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I. Benzylguanine-Mediated Tumor Sensitization with Chemoprotected Autologous Stem Cells for Patients with Malignant Gliomas

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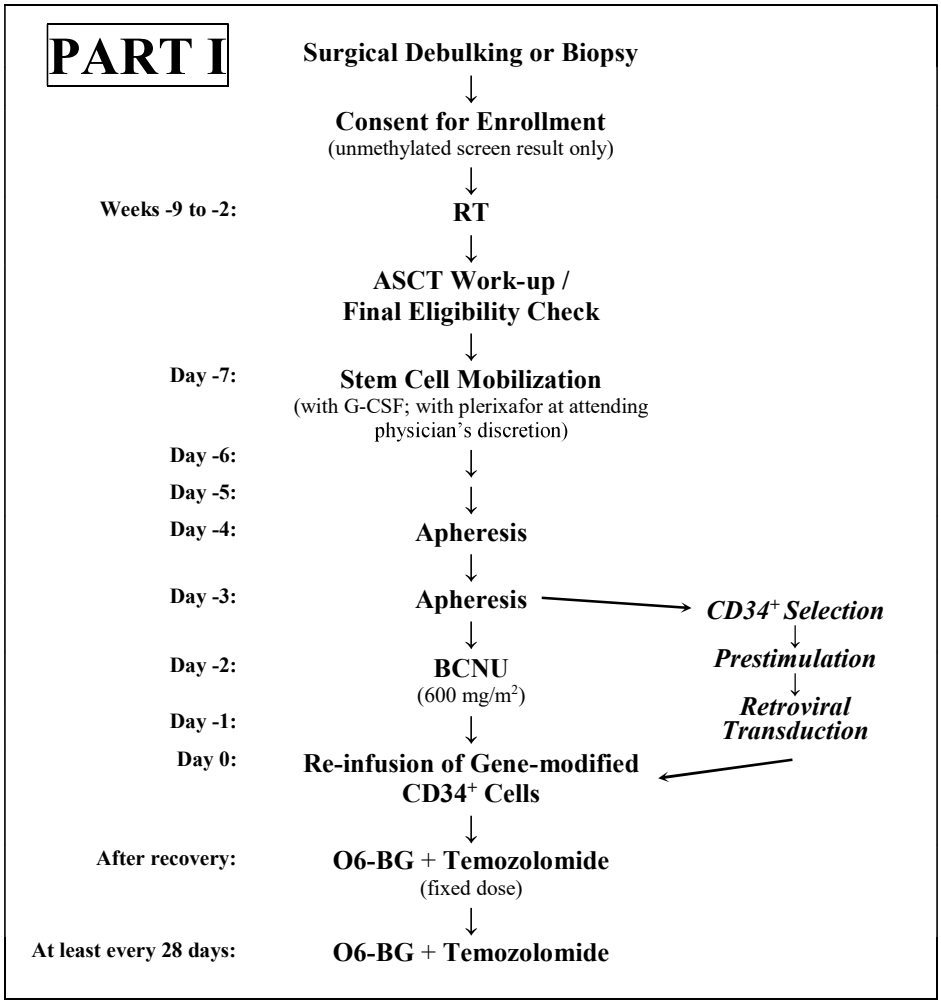
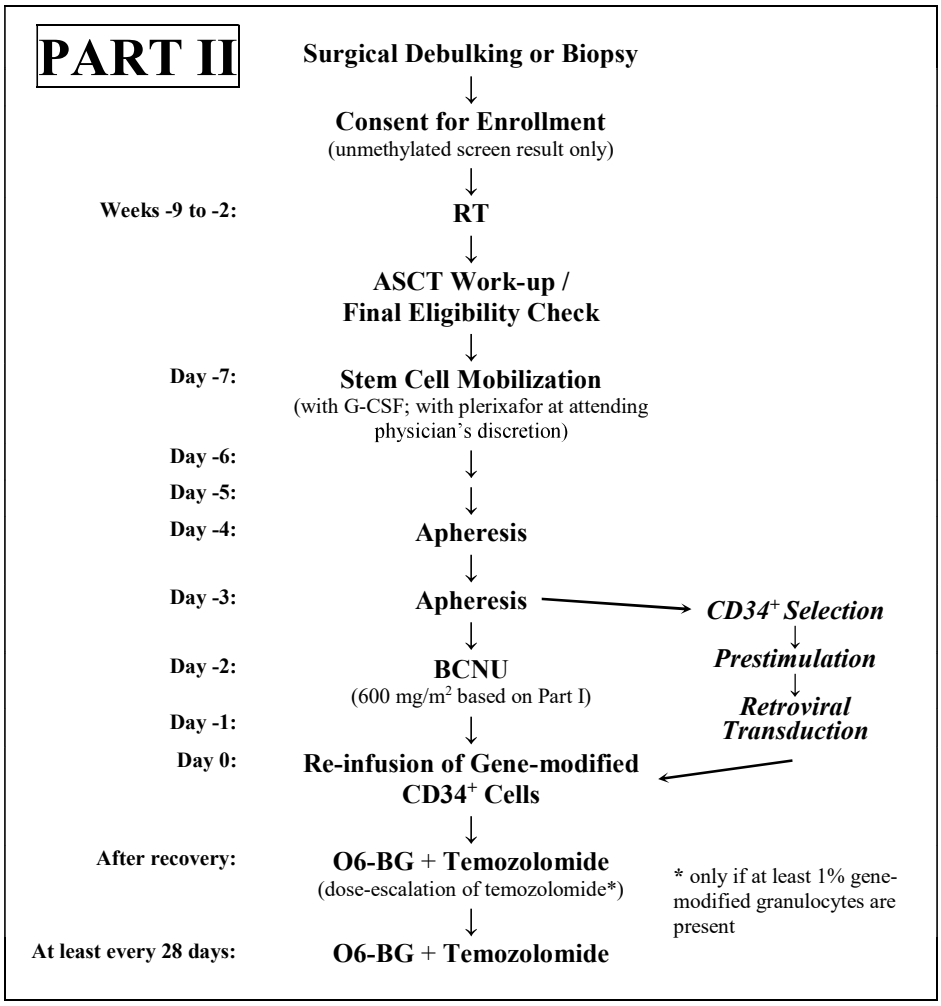
II. Introduction

Glioblastoma is the most common subtype of primary brain tumors in adults. These tumors are highly invasive. Median survival after diagnosis is approximately 12 months. Several significant prognostic factors have been identified, but even in the most favorable groups survival beyond 2 years is unusual. Radiation therapy prolongs survival after the initial surgical resection. Chemotherapy with a nitrosourea, methylating agents such as procarbazine or temozolomide, or other agents is effective and can prolong survival. However, a significant obstacle to the treatment has been the hematopoietic toxicity of chemotherapeutic agents like BCNU and temozolomide which has limited the ability to dose-escalate the drugs. This hematopoietic toxicity is even more pronounced when BCNU or temozolomide are combined with O^6-benzylguanine (O6-BG), an agent which inhibits O^6-alkylguanine DNA alkyltransferase, the major resistance mechanism in gliomas. We have recently demonstrated in a clinically relevant large

animal model that genetic modification of hematopoietic stem cells with the P140K mutant of methylguanine methyltransferase (MGMT and also known as alkyl-guanine transferase (AGT)) results in chemoprotection and the ability to administer higher doses of temozolomide and BCNU when given in combination with O6-BG. Based on these findings we hypothesize that chemoprotection with MGMT (P140K) will allow the administration of higher doses of temozolomide and thus potentially improve the efficacy of this drug in the treatment of patients with high-grade gliomas. Since efficient engraftment of gene-modified cells is important for the success of this strategy, we propose a 2-part approach. In the first part, we propose a dose-escalation of BCNU with gene-modified stem cell support followed by fixed doses of temozolomide and O6-BG. The initial dose of BCNU will be based on the range of doses used in prior studies of high dose BCNU and autologous stem cell transplant. The initial dose (600 mg/m^2) will be slightly lower than the lower end of the range of typical doses used for the prior studies (800−1350 mg/m^2), because the patients in the current study will be receiving gene modified stem cells, and will have already undergone treatment with radiation. In the second part of the study, we will use the MTD of BCNU with support of gene-modified peripheral blood stem cells, followed by a dose-escalation of temozolomide with a fixed dose of O6-BG. We will use our optimized transduction conditions using the Phoenix-GALV-pseudotype packaging cell line generated by the principal investigator of this study to generate MGMT (P140K) expressing vectors. Thus, this study will address the feasibility, toxicity and tumor response of temozolomide dose escalation with chemoprotected stem cells.





III. Background

A. Glioblastoma

Glioblastoma is an almost uniformly fatal disease. Median survival after diagnosis is approximately 12 months [1,2]. Several significant prognostic factors have been identified, but even in the most favorable groups survival beyond 2 years is unusual [3,4]. Radiation prolongs survival after the initial surgical resection. Chemotherapy with a nitrosourea, a methylating agent (such as procarbazine or temozolomide), or other agents is clearly effective. MGMT expression has been shown to be important in mediating resistance in human CNS tumors. In a retrospective study of patients with anaplastic gliomas who had been treated on various protocols with radiation therapy and BCNU, Belanich et al. [1] found that patients whose tumors were found to have low MGMT activity had a significantly better outcome than patients with high MGMT activity [1]. Patients with glioblastoma and high MGMT levels showed a four-month shorter time to treatment failure (TIF) than patients with low MGMT activity. Similarly, patients with glioblastoma and high MGMT activity showed a two-month shorter median survival time (MST) than patients with low activity. For glioblastoma multiforme, survival was correlated more strongly with MGMT activity than with age. This relationship remained statistically significant when other known prognostic factors were considered. However, due to the irreversible degradation of each MGMT molecule

after a single enzymatic reaction, correlating MGMT protein levels with MGMT activity and tumor response has proven difficult [5,6].

O^6-benzylguanine (O6-BG) has been shown to deplete MGMT activity. O6-BG is not toxic as a single agent according to the Phase I trials of O6-BG that have been reported [2]. Friedman et al. conducted a Phase I trial to define the presurgical dose required to deplete tumor MGMT activity in patients with malignant glioma. Patients were treated 18 hours before craniotomy with intravenous doses that ranged between 40 and 100 mg/m^2 given over 1 hour. Resected tumor was snap-frozen in liquid nitrogen and MGMT activity analyzed by high-pressure liquid chromatography. Up to 13 patients were treated at a specific dose of O6-BG, with a target end point of minimally 11 of 13 patients with undetectable tumor MGMT levels (<10 fmol/mg protein). Thirty patients were enrolled, with 11 of 11 patients treated at 100 mg/m^2 O6-BG demonstrating tumor MGMT levels less than 10 fmol/mg protein. No toxicity was noted in any patient treated. These results indicate that 100 mg/m^2 of O6-BG can maintain tumor MGMT levels less than 10 fmol/mg protein for at least 18 hours after treatment, a time interval in which BCNU-induced chloroethyl adducts are fully converted into interstrand cross-links.

Spiro et al. performed a dose escalation clinical trial in which the dose of O6-BG required to deplete MGMT to undetectable levels in sequential CT-guided tumor biopsies before O6-BG and 18 hours after O6-BG was determined [7]. Thirty patients received doses of O6-BG ranging from 10 to 120 mg/m^2. MGMT depletion below the level of detection in all cases was demonstrated at 120 mg/m^2 dose. These results have been extended in a Phase I study in which 100 mg/m^2 of O6-BG were used in combination with BCNU to determine the maximal-tolerated dose of BCNU [8]. In this study, 23 patients with recurrent or progressive glioma were treated at different dose levels. The dose limiting toxicity was found to be myelosuppression, with a maximum tolerated dose of 40 mg/m^2 for BCNU. In a recently published Phase II study, 18 patients with recurrent or progressive glioma resistant to nitrosoureas were treated with 120 mg/m^2 of O6-BG followed by 40 mg/m^2 of BCNU. There were no objective responses, however, 5 out of 18 patients experienced stable disease of 6–18 weeks duration before progression. The dose limiting toxicity in this study was hematopoietic, with no reported pulmonary toxicity. Of note, 3 of the 5 patients with stable disease had to discontinue treatment due to hematologic toxicity; their disease progressed after discontinuation of chemotherapy.

In a recent Phase I study, Quinn et al. have reported dose-escalation of O6-BG and temozolomide [9]. Temozolomide is an imidazole tetrazinone, and its precise mechanism of action is similar to that of dacarbazine, both being converted to the active methylating agent MTIC. Unlike dacarbazine, however, which requires metabolic dealkylation (a relatively inefficient process in humans compared with rodents) to form MTIC, temozolomide undergoes chemical conversion to MTIC under physiologic conditions [10]. The recent observation that temozolomide produced responses in patients with high-grade glioma [11] provides the rationale for a dose escalation study with chemoprotected stem cells. We propose to give temozolomide as a single dose 6 hours after the start of the 48 hour O6-BG infusion. The dose of temozolomide will be escalated according to the study design until the maximally tolerated dose has been reached. Starting dose will be the maximally tolerated dose used in the Duke Phase I study (472 mg/m^2) [12]. We hypothesize that in the absence of MGMT in the tumor tissue (caused by O6-BG) additional doses of temozolomide for the formation of DNA methyl adducts may not be necessary, but may enhance hematopoietic toxicity and thus hamper the optimal delivery of both drugs in combination.

In addition, studies by Stupp et al. and Hegi et al. [13,14] it was shown that only patients with low levels of MGMT had a significant benefit from the combination of radiation (RT) and temozolomide. Thus, for patients with an unmethylated MGMT promoter and high levels of MGMT expression (the target patient population of this study), improved therapies are required. In patients with low MGMT levels, the median survival was 21.7 months from temozolomide + RT versus 12.7 months for patients with high

MGMT levels. This predictive value of MGMT promoter methylation status was substantiated in a clinical study by the same author [15]. More recently, a high-throughput, real-time methylation-specific PCR-based method to determine MGMT promoter methylation status was validated and made commercially available for screening patient tumor tissues [16]. Thus, a realistic goal of this work would be to provide patients with an unmethylated MGMT promoter (indicative of de novo high MGMT activity) an outcome as good as the patients with low MGMT activity (a methylated MGMT promoter), i.e., increase median survival from 12.7 to 21.7 months.

