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ORIGINAL ARTICLE

A Phase III study of radiation therapy (RT) and O^6-benzylguanine + BCNU versus RT and BCNU alone and methylation status in newly diagnosed glioblastoma and gliosarcoma: Southwest Oncology Group (SWOG) study S0001

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Abstract
Aims To determine the efficacy of methylguanine methyltransferase (MGMT) depletion + BCNU [1,3-bis(2-chloroethyl)-1- nitrosourea: carmustine] therapy and the impact of methylation status in adults with glioblastoma multiforme (GBM) and gliosarcoma.
Methods Methylation analysis was performed on GBM patients with adequate tissue samples. Patients with newly diagnosed GBM or gliosarcoma were eligible for this Phase III open-label clinical trial. At registration, patients were randomized to Arm 1, which consisted of therapy with O^6-benzylguanine (O^6-BG) + BCNU 40 mg/m^2 (reduced dose) + radiation therapy (RT) (O6BG + BCNU arm), or Arm 2, which consisted of therapy with BCNU 200 mg/m^2 + RT (BCNU arm).
Results A total of 183 patients with newly diagnosed GBM or gliosarcoma from 42 U.S. institutions were enrolled in this study. Of these, 90 eligible patients received O^6-BG + BCNU + RT and 89 received BCNU + RT. The trial was halted at the first interim analysis in accordance with the guidelines for stopping the study due to futility (<40 % improvement among patients on the O6BG + BCNU arm). Following adjustment for

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stratification factors, there was no significant difference in overall survival (OS) or progression-free survival (PFS) between the two groups (one sided $p = 0.94$ and $p = 0.88$, respectively). Median OS was 11 [95 % confidence interval (CI) 8–13] months for patients in the O6BG + BCNU arm and 10 (95 % CI 8–12) months for those in the BCNU arm. PFS was 4 months for patients in each arm. Adverse events were reported in both arms, with significantly more grade 4 and 5 events in the experimental arm.

Conclusions The addition of O^6-BG to the standard regimen of radiation and BCNU for the treatment patients with newly diagnosed GBM and gliosarcoma did not provide added benefit and in fact caused additional toxicity.

Keywords Glioblastoma · SWOG · Methylation · MGMT · BCNU · Carmustine

Background

Glioblastoma multiforme (GBM) is the highest grade glial tumor and the most frequent type of primary malignant brain tumor in adults. Standard radiation therapy (RT) doubles median survival [1, 2], and the addition of chemotherapy plays a significant role in further enhancing patient longevity [3, 4]. In recent years, median overall survival (OS) of GBM patients has increased to 14.6 months with first-line therapy of radiation and temozolomide (TMZ) [3]. Over the past decade, certain tumor molecular and epigenetic characteristics, such as methylguanine methyltransferase (MGMT) methylation, have been identified as important predictive factors of patient survival and glioma response to the treatment [5–7]. It has long been recognized that approximately 30 % of patients with GBM respond favorably to alkylating chemotherapy [8, 9]. Later work has shown that this percentage correlates with promoter methylation of the MGMT enzyme, which repairs tumor DNA damaged by alkylating therapy [10]. Patients whose tumors lack MGMT methylation are less likely to respond to standard alkylating chemotherapy.

O^6-benzylguanine (O^6-BG), which is inert and nontoxic when administered alone, is a potent inhibitor of MGMT. In animal models of MGMT-active (nonmethylated), BCNU [1,3-bis(2-chloroethyl)-1- nitrosourea: carmustine]-resistant tumors, MGMT activity is inhibited for several hours after exposure of the animal to O^6-BG, during which time the tumor becomes highly sensitive to BCNU [11]. Likewise, MGMT-deficient human central nervous system (CNS) tumor-derived xenografts are more sensitive to alkylating drugs [12].

