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ORIGINAL ARTICLE

O^6-benzylguanine and BCNU in multiple myeloma: a phase II trial

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Abstract
Purpose Carmustine (BCNU) is known to have modest activity in multiple myeloma; however, resistance to BCNU manifests by the activity of O^6-methylguanine methyltransferase (MGMT). The objective of this study was to determine the safety and efficacy of depletion of MGMT activity in plasma cells using O^6-benzylguanine (O^6-BG) with BCNU in patients with multiple myeloma.
Methods Patients with previously treated or untreated multiple myeloma were eligible. Cycles of O^6-BG at a dose of 120 mg/m^2 and BCNU at a dose of 40 mg/m^2 were repeated every 6 weeks.
Results Seventeen patients were enrolled on the study, with a median follow-up of 24.5 (range 5–69) months. One complete response (7%) and 3 partial responses (20%) were observed. Nine patients (60%) had stable disease. Bone marrow studies demonstrated 94% depletion of MGMT activity in CD38+ marrow cells. The most frequent grade 3 and 4 adverse events were neutropenia (71%), lymphocytopenia (53%), and thrombocytopenia (53%).
Conclusions Chemotherapy utilizing the MGMT inhibitor O^6-benzylguanine and BCNU results in inhibition of MGMT activity in malignant plasma cells and produces meaningful responses in a modest proportion of patients with multiple myeloma. Hematologic toxicity with this regimen is significant and dose-limiting.

Keywords O^6-benzylguanine · Methylguanine methyltransferase · BCNU · Multiple myeloma

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Introduction

Multiple myeloma (MM) is the second most-common hematologic malignancy, and was estimated to affect approximately 16,000 patients in the United States in 2005 and result in approximately 11,000 deaths [19]. Despite many therapeutic advances, it remains incurable, with a median survival of three years in patients receiving chemotherapy [12].

Nitrosoureas and related alkylating agents have a broad spectrum of antitumor activity, but resistance can be intrinsic or acquired. The nitrosourea carmustine (BCNU) has long been known to have some activity in myeloma. When combined with prednisone it produces response rates (20–40%) and survival (30 months) similar to treatment with melphalan and prednisone [20]. As a single agent in patients with relapsed disease, it produces response rates of 5–15% [20]. The cytotoxic action of BCNU occurs through the formation of alkyl DNA adducts at the O^6 position of guanine, which produce cross-links that disrupt DNA synthesis [21]. However, resistance to BCNU is common, and

is in large part mediated by DNA repair activity [13]. The DNA repair suicide enzyme O6-methylguanine methyltransferase (MGMT) mitigates toxicity of BCNU by repairing adducts at the O^6 position of guanine [7]. This process irreversibly inactivates MGMT [16], making it a desirable target for biochemical modulation. MGMT is expressed in most normal human tissues, but is overexpressed in all types of human tumors, including colon cancer, glioma, lung cancer, breast cancer, leukemias, lymphomas, and myeloma [14]. Previous studies have shown that O^6-benzylguanine (O^6-BG) is a potent MGMT-inactivating agent, which renders tumor cells more sensitive to BCNU in non-toxic doses [9]. In addition, in vitro studies demonstrated that a MM cell line that manifests reduced MGMT activity was hypersensitive to BCNU, thus providing rationale for the clinical use of combination O^6-BG and BCNU [6, 8].

Our institution has completed a phase I clinical trial in patients with advanced solid tumors in which MGMT activity was depleted with O^6-BG prior to treatment with BCNU [21]. This trial employed pharmacodynamic endpoints to monitor drug activity at a cellular level, including assays for MGMT activity in tumor biopsies, peripheral blood mononuclear cells (PBMCs), and pharmacokinetic monitoring. A non-toxic dose of 120 mg/m^2 of O^6-BG completely inhibited tumor tissue MGMT. Combined with O^6-BG, this trial determined the maximum tolerated dose (MTD) of BCNU to be 40 mg/m^2, with the dose-limiting toxicities primarily related to myelosuppression. Here we present the results of a multicenter open-label phase II clinical trial conducted to assess the efficacy of O^6-BG in combination with BCNU in patients with multiple myeloma, either previously treated or untreated, coupled with a determination of biochemical measurement of MGMT depletion in malignant plasma cells to evaluate the therapeutic strategy of knocking out a major drug resistance pathway in myeloma.