In summary, the above-cited studies demonstrate that an increased median survival time correlates with low MGMT activity and that O6-BG can be administered at doses that show no significant toxicity while effectively depleting MGMT. This provides a good rationale for using the combination of O6-BG and BCNU or temozolomide. The published data further demonstrate that the dose limiting toxicity of this combination is mainly hematopoietic. Thus, the main objective of this protocol is to determine whether the dose of temozolomide in combination with O6-BG can be safely escalated without inducing myelosuppression by using genetically modified, P140K expressing stem cells.

B. Clinical Trials of BCNU for Malignant Glioma

The use of BCNU has been shown to be effective in patients with malignant gliomas. One retrospective analysis reviewed the course of 114 patients with high-grade malignant gliomas who received high dose BCNU and underwent autologous transplant over a ten year period [17]. The patients received high dose BCNU at 800 mg/m^2 about a month after surgical resection, followed by infusion of autologous bone marrow or peripheral blood stem cells. They then underwent radiation therapy to a total of 60 Gy. There were 4 deaths attributed to therapy, only in patients with a Karnofsky performance status under 70%. Six patients developed interstitial pneumonitis, one lethal (included in the aforementioned description of 4 deaths). There were a couple of long-term survivors, and the overall survival was 12 months for glioblastoma multiforme. An early study examined doses from 1050−1200 mg/m^2 in eleven patients with malignant glioma, who then received autologous bone marrow transplants [18]. One patient died after a second 1200 mg/m^2 dose, and another during the blood count nadir. Other complications included steroid responsive interstitial pneumonitis, centrilobular hepatic necrosis with resolution, and deep vein thrombosis. Another subsequent trial included 36 patients with malignant glioma who received 1050, 1200 or 1350 mg/m^2 BCNU followed by infusion of autologous bone marrow [19]. The overall response rate was 44% amongst those with progressive disease prior to transplant. Two of these remained alive at 60 and 84 months after transplant. A later trial in 25 patients included high dose BCNU at 1050 mg/m^2, then autologous bone marrow transplant, then 60 Gy whole-brain irradiation [20]. Toxicities included mild to moderate nausea and vomiting, mild neutropenia with 6 patients never reaching an absolute neutrophil count of less than 500/μl, and four deaths due to seizure (1), infections (2), or pulmonary hemorrhage (1). Lastly, a large retrospective study was performed by the EBMT of 217 patients with high-grade glioma who underwent treatment with 800 mg/m^2 BCNU followed by autologous transplant and 40 Gy radiation [21]. There was a treatment related mortality of 4.5%, and a median overall survival of 20 months.

Thus we propose to use the 600 mg/m^2 dose of BCNU (as determined in Part I, initial cohort of 3 patients) for our conditioning regimen to provide potentially increased tumor efficacy and significant myelosuppression, which should facilitate the engraftment of gene-modified stem cells.

C. Preclinical Studies Using Retroviral Transfer of MGMT to Hematopoietic Stem Cells

Vector-containing medium harvested from murine packaging cell lines has been shown to contain factors which can negatively influence the transduction and maintenance of hematopoietic stem cells. Thus, we

generated a human packaging cell line with a gibbon ape leukemia virus pseudotype (Phoenix-GALV), and we evaluated vectors produced by Phoenix-GALV for their ability to transduce hematopoietic progenitor/stem cells. In three baboons, we used a competitive repopulation assay to directly compare GALV-pseudotype retrovirus vectors produced by either Phoenix-GALV or by the NIH 3T3-derived packaging cell line, PG13. In three additional baboons we compared Phoenix-GALV-derived vectors to more recently developed lentiviral vectors. Gene transfer efficiency into hematopoietic repopulating cells was assessed by evaluating the number of genetically modified peripheral blood and marrow cells using flow cytometry and real-time PCR. Transduction of hematopoietic repopulating cells was significantly higher using the Phoenix-GALV–derived vector as compared to the PG13-derived vectors or lentiviral vectors [22]. Highest gene transfer with Phoenix-GALV was achieved when cells were transduced in the presence of IL-3, IL-6, SCF, M-GDF, Flt3-L, and G-CSF. Two animals were followed for more than 1 year. Flow cytometric analysis of hematopoietic subpopulations in these animals revealed transgene expression in $CD13^+$ granulocytes, $CD20^+$ B lymphocytes, $CD3^+$ T lymphocytes, $CD61^+$ platelets, as well as red blood cells, indicating multilineage engraftment of cells transduced by Phoenix-GALV-pseudotype vectors. In addition, transduction efficiency into human $CD34^+$ cells was significantly higher than transduction of baboon $CD34^+$ cells, suggesting that Phoenix-GALV-derived gammaretroviral vectors may be more efficient in human stem cell gene therapy applications. More recent follow up in animals that received $CD34^+$ cells transduced with Phoenix-GALV derived vectors confirmed the efficient and sustained marking. In addition we have shown that the use of G-CSF mobilized peripheral blood $CD34^+$ cells results in highly efficient gene transfer to long-term repopulating cells when Phoenix-GALV derived vectors are used [23].

Successful genetic chemoprotection of hematopoietic stem cells using wild type MGMT as a selectable marker gene has been demonstrated in murine models. MGMT mutants highly resistant to the inhibitory action of O6-BG have been described. The use of these mutants has been shown to allow for in vivo enrichment of gene-modified cells and protection from O6-BG and BCNU-induced myelosuppression in mice. Recently, we were able to demonstrate in vivo selection and successful chemoprotection in a clinically highly relevant canine model. CD34-enriched marrow cells were transduced with a retroviral vector encoding the P140K mutant of the DNA repair enzyme methylguanine-methyltransferase and enhanced green fluorescent protein (EGFP) [24]. Transduced cells were infused into two recipients conditioned with 920 cGy of total body irradiation. Gene marking in hematopoietic repopulating cells was assessed by flow cytometry and real-time PCR. In the first dog (G069), we administered 5 mg/kg O6-BG and 0.4 mg/kg BCNU at 16 weeks after transplantation. We observed an increase in the proportion of gene-modified granulocytes from 15% to 55−65%. Selection was also observed in red blood cells (from a baseline of 12% to approximately 20%) and platelets (from a baseline of 9% to approximately 40%). Increases in gene marking in the different lineages were stable for 12 months after the first round of drug selection. After 12 months, we re-treated the dog with an identical dose of O6-BG and BCNU. Marking rose to levels of 98% in granulocytes, 59% in red blood cells and 30% in platelets, with a follow-up of currently 6 weeks after the second drug cycle. In contrast to the first drug cycle, there was no myelosuppression associated with the second cycle of chemotherapy, indicating successful marrow protection. The second dog (G154) received three cycles of O6-BG and a reduced dose of BCNU to avoid the pronounced myelosuppression associated with the first treatment cycle in G069. This dog showed in vivo selection to levels of approximately 55% in granulocytes. Selection was also observed in red blood cells and platelets. The multi-lineage increase in gene marking was maintained for 7 months after the last drug cycle. We re-treated G154 with the same dose of BCNU (0.4 mg/kg) that had produced profound myelosuppression in G069. Peripheral blood counts remained within a normal range, indicating marrow protection. This dog showed in vivo selection to levels of approximately 95% in granulocytes. As before, selection was also observed in red blood cells and platelets. The multi-lineage

increase in gene marking was maintained for ~67 months after the last drug cycle. Toxicity in both animals was minimal, with myelosuppression after the initial but not later cycles of chemotherapy being the main side effect. Dog G069 had preexisting elevation of transaminases and cholestasis parameters that waxed and waned independent of the drug administration. The etiology of this finding is unclear. Both animals maintained their weight within ± 10% of the weight before the first dose of chemotherapy during all cycles. There were no clinical signs of toxicity. A morphological analysis of the bone marrow in both animals 12 months (G069) or 7 months (G154) after the first cycle of chemotherapy showed trilineage hematopoiesis with no indication of leukemia or myelodysplasia. In 2 additional dogs we studied whether MGMT-transduced stem cells would also mediate chemoprotection with O6-BG and temozolomide. After several cycles with slowly escalating drug doses, more than 80% of granulocytes and platelets expressed MGMT. When these animals received 5 mg/kg O6-BG and 800 or 900 mg/m^2 of temozolomide, a dose that causes substantial thrombocytopenia in control dogs (platelets <15,000/μl), no significant myelosuppression was observed. Extrahematopoietic toxicity of these treatments was minimal.

Conclusions: These data from a clinically relevant canine model demonstrate that MGMT(P140K)-transduced stem cells can provide effective marrow protection from intensive chemotherapy. These findings should have important implications for the use of dose-dense or dose-intensive chemotherapy in patients with malignant diseases.

IV. Objectives

A. Primary Objectives

1. Determine the safety and feasibility of infusing autologous G-CSF mobilized stem cells transduced with a Phoenix-GALV-pseudotype vector expressing MGMT(P140K).
2. Define the dose of BCNU that results in efficient engraftment of gene modified cells when given with peripheral blood stem cell support.

B. Secondary Objectives

1. Determine the engraftment of gene-modified cells after conditioning with BCNU.
2. Determine the ability to select gene-modified cells in vivo with this regimen.
3. Evaluate the molecular and clonal composition of gene-modified cells after chemotherapy with temozolomide.
4. Observe patients for clinical anti-tumor response.
5. Determine the correlation of the level of MGMT(P140K) marking with toxicity, temozolomide dose achieved, and response.
6. Characterize the toxicity associated with this regimen.

V. Patient Selection

A. Inclusions

1. Patients with glioblastoma multiforme or gliosarcoma.
2. The patient or legal guardian must be able to comprehend the informed consent form and sign prior to patient enrollment.

3. Karnofsky performance status at time of study entry must be ≥70%.
4. Patient must be at least 18 years old.
5. Life expectancy of ≥3 months.
6. Patients must agree to undergo repeat clinical neurological examinations and brain MRI with appropriate contrast after every other cycle of chemotherapy.
7. WBC >3000/µl, ANC >1500/µl, platelets >100,000/µl, hemoglobin >10 gm/100ml.
8. Total and direct bilirubin <1.5 times upper limit of laboratory normal. SGOT and SGPT ≤3 times upper limit of laboratory normal. Alkaline phosphatase ≤3 times upper limit of laboratory normal.
9. BUN and serum creatinine <1.5 times upper limit of laboratory normal.
10. LVEF ≥40%, however, subjects with an LVEF in the range of 40−49% should have cardiology clearance prior to intervention.
11. MGMT promoter methylation analysis of surgically resected tumor or tumor biopsy must demonstrate an unmethylated or hypomethylated MGMT promoter status.

B. Exclusions

1. Patients with cardiac insufficiency and an LVEF of <40%. History of coronary artery disease or arrhythmia, which has required or requires ongoing treatment.
2. Patients with active pulmonary infection and/or pulse oximetry <90% and a corrected DLCO <70% of predicted.
3. Active systemic infection.
4. Patients who are HIV positive.
5. Pregnant or lactating women. A beta-HCG level will be obtained from women of child-bearing potential. Fertile men and women should use effective contraception.
6. Previous chemotherapy for any malignancy including temozolomide, DTIC or prior nitrosourea.
7. Diabetes mellitus.
8. Bleeding disorder.
9. Methylated or hypermethylated MGMT promoter status within tumor tissue.
10. Medical or psychiatric condition which in the opinion of the protocol chairman would compromise the patient's ability to tolerate this protocol.
11. Prior interstitial radiotherapy, stereotactic or gamma knife surgery or implanted BCNU-wafers.

VI. Evaluation and Counseling of Patient

Patients who are referred for consultation will be evaluated completely. The protocol will be discussed thoroughly with the patient and family, and all known risks will be described. The chemotherapy and the gene transfer will be described in detail. Alternative treatment forms will be presented and discussed. A summary of the conference detailing what was covered in the conference will be dictated for the medical record.

VII. Protocol Registration

Patients who have had their tumor resected or biopsied, who meet MGMT promoter methylation criteria, and who are deemed fit for trial admission by the attending neuro-oncologist and who wish to participate will sign the consent form and begin or continue radiation therapy. At the completion of radiation therapy,

an eligibility conference will be held with the Auto Team to discuss each patient's suitability for enrollment and receipt of experimental procedures.

VIII. Plan of Treatment

This Phase I/II nonrandomized, dose escalation study will enroll patients with newly diagnosed glioblastoma multiforme or gliosarcoma. Each subject will have standard-of-care surgery and radiation therapy (without concomitant temozolomide, since this is of no added benefit to patients with an unmethylated MGMT promoter status [14,15,19,20,25]) followed by stem-cell mobilization and apheresis. The stem cells will be genetically modified in the laboratory to make them resistant to the chemotherapy. After re-infusion of the stem cells, patients can receive up to 24 cycles of concomitant O6-BG and temozolomide.

In Part I, the optimal dose of BCNU for conditioning, with genetically modified PBSC support, will be established. The optimal dose of BCNU will be used for the conditioning regimen in Part II. In Part II, following infusion of transduced cells and engraftment, the dose of O6-BG will be fixed, and the dose escalation of temozolomide in each cohort will go forward if there are at least 1% MGMT(P140K)$^+$ granulocytes. Dose escalation of temozolomide in Part II will follow a standard 3+3 design, with each cohort of 3 subjects receiving a higher dose of temozolomide in the subsequent chemotherapy cycles in Part II.