MGMT expression has been shown to play an important role in human CNS tumors. Several retrospective studies of patients with anaplastic gliomas who were treated on various protocols with RT and BCNU showed a strong correlation with low MGMT activity (stronger than other prognostic factors such as age) and improved survival [13]. Friedman and colleagues conducted a Phase I trial to define the presurgical dose required to deplete tumor MGMT activity in patients with malignant glioma and found that O^6-BG was not toxic when administered as a single agent [14]. Subsequently, Spiro et al. performed a dose escalation clinical trial in 30 patients to determine the dose of O^6-BG required to deplete alkyl guanine alkyltransferase (AGT; a previously used nomenclature for MGMT) to undetectable levels with acceptable toxicity. Sequential computed tomography (CT)-guided biopsies were performed before and 18 h after exposure to O^6-BG [15]. MGMT depletion below the level of detection was demonstrated at exposure levels of 120 mg/m^2; hence, the recommended dose of 120 mg/m^2 of O^6-BG was infused over 1 h in the Phase II trials.

Studies with O^6-BG combined with increasing doses of BCNU at 13.5, 27, 40, and 55 mg/m^2 established 40 mg/m^2 as the optimal dose of BCNU. Higher doses were associated with grade 3 and 4 myelosuppression (thromboytopenia and neutropenia) [16]. Depletion of AGT activity to undetectable levels in peripheral blood mononuclear cells occurs at lower doses and is not a predictor of tumor cell depletion [17]. A higher dose of BCNU is required to deplete AGT activity in tumor cells, compared to the dose that depletes activity in peripheral blood cells (and renders myelosuppression, the primary toxicity of BCNU "optimization").

Improved survival is correlated with low MGMT levels, and O^6-BG could be administered at doses without significant toxicity while effectively depleting MGMT. The aim of our study was to determine whether there is benefit to therapy of MGMT depletion + BCNU in patients with grade IV astrocytomas.

Methods

Patient eligibility

Patients from 42 U.S. institutions with a histologically confirmed diagnosis of GBM or gliosarcoma [World Health Organization (WHO) grade IV astrocytoma] were enrolled in the Southwest Oncology Group (SWOG) study S0001 (ClinicalTrials.gov Identifier: NCT00017147) between 2001 and 2005. Biopsy or surgical resection was required within 28 days prior to registration. Eligible patients were aged ≥18 years with a Zubrod performance status of ≤2. Documentation of adequate renal function [serum creatinine of ≤1.5× the institutional upper limit of normal

(ULN) or creatinine clearance of ≥60 ml/min] and of prothrombin time/partial thromboplastin time ratio of ≤120 % of the ULN was required within 28 days of registration. Documentation of pulmonary function [diffusing capacity of the lung (DLCO) ≥70 % of predicted] within 42 days of registration was also required.

Patients were excluded from the study if they had received or were currently receiving cranial radiation or chemotherapy outside of the protocol treatment or if there were three or more noncontiguous sites of tumor on T2 magnetic resonance imaging (MRI) or CT. Patients with known allergies to the study drugs, human immunodeficiency virus-positive status, or medical illnesses not adequately controlled with therapy were also ineligible. A history of prior malignancy (other than adequately treated basal cell or squamous cell skin cancer, in situ stage I or II cervical cancer in complete remission, or any other cancer from which the patient had been disease-free for ≥5 years) was a criterion for ineligibility. Pregnant or nursing women were not eligible for entry to the study, and women of reproductive potential needed to agree to effective contraception methods.

A post-operative MRI was required prior to registration for tumor removal that involved more than simple biopsy (pre-operative MRI was allowed for biopsy only). Patients unable to undergo MRI for medical reasons were eligible if they underwent a CT scan with intravenous contrast. Documentation of stable or decreasing corticosteroid usage was required prior to the preregistration MRI/CT.

All participating centers had formal institutional review board approval of the protocol, and all participants provided signed informed consent prior to registration and treatment.

Central pathology review for eligibility determination was performed by the Neuropathology Coordinator (EJR). Specifically, microscopic preparations, which consisted of hematoxylin and eosin (H&E) stained sections, were reviewed and classified according to WHO criteria [18].

Study design and treatment plan

At registration, patients were randomized by the SWOG Statistical Center to receive O^6-BG + low-dose BCNU + RT or BCNU (at standard dose) + RT (BCNU arm) using a dynamic balancing algorithm program stratified by age (<50 vs. ≥50 years), performance status (0–1 vs. 2), and surgery (biopsy only vs. resection). Treatment was begun within 5 working days of registration.

