Immunohistochemical analysis of MGMT

Representative samples of each tumor were assessed for MGMT expression. Positivity of expression was defined as >10% of counted cells having the presence of MGMT reaction product. The analysis showed that 1 of 2 patients responding to this therapy was negative for MGMT. Only one of the nonresponders, a patient in the 9 MU/m² dose

group, was interpreted as negative for MGMT. The remaining eight patients were positive for MGMT.

Microarray gene expression profiling

The two responding cases and four randomly selected nonresponding cases were selected to assess the feasibility of this analysis. Of the 39,000 markers assessed on the original chip, 994 gene markers had a 2-fold difference and 202 gene markers had a ≥ 4 -fold difference in signal between responders and nonresponders ($P < 0.05$). Those in the group with a ≥ 4 -fold difference included genes known to possibly be important in glial neoplasm progression, such as *AKT*, and *FGF1*, as well as genes not generally associated with glial neoplasms [23, 24]. Despite careful searching, no obvious difference was observed between responders and nonresponders for genes related to interferon-alfa metabolism and signaling or resistance to alkylating drugs.

Discussion

In this population of relatively ill and heavily pretreated patients, the main observed toxicity of surgery, BCNU-wafer implantation, and IFN α_{2b} was fatigue. In fact, fatigue proved to be dose limiting at the highest level of treatment with IFN α_{2b} , 9 MU/m². Therefore, the maximum tolerated

dose of IFN α_{2b} within this multimodal treatment strategy was concluded to be 6 MU/m².

Fatigue is a well known problem with systemic administration of IFN α_{2b} [25], and thus, this observation in all dose groups and it eventually being the dose-limiting characteristic was not unexpected. Other toxicities observed during this study were consistent with previous reports of interferon-alfa therapy. [19, 26, 27]. Though this was a phase I study, two responses were observed, which is somewhat unexpected in such a small group of patients. Furthermore, these responses occurred at the lowest dose utilized (3 MU/m²) at which clinical toxicity was grade 1 or 2 and reversible over time. These responses have been durable, with no additional therapy needed since treatment.

Correlative analyses of MGMT expression and microarray gene expression were carried out and confirm the feasibility of such studies in this patient population. Neither the MGMT data nor the microarray data provided insights into the likelihood of treatment toxicity or response. This is likely because of the small patient numbers. However, the technical aspects of the analyses are straight forward and reproducible and could be useful in larger studies [28]. In the population under study, there clearly was variation in the information provided from tumor to tumor. Though significant differences in signal from a number of genes was noted between responders and nonresponders, that number was quite large and does not lend itself to any meaningful form of predictive pattern recognition. Although this study did not formally assess the relationship between immunomodulation with IFN α_{2b} and treatment response, the lack of change in the combined white blood count in these patients suggested that immunomodulation did not play a significant role in treatment response. In spite of the recognized limitation in this phase I report, these findings are promising as they suggest that with a larger population, data could be obtained that may be more informative.

Development of surrogates for determining whether or not a given tumor has a reasonable likelihood of responding to the regimen described here is a worthy goal. This phase I work has shown the feasibility of relatively simple analyses. A phase II study enrolling a greater number of individuals in whom MGMT, gene expression profiling or other assessments could be carried out is more likely to provide meaningful information about the value of such methods for predicting responsiveness to certain treatments. Ideally, a practitioner would be able to assess tumor tissue from an individual having undergone craniotomy for tumor debulking and BCNU wafer implantation and determine whether proceeding with treatment cycles of interferon alfa in one form or another would be beneficial.

The data here suggest the relative safety and potential value of this multimodal treatment with surgery, BCNU

wafers, and IFN α_{2b} in the setting of recurrent malignant glioma. The phase I nature of the work and the small number of patients preclude these data supporting the use of this treatment strategy outside of a treatment option or investigational context [29]. The mechanism of the benefit from such a combination remains to be determined. The proposed value of high local concentrations of BCNU [30] combined with the antiangiogenic or immunologic properties of IFN α_{2b} [16] are all possibilities. Whether these properties have any synergistic or additive effects on tumor control cannot be determined with the data in hand.

In conclusion, the data reported here support further assessment of surgery, BCNU wafers, and IFN α_{2b} for recurrent malignant glioma in a phase II study utilizing the maximum tolerated dose of 6 MU/m². Alternatively, as the responses were seen at the dose of 3 MU/m², it also would be reasonable to carry out a phase II study with this dose to decrease the likelihood of toxicity.

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