Surgical debulking or tumor biopsy*
↓
Enrollment Consent
↓
Radiation Therapy
↓
Evaluation of eligibility criteria/Enrollment for intervention
↓
G-CSF stem cell mobilization and apheresis
↓
CD34$^+$ Separation and Transduction
↓
BCNU (600mg/m^2)
↓
Infusion of transduced stem cells at least 24 hours after BCNU
↓
Blood cell recovery (engraftment) and recovery from any BCNU-related acute toxicity achieved
↓
O6-BG and temozolomide starting at least 4 weeks after conditioning, once engraftment and BCNU recovery achieved ‡

* MGMT promoter methylation status will be provided by the University of Washington and available to the PI to determine eligibility.

‡ Dose escalation for temozolomide will begin once BCNU dose for the conditioning regimen has been defined. Dose escalation will only be started if at least 1% MGMT(P140K)$^+$ granulocytes are detected in the peripheral blood and the ANC is within retreatment criteria (>1.5 10^3/ml).

Prior to each cycle, patients will be evaluated for safety. See section 9.0.

We propose here in Part I to treat a total of 6–9 patients (an additional 3–6 patients) with the 600 mg/m^2 dose of BCNU, followed by intra-patient dose escalation of temozolomide in combination with a fixed dose of O6BG. The following define the initial dose of temozolomide post-conditioning, as well as allowable dose levels for intra-patient dose escalation of temozolomide in Part I. Conditioning is the first chemotherapy using BCNU. Only one dose level of BCNU (600mg/m^2) will be studied in Part I,

as this dose was found to allow for efficient engraftment and minimize extrahematopoietic toxicities before the infusion of gene-modified cells in the first three patients enrolled in the study. Patients enrolled in Part I of the study will receive O6-BG and a fixed dose of temozolomide at 472 mg/m^2 without any dose escalation for a minimum of two cycles (see Part I, subpart b and Section 15. Statistical Considerations, Subsection 8 regarding intra-patient dose-escalation). Patients can be retreated with O6-BG and 472 mg/m^2 of temozolomide at least every 4 weeks up to 96 weeks or 24 cycles if they meet the retreatment criteria defined in the protocol (See Section 9. Evaluation, subsection C). Provided patients tolerate this dose for at least two cycles, as indicated by hematopoietic recovery consistent with retreatment criteria (included below) and at least 1% gene modified granulocytes in the peripheral blood within 28−42 days from the previous treatment, dose escalation in 25% increments, beginning with 590 mg/m^2 and not exceeding 925 mg/m^2 temozolomide. If recovery occurs later than 42 days from the previous treatment, the previous dose should be reduced by 25%. If retreatment criteria are achieved within 28−42 days following the dose reduced cycle, escalation to the previous dose that caused delay is permitted, but must be tolerated for two consecutive cycles before further dose escalation may be attempted in that patient. Dose levels will be closed to intra-patient escalation if more than one patient experiences a DLT at that dose, or if one patient experiences a DLT at the escalated dose and a second patient experiences a DLT at a lower dose. If no additional patients experience a DLT at the same dose, escalation to that dose level may continue, but no further dose escalation may be attempted in the patient experiencing the DLT.

Dose escalation in Part II will follow a standard 3+3 design. Three patients will initially be enrolled at a dose level. If no patient experiences DLT, then escalation to the next level is permitted. Dose escalation/de-escalation will be by approximately 25%. If it becomes possible to have more than three patients enrolled within a given treatment period, the third patient treated will be observed for seven days before additional patients are treated at the next dose level. If 1 of 3 patients experiences DLT, then an additional 3 patients will be enrolled at the same dose level. If no further DLT is seen, then escalation to the next level is permitted. A second DLT in any number of patients will halt enrollment to that dose level, and the next lower dose level will be defined as the MTD. The MTD dose level cohort will then be similarly expanded to six patients. We anticipate enrollment of approximately 6−9 patients for Part I, and 12−15 patients for Part II.

Part I

1. **Conditioning dose of BCNU**
   Dose level 1 BCNU 600 mg/m^2 IV
2. **Dose of temozolomide during O6-BG/temozolomide treatment after infusion of chemoprotected stem cells is initiated at 472 mg/m^2 during this part of the study.**
   Intra-patient dose escalation of temozolomide is permitted after the first two doses of O6-BG and temozolomide, provided that at least 1% MGMT(P140K)$^+$ granulocytes are detected in the peripheral blood and criteria as outlined in Section 8, Part II, Criteria for Retreatment (below) are met. The escalated dose levels will be the same as for the inter-patient escalation of the initial temozolomide dose in Part II (590, 740, and 925 mg/m^2). Please also see 15. Statistical Considerations.

Part II

1. **Conditioning dose of BCNU as defined in Part I (fixed at 600mg/m^2)**

2. **Dose of temozolomide after conditioning and infusion of chemoprotected stem cells (during O6-BG/temozolomide treatment)**

   Dose level 1 590 mg/m^2 PO
   Dose level 2 740 mg/m^2 PO
   Dose level 3 925 mg/ m^2 PO

   Dose escalation will only start if 1% MGMT(P140K)$^+$ granuloctye cells are present with ANC $\geq 1.5\times10^3/\mu l$ as defined in retreatment criteria below. If these conditions are not met, the temozolomide dose will remain at 472 mg/m^2.

A. Radiation Therapy

Radiation therapy should start within 35 days of surgery but can begin later if tumor stability is confirmed. Standard 3-D conformal and Intensity Modulated Radiation Therapy techniques will be allowed on this study. A total dose of 60.0 Gy delivered by single dose of 2 Gy given daily 5 days per week over the course of six weeks will be delivered with the combination of initial fields and boost field techniques. Patients will not receive concomitant temozolomide.

1. **Equipment**

   Radiation will be administered by a linear accelerator with an isocentric treatment gantry that delivers x-rays of at least 6 MV. Radiation may also be delivered using proton radiotherapy by either passive scattering or pencil beam techniques. If proton therapy is used, the dose will be reported in Gy(RBE), where 1 Gy(RBE) = proton dose Gy x RBE (radiobiological effective dose), RBE = 1.1. Treatment planning CT or MRI scans shall be obtained on all patients, and simulation will be used for design and verification of fields.

2. **Treatment Technique**

   Three dimensional (3D) treatment techniques which deliver the appropriate dose to the planning target volume (PTV) are required. Coplanar and non-coplanar techniques are allowed. A treatment planning CT is required. Ideally, preoperative MRI and immediate postoperative MRI (within 48 hours following surgery) are used for treatment planning purposes after being superimposed on the treatment planning CT. In the absence of MRI, the treatment volumes should be determined by pre- and post-operative CT scans. Head immobilization through the use of a thermoplastic mask is strongly encouraged. Cerrobend blocks or multileaf collimators (MLC) are to be used for field shaping.

3. **Treatment Delivery**

   Patients will be treated five days per week (Monday through Friday), excluding holidays. Patients are to be treated at least two times during the first week of treatment. Thus patients started on a Friday will require a Saturday treatment. The dose per fraction will be 2.0 Gy per day delivered to the isocenter. All fields are to be treated each day. Both isocenter and field verification films will be obtained prior to starting treatment. Field verification films or isocenter films will be obtained weekly unless there is a clinical indication to obtain more frequently.

4. **Target Volumes**

   The initial gross tumor volume (GTV1) is defined as the T2 signal or FLAIR abnormality on a preoperative MRI. Two planning target volumes (PTV) will be defined, as outlined below.

   a. *Initial Planning Target Volume (PTV1)*

   The initial clinical tumor volume (CTV1) is defined as the GTV1 plus a 2 cm margin of brain

parenchyma. The CTV1 margin may be reduced to 0.5cm around natural barriers to tumor growth such as the skull, ventricles, falx, etc., and also to allow sparing of the optic nerve/chiasm, if necessary. If no edema is present, the initial planning target volume (PTV1) shall be 0.3−0.5cm beyond CTV1 (this is equivalent to the GTV1 plus 2.3−2.5 cm), depending upon localization method and reproducibility, at each center. PTV margins account for variations in set-up and reproducibility. Reducing PTV margins to modify an organ at risk (OAR) dose(s) is not generally permissible. However, an OAR must be defined, along with a planning risk volume (PRV) for each OAR. Each PRV will be its OAR plus 3mm. In the event that an OAR is in immediate proximity to a PTV such that dose to the OAR cannot be constrained within protocol limits, a second PTV ($PTV_{overlap}$), defined as the overlap between the PTV2 and the particular PRV of concern, may be created (the overlap is the intersection between the PTV1 and the PRV). Dose to the $PTV_{overlap}$ must be as close as permissible to 46 Gy while not exceeding the OAR dose limit.

b. *Boost Planning Target Volume (PTV2)*

The boost gross tumor volume (GTV2) shall correspond to the region of gadolinium enhancement on a postoperative T1 MRI and must include the surgical cavity margins. The boost clinical tumor volume (CTV2) is GTV2 plus 2.0 cm. The CTV2 margin may be reduced to 0.5cm around natural barriers to tumor growth, such as the skull, ventricles, falx, etc., and also to allow sparing of the optic nerve/chiasm, if necessary. The boost planning target volume is 0.3−0.5 cm beyond this (equivalent to GTV2 plus 2.0 cm), depending on localization method and reproducibility, at each center. PTV margins account for variations in set-up and reproducibility. Reducing PTV margins to modify an organ at risk (OAR) dose(s) is not generally permissible. However, an OAR must be defined, along with a planning risk volume (PRV) for each OAR. Each PRV will be its OAR plus 3 mm. In the event that an OAR is in immediate proximity to a PTV such that dose to the OAR cannot be constrained within protocol limits, a second PTV ($PTV_{overlap}$), defined as the overlap between the PTV2 and the particular PRV of concern, may be created (the overlap is the intersection between the PTV1 and the PRV). Dose to the $PTV_{overlap}$ must be as close as permissible to 14 Gy while not exceeding the OAR dose limit.

The initial target volume shall be treated to a dose of 46 Gy in 23 fractions. The boost target volume will be treated to a dose of 14 Gy (total dose 60 Gy) with 7 additional fractions of 2Gy each. For all patients, as mentioned above, two PTV prescriptions, PTV1 and PTV2 will be used and the prescription isodose (46 Gy for PTV1 and 14 Gy for PTV2) must cover >95% of the PTV volume; therefore, the total dose in the PTV2 volume will be 60 Gy. The minimum acceptable dose within PTV1 will be 41.4 Gy (90% of 46 Gy), and in the PTV2 volume, it will be 54 Gy (90% of 60 Gy). If the minimum dose falls below these parameters, a minor protocol deviation will be assigned. The maximum dose for the PTV1 (not including the PTV2 boost volume) should not exceed 50.6 Gy (110% of 46 Gy) and for the PTV2 dose should not exceed 66 Gy (110% of 60 Gy). If the maximum doses exceed these parameters, a major protocol deviation will be assigned.

Up to 5 days of treatment interruption will be permitted for any reason. Interruptions of 6 to 7 treatment days will be considered a minor protocol deviation. Interruptions of 8 treatment days or greater will be considered a major protocol deviation.

5. **Dose Limitation**

In addition to the above defined GTVs, CTVs and PTVs, the lenses of both eyes, both retinae, both optic nerves, the optic chiasm, and the brainstem must be defined. The maximum point (defined

as a volume greater than 0.03 cc) doses permissible to the structures are listed in the table below. Proton dose tolerances will be converted to RBE equivalent photon doses−i.e., 7 Gy/1.1 = 6.36 Gy proton.

Critical Structure	Maximum Dose
Lenses	7 Gy
Retinae	50 Gy
Optic Nerves	55 Gy
Optic Chiasm	56 Gy
Brainstem	60 Gy

6. **Withholding Radiation**

   Radiation treatment shall be delayed for WBC <1000 or platelets <20,000, and shall be resumed when the WBC reaches at least 1000 and platelets reach at least 20,000.

7. **Reporting Radiation Treatment**

   Pre- and post- operative scans, simulation films, and port films will be submitted to Radiation Oncology attending for review. Copies of the treatment plan with isodose curves will be submitted as well. The images submitted should be at 0.5 cm intervals starting 0.5 cm inferior to the PTV1 through 0.5 cm superior to PTV1. The image containing the isocenter should be submitted as well. Dose volume histograms (DVH's) for the GTV's and PTV's are to be submitted as well as DVH for any critical structure.

B. Peripheral Blood Stem Cell Mobilization

Upon completing radiation therapy, verification of eligibility criteria and enrollment for intervention, patients will receive G-CSF 16 μg/kg subcutaneously daily, beginning on Day -7, to mobilize stem cells into the peripheral blood. To maximize the stem cell collection, apheresis will be performed on Days -4 and -3 (and possibly on Day -2) with concurrent G-CSF administration. Peripheral blood $CD34^+$ counts will be obtained daily beginning on Day -5 and will stop when G-CSF administration stops.

A minimum of 2×10^6 unmanipulated $CD34^+$ cells/kg should be cryopreserved as backup and a minimum of 2.5×10^6 $CD34^+$ cells/kg should be obtained following CD34 selection. Maximum cell capacity per CD34 selection is 120×10^9 total nucleated cells (TNCs).

Following the first apheresis, both a total nucleated cell (TNC) count and a $CD34^+$ cell count should be obtained. If $>2.0\times10^6$ $CD34^+$ cells/kg were obtained, volume equivalent to 2×10^6 $CD34^+$ cells/kg should be cryopreserved as unmanipulated backup according to CTL standard operating procedures (SOPs). Remaining product should be held overnight according to CTL SOPs. If $CD34^+$ content after the first apheresis is $<2\times10^6$ $CD34^+$ cells/kg or if the peripheral blood $CD34^+$ cell count is <5/μl on day 3 of mobilization with G-CSF alone, all product should be held overnight and the G-CSF dose should be increased to 16μg/kg B.I.D.