Patients were randomized to Arm 1, consisting of therapy with O^6-BG + BCNU + RT (O6BG + BCNU arm), or Arm 2, consisting of therapy with BCNU + RT (BCNU arm). Chemotherapy began concurrently with the RT. The Arm 1 treatment group received 40 mg/m^2 BCNU 6 h after the administration of 120 mg/m^2 O^6-BG intravenously over 1 h every 6 weeks. The Arm 2 group received BCNU 200 mg/m^2 intravenously over 1 h every 6 weeks. A maximum of seven treatment were allowed.

RT was administered once per day, 5 days per week with a linear accelerator using X-ray energy of at least 4 MV. The initial gross target volume (GTV1) was defined as T2 signal abnormality on a postoperative MRI. The boost GTV (GTV2) was defined by the resection cavity plus gadolinium enhancement on T1 MRI. Multiple conformal fields (without intensity modulation) were used, with field margins encompassing GTV1 and GTV2 by 2 and 2.5 cm, respectively. The initial volume was treated to 5,040 cGy in 28 fractions, and the boost volume was treated for an additional 1,080 cGy in six fractions (cumulative dose of 6,120 cGy). Doses were prescribed to the 100 % isodose at the isocenter, and each GTV was covered by at least the 95 % isodose. The central radiation review for adherence to treatment protocol was performed by the Radiation Therapy Study Coordinator (KJS).

Dose modifications were specified for hematologic, nonhematologic, and pulmonary toxicities as follows. BCNU was maintained for hematologic toxicity of CTC (common toxicity criteria) grade ≥2, until recovery to ≤ CTC grade 1. For CTC grade 3 toxicity resolving within 8 weeks of dosing, BCNU was continued without dose reduction once the CTC grade was ≤1. If the time to recovery of normal bone marrow function exceeded 8 weeks, the BCNU dose was reduced by 25 %. For hematologic CTC grade 4, the BCNU dose was reduced by 50 % (after recovery to CTC grade ≤1). Failure to recover to CTC grade 1 within 10 weeks of therapy mandated removal of the patient from the protocol treatment. Similar dose reduction criteria were dictated for nonhematologic toxicities: 25 % dose reduction of BCNU for CTC grade 2 toxicity delaying treatment for >2 weeks, and 50 % dose reduction for any CTC grade ≥3 toxicity. Patients were removed from the protocol treatment for clinical or radiographic evidence of pulmonary fibrosis or DLCO <50 % of the upper limit of normal. Radiation therapy was withheld for WBC <1,000/μl or platelets <20,000/μl until recovered.

Patients were removed from the study at completion of therapy (7 cycles), tumor progression (defined as a 25 % increase in the bidimensional sum of the tumor areas, clear worsening of evaluable, but not measurable disease, appearance of a new lesion, or reappearance of a prior lesion), unacceptable toxicities not manageable with dose reduction, delay in treatment of >4 weeks due to toxicity, administration of other antitumor treatment, or patient-initiated withdrawal for any reason. Patients were followed for 5 years after randomization or until death.

MGMT promoter methylation assay

Unstained slides prepared from formalin-fixed paraffin-embedded tissue blocks were used for MGMT activity assays. H&E stained slides were marked by a neuropathologist (EJR) to demarcate the tumor from uninvolved tissue on each slide. DNA was isolated from archived paraffin-embedded formalin-fixed unstained slides from tumor areas identified by the pathologist, subsequently treated with bisulfite, and then assayed by methylation-sensitive PCR using bisulfite- and methylation-sensitive DNA primers [19]. The original aim of the study was to analyze MGMT expression by immunohistochemistry (IHC) staining; however, this method was subsequently found to be less reliable than PCR analysis [20]. IHC assays may be difficult to interpret due to "noise" from non-neoplastic cells in the tumor sections, including endothelial cells, lymphocytes, and macrophages, which normally express MGMT and can alter the results.