Patients and methods

Patient selection and eligibility

Patients were eligible to enroll in the study if they had previously treated or untreated multiple myeloma with measurable disease, defined as any detectable monoclonal immunoglobulin on serum or urine protein electrophoresis, or other measurable disease in patients with nonsecretory myeloma. No more than one previous chemotherapy regimen containing an alkylating agent was allowed. At least 4 weeks were required to have elapsed since the last cycle of chemotherapy for patients who had prior chemotherapy. Eligible patients had a white blood cell count >3,500/μl, absolute neutrophil count >1,500/μl, platelet count >100,000/μl, hemoglobin concentration more than 9 g/dl, ALT and AST $\leq 2x$ the upper limit of normal, serum bilirubin $\leq 1.5x$ the upper limit of normal, serum creatinine <2.0 mg/dl, and serum calcium < 14 mg/dl. Adequate pulmonary function with a corrected DLCO $\geq$ 60% of predicted, ECOG performance status of 0–2, and life expectancy greater than 12 weeks were also required. Patients who were pregnant or lactating, had uncontrolled diabetes mellitus, or who had previously had pelvic irradiation or irradiation involving >25% if the bone marrow space were excluded. All patients of child-bearing or child-fathering potential were required to use adequate contraception. Written informed consent was obtained from all patients prior to enrollment on the study. Approval of the study and consent form by the institutional review boards of the participating institutions was obtained in accordance with federal regulations.

Treatment regimen

Administration schedule

O^6-BG was given at a dose of 120 mg/m^2 IV over 1 h. BCNU was given 2 h after the start of O^6-BG at a dose of 40 mg/m^2 IV over 1 h. Treatment cycles were repeated every 6 weeks, and treatment was continued for at least 2 cycles beyond attainment of best response. Patients were hospitalized for cycle 1 only, for limited pharmacokinetic sampling.

Toxicity management

Patients were allowed retreatment as long as toxicities had recovered to ≤grade 1 for thrombocytopenia, ≤grade 2 for neutropenia, and ≤grade 1 for non-hematologic toxicity, according to the National Cancer Institute Common Toxicity Criteria (version 2.0). Up to two dose reductions for toxicity were allowed, and patients with ≥grade 2 neutropenia despite two dose reductions were administered G-CSF or pegylated G-CSF. Dose delay of up to two weeks was allowed for resolution of cytopenias. Antiemetics were used to manage nausea/emesis. Blood product transfusions and erythropoietin were administered at the treating physician's discretion.

Criteria for removal from study

Patients were removed from the study for any of the following: tumor progression, hematologic toxicity ≥grade 4 after two dose reductions and use of G-CSF or not resolving within a 4 week treatment delay period, treating physician's discretion, or patient request.

Response and toxicity assessments

The primary endpoint was the overall rate of response to the combination of O^6-benzylguanine and BCNU. Evaluation of response occurred after every cycle, unless the bone marrow was the sole measure of disease, in which case assessment of response was made after every 2 cycles.

Modified ECOG response and progression criteria were used. An objective response required a ≥50% decrease in serum monoclonal paraprotein and 24 h urinary light chain excretion (≥90% decrease was required if response was based solely on urine myeloma protein). A similar reduction in soft tissue plasmacytomas and the absence of new lytic bone lesions, if present, were also required for response. Paraprotein responses were verified on 2 consecutive determinations at least 2 weeks apart. A complete response (CR) was characterized by complete disappearance of myeloma protein from the serum and urine confirmed by electrophoresis, normal quantitative immunoglobulins, and a bone marrow biopsy demonstrating ≤3% plasma cells. A near-complete-response (NCR) was a response meeting criteria for a complete response, except lacking a repeat bone marrow biopsy, or with evidence of residual myeloma on bone marrow biopsy (>3–6% plasma cells or a marrow aspirate or biopsy showing sheets or clusters of malignant plasma cells). A partial response (PR) was an objective response that did not meet criteria for CR or NCR. For non-secretory myeloma, $a \geq 50\%$ decrease in bone marrow plasma cells was required to define a partial response. Disease progression was defined as a 50% increase in the serum or urine myeloma protein over the lowest response level on two separate determinations, the appearance of new lytic bone lesions, or an increase in the size of existing lesions by at least 50%. In addition, hypercalcemia, a hemoglobin decrease of 2 gm/dl (not due to chemotherapy), an increase of bone marrow plasma cell percentage of >50%, or generalized bone pain in patients who met only the serum or urine paraprotein criteria above also constituted progressive disease. Disease that did not meet criteria for any response or progression as defined above was considered stable disease. Adverse events were assessed at each visit and graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0).