Plerixafor (AMD3100; Mozobil) may be administered if the peripheral blood $CD34^+$ cell count is <5/μl on day 3 of mobilization with G-CSF alone or after the first apheresis collection, at the discretion of the attending physician. Plerixafor should be administered at a dose of 240μg/kg subcutaneously within 1 hour following administration of the evening G-CSF dose, if evening dose of G-CSF is given. Administration of plerixafor should be discontinued after the last apheresis procedure such that no more than a total of 3 doses are given.

If >2.0×10^6 CD34$^+$ cells/kg were obtained and sufficient backup was cryopreserved during the first collection, product from the second apheresis on Day -3 will be analyzed for TNC only. If <2.0×10^6 CD34$^+$ cells/kg were obtained during the first collection, a CD34$^+$ count will also be performed and combined products from both apheresis collections equivalent to 2×10^6 CD34$^+$ cells/kg should be cryopreserved as unmanipulated backup according to CTL SOPs.

Any portion of apheresis product that was held overnight should be applied toward the TNC maximum first. If >120×10^9 TNCs are available from the remainder of the first collection, CD34 selection should proceed with 120 × 10^9 TNCs and any remaining product should be cryopreserved as additional backup according to CTL SOPs. Product from the second collection on Day -3 should be held until completion of CD34 selection to verify that 2.5×10^6 CD34$^+$ cells/kg are obtained post-selection. If ≥2.5×10^6 CD34$^+$ cells/kg are obtained following selection, all remaining product should be cryopreserved as backup. If <2.5×10^6 CD34$^+$ cells/kg are obtained following selection, apheresis product from the second collection on Day-3 should be submitted for additional CD34 selection.

Summary of Mobilization, Collection and Selection Procedures:

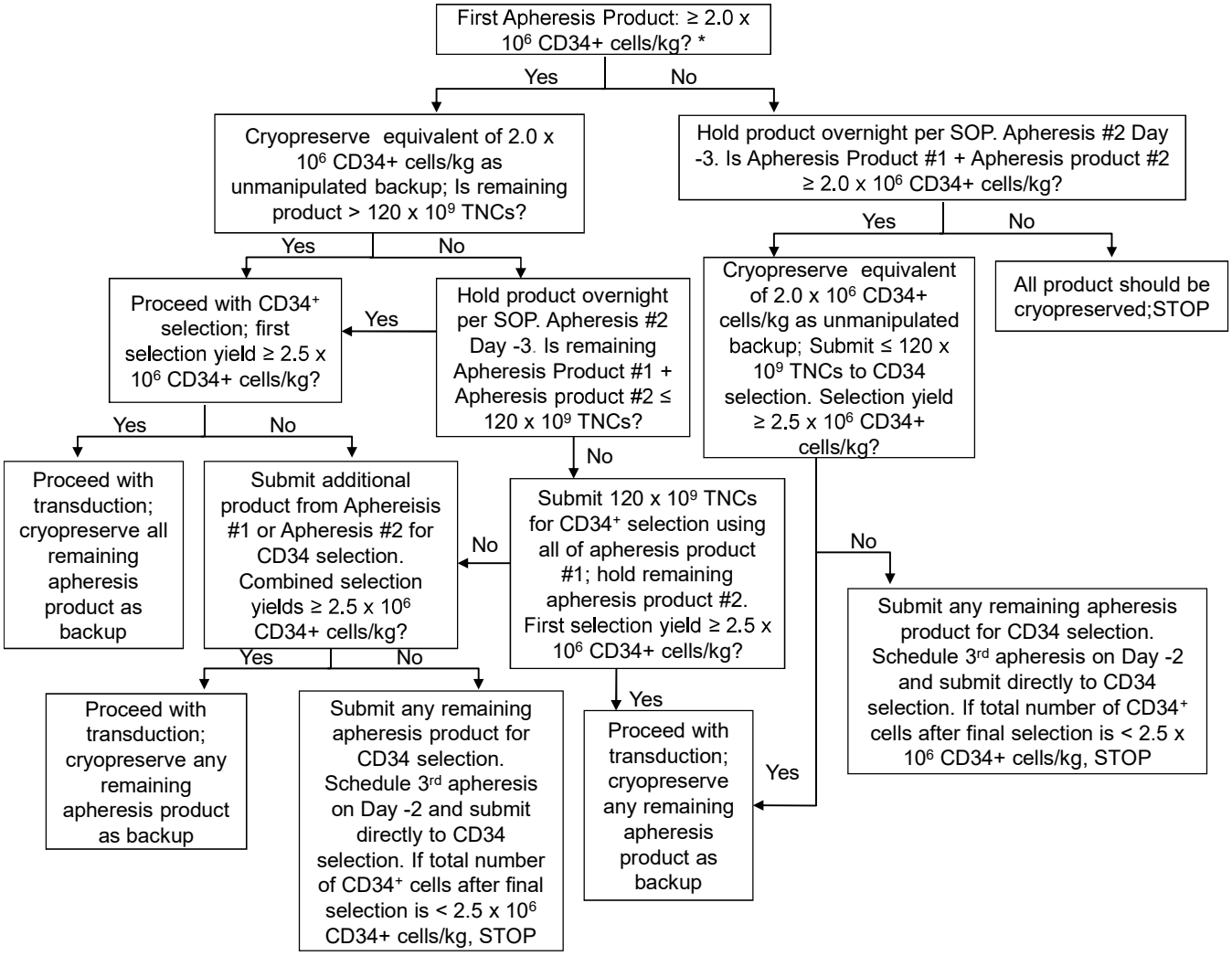
Day	Procedure(s)
-7	G-CSF
-6	G-CSF
-5	G-CSF (Possible plerixafor) / PBL CD34$^+$ count
-4	G-CSF (Possible plerixafor) / PBL CD34$^+$ count / Apheresis #1
-3	G-CSF (Possible plerixafor) / PBL CD34$^+$ count / Apheresis #2
-2	BCNU **or** Apheresis #3*
-1	Rest **or** BCNU*
0	Infusion of gene modified cells (or backup)

* If <2.5×10^6 CD34$^+$ cells/kg were obtained for transduction following selection of Apheresis products #1 and #2.

If <120×10^9 TNCs remain within held overnight product from the first collection, the deficit can be filled by equivalent product from the second collection on Day -3 and pooled product should be submitted for CD34 selection. Any remaining product from the second collection on Day -3 should be held until completion of CD34 selection to verify that 2.5×10^6 CD34$^+$ cells/kg are obtained post-selection. If ≥2.5×10^6 CD34$^+$ cells/kg are obtained following selection, all remaining product should be cryopreserved as backup. If <2.5×10^6 CD34$^+$ cells/kg are obtained following selection, remaining apheresis product from the second collection on Day-3 should be submitted for additional CD34 selection.

If less than 2.5×10^6 CD34$^+$ cells/kg are obtained after 2 aphereses and subsequent CD34 selection, a third apheresis will be performed. If, after the third apheresis and selection there are less than 2.5×10^6 CD34$^+$ cells/kg available for transduction, the patient will be eligible for BCNU administration with unmanipulated stem cell support at the discretion of the attending physician, but will not be eligible to continue on the protocol for the administration of gene modified cells.

Collection, Cryopreservation and Selection Decision Tree:



C. Apheresis

Intermediate or large volume apheresis will be performed according to standard operating procedures established at the SCCA and UW. The Apheresis Team will make the determination whether subjects have adequate venous access or need a central line placed.

D. CD34$^+$ Cell Selection

To separate and enrich CD34$^+$ cells from PBSC for transplantation, we will use the CliniMACS device, which is a continuous flow system that utilizes super-paramagnetic MicroBeads conjugated to monoclonal mouse anti-human CD34 antibody. The cells are incubated with the MicroBeads. The CD34$^+$ cells bind to the beads and are trapped with a magnetic field. The CD34$^+$ cells pass through the field. The magnetic field is released and the CD34$^+$ cells are collected. This selection strategy typically results in a CD34$^+$ cell recovery of ~50% with a purity of ~95% [26].

E. Retroviral Transduction

$CD34^+$ cells will be transduced in our GMP facility. We will transduce G-CSF-mobilized human peripheral blood CD34-enriched cells with concentrated Phoenix-GALV pseudotype vector provided by the National Gene Vector Laboratories. Availability of adequate amounts of viral stock will be verified prior to patient enrollment. After the first transduction, cells will be cultured in growth medium overnight, and the cells will be transduced again the following morning. Immediately after the second transduction, the cells will be harvested. Aliquots of the transduced and mock-transduced cells will be plated into liquid culture and into a soft agar CFC assay. Transduction efficiency into the bulk CD34-enriched population will be assessed by quantitative PCR and by flow cytometry for MGMT positive cells on day 3 after transduction. Colony growth will be used as an indication of the health of the cultured CD34-enriched population. Single colonies will be picked from the soft agar on day 14 after plating and DNA isolated from these colonies will be subjected to PCR in order to determine gene transfer efficiency into progenitors. Aliquots of transduced and mock-transduced cells and the supernatants from the prestimulation and the virus exposures will be cryopreserved for sterility and RCR testing.

F. BCNU Escalation and Administration for the Conditioning Regimen in Part I

One day after completion of stem cell collection, high dose chemotherapy with BCNU will be initiated. BCNU should be given IV over 3 hours with recommended premedication including dexamethasone (20 mg IV), diphenhydramine (1 mg/kg up to a maximum of 50 mg IV) and ranitidine (50 mg IV) for prevention of cytokine release syndrome during administration. BCNU dosing for the conditioning regimen will be 600 mg/m^2.

G. Reinfusion of Genetically-Modified Cells

The re-infusion should occur no less than 24 hours after the conditioning chemotherapy with BCNU and after cells have tested negative for bacterial contamination by gram stain and endotoxin assay. Aliquots of gene-modified cells will also be tested for bacterial cultures, mycoplasma and replication competent retrovirus (RCR). The RCR assay result is not required to be completed prior to infusion for cells in culture less than 96 hours.

No toxicities are expected, but patients will be closely monitored. Twelve patients have received infusions of gene-modified hematopoietic progenitor cells at this institution: five patients enrolled in this study, two patients after autologous transplantation for malignancy, two patients with leukocyte adhesion deficiency, and three patients with Gaucher's disease; no complications were observed. The infusion of gene-modified cells will be performed under physician supervision. Patients will be monitored for 2 hours after infusion. Toxicity will be assessed according to NCI Common Toxicity Criteria (CTC) version CTCAEv3. The study will be stopped and re-evaluated for any grade 4 toxicity.

1. **Infusion Procedures (Treated as Fresh Hematopoietic Progenitor Cells):**
   a. *Timing*
   Cells are infused no earlier than 24 hours following administration of BCNU as conditioning chemotherapy and should be infused as soon as possible upon arrival to the infusion site.
   b. *Volume*
   The volume of gene-modified cells for infusion should be 200 ml.
   c. *Medications*
      i. To avoid allergic reactions to the cell product, the following medication must be administered 15 minutes before the start of the infusion:

Diphenhydramine, 50 mg IV.
Acetaminophen (500−650 mg, PO) and Hydrocortisone (1 mg/kg) may also be given. If there is a history of true anaphylaxis to diphenhydramine (Benadryl), the patient must be treated with an alternative histamine 1 receptor blocker to prevent the potential allergic reactions to the cell product. The alternative for adults is promethazine (Phenergan) at a dose of 12.5−25 mg IV.

ii. Anti-emetics – optional.
iii. Have emergency anaphylaxis medications available at the bedside as per standard practice guidelines.

d. *Filtration*

Filter through a blood or platelet administration set with 150-260 micron mesh size.

e. *Product Infusion Rate*

Infuse cell product at 0.5 times maintenance rate for the first 15 minutes (do not "piggyback" into any other fluids). Increase as tolerated, up to a maximum of 2 times maintenance, until completed.

f. *Monitoring*

i. Emergency equipment and medications (diphenhydramine, epinephrine, hydrocortisone, etc.) must be immediately available.
ii. A nurse familiar with adverse signs of fresh or cryopreserved cell product infusion (rash, fever, chills, dyspnea, bronchospasm, hypotension, cyanosis, chest or back pain, etc.) must attend the patient during infusion.
iii. A physician or mid-level provider familiar with the effects of fresh or cryopreserved cell product infusion must be available for emergency consultation.
iv. Complete vital signs (BP, pulse, respirations, temperature) and body weight must be obtained before the start of the infusion. Vital signs should be obtained after 15 minutes, then q60 minutes until 2 hours after the completion of infusion. At any suspicion of cardiac problem, obtain ECG.
v. After the infusion, flush the central catheter with normal saline.

g. *Concomitant Infusions:*

No medications or fluids should be given "piggy-back" with the infusion of stem cell product (fresh or cryopreserved), although they may be given through the other lumen of the catheter. Amphotericin, antibodies, investigational medications, or blood products should not be given concomitantly because of difficulty in evaluating reactions. Cells must not be infused during plasmapheresis or dialysis.

h. *Reactions:*

Some patients may develop a reaction to the cultured, gene-modified cell product which may cause severe hypotension, cramping, dyspnea, nausea, diarrhea, and cardiac conduction abnormalities. Anaphylaxis occurs rarely. Bradycardia (rarely heart block) and hypertension have been reported to occur within 2-6 hours after the infusion. Blood pressure elevations are often transient and asymptomatic.

Allergic Reaction: Anaphylactic reactions are rare, but dramatic, and may occur with the start of the infusion. If a reaction occurs, stop the infusion and treat the patient with therapy for this type of reaction. See Anaphylaxis Emergency / Drug Chart Reference.

Pulmonary Micro-Embolism: Clumps of cell debris may occur. Therefore, all products should be infused through a filter. If the patient complains of chest pain, dyspnea or coughing, slow

the infusion or complete the bag and administer oxygen. If symptoms persist, obtain ECG and notify physician. If clumping is excessive, acid-citrate-dextrose (ACD) may be added by the Cellular Therapy laboratory technician (watch for signs of hypocalcemia).