Statistical analysis

The primary endpoint was OS. Assuming a 12-month median survival of patients receiving standard therapy, a total enrollment of 375 patients was expected to show a 40 % improvement with the addition of O^6-BG (Arm 1) to the therapy for a one-sided 0.05 level test with 92.5 % power.

Interim analysis was planned after the entrance of half the patients into the study, and a second interim analysis was planned after complete accrual. Early termination of the trial and the conclusion that the O6BG + BCNU arm is not superior would occur if the alternative hypothesis of a 40 % improvement in survival with the combination arm was rejected at the 0.005 level using an extension of the log-rank test.

All eligible patients were included in the survival analyses by assigned treatment according to the intent-to-treat principle. Patients who were never treated were not included in the toxicity analyses. All survival curves and estimates were calculated using the Kaplan–Meier product limit method [21].

Response was assessed in a post hoc fashion by the study chair for all patients who showed evidence of measurable or nonmeasurable disease using the Macdonald criteria [22]. Additional analysis of the prognostic value of MGMT expression as assessed by immunohistochemical assay of MGMT on OS and progression-free survival (PFS) was planned. Subgroup analysis (based on MGMT levels) of treatment groups was also planned. Based on data from SWOG study-9218, an assumption of 70 % Mer expression was expected, and statistical models and sample sizes were calculated accordingly [8].

Results

This study was terminated in November 2005, at the time of the initial interim analyses, per recommendation of the Data and Safety Monitoring Committee. The hypothesis that O^6-BG added benefit to therapy with BCNU + RT was judged to be untenable. A 40 % OS improvement in the O6BG + BCNU arm was ruled out ($p = 0.002$). At the time of study closure, 183 patients were registered. Four patients were ineligible due to prior diagnosis of low-grade brain tumors (2 patients), surgery more than 28 days prior to registration (1 patient), and pre-study MRI done without contrast (1 patient). Patient characteristics were balanced between the two treatment arms (Tables 1, 2).

The best response data are shown in Table 1, and graphs of OS and PFS are represented in Fig. 1. The patients receiving BCNU + RT (Arm 2) had a median PFS of 4 [95 % confidence interval (CI) 4–5] months and OS of 10 (95 % CI 8–12) months.

Patients in the O6BG + BCNU group had a median PFS of 4 (95 % CI 4–5) months and OS of 11 (95 % CI 8–13) months.

The one-sided tests of differences between arms were not significant for OS ($p = 0.94$ adjusted for stratification factors of age, performance status, and type of surgery) or for PFS ($p = 0.88$ adjusted for stratification factors). The BCNU/O6BG+BCNU hazard ratio for OS was 0.77 (99 % CI 0.51–1.18; i.e., upper bound of improvement due to addition of O^6-BG was 18 %). The BCNU + RT/O^6-BG + BCNU + RT hazard ratio for PFS was 0.83 (99 % CI 0.54–1.26).

MGMT analysis

Tissue from 84 GBM patients was evaluated for methylation status of MGMT. Results were obtainable in 41 patients (49 %), with one patient ultimately being ineligible, leaving tissue samples from 40 patients for evaluation. Thirteen samples (33 %) were found to be methylated and 27 (67 %) were unmethylated.

Regardless of treatment arm, patients with methylated DNA had a median OS of 13 (95 % CI 8–16) months and median PFS of 4 (95 % CI 3–6) months. Patients with unmethylated MGMT had an OS of 11 (95 % CI 9–13) months and median PFS of 3 (95 % CI 3–5) months.

OS and PFS by methylation status and arm are shown in Table 3.

Treatment delivery and tolerability

Of the 90 patients eligible to receive treatment with O^6-BG + BCNU + RT (Arm 1), ten patients were removed from the arm due to adverse events, primarily hematologic

Table 1 Patient, tumor, and treatment characteristics, and best response per treatment arm