Measurement of biochemical effect

All patients had a pretreatment bone marrow aspirate and biopsy analyzed for MGMT activity. Three patients also had a bone marrow biopsy performed 18 h after O^6-BG to assess depletion of MGMT, according to the method previously described [21]. Briefly, CD38+ cells were selected from the marrow aspirates using immunomagnetic beads. CD38+ cell extracts were incubated with DNA substrate containing O^6-methylguanine (O^6mG) and N^7-methylguanine (N^7mG) adducts that were generated by methylating calf thymus DNA with *N*-[^{3}H]methyl-*N*-nitrosourea. The [^{3}H]O^6mG and [^{3}H]methyl-N^7-guanine bases in reactant were separated by HPLC and quantified by liquid scintillation. MGMT activity was measured by the amount of [^{3}H]methyl group removed from [^{3}H]O^6mG adducts. Pre- and post- O^6-BG samples were compared for MGMT activity level, and successful depletion of MGMT was defined by post-O^6-BG MGMT levels of less than 1 fmol/μg DNA.

Statistical methods

The primary endpoint for this study was objective response rate. The secondary endpoint was toxicity. Response rate and incidence of toxicity were estimated using binomial distribution theory and their confidence intervals were estimated using Wilson's method [5]. The difference of MGMT activity performed before treatment and 18 hours following administration of O^6-BG was tested by paired *t* test; a P value <0.05 was considered significant.

Results

Seventeen patients were enrolled on the study between January 2000 and October 2003. All patients were evaluable with respect to toxicity; two patients had insufficient follow-up data to assess response. Baseline characteristics are shown in Table 1. 53% (9/17) of patients met criteria for Durie-Salmon stage III at the time of enrollment. All had an ECOG performance status of 0–2. The majority of patients (53%) had received one prior therapy for their multiple myeloma. Prior regimens included mephalan-prednisone, doxorubin liposomal–vincristine–dexamethasone (DVD), vincristine–adriamycin–dexamethasone (VAD), single agent dexamethsone, and thalidomide–dexamethasone. The median follow-up was 24.5 (range 4–69) months for all patients and 39 (range 5–69) months for survivors.

Response

Fifteen patients were evaluable for response. Patient responses included one complete response and three partial responses, representing an overall response rate of 27% (95% CI: 0.11–0.52) (Table 2). These responses all occurred in patients that had not received prior chemotherapy for multiple myeloma. Nine patients achieved stable disease, including five patients who had received prior chemotherapy for multiple myeloma, for an overall clinical benefit of 87% (95% CI: 0.62–0.96). One of these patients did demonstrate a slight increase in serum paraprotein following the first

Table 1 Patient characteristics

Characteristic		Percentage
Age (median)	57	
Min	38	
Max	78	
Sex (Percentage of male)	8	47
Race		
Caucasian	9	53
African American	7	41
Middle Eastern	1	6
ECOG performance status		
0	8	47
1	7	41
2	2	12
Prior chemotherapies		
0	8	47
1	9	53
IgG paraprotein	12	71
IgG < 5.0 g/dl	10	59
IgA < 3.0 g/dl	3	18
Beta-2 microglobulin > 2.5 mg/l	10	59
Hemoglobin > 10.0 g/dl	14	82
Plasma calcium < 12 mg/dl	17	100
Serum creatinine > 1.5 g/dl	3	18
Durie–Salmon stage III	9	53

Min minimum, *Max* maximum, *ECOG* Eastern Cooperative Oncology Group

Table 2 Best response for patients receiving O^6-BG/BCNU

Best response	No. of cases	Rate (95% CI)[a]
CR	1	0.07 (0.007, 0.27)
PR	3	0.2 (0.07, 0.45)
SD	9	0.6 (0.36, 0.8)
PD	2	0.13 (0.037, 0.38)
Unassessable	2	
CR + PR	4	0.27 (0.11, 0.52)
CR + PR + SD	13	0.87 (0.62, 0.96)

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

[a] The denominator for calculating the rate is 15 (excluding two cases not evaluable for response)

cycle of treatment, but then achieved stable disease for an additional 11 cycles before disease progression. The remaining two patients had no response to therapy.

Dose administration and toxicity

A total of 59 cycles of O^6-BG and BCNU were administered. The median number of cycles administered per patient was 3 (Table 3). Grade 3–4 toxicity was primarily hematologic (Table 4). Treatment delays for hematologic toxicities were common, occurring in 9 (53%) of patients, and 41% (7/17) had hematologic toxicity requiring at least one dose-reduction during the trial. G-CSF was administered to five (29%) patients due to recurrent grade 3 or 4 neutropenia despite two dose reductions. One patient developed an infectious pneumonia, which responded to appropriate treatment. Anemia severe enough to require exogenous erythropoietin administration occurred in 10 (59%) patients. Six (35%) patients were removed from the study due to persistent adverse hematologic events. No patients were discontinued from the study for non-hematologic toxicities.