2. **Use of "Backup" Cells (Unmanipulated peripheral blood stem cells that were cryopreserved)**

   Back-up cells may be infused instead of gene-modified cells if the gene-modified cells do not meet infusion criteria, for example, if the gene-modified cells have a high level of endotoxin or are positive on Gram stain. The clinical condition of the patient, as determined by the attending, (i.e., active infection with prolonged neutropenia) will also dictate whether back-up cells should be employed. Infusion of back-up cryopreserved cells should proceed according to standard practice guidelines for infusion of cryopreserved hematopoietic progenitor cells.

   Back-up cryopreserved, stored cells may be infused if the patient does not engraft. Criteria for failure to engraft would be an absolute neutrophil count of <500 at Day 28 after transplantation. Back-up cell infusion would also be considered for prolonged thrombocytopenia, defined as platelet count not recovered to 25,000 by day 40, independent of transfusion. Back-up cells will be cryopreserved and made available for patients as medically indicated and at the discretion of the clinician.

H. Part II: Temozolomide Dose Escalation and Administration in combination with O6-BG after Part I is completed.

Doses of drugs for Part II will proceed as follows:

1. **Conditioning dose of BCNU as defined in Part I (600mg/m^2)**
2. **Dose of temozolomide after conditioning and infusion of chemoprotected stem cells (during O6-BG/temozolomide treatment)**

   Dose level 1 590 mg/m^2 PO
   Dose level 2 740 mg/m^2 PO
   Dose level 3 925 mg/ m^2 PO

   Note: Dose escalation will only start if 1 % MGMT(P140K)$^+$ granuloctyes cells are present and criteria as outlined in Section 8, Part II, Criteria for Retreatment (below) are met. If these conditions are not met, the temozolomide dose will remain at 472 mg/m^2.

Thus, temozolomide dosing for cycles after conditioning and stem cell infusion will begin at 590 mg/m^2. The temozolomide dose will be increased in approximately 25% increments in subsequent cohorts until the MTD is reached (see Statistical considerations, Table 1). Intra-patient dose escalation will be permitted after the first two doses of O6-BG and temozolomide, provided that at least 1% of peripheral blood granulocytes are positive for the MGMT(P140K) gene and criteria as outlined in Section 8, Part II, Criteria for Retreatment (below) are met. No escalation of temozolomide above 925 mg/m^2 is permitted. Should a patient experience DLT at an escalated dose of temozolomide, additional courses of temozolomide will be given at the previous dose without DLT, and no further intra-patient escalation may occur in that patient. Please also see 10. Primary Endpoints and 15. Statistical Considerations.

Treatment courses may be repeated at least every 28 days for a total of 24 courses (96 weeks). The body surface area (BSA) will be calculated before the start of each cycle to determine the dose of temozolomide to be administered for that cycle. The height obtained at the pretreatment visit and the weight obtained at each study visit prior to dosing will be used to calculate BSA.

I. O6-BG and Temozolomide Administration

O6-BG should be administered intravenously over 1 hour at a dose of 120 mg/m^2, followed immediately by O6-BG 30 mg/m^2/day for approximately 48 hours (equivalent to 1.25 mg/m^2/hr×48 hours). If the infusion is interrupted for less than 2 hours, it can be continued at the same rate to complete the planned dose. Temozolomide should be administered orally, in a fasting state, within one hour of the end of the bolus O6-BG infusion.

1. **G-CSF/Filgrastim**

   Treatment may be instituted for a grade 4 febrile neutropenia or asymptomatic grade 3 or 4 neutropenia. The dose of G-CSF will be 5 μg/kg daily given either SQ or IV. Patients will continue to receive G-CSF until ANC >5000 cells/mm^3.

2. **Criteria for Retreatment**

   Patients must have evidence of recovery from prior course toxicity, as indicated by:
   - i. ANC ≥1500/μl (off G-CSF for at least 2 days)
   - ii. Platelets ≥100,000/μl (transfusion independent)
   - iii. Hgb >8 g/dl (transfusion independent)
   - iv. SGOT <5.0 times the upper limit of normal and SGPT <5.0 times the upper limit of normal
   - v. Total bilirubin <1.5 times upper normal except in patients with a history of Gilbert's syndrome. In patients with Gilbert's syndrome, retreatment can occur as long as total bilirubin is <1.5 times the upper limit typically observed in that patient.
   - vi. Creatinine <1.5 times upper normal
   - vii. Non-hematological toxicities associated with the BCNU course, infusion of gene-modified cells, or previous cycle of O6-BG/temozolomide (with the exception of nausea, vomiting, and alopecia) should have resolved to grade 1 or baseline function (see CTC).

3. **Duration of Therapy**

   O6-BG/temozolomide treatments will start after hematopoietic recovery from BCNU course, indicated by an ANC of ≥1500/μl and platelet count ≥100,000/μl, provided no additional non-hematologic toxicities associated with the infusion of gene-modified cells or BCNU conditioning are evident (with the exception of nausea, vomiting, and alopecia; see "2. Criteria for Retreatment" above). Minimum cycle length for chemotherapy is 4 weeks. O6-BG/temozolomide cycles may be repeated every 28 days. Patients may continue O6-BG/temozolomide therapy for a maximum of 24 courses (96 weeks) if they do not exhibit unacceptable toxicity and there is no clinical and radiographic evidence of unequivocal progressive disease. Any patient with unequivocal progressive disease, as assessed by the attending neuro-oncologist or the neuro-oncology tumor board will be removed from study.

   Subjects who do not complete the study protocol will be considered to have prematurely discontinued the study. The reasons for premature discontinuation must be recorded on the case report form. Potential reasons for premature discontinuation include:

   - i. The development of a life-threatening infection.
   - ii. The judgment of the treating physician that the patient is too ill to continue.
   - iii. Patient noncompliance with study therapy and/or clinic appointments.
   - iv. Pregnancy.

v. Voluntary withdrawal; a patient may remove him/herself from the study at any time without prejudice.
vi. Significant and rapid unequivocal progression of disease as assessed by the attending neuro-oncologist or the neuro-oncology tumor board. (See definition of Progressive Disease in section 10.B.1. **Response**.)
vii. Grade 3 or 4 toxicity judged to be possibly or probably related to genetically modified stem cell infusion.
viii. A delay of more than 14 days in the administration of treatment.
ix. Termination of the study by the principal investigator, the IRB, or the Food and Drug Administration.

4. **Supportive Care Guidelines for Both BCNU and O6BG/temozolomide**

Supportive care measures should be initiated as per institutional practice or in accordance with the guidelines below.

a. *G-CSF*

Administration of G-CSF will be allowed.

b. *Blood products*

All blood products should be administered according to standard practice guidelines.

c. *Platelets*

Transfuse as necessary to maintain the platelet count at >20,000/mm^3 following chemotherapy.

d. *Red blood cells*

Therapy induced anemia and reticulocytopenia are expected. Transfuse with irradiated packed red blood cells to maintain hematocrit >20−25%.

e. *Pneumocystis prophylaxis (PCP)*

All patients should receive PCP therapy as per standard practice guidelines during transplant and chemotherapeutic administration.

f. *Neutropenic fever*

Patients with fever (>38° C) and ANC <500 will be evaluated promptly, cultured and treated with broad spectrum antibiotics, with an attempt to avoid nephrotoxic drugs. Antifungal treatment should be given to those patients who have received at least 5−7 days of IV broad spectrum antibiotics with persistent fevers and those with documented fungal colonization or CT evidence of systemic fungal disease. Patients will be treated per Standard Practice Guidelines: “Antibiotic Indications (Prophylactic Systemic and Empiric Regimens).”

g. *Nutritional management*

Any patients with greater than 10% weight loss from pretreatment baseline should begin nutritional support.

h. *Antiemetics*

The preferred anti-emetic for glioma patients receiving O6-BG/temozolomide chemotherapy is Ondansetron 12 to 16 mg PO or IV one half hour before chemo, then 8 to 16 mg Q8 hours up to 36 hours, then 8 mg Q8 hours PRN. Antiemetics for BCNU chemotherapy should be those recommended per standard practice guidelines.

IX. Evaluation

A. Before G-CSF and possible plerixafor mobilization, Apheresis and BCNU Treatment

1. Medical history as per standard practice guidelines.
2. Physical Exam including neurological, vital signs, weight and height
3. MGMT promoter methylation status in tumor cells must be unmethylated or hypomethylated.
4. Laboratory work per standard practice guidelines for transplant patients:
    i. CBC with differential
    ii. Comprehensive metabolic panel (SCOMP) and Hematopoietic stem cells Transplant panel (SHSCT)
    iii. CMV titer
    iv. HSV, VZV titers
    v. ABO, Rh typing and antibody screen
    vi. Recipient/Patient Battery
    vii. HIV-1 P24 Antigen Quantitation
    viii. HCG (quantitative pregnancy) if clinically indicated
    ix. Urinalysis
    x. Anticonvulsant level as clinically indicated on Day 0 of transplant
5. Procedures per standard practice guidelines for transplant patients (with exception of Brain MRI):
    i. Brain MRI (performed as follow-up to completion of radiation therapy per standard neuro-oncology/radiation oncology guidelines).
    ii. MUGA scan (multiple gated acquisition scan or Echocardiogram to assess heart function
    iii. 12-lead ECG, Chest x-ray (PA and LAT)
    iv. Pulmonary Function Test (DLCO)
    v. Oral Medicine Exam including Panarex if clinically indicated
    vi. Gynecology consultation (female subjects)
    vii. Unilateral bone marrow aspiration and biopsy for pathology review, cytogenetics and flow cytometry

B. Evaluation prior to infusion of genetically modified cells

Evaluation before the infusion of genetically modified cells will be done as per FDA guidelines. Cells need to be negative by Gram stain before re-infusing. Endotoxin test must also be completed.

C. Evaluation during O6-BG/temozolomide treatment

1. Physical exam including neurological, vital signs, and weight before each cycle
2. CBC with differential weekly
3. Comprehensive metabolic panel (sodium, potassium, chloride, carbon dioxide, glucose, BUN, creatinine, calcium, total protein, albumin, bilirubin, AST/ALT, alk phos, globulin, A/G ratio) – every 2 weeks and before each treatment
4. Anticonvulsant level(s) as clinically indicated
5. Urinalysis – before each treatment.
6. Pulmonary function test as clinically indicated.
7. MRI of brain – after every other treatment cycle or more frequently if clinically indicated.
8. Blood will be evaluated before each treatment for the presence of the transgene.
9. If feasible, serum levels of temozolomide and O6-BG will be monitored following dose escalation to verify physiological dose achieved.
10. Long term follow-up as outlined below.

D. Testing for Replication Competent Retrovirus

In compliance with FDA Guidance, "Supplemental Guidance on Testing for Replication Competent Retrovirus in Retroviral Vector Based Gene Therapy Products and During Follow-up of Patients in Clinical Trials Using Retroviral Vectors" (November 28, 2006), patients' samples will be obtained for testing for replication competent retrovirus (RCR) pretreatment, and at 3, 6, and 12 months for the first year and annually thereafter. If all post-treatment assays are negative during the first year, subsequent yearly samples will be archived. Samples will be archived with appropriate safeguards to ensure long-term storage and an efficient system for the prompt linkage and retrieval of the stored samples with the medical records of the patient and the production lot records. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with CBER.

E. Long-Term Follow-up

Patients will be followed for 15 years, in compliance with the FDA Guidance, "Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events" (November 28, 2006). As per this guidance, viruses that have a potential to integrate, including gammaretroviruses, "present sufficient risk that long-term follow-up observations are necessary to mitigate long-term risks to subjects receiving these vectors." The patients on this study will have follow-up clinical visits every 1−3 months for the first two years post infusion, then every 3−6 months through year five. A minority of patients with high-grade glioma survive to beyond two years with current treatment.

At clinical follow-up visits, patients will be examined for clinical evidence suggestive of retroviral disease, such as cancer, neurologic disorders, or other hematologic disorders. Additionally, samples will be collected to determine levels of gene modified cells in peripheral blood. Suspect clinical symptoms or findings may trigger RCR analysis of archived samples and/or taking additional samples, in consultation with CBER. If patients die or develop neoplasms, every effort will be made to assay for RCR in a biopsy sample of the neoplastic tissue or the pertinent autopsy tissue. At clinic visits (as described above), patients will undergo physical examination and laboratory testing including CBC with differential, comprehensive metabolic panel and levels of gene modified cells. In case histories, physicians will record details of all exposures to mutagenic agents and other medications. Physicians will record the emergence of new clinical conditions, including new malignancies, new incidence or exacerbation of a pre-existing neurologic disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, and new incidence of a hematologic disorder. Study subjects and health providers will be asked to cooperate in reporting delayed adverse events, including unexpected illness and hospitalization.

For those patients who survive beyond five years, patients will be contacted at least annually. Patients will generally be seen by their doctors in their local area. However, for visits that are purely study-related, the University of Washington General Clinical Research Center (which has approved this trial) is available to assist in the long-term follow-up of participants in NIH-funded gene transfer clinical trials (per NCRR).