Patient characteristics	Study arm	
	Arm 2: BCNU + RT ($n = 89$)[a]	Arm 1: O^6-BG + BCNU + RT ($n = 90$)[a]
Age (years)	56 (24–76)	55 (19–73)
≤50 years	25 (28 %)	27 (30 %)
>50 years	65 (72 %)	62 (70 %)
Sex		
Male	56 (62 %)	51 (57 %)
Female	34 (38 %)	38 (43 %)
Race		
White	88 (98 %)	87 (98 %)
Black	1 (1 %)	1 (1 %)
Asian	0 (0 %)	1 (1 %)
Native American	1 (1 %)	0 (0 %)
Ethnicity		
Non-Hispanic	85 (94 %)	84 (94 %)
Hispanic	2 (2 %)	2 (2 %)
	3 (3 %)	3 (3 %)
Performance status		
0–1	79 (88 %)	77 (87 %)
2	11 (12 %)	12 (13 %)
Type of initial surgery stratification		
Biopsy only	20 (22 %)	16 (18 %)
Resection	70 (78 %)	73 (82 %)
Institutional report		
Excisional biopsy	20 (22 %)	16 (18 %)
Partial resection	29 (32 %)	32 (36 %)
Complete resection	38 (42 %)	36 (40 %)
Other	0 (0 %)	4 (4 %)
Not reported	3 (3 %)	1 (1 %)
Central review of operative report[b]		
Biopsy only	20 (22 %)	13 (15 %)
Sub-total resection	52 (58 %)	50 (56 %)
Gross total resection	17 (19 %)	22 (25 %)
Insufficient information	1 (1 %)	4 (4 %)
Subsequent surgery	3 (3 %)	7 (8 %)
Histology		
Glioblastoma multiforme	88 (98 %)	88 (99 %)
Gliosarcoma	0 (0 %)	0 (0 %)
Other	2 (2 %)	1 (1 %)
Hemisphere of tumor		
Right	42 (47 %)	43 (48 %)
Left	41 (46 %)	39 (44 %)
Both	2 (2 %)	5 (6 %)
Midline	5 (6 %)	0 (0 %)
Infratentorial	0 (0 %)	1 (1 %)
Not reported	0 (0 %)	1 (1 %)
Baseline MMSE score[c]		
30	24 (29 %)	37 (45 %)
27–29	36 (44 %)	31 (37 %)
<26	22 (27 %)	15 (18 %)
Unknown		
Disease status at baseline[b]		
Measurable	32 (36 %)	28 (32 %)
Non-measurable	49 (55 %)	57 (65 %)
Unknown	9 (10 %)	4 (5 %)
Best response[b, d]		
Complete response	1 (1 %)	0 (0 %)
Partial response	9 (11 %)	4 (5 %)
Stable disease	25 (31 %)	33 (39 %)
Increasing disease	33 (41 %)	27 (32 %)
Not assessable	13 (16 %)	21 (25 %)

Data are reported as the number of patients, with the percentage in parenthesis, with the exception of the data on"Age", which are presented as the mean with the range in parenthesis

[a] Arm 1 consisted of therapy with O^6-BG (O^6-benzylguanine) + BCNU [1,3-bis(2-chloroethyl)-1- nitrosourea: carmustine] + radiation therapy (RT) (O6BG + BCNU arm); Arm 2 consisted of therapy with BCNU + RT (BCNU arm)

[b] Ascertained by Study Chair by post hoc chart review of clinical notes and imaging reports

[c] Maximum score on the Mini-Mental Stat Examination (MMSE) is 30, and scores of >26 are considered to indicate normal mental status

[d] Among patients with measurable or non-measurable disease

in nature. Four patients had major protocol deviations (two due to chemotherapy dosing errors and two due to deviations in RT). Assessment of these 90 patients for adverse events revealed three treatment-related deaths (sepsis, febrile neutropenia, renal failure and acute respiratory distress syndrome). Grade 4 toxicities, primarily hematologic events, were present in an additional 45 patients (Table 4).

Of the 89 patients eligible to receive treatment with BCNU + RT, seven refused the protocol treatment, two experienced major protocol deviations with regard to RT, and one received a decreased dose chemotherapy. Nine patients were removed from the treatment protocol due to toxicity. A total of 82 patients treated with BCNU + RT were assessed for toxicity, with four treatment-related deaths (infection, acute respiratory distress syndrome, and sudden death possibly related to infection). Eighteen additional patients had grade 4 toxicities.