MGMT depletion

During protocol development, we determined the MGMT activity at the time of initial diagnosis in six patients with active myeloma and seven normal donors. In the mononuclear fraction from the marrow aspirate, the mean MGMT

Table 3 Treatment summary for CWRU 1A96

O6-BG cycles		Percentage
Median	3	
Min	1	
Max	13	
Total	59	
Patients requiring treatment delays	9	53
Patients requiring dose reduction	7	41
Patients requiring G-CSF	5	29
Patients requiring erythropoietin	10	59
Off-study for hematologic toxicity	6	35

Min minimum, *Max* maximum

Table 4 Grade 3–4 toxicity observed during CWRU 1A96

Grade 3–4 toxicities	Number	Percentage
Nonhematologic		
Fatigue	1	6
Anorexia	1	6
Infection	1	6
Hyponatremia	1	6
GGT	1	6
Hematologic		
Neutropenia	12	71
Lymphopenia	9	53
Anemia	6	35
Thrombocytopenia	9	53

GGT gamma glutamyl transpeptidase

Table 5 MGMT activity in CD38+ marrow cells before and after treatment with O^6-BG. There was significant reduction of MGMT activity after treatment ($P = 0.024$)

Patient number	MGMT activity pre-O^6-BG[a]	MGMT activity post-O^6-BG[a]	Percentage of decline in MGMT activity after O^6-BG
1	5.7	0.25	96
2	9.2	0.3	97
3	6.4	0.4	94

O^6 -BG, O^6 -benzylguanine

[a] MGMT activity expressed as fmol/microgram of cellular DNA

activity in patients was 10.3 ± 2.6 fmol/μg DNA versus 3.0 ± 1.6 fmol/μg DNA in normal donors. Since the myeloma samples might include a mixture of normal hematopoietic cells and plasma cells, analysis of MGMT activity in patients placed on this study was performed on separated CD38+ cells to enrich for plasma cells.

The first three patients enrolled in this study had bone marrow aspirates and biopsies for MGMT activity performed before treatment and 18 hours following administration of O^6-BG. The results of these assays are shown in Table 5, and demonstrate near-complete depletion of MGMT activity following O^6-BG ($P = 0.024$).

Duration of response

The duration of best response for the patient with a complete response was 44 months. The median duration of best response for the patients with partial responses was 9 (range 6–10) months. Patients with stable disease had a median duration of response of 5 (range 3–22) months.

Discussion

This paper represents the first report of a clinical trial utilizing MGMT depletion with O^6-benzylguanine to augment the cytotoxic activity of an alkylating agent in patients with multiple myeloma. The results demonstrate proof-of-principle that MGMT depletion can be achieved in vivo in CD38+ malignant plasma cells in patients with multiple myeloma. Furthermore, they suggest that MGMT depletion in multiple myeloma may overcome tumor cell resistance to alkylating agents, such as BCNU, based on a response rate somewhat higher than published responses for single agent BCNU in myeloma [20]; however, the small sample size, the heterogeneity of patients enrolled in this study, and comparison to historical controls limit the ability to determine a causal relationship between MGMT depletion and response to BCNU.

Over 25% of patients in this study did have an objective response, including one complete response, despite the fact that study subjects were not selected based on pre-treatment MGMT activity. However, these response rates are considerably lower than those reported for untreated patients using regimens available at the time this study was conceived, such as melphalan/prednisone [15] and vincristine, doxorubicin, and dexamthasone (VAD) [1], as well as newer approaches, including thalidomide/dexamethasone [18] and bortezomib [11]. All of the objective responses observed in this study occurred in previously untreated patients, and only half of these patients had an objective response. The lack of any objective response in previously treated patients may reflect differential expression of MGMT activity in previously treated versus untreated patients, or it may represent bias introduced by the small number of patients enrolled.