X. Primary Endpoints

A. Safety

1. **Definition of dose-limiting toxicity**
   a) *Non-Hematologic Dose-Limiting Toxicity*
      - Dose-limiting toxicity (DLT) will be defined as any Grade 4 non-hematologic toxicity that

is likely related to the investigational procedures

b) *Hematologic dose-limiting toxicity for dose escalation of temozolomide during Part I*
- Grade 4 neutropenia of >7 days duration (despite G-CSF administration)
- Grade 4 thrombocytopenia >7 days duration

c) *Hematologic dose-limiting toxicity for dose escalation of temozolomide during Part II:*
- Grade 4 neutropenia of >7 days duration (despite G-CSF administration)
- Grade 4 thrombocytopenia >7 days duration
- Delay of >14 days beyond the planned interval between treatment courses due to hematologic toxicity after the first cycle of O6-BG and temozolomide

2. **Development of replication competent retrovirus or leukemia**
- Any development of RCR or leukemia

B. Efficacy

MRI with and without gadolinium contrast will be the imaging procedure used to judge response as below. Note that contrast-enhanced CT may be used for patients who cannot undergo MRI (for example, those patients with embedded metal fragments, cardiac pacemakers). Due to the high incidence of pseudoprogression, defined as an increase in enhancement within the irradiated field that spontaneously subsides without new antitumor treatments, in GBM patients within the first 12 weeks following completion of radiotherapy or more commonly chemo-radiotherapy, and given the primary dose-finding endpoint of this study, enrolled patients will not be considered evaluable for tumor response until 12 weeks following the completion of radiotherapy [27-31]. For the purpose of this study, we are utilizing modified RANO criteria [32]. RANO criteria describe response assessment in patients following chemo-radiotherapy, however, pseudo-progression can occur after radiation only. We will be using RANO criteria for this study with the understanding that all subjects receive radiotherapy alone, followed by chemotherapy.

Table 1. Criteria for Determining First Progression Depending on Time from Initial Chemoradiotherapy [32]*

First Progression	Definition
Progressive disease <12 weeks after completion of chemotherapy	Progression can only be defined using diagnostic imaging if there is new enhancement outside of the radiation field (beyond the high-dose region or 80% isodose line) or if there is unequivocal evidence of viable tumor on histopathologic sampling (e.g., solid tumor areas [i.e., >70% tumor cell nuclei in areas], high or progressive increase in MIB-1 proliferation index compared with prior biopsy, or evidence for histologic progression or increased anaplasia in tumor). Note, Given the difficulty of differentiating true progression from pseudoprogression, clinical decline alone, in the absence of radiographic or histologic confirmation of progression, will not be sufficient for definition of progressive disease in the first 12 weeks after completion of radiation* or concurrent chemoradiotherapy
Progressive disease ≥12 weeks after chemoradiotherapy completion	1. New contrast-enhancing lesion outside of radiation field on decreasing, stable, or increasing doses of corticosteroids. 2. Increase by ≥25% in the sum of the products of perpendicular diameters between the first post-radiotherapy scan, or a subsequent scan with smaller tumor size, and the scan at 12 weeks or later on stable or increasing doses of corticosteroids. 3. Clinical deterioration not attributable to concurrent medication or comorbid conditions is sufficient to declare progression on current treatment but not for entry onto a clinical trial for recurrence. 4. For patients receiving antiangiogenic therapy, significant increase in T2/FLAIR nonenhancing lesion may also be considered progressive disease. The increased T2/FLAIR must have occurred with the patient on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy and not be a result of comorbid events (e.g., effects of radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects).†

Abbreviation: FLAIR, fluid-attenuated inversion recovery.
*Modified for use in this study to evaluate and distinguish progressive disease from pseudoprogression after radiation and/or chemotherapy.
†Criterion number four will not be applicable to this study as anti-angiogenic therapy will not be allowed while the patient is on the protocol.

Reprinted from Wen et al., Updated Response Assessment Criteria for High-Grade Gliomas: Response Assessment in Neuro-Oncology Working Group. *J Clin Oncol* 28:1963-1972, 2010.

1. **Response**
   a) *Complete Response (CR)*

Requires all of the following: complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks; no new lesions; stable or improved non-enhancing (T2/FLAIR) lesions; patients must be off corticosteroids (or on physiologic replacement doses only); and stable or improved clinically. Note: Patients with non-measurable disease only cannot have a complete response; the best response possible is stable disease.

b) *Partial Response (PR)*
Requires all of the following: >50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks; no progression of non-measurable disease; no new lesions; stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; the corticosteroid dose at the time of the scan evaluation should be no greater than the dose at time of baseline scan; and stable or improved clinically. Note: Patients with non-measurable disease only cannot have a partial response; the best response possible is stable disease.

c) *Stable Disease (SD)*
Requires all of the following: does not qualify for complete response, partial response, or progression; stable non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

d) *Progressive Disease (PD)*
Note: The criteria for establishing progressive disease within the first 12 weeks from completion of radiotherapy or chemo-radiotherapy (described above).

Progressive disease is defined by any of the following: >25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids; significant increase in T2/FLAIR non-enhancing lesion on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy not caused by comorbid events (e.g., radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects); any new lesion; clear clinical deterioration not attributable to other causes apart from the tumor (e.g., seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose; failure to return for evaluation as a result of death or deteriorating condition; or clear progression of non-measurable disease.

e) *Brain MRI*

Standard MRI scan of the brain without and with administration of intravenous contrast will be performed on all patients at the times specified above. Standard 2 dimensional (2D) measurements for response assessment will be obtained. In addition, volumetric analysis may be performed if available.

Once evaluable, duration of response will be measured from the onset of temozolomide therapy through the date at which unequivocal disease progression is determined by the attending neuro-oncologist or the neuro-oncology tumor board. At that time, the patient will be removed

from the protocol and can be treated with other appropriate therapy. Survival will be measured from the first day of treatment until death. Patients will be followed until death.

Time to progression (TTP) will be measured from the first day of treatment until unequivocal progression is documented.

In order to be evaluable, a patient:

i. Must have an MRI scan at appropriate time intervals, as clinically indicated or at a minimum of every other chemotherapy cycle.
ii. Must receive at least one full dose of temozolomide in cycle 1.
iii. Must remain off steroids or on stable dose of steroids (see criteria above).
iv. Must have study parameter data set complete.

2. **Gene transfer efficiency and in vivo selection**

Gene marking in peripheral blood and marrow will be assessed on a monthly basis.

3. **Chemoprotection**

The ability to increase the temozolomide dose beyond 472 mg/m^2.

XI. Chemotherapy Medications and Growth Factors

A. Drug Formulations

1. BCNU

(1,3-bis (2-chloroethyl)-1 -nitrosourea) (NSC-409962) (Carmustine) (BiCNU). BCNU is a lipid soluble agent which has alkylating properties, plus an isocyanate metabolite which interferes with DNA and RNA synthesis.

- **Toxicities:** The most frequent and most serious toxicity of BCNU is delayed myelosuppression. Pulmonary infiltrates and/or fibrosis, dry cough and difficulty breathing have been reported. Cases of fatal pulmonary toxicity with BCNU have been reported. Nausea and vomiting are very common. A reversible hepatic toxicity is demonstrated by elevated SGOT, alkaline phosphatase and bilirubin. Renal abnormalities consisting of progressive azotemia, decrease in kidney size and renal failure have been reported. Thrombophlebitis and local ulceration occur if extravasation occurs. Venous pain and flushing during injection and a brownish discoloration of skin on contact have also occurred. Rapid infusion may produce intensive flushing. Neuroretinitis has been reported. The occurrence of acute leukemia and bone marrow dysplasia has been reported in patients following long-term nitrosourea therapy. The occurrence of acute leukemia has been reported rarely in patients treated with anthracycline/alkylator combination chemotherapy.
- **Kinetics:** IV BCNU is rapidly degraded to active metabolites. Sixty to 70% is excreted in the urine in 96 hours and 10% as respiratory CO. Because of the high lipid solubility, it crosses the blood-brain barrier readily.
- **Formulation:** BCNU is supplied as a lyophilized powder containing no preservatives. Due to its water insolubility, it is to be reconstituted with the diluent provided (3 ml of absolute ethanol) and then with either 27 ml or 17 ml of sterile water for injection to provide a resulting concentration of 3.3 mg/ml or 5 mg/ml, respectively.
- **Storage and Stability:** Stability upon reconstitution (less than 8% potency lost) is eight hours at room temperature or 24 hours if refrigerated and protected from light. BCNU solution may

be further diluted with 500 ml of 5% Dextrose OR sodium chloride injection, USP, and will be stable for 8 hours. Diluted solution should be protected from light. Unopened vials are stable under refrigeration for two years. BCNU has a low melting point **requiring refrigeration at all times prior to reconstitution. Do not use if an oil film is present at the bottom of vial.**

- **Administration:** BCNU should be administered by IV drip over three hours. Absorption of BCNU to plastics has been documented. Thus, PVC free bags and polyethylene tubing should be used during the administration of BCNU.

2. **Temozolomide (Capsules)**

Temozolomide is commercially available from Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., under the trade name Temodar. Temozolomide is not directly active but undergoes rapid nonenzymatic conversion at physiologic pH to the reactive compound 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC). The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O^6 and N^7 positions of guanine.

- **Chemical Name:** 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-*as*-tetrazine-8-carboxamide.
- **Kinetics:** The drug is rapidly and completely absorbed after oral administration; peak plasma concentrations occur in a median T_{max} of 1 hour. Food reduces the rate and extent of temozolomide absorption. Mean peak plasma concentration and AUC decrease by 32% and 9%, respectively, and T_{max} increased 2-fold (from 1.1 to 2.25 hours) when temozolomide was administered after a high fat breakfast. Investigators may refer to the package insert for further details.
- **Storage:** Temozolomide capsules should be stored in amber glass bottles at 25°C; excursions permitted to 15-30°C. Temozolomide may be dispensed to the patient in amber plastic containers.
- **Formulation:** Temozolomide is available in 5 mg (opaque white bodies with green caps), 20 mg (opaque white bodies with yellow caps), 100 mg (opaque white bodies with pink caps), 140 mg (opaque white bodies with blue caps), 180 mg (opaque white bodies with orange caps) and 250 mg (opaque white bodies with white caps) capsules. Refer to package insert for contents of the formulation. The commercial product has an expiration date on the label of the bottle. Temozolomide is administered orally, with a glass of water. Tablets should not be chewed. To avoid nausea, other than water, it should be taken on an empty stomach. The drug is approximately 100% bioavailable. The dose should be rounded to the nearest 5 mg. Temozolomide should be administered every 28 days no sooner than 6 hours following the IV bolus dose of O6-BG.
- **Adverse Reactions:**
  - The most common adverse reactions (≥10% incidence) are: alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, abnormal coordination, viral infection, amnesia and insomnia.
  - The most common Grade 3 or 4 hematologic laboratory abnormalities (≥10% incidence) that have developed during treatment with temozolomide are: lymphopenia, thrombocytopenia, neutropenia, and leukopenia.
  - Allergic Reactions have also been reported.
- **Contraindications:** Temozolomide is contradicted in patients who have a history of hypersensitivity reaction (such as urticarial, allergic reaction including anaphylaxis, toxic epidermal necrolysis, and Stevens-Johnson syndrome) to any of its components. Temzolomide

is also contraindicated in patients who have a history of hypersensitivity to dacarbazine (DTIC), since both drugs are metabolized to 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC).

- **Warnings and Precautions:**
  1) Myelosupression: Patients treated with temozolomide may experience myelosupression, including prolonged pancytopenia, which may result in aplastic anemia, which in some cases has resulted in fatal outcome.
  2) Myelodysplastic Syndrome: Cases of myelodysplastic syndrome and secondary malignancies, including myeloid leukmia, have been observed.
  3) *Pneumocytosis* Pneumonia (PCP): There may be a higher occurrence of PCP when temozolomide is administered during a longer dosing regimen. All patients receiving temozolomide, particularly patients receiving steroids, should be observed closely for the development of PCP regardless of the regimen.
  4) Hepatotoxicity: Fatal and severe hepatotoxicity have been reported in patients receiving temozolomide.
  5) Use in Pregnancy (Pregnancy Category D): Temzolomizde can cause fetal harm when administered to a pregnant woman. Women of childbearing potential should be advised to avoid becoming pregnant during therapy with temozolomide.

3. **O^6-Benzylguanine**

O6-BG is a potent inhibitor of O6-benzylguanine-DNA akyltransferase (MGMT), a DNA repair protein that plays an important role in protecting cells from the lethal effects of chloroethylnitrosoureas and triazines. O6-BG is supplied by University Hospitals Case Medical Center in a dual pack with diluent. 100 mg of the active drug is supplied as a white lyophilized powder with 670 mg mannitol, USP, and sodium hydroxide to adjust the pH to 7-8.5. The 30 mL vial of diluent (NSC 659805) is a sterile solution of 40% polyethylene glycol 400 in pH 8 phosphate buffer (106 mg dibasic sodium phosphate and 102 mg monobasic potassium phosphate in sterile water for injection, USP).

- **Chemical Name:** O6-benzylguanine, 6-(phenylmethoxy)-1H-purin-2-amine.
- **How Supplied**: O6-BG is supplied by University Hospitals Case Medical Center in a dual pack with diluent. 100 mg of the active drug is supplied as a white lyophilized powder with 670 mg mannitol, USP, and sodium hydroxide to adjust the pH to 7-8.5. The 30ml vial of diluent (NSC 659805) is a sterile solution of 40% polyethylene glycol 400 in pH 8 phosphate buffer (106 mg dibasic sodium phosphate and 102 mg monobasic potassium phosphate in sterile water for injection, USP).
- **Preparation**: Withdraw 30ml from the diluent vial; add to the vial of O6-BG. Shake until complete solution is observed. Each milliliter of the resulting solution will contain 3.3 mg O^6-Benzylguanine, 22 mg of mannitol, UPS, 0.4 ml of polyethylene glycol 400 and approximately 0.6 ml pH 7 phosphate buffer. After reconstitution, filter the 30ml suspension of BG through a sterile MILLEX GV 0.22 micron filter into an empty Viaflex container.
- **Storage**: Refrigerate dual pack or intact vials (2-8 deg C).
- **Stability and Handling**: Shelf-life of intact vials is on-going. Samples from a pilot lot of freeze-dried vials showed significant loss of potency after one month's storage at 50deg C.