Grade 4 or higher toxicities were seen in 53 % of the patients in the O6BG + BCNU arm compared to 27 % in the BCNU arm (χ^2: $p = 0.0004$), primarily hematologic. There was no difference between arms with respect to grade 4 and 5 non-hematologic events (χ^2: $p = 0.40$).

Discussion

Historically, approximately one-third of GBM patients seem to benefit from treatment with alkylating chemotherapy. This minority may correspond to the tumor status of O^6-MGMT activity, as this enzyme repairs tumor DNA damaged by chemotherapy and allows tumors to progress after exposure to treatment. Patients with inactive MGMT are more likely to respond favorably to treatment with alkylating agents, as tumor DNA is not repaired by the enzyme [23].

Since hypermethylation of the MGMT promoter region had been shown to be associated with improved outcome and treatment response in GBM patients, we attempted to exogenously influence the MGMT-methylation status. Our hypothesis was that the addition of O^6-BG to the treatment protocol would render unmethylated tumors functionally "methylated" and thereby improve response to alkylating chemotherapy with BCNU. At its inception, this Phase III study was designed to accrue 375 patients; however, the study was halted at the interim analysis due to negative results.

Although the treatment protocol that included O^6-BG proved to be nonbeneficial to GBM patients, there may still be an application for O^6-BG at the proper dose and in the appropriate clinical setting.

We may have been limited in showing the efficacy of the treatment by using too small a dose. BCNU dosing was limited by systemic toxicity (primarily myelosuppression) and capped at a maximum of seven cycles due to concerns of pulmonary and bone marrow toxicities from cumulative nitrosourea toxicity [24–26]. Although many pathways of cell survival and repair mechanisms are involved in the process of GBM proliferation, the strongest pathway confirmed prospectively is the methylation status of MGMT [27]. For more than 60 % of GBM patients with unmethylated MGMT, the optimal strategy to improve outcome would be to effectively change their biologic functional status to that of their methylated counterparts. An important consideration is that the maximum effective alkylator dose used in combination with O^6-BG is limited by systemic toxicities.

Nitrosoureas are known to cause delayed and cumulative myelosuppression. These compounds affect the desired chemotherapeutic result through DNA cross-linking by alkylation, which destabilizes tumor cells. The use of lipid-soluble nitrosoureas, such as carmustine (BCNU), may lead to tumor response in some patients (particularly those with hypermethylation of MGMT) by this alkylating mechanism. While carbamoylation may be the reason for other organ toxicities (such as pulmonary fibrosis) seen cumulatively with BCNU exposure, alkylation of myeloid cell DNA is the likely mechanism responsible for the myelosuppressive toxicity from BCNU, via a decrease of glutathione reductase enzyme function in the bone marrow cells [28, 29]. In vitro and in vivo brain tumor models have shown this myeloid cytotoxicity to be increased by at least two-fold when pretreatment with O^6-methylguanine preceded nitrosourea exposure [30]. Hence, while we hoped for improved tumor cell response, we were limited by the cytotoxicity of the BCNU/O^6-BG combination, manifested by an unacceptable rate (nearly 50 %) of grade 4 hematologic toxicity in this study.

Carmustine wafers implanted into the surgical cavity was studied as a strategy to avoid systemic toxicity by combining O^6-BG with a local therapy, with promising results [31, 32]. However, this approach was associated with complications, including cerebrospinal fluid (CSF) leak and CNS/CSF infections [32].

The rationale for the addition of O^6-BG to alkylating chemotherapy in order to overcome MGMT resistance in malignant gliomas should apply to TMZ as well. A Phase II trial using TMZ with O^6-BG in the setting of recurrent TMZ-resistant malignant glioma demonstrated that anaplastic glioma, but not GBM, did show some response to the combination, with nearly 50 % of patients developing

Table 2 Characteristics of pre-surgery magnetic resonance imaging[a]

Characteristics	Study arm	
	Arm 2: BCNU+RT ($n = 89$)	Arm 1: O^6BG+ BCNU+RT ($n = 89$)
Evidence of necrosis		
Yes	59 (68 %)	50 (60 %)
No	28 (32 %)	33 (40 %)
Evidence of cyst/cystic		
Yes	14 (16 %)	17 (20 %)
No	71 (84 %)	66 (80 %)
Evidence of hemorrhage/blood		
Yes	9 (11 %)	8 (10 %)
No	76 (89 %)	75 (90 %)