Overall efficacy conclusions reached from this study are somewhat limited due to the small number and heterogeneity of patients enrolled, as well as less stringent response criteria than observed in many contemporary studies. This study was designed prior to the advent of myeloma therapy utilizing biological response modifiers such as thalidomide and bortezomib, and accrual dropped off sharply midway through the intended enrollment as a result of enthusiasm for the activity, and lesser toxicity, seen with these agents in relapsed and refractory myeloma. A study design limiting patient enrollment to patients with BCNU-resistant multiple myeloma would have strengthened efficacy conclusions; however, BCNU was not commonly used for the treatment of multiple myeloma at the time this study was conceived, which would have made patient accrual difficult. The protocol regimen was selected due to limited efficacy of contemporary myeloma regimens and our previous phase I experience with this combination in solid tumors [21]. A mixed patient population (previously treated and untreated) was deemed reasonable at the time the study was developed, given the fairly limited efficacy of other therapies available at the time. The response criteria differ from other response criteria in use with respect to the time interval between consecutive determinations and the requirement of immunofixation to confirm complete response. In practice, although the protocol stipulated only a 2-week interval between determinations, patients actually received response determinations prior to each cycle (6-week intervals). The requirement for immunofixation to confirm responses was included in the original protocol, but immunofixation data was not available for a subset of patients because this test was not reflexively performed in the laboratory at one of the participating institutions. As a result, the requirement was deleted at the time of data analysis. Reclassification of responses according to the criteria of the European Group for Blood and Marrow Transplanta-

tion (EBMT) [3] would result in a similar response rate to that reported in this manuscript; however, the patient who experienced a complete response by study criteria would be reclassified as a near-complete response due to immunofixation consistent with a residual amount of paraprotein that was too small to quantify.

The most notable feature of the data from this study relates to toxicity of the O6-BG/BCNU combination. While this regimen demonstrated little non-hematologic toxicity, the anticipated hematologic toxicities were not trivial. Myelosuppression was the primary reason for removal from the study among responders, despite dose reductions, and accounted for treatment delays in more than half of the patients enrolled, as well as discontinuation of treatment in 35%. Efforts to limit systemic myelosuppression might improve the therapeutic efficacy of this combination, but this may prove difficult in patients with myeloma, since disease-related bone marrow impairment is common.

This study was stopped prematurely for the reasons noted, but is being reported because of the value it brings to the field of biochemical modulation of drug resistance as a therapeutic approach to the treatment of cancer. In addition, not only were responses seen, but patient toxicity, aside from the hematologic toxicity noted, was modest and not long lasting, allowing for subsequent therapy in most patients. A majority of patients treated on this protocol went on to receive other chemotherapeutic regimens (data not shown), which prevents meaningful reporting of survival data. Some of these patients experienced durable responses to their subsequent therapy lasting 2 years or more. Four patients were subsequently considered for autologous stem cell transplantation, which increases the likelihood of a complete response, prolongs disease free survival, and has become the standard therapy for myeloma patients with good performance status [2, 4, 12]. Alkyating agents are thought to be best avoided in these patients because they can prevent adequate stem cell mobilization [10]; however, three patients in this study subsequently underwent successful stem cell mobilization and autologous transplantation after receiving at least 2 cycles of protocol therapy. These data suggest that autologous transplantation may be feasible after treatment with O^6-BG and BCNU without excess toxicity or inability to collect sufficient hematopoietic stem cells; however, this conclusion should be viewed with caution given the small number of patients involved, especially since the fourth patient considered for stem cell transplantation failed to mobilize sufficient stem cells after completing seven cycles of the study therapy.

Newer alkylating agents that may have lesser hematologic toxicities, such as temozolomide, are being studied in conjunction with O^6-BG in other malignancies. A phase I study with this combination was recently reported in patients with malignant gliomas, demonstrating that this combination is tolerable in patients with these disorders [17]. Combination therapy with an O^6-BG/alkylating agent combination plus another agent or agents with activity in myeloma, such as dexamethasone, thalidomide, bortezomib, or lenalidomide may produce higher response rates than seen in this study and may be worth studying; however careful attention should be given to the possibility of significant additive hematologic toxicities.

In conclusion, chemotherapy utilizing the MGMT inhibitor O^6-benzylguanine and BCNU results in inhibition of MGMT activity in malignant plasma cells, which have higher levels of drug resistance than normal hematopoietic progenitors, and produces meaningful responses in a modest proportion of patients with multiple myeloma at the cost of considerable hematologic toxicity. The availability of more efficacious, and less toxic, regimens for both previously treated and untreated patients with multiple myeloma makes it difficult to recommend the treatment strategy presented here for most patients, at least until these other modalities have been exhausted. Future studies of O^6-benzylguanine/alkylating agent combinations alone or in concert with other active agents in myeloma might benefit from pre-treatment MGMT activity determinations to aid proper patient selection, and investigators should be aware of the possibility of significant hematologic toxicity.

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