  Solutions of drug, reconstituted as above, were stable for at least 24 hours when stored at room temperature. The drug, further diluted to 0.04 mg/ml with 0.9% saline or dextrose 5% in water,

was also stable for at least 24 hours at room temperature. However, at many concentrations between those two extremes, an inconsistent precipitation problem has been documented. Therefore, based on current doses, the University Hospitals Case Medical Center recommends that the 3.3 mg/ml concentration be used for patient administration.

CAUTION: The single-use lyophilized dosage form contains no antibacterial preservatives. Therefore, it is advised that the reconstituted product be discarded 8 hours after initial entry.

- **Sterility:** The sterility of clinical grade O6BG after filtration through a 0.2 micron membrane has been demonstrated (Appendix B) and is safe for use in patients.
- **Route/Frequency of Administration:** Intravenous bolus infusion (loading dose) over 1 hour followed by continuous infusion over approximately 48 hours. O6-BG should be administered every 28 days concurrently with temozolomide.
- **Known Human Toxicities:** The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. *Frequency is provided based on 265 patients.* See Table 2. CAEPR for O-6-Benzylguanine.

  Also reported on O-6-Benzylguanine trials but with the relationship to O-6-Benzylguanine still undetermined:
  - **Cardiac disorders:** Palpitations; Supraventricular tachycardia
  - **Ear and labyrinth disorders:** Ear pain
  - **Eye disorders:** Flashing lights
  - **Gastrointestinal disorders:** Abdominal pain; Dyspepsia; Gastrointestinal hemorrhage (includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC); Mucositis oral
  - **General disorders and administration site conditions:** - Injection site reaction
  - **Infections and infestations:** Infection (includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC)
  - **Injury, poisoning and procedural complications:** Bruising
  - **Investigations:** Creatinine increased; GGT increased; Weight gain; Weight loss
  - **Metabolism and nutrition disorders:** Dehydration; Hypercalcemia; Hyperkalemia; Hypermagnesemia; Hypernatremia; Hypoglycemia; Hypomagnesemia
  - **Musculoskeletal and connective tissue disorders:** Generalized muscle weakness; Myalgia
  - **Nervous system disorders:** Depressed level of consciousness; Dysphasia; Intracranial hemorrhage; Peripheral motor neuropathy; Peripheral sensory neuropathy
  - **Psychiatric disorders:** Anxiety; Confusion
  - **Renal and urinary disorders:** Urinary frequency; Urinary retention; Urinary tract pain
  - **Respiratory, thoracic and mediastinal disorders:** Cough; Dyspnea; Epistaxis; Hypoxia; Pharyngeal mucositis; Pleural effusion; Pneumonitis
  - **Skin and subcutaneous disorder:** Pruritus; Purpura; Rash maculo-papular; Skin hyperpigmentation

- **Vascular disorders:** Flushing; Hypotension; Phlebitis; Vascular disorders - Other (hemorrhage with thrombocytopenia)

Note: O-6-Benzylguanine in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

Version 2.1, August 12, 2013

Adverse Events with Possible Relationship to O-6-Benzylguanine (CTCAE 4.0 Term) [n= 265]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 3)***
	Febrile neutropenia		
GASTROINTESTINAL DISORDERS			
	Constipation		
	Diarrhea		
Nausea			***Nausea (Gr 2)***
Vomiting			***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
	Fever		
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 2)***
	Alkaline phosphatase increased		
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 2)***
	Blood bilirubin increased		
	Carbon monoxide diffusing capacity decreased		
Lymphocyte count decreased			***Lymphocyte count decreased (Gr 2)***
Neutrophil count decreased			***Neutrophil count decreased (Gr 4)***
Platelet count decreased			***Platelet count decreased (Gr 4)***
White blood cell decreased			***White blood cell decreased (Gr 4)***
METABOLISM AND NUTRITION DISORDERS			
	Acidosis		
	Anorexia		
	Hyperglycemia		
	Hypoalbuminemia		
	Hypocalcemia		
	Hypokalemia		
	Hyponatremia		
	Hypophosphatemia		
NERVOUS SYSTEM DISORDERS			
	Dizziness		
	Headache		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		

4. **G-CSF**

Recombinant methionyl human G-CSF (Filgrastim) is produced in E. coli transfected with the human G-CSF gene. The E. coli-derived protein consists of 175-amino acids with a molecular

weight of about 18,800 daltons and differs from the natural protein in that the N-terminal amino acid is a methionine and the protein is not O-glycosylated.

The E. coli transfected with the human G-CSF gene are grown in culture under defined and controlled conditions. The bacteria are harvested, and Filgrastim extracted and purified via a series of proprietary processing and chromatographic steps. The resulting purified Filgrastim is formulated in an aqueous buffer before undergoing sterile filtration and filling.

- **Formulation and Stability:** Criteria for release of Filgrastim for use in the clinic are stringent. These include passing the USP pyrogen test, the limulus amebocyte assay, a sterility test, and the general safety test (Code of Federal Regulations, Title 21 Section 610.11). The nucleic acid content can be no greater than 10 pg/unit dose. The final product is a clear, colorless, sterile protein solution free of particulates; Filgrastim is not less than 95% pure.

  G-CSF is a sterile, clear, colorless, preservative-free liquid for parenteral administration. Each single-use vial of G-CSF contains 300 µg/ml of filgrastim at a specific activity of $1.0 \pm 0.6 \times 10^8$ U/mg. The product is formulated in a 10 mM sodium acetate buffer at pH 4.0, containing 5% mannitol and 0.004% Tween 80. Both 1.0 and 1.6 ml vials are available. Store between 2 and 8° C. Do not freeze. Avoid shaking. Drug administered SQ should not be diluted and can be drawn directly from the vial and administered.

  Dilution for IV infusion should be performed in D5W. If the final concentration of G-CSF is <15 µg/ml, albumin (human) at a final concentration of 2 mg/ml should be added to the D5W prior to addition of G-CSF to prevent adsorption to the components of the drug delivery system. Do not dilute G-CSF <5 µg/ml under any circumstances. IV drug can be infused over 30 minutes.

  G-CSF contains no antibacterial preservative and therefore should be administered as soon as possible and no longer than 6 hours following dilution for IV infusion. The solution should be stored under refrigeration at 2-8°C. In the absence of compatibility and stability information, no other medication should be added to infusion solutions containing G-CSF.
- **Supplier:** Commercially available from Amgen.
- **Route of Administration:** IV or subcutaneously.
- **Toxicities:** The predominant toxicity from G-CSF is mild medullary bone pain, which is usually easily manageable with analgesics. Additional possible side effects of G-CSF are fatigue, muscle aches, abnormal liver function, pain or redness at the injection site, nausea, insomnia, and headache. Vasculitis is a rare side effect.

5. **Plerixafor**

   Plerixafor (previously known as AMD3100, and sold commercially as Mozobil™) is an inhibitor of CXCR-4. CXCR-4 is the receptor that is involved in retention of stem cells in the marrow through interaction with SDF-1. Plerixafor was approved by the Food and Drug Administration in December 2008, as a drug intended to be used in combination with G-CSF to mobilize hematopoietic stem cells to the bloodstream for subsequent autologous transplantation in patients with non-Hodgkin's lymphoma and multiple myeloma.

- **Supplier:** Sanofi/Genzyme
- **Formulation and Storage:** Plerixafor is supplied as a solution of 20 mg/ml per 1.2 ml glass vial. Plerixafor will be stored at room temperature (25ºC); excusions permitted to 15−30 ºC.

- **Route of Administration:** The amount of drug to be administered will be withdrawn from the vial using a syringe that will allow the drug to be drawn up in milliliters to one decimal place. The amount of drug to be administered will be calculated based on the patient's weight.
- **Toxicities:** Plerixafor is a novel mobilizing agent which seems to be well-tolerated with few side effects. There is a significant difference in the toxicity profile of plerixafor as compared to G-CSF, which likely reflects their different effects on the marrow. In contrast to the substantial marrow hyperplasia induced by G-CSF and the consequent bone pain, plerixafor does not produce bone pain.

  The most common side effects of plerixafor are gastrointestinal, including bloating, increased stool frequency, and loose stools. Other plerixafor-related adverse effects are headaches, erythema at the injection site, and mild thrombocytopenia.

  Since plerixafor is a relatively new drug, it is pertinent to summarize the most important studies that were published on this drug (at which point it was designated as AMD3100).

  In four studies with healthy volunteers, plerixafor was given to 82 healthy volunteers, either alone or after G-CSF. The most common side effects reported that were thought to be related to plerixafor were abdominal distension (7.3%), abdominal pain (7.3%), diarrhea (8.5%), nausea (23.2%), burning at the injection site (9.8%), erythema at the injection site (45.1%), swelling at the injection site (6.1%) dizziness (7.3%), headache (19.5%), paresthesias (11%), paresthesias around the mouth (11%), hypothesias (8.5%), and chest tightness (8.5%). All of these symptoms were mild and subsided after treatment. White blood cell counts were elevated in all subjects and returned to baseline within 24 hours after plerixafor was discontinued. Plerixafor has been administered to over 40 patients with Non-Hodgkins Lymphoma (NHL) and multiple myeloma (MM) on clinical trials, and 115 patients with NHL, Hodgkin's disease, and MM on a compassionate use protocol currently underway. The events reported to date are similar to those reported in healthy volunteers. In a study with 40 HIV^+ patients, one of the first eight patients developed a severe, but temporary thrombocytopenia after 6 days of continuous intravenous infusion. Another had premature ventricular contractions (unexpected AE) after 52 hours of drug.

XII. Protocol Enrollment and Special Considerations

A. Projected Target Accrual

Table 3. Ethnic and Gender Distribution Chart

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
	Sex / Gender		
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	1	1	2
Not Hispanic or Latino	11	11	22
Ethnic Category Total of All Subjects	12	12	24
Racial Categories			
American Indian / Alaska Native	0	0	0
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	2	2	4
White	9	9	18
Racial Categories: Total of All Subjects	12	12	24

XIII. Guidelines for Adverse Event Reporting

All unexpected and serious adverse events, which may be due to study treatment or intervention, will be reported to the FHCRC Institutional Review Office as soon as possible but within at least 7 calendar days of the investigator learning of the event.

A. Definitions

1. **Adverse Event (AE)**

   Any sign, symptom or illness that appears or worsens during the study period regardless of the relationship to the study agent will be considered an adverse event.

2. **Life-threatening Adverse Event**

   Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction.

3. **Unexpected Adverse Event**

   An adverse event, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package

insert/summary of product characteristics for an approved product). If applicable product information is not available, such as for studies that do not involve pharmaceutical products or devices, an unexpected adverse event is an adverse event that was not described in the study protocol or informed consent.

4. **Serious Adverse Event (SAE)**

   Any adverse event occurring that results in any of the following outcomes:

   - Patient death, regardless of cause;
   - a life-threatening adverse event (real risk of dying);
   - inpatient hospitalization or prolongation of existing hospitalization;
   - a persistent or significant disability/incapacity;
   - congenital anomaly in offspring conceived after initiation of study;
   - requires intervention to prevent permanent impairment of damage or events that, in the investigator's judgment, jeopardized the patient or required significant medical treatment to prevent one of the above outcomes.

   A life-threatening event is defined as having placed the patient, in the view of the Investigator, at immediate risk of death from the adverse event as it occurred. It does not include an adverse event that had it occurred in a more serious form, might have caused death. All adverse events that do not meet at least one of the above criteria are defined as non-serious.

B. Adverse Event Reporting Guidelines

All adverse events occurring during the study that are observed by the treating physician or reported by the patient after the initiation of G-CSF will be recorded until 30 days after the last infusion of O6BG/TMZ or until the patient is removed from the study, whichever comes later. All adverse events will be scored based on the criteria delineated in the NCI Common Terminology Criteria for Adverse Events (CTCAE Version 3.0).

C. Procedure for Reporting Adverse Events

Regulations defining the responsibilities for reporting serious and unexpected adverse reactions are defined in 21CFR312.32 (IND Safety Reports). Serious and unexpected adverse events noted by the the treating physician will be reported to the FDA, the FHCRC IRB, the Data and Safety Monitoring Board for this study, the UW Institutional Biosafety Committee, the SCCA Institutional Biosafety Committee, and the RAC (NIH/OBA). Copies of all adverse event reports submitted to the FDA should be sent to Case Hospitals Case Medical Center if reasonable causality of the unexpected serious adverse event is attributed to O6BG.

Fatal or life-threatening serious and unexpected adverse events that may be associated with study treatment will be reported as soon as possible, and always within 7 days, by telephone or fax to the agencies and committees indicated. The immediate report must be followed by a detailed written report within 15 working days. Serious and unexpected adverse events that are not fatal or life-threatening will be reported within 7 days to the FHCRC IRB and the DSMB, and within 15 days to the additional agencies and committees noted above. The report should include the date of onset, severity and duration of the event, the relationship to the genetically modified stem cell infusion, the treatment given, and the eventual outcome.

Appropriate clinical, diagnostic, and laboratory measure to attempt to delineate the cause of the adverse reaction in question must be performed and the results reported. All tests that reveal an

abnormality considered to be related to the genetically modified stem cell infusion will be repeated at appropriate intervals until the course is determined or a return to normal or baseline values occurs. All AEs related to the genetically modified stem cell infusion will be followed until resolution or until the subject's death.

D. Data Safety and Monitoring Plan (DSMP)

In compliance with the DSMP, mandated by the NIH/NCI, consortium investigator initiated studies not monitored by other entities that are treating subjects at Consortium sites are eligible for monitoring by the Clinical Trials Support Office (CTSO). According to the DSMP, clinical trials that meet the criteria for "High Risk" (i.e. investigator-sponsored IND, Phase I or gene therapy studies) will begin monitoring no later than 6 months after the first subject has completed study treatment. Thereafter, high risk studies are monitored at least twice annually when subjects are enrolling and receiving study intervention. See Appendix A for the complete DSMP for this protocol.