Data are reported as the number of patients, with the percentage in parenthesis

[a] Ascertained by Study Chair

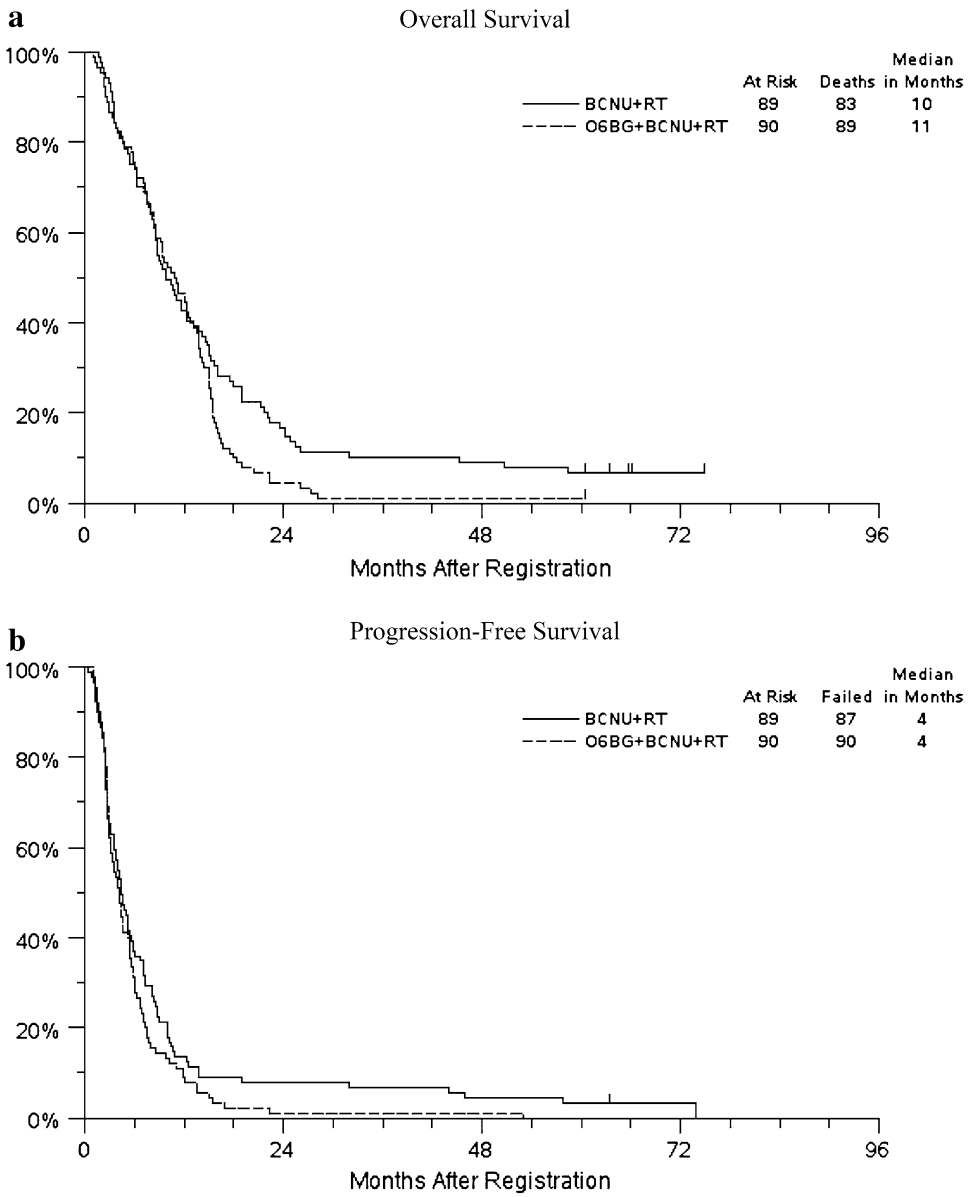

Fig. 1 Results for overall survival (**a**) and progression-free survival (**b**) in patients treated with bis-chloroethylnitrosourea (carmustine) + radiation therapy (Arm 2: *BCNU+RT*; *solid line*) and patients treated with O^6-BG (O^6-benzylguanine) + BCNU + RT (Arm 1:*O6BG+BCNU+RT*; *dotted line*). Median overall survival was 10 and 11 months for patients in Arm 2 and Arm 1, respectively (not significantly different); mean PFS was 4 months for both groups of patients

Table 3 Overall survival and progression-free survival by methylguanine methyltransferase methylation status and study arm

Study arm	Overall survival		Progression-free survival	
	Methylated status ($n = 13$)	Unmethylated status ($n = 27$)	Methylated status ($n = 13$)	Unmethylated status ($n = 27$)
Arms combined	13 (8–16) months	11 (9–13) months	4 (3–6) months	3 (3–5) months
O6BG + BCNU (Arm 1)	13 (9–14) months; $n = 9$	13 (8–14) months; $n = 14$	4 (4–6) months; $n = 9$	3 (3–4) months; $n = 14$
BCNU (Arm 2)	19 (3–19) months; $n = 4$	10 (8–11) months; $n = 13$	5 (1–5) months; $n = 4$	4 (3–8) months; $n = 13$

grade 4 hematologic toxicity [33]. More recent studies have shown that there may be an advantage to combining additional agents, concurrently or in rotation, with carmustine and O^6-BG for optimal anti-tumor effect [31, 34, 35].

Conclusion

Based on the doses and treatments given to this cohort of patients, the results of SWOG-S0001 do not support the

Table 4 Adverse events

Adverse event	Arm 1: O^6BG + BCNU + RT ($n = 90$)							Arm 2: BCNU + RT ($n = 82$)						
	Grade							Grade						
	Unknown	0	1	2	3	4	5	Unknown	0	1	2	3	4	5
Adverse drug reaction	0	90	0	0	0	0	0	0	81	0	0	0	0	1
Cardiovascular	0	67	10	10	3	0	0	0	63	4	10	4	1	0
Clotting	0	89	0	0	1	0	0	0	82	0	0	0	0	0
Dermatologic	0	38	24	27	1	0	0	0	33	30	16	3	0	0
Ear	0	85	2	2	1	0	0	0	72	9	1	0	0	0
Endocrine	0	85	0	5	0	0	0	0	78	1	3	0	0	0
Eye	0	70	6	14	0	0	0	0	71	3	7	1	0	0
Flu-like symptoms	0	27	23	31	8	1	0	0	23	26	28	5	0	0
Gastrointestinal	0	28	32	28	2	0	0	0	27	31	20	3	1	0
Hematologic	0	2	4	7	34	43	0	0	2	12	24	30	14	0
Hemorrhage	0	79	7	3	1	0	0	0	79	2	0	1	0	0
Immunological	0	87	1	2	0	0	0	0	81	1	0	0	0	0
Infection	0	57	2	5	17	7	2	0	69	0	4	6	1	2
Liver	0	44	34	7	3	2	0	0	61	12	7	2	0	0
Lung	0	57	10	13	8	1	1	1	61	4	8	5	2	1
Metabolic	0	57	21	4	6	2	0	0	64	13	2	2	1	0
Musculoskeletal	0	79	3	7	0	1	0	0	76	2	2	2	0	0
Neurologic	0	42	11	24	12	1	0	2	40	6	17	14	3	0
Pain	0	36	23	24	7	0	0	1	38	19	16	7	1	0
Renal/bladder	0	74	10	4	1	0	1	0	76	4	1	0	1	0
Sexual/reproductive function	0	87	0	1	2	0	0	0	81	0	1	0	0	0
Maximum grade any adverse event number	0	0	0	9	33	45	3	0	0	1	24	35	18	4

Adverse events unlikely or not related to treatment excluded

hypothesis that extrinsic depletion of MGMT renders GBM more sensitive to alkylating therapy with BCNU.

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Conflict of interest The authors declare that they have no conflict of interest.

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