E. Data Safety and Monitoring Board (DSMB)

This protocol will have a DSMB that will be responsible for reviewing protocol data and safety endpoints. See Appendix A for DSMB details. Reports will be submitted to the Institutional Review Board, PDMC Chairman, and the Protocol Office.

XIV. Records

Clinical Statistics maintains a patient database at FHCRC to allow storage and retrieval of patient data collected from a wide variety of sources. The investigator will ensure that data collected conform to all established guidelines for coding, collection, key entry and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Patients will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. Patient research files are kept in a locked room. They are maintained by the FHCRC data collection staff which is supervised by an A.R.T. Access is restricted to personnel authorized by the Division of Clinical Research.

XV. Statistical Considerations

Dose limiting toxicity, defined as any grade 4 non-hematopoietic toxicity that is likely related to the investigational procedures.

The following define the allowable dose level for BCNU during conditioning. Conditioning is the first chemotherapy using BCNU.

Part I – Conditioning dose of BCNU

Dose level 1 BCNU 600 mg/m^2

Part II – Escalation of the initial dose of temozolomide after conditioning (conditioning dose of BCNU is fixed at 600mg/m^2)

Dose level 1	590 mg/m^2
Dose level 2	740 mg/m^2
Dose level 3	925 mg/m^2

In Part I, patients can be retreated with O6-BG and 472 mg/m^2 of temozolomide at least every 4 weeks up to 96 weeks or 24 cycles if they meet the retreatment criteria defined in the protocol (See Section 9. Evaluation, Subsection C). Provided patients tolerate this dose for at least two cycles, as indicated by hematopoietic recovery consistent with retreatment criteria (included below) and at least 1% gene modified granulocytes in the peripheral blood within 28-42 days from the previous treatment, dose escalation in 25% increments, beginning with 590 mg/m^2 and not exceeding 925 mg/m^2 temozolomide. If recovery occurs later than 42 days from the previous treatment, the previous dose should be reduced by 25%. If retreatment criteria are achieved within 28-42 days following the dose reduced cycle, escalation to the previous dose that caused delay is permitted, but must be tolerated for two consecutive cycles before further dose escalation may be attempted in that patient. Dose levels will be closed to intra-patient escalation if more than one patient experiences a DLT at that dose, or if one patient experiences a DLT at the escalated dose and a second patient experiences a DLT at a lower dose. If no additional patients experience a DLT at the same dose, escalation to that dose level may continue, but no further dose escalation may be attempted in the patient experiencing the DLT.

In Part II, dose escalation between dose levels will follow a standard 3+3 design. Three patients will initially be enrolled at a dose level. If no patient experiences DLT, then escalation to the next level is permitted. If it becomes possible to have more than three patients enrolled within a given treatment period, the third patient treated will be observed for seven days before additional patients are treated and the additional patients must be treated at the same dose level. If 1 of 3 patients experiences DLT, then an additional 3 patients will be enrolled at the same dose level. If no further DLT is seen, then escalation to the next level is permitted. A second DLT in any number of patients will halt enrollment to that dose level, and the next lower dose level will be defined as the MTD. The MTD dose level will then be similarly expanded to six patients.

Subjects will be followed for dose limiting toxicities for approximately 6 weeks after infusion of the genetically modified stem cells in Part I before advancing to Part II. Subjects in Part II will be observed for 28 days after the first escalated dose of O6-BG and temozolomide, and an additional 28 days following the second escalated dose of O6-BG and temozolomide (for a total of 56 days) before advancing to the next cohort.

The percentage of MGMT(P140K) containing peripheral blood granulocytes will be assessed prior to every post-conditioning cycle of temozolomide + O6-BG. At least 1% gene modified cells (average proviral copy number of 0.01) are required before escalation above 472 mg/m^2 temozolomide is permitted. If the level of MGMTP140K$^+$ neutrophils after conditioning is less than 1%, then the dose of temozolomide will remain at 472 mg/m^2 until the level is at least 1%, at which time the appropriate escalated dose of temozolomide will be given. If the level of MGMT(P140K)$^+$ neutrophil remains below 1%, then dose escalation will not be permitted in this patient, and this patient will not count for purposes of defining the post-conditioning MTD.

Should the maximum dose level defined in Part II be evaluated without defining an MTD, then that dose level will be defined as the MTD, unless the protocol is amended to define additional higher dose levels.

Should a second DLT occur at the first dose level defined in Part II, then the dose will be de-escalated by 25%.

In Parts I and II, intra-patient dose escalation of temozolomide is permitted after the first two doses of O6-BG and temozolomide, provided that at least 1% MGMT(P140K)$^{+}$ granulocytes are detected in the peripheral blood and retreatment critera, as defined in Section 9. Evaluation, Subsection C, are met. The escalated dose levels will be the same as for the inter-patient escalation of the initial temozolomide dose in Part II (590, 740, and 925 mg/m^2). Further intra-patient escalation may also occur after two doses have been given without DLT, and with the same conditions as for the first intra-patient escalation. No escalation of temozolomide above 925 mg/m^2 is permitted. Following dose escalation, serum levels of temozolomide will be measured to verify physiological dose achieved if feasible. Should a patient experience DLT at an escalated dose of temozolomide, additional courses of temozolomide will be given at the previous dose without DLT, and no further intra-patient escalation may occur in that patient.

DLT's observed during intra-patient dose escalation will not be formally counted as part of the inter-patient dose escalation of the initial dose of temozolomide in Part II. Nevertheless, if the experience at a particular dose given during intra-patient escalation suggests that this dose would also be intolerable as an initial dose, then the protocol will be modified to eliminate that dose as a possibility.

MGMT expression will be assessed post-conditioning and prior to every cycle of temozolomide. We will evaluate the correlation of the level of MGMT marking with toxicity, temozolomide dose achieved, and response, using linear regression (for quantitative outcomes) and logistic regression (for binary outcomes). For example, we might hope to see a suggestion as to whether the tolerable dose of post-conditioning temozolomide can be determined based on the level of marking achieved after conditioning. However, given the small sample size for this study and low probabilities of DLT and response, there will be limited power to detect such relationships.

All patients will be evaluated for tumor response and time to progression; however, the primary objective of this study is feasibility and dose-finding. As no more than 6 patients will be treated at the MTD, we will be unable to draw any conclusions regarding the potential efficacy of this treatment strategy. If the approach is feasible, and we can successfully administer higher doses of temozolomide than would otherwise be possible, then a sole Phase II study in which patient numbers could be used to draw statistical conclusions regarding potential efficacy would be planned.

STOPPING RULES:

The study will terminate after all cohorts have been filled and all patients have completed treatment. The study will be stopped if any of the following events occur:

1. Any development of replication competent retrovirus (RCR).
2. Any development of leukemia due to insertional mutagenesis.
3. Inability to detect gene-modified cells (that is, fewer than 1% gene modified granulocytes are detectable) in 5 or more patients after the first cycle of O6-BG and temozolomide.
4. Any grade 4 treatment-related non-hematological toxicity
5. Two out of the first six patients need backup cells. We would hold accrual for discussion with the DSMB regarding a possible change in study design.

The Principal Investigator reserves the right to terminate the study at any time The FDA may also terminate the study.

A stopping rule will be imposed for any grade 3/4 toxicity related to the infusion of modified stem cells. This rule will be applied across all dose levels and both parts of the study. We will stop the study for infusion toxicity if there exists reasonable evidence that the true rate of this event exceeds 0.30. Reasonable evidence will be taken to mean that the lower bound of a one-sided 80% confidence interval for the true rate of grade 3/4 infusion toxicity exceeds 0.30. Operationally, this rule will be evaluated at least every 6th patient, and will be triggered if 4 or more of 6 patients, 6 or more of 12 patients, 8 or more of 18 patients, or 10 or more of 24 patients experience the event. The probability of triggering this stopping rule is 61% if the true rate of grade 3/4 infusion toxicity is 0.40, and 88% if it is 0.50.

Development of replication competent retrovirus or leukemia. A toxicity here is defined as finding an evidence of replication competent retrovirus. We will use extended S+L- assays and PCR for GALV envelope sequences. An early stopping rule for this study will be invoked should 1 toxicity event occur. The properties (based on sequential Bernoulli outcomes) of this rule are as follows (**Table 4**):

Table 4. Probability of stopping at or before indicated number of patients due to single RCR event.

	True rate of RCR		
Number of patients	**.05**	**.10**	**.15**
5	23%	41%	56%
10	40%	65%	80%
15	54%	79%	91%
20	64%	88%	96%

For example, if the true toxicity rate is 10% or higher, then the chance of terminating the trial at or before the 10th patient because of observing one toxicity exceeds 65%.

XVI. References

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XVII. APPENDIX A − DATA AND SAFETY MONITORING PLAN

A. Protocol Review and Approval

The Fred Hutchinson Cancer Research Center (FHCRC) is an NCI comprehensive cancer center and consequently has an approved Protocol Review and Monitoring System (PRMS) in place that serves as a framework for this protocol. The key components of the PRMS are the Clinical Investigators Meeting (CIM), which provides an open forum for presentation and discussion of proposed clinical protocols; the Scientific Review Committee (SRC), which provides formal internal peer review of all protocols and general scientific oversight of clinical research; and the ancillary Protocol and Data Monitoring Committee (PDMC), which monitors the progress of ongoing research protocols. Data and safety monitoring are carried out under the umbrella of the PRMS committees in cooperation with the Institutional Review Board (IRB). This protocol has been and continues to be reviewed by the CIM and SRC of the Fred Hutchinson Cancer Research Center. This protocol has been and continues to be reviewed by the PDMC, and IRB of the Fred Hutchinson Cancer Research Center. In addition, it has been reviewed and approved by the National Cancer Institute, the Recombinant DNA Advisory Committee of the Office of Biotechnology Activities, the University of Washington Institutional Biosafety Committee, the University of Washington Radiation Safety Committee, and the University of Washington Clinical Research Center Scientific Advisory Committee. An Investigational New Drug (IND) Application for this protocol under the U.S. Food and Drug Administration, Center for Biologics Evaluation and Research (CBER) is in effect with a birthdate of December 23, 2008. This protocol has been and continues to be reviewed by the Recombinant DNA Advisory Committee of the National Institutes of Health (NIH) Office of Biotechnology Activities (OBA), as well as the National Cancer Institute (NCI) Cancer Therapeutics Evaluation Program (CTEP).

B. Monitoring the Progress of the Trial and Safety of Participants

The first level of trial oversight for this protocol will be provided by the IND sponsor, Dr. Hans-Peter Kiem; and Dr. Maciej Mrugala from the Department of Neurology at the University of Washington, who will provide continuous oversight of the trial. These individuals will meet regularly to review recently acquired data, stopping rules, and adverse events. Serious adverse events will be reviewed upon occurrence to ensure immediate and accurate reporting to the appropriate committees and regulatory agencies as described in section 13 of the protocol. Dr. Kiem and all other investigators on the protocol have received formal training in the ethical conduct of human research.

Additionally, institutional support of trial monitoring is provided in accordance with the Fred Hutchinson/University of Washington Cancer Consortium Data and Safety Monitoring Plan (current version dated July 31, 2009). Under the provisions of this plan, protocols must obtain annual re-approval by the Institutional Review Board (IRB) to continue accrual and are reviewed at least annually during this re-approval by the Protocol and Data Monitoring Committee (PDMC) if the protocol is subject to SRC review at the time of annual renewal. The PDMC reviews accrual, adverse events, stopping rules, and adherence to the data and safety-monitoring plan. The FHCRC IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects.

Approval of both committees is necessary to continue the study. Because this project involves an investigator-sponsored IND, and because it is a gene therapy trial, it is identified as “high risk” under the provisions of the FHCRC Monitoring Plan. This designation requires periodic monitoring visits for data, safety and protocol compliance and written reports to the PDMC. Dr. Kiem has formally delegated responsibility for these monitoring visits to the FHCRC/University of Washington Cancer Consortium

Research Trials Office (RTO) with a transfer of obligation agreement. Monitoring visits are conducted by a monitor identified by the RTO. Further details of monitoring visits are addressed under item 6 below.

An independent Data and Safety Monitoring Board (DSMB) has been established to monitor the conduct of this trial. None of the members of the DSMB collaborate with Dr. Kiem or Dr. Mrugala and none will be affiliated with the current trial. The membership of this independent board has been approved by Dr. Barry Storer, Chairman of the Fred Hutchinson Cancer Research Center, Protocol Data Monitoring Committee. The DSMB will initially meet after three (3) subjects have been enrolled or after six months (whichever comes first). After that, the DSMB will meet a minimum of once every six months during the course of the study and more frequently if serious and unexpected adverse events (SAEs) are encountered. Teleconference meetings are acceptable. Dr. Kiem will be responsible for ensuring that meetings occur as planned and that data summaries are provided to DSMB members prior to the meetings. The DSMB meetings will consist of an open session in which Drs. Kiem\ and Mrugala present the interim data and answer questions, followed by a closed session in which DSMB members meet without the study investigators to discuss accrual, patient eligibility, protocol compliance, adverse events, deaths, safety, monitoring results, stopping rules and other pertinent issues. Written summaries of the DSMB meetings will be transmitted to the FHCRC Protocol Data Monitoring Committee and will include a recommendation for continuation, modification or termination of the study.

C. Plans for Ensuring Compliance with Requirements for Reporting, in Regards to Adverse Events

All toxicities and serious adverse events will be reported as noted in section 13 of the protocol. All AE reporting will be monitored by the RTO monitor at each visit.

All serious adverse events and other pertinent safety information will be reported at least annually by number and summary information to the FHCRC PDMC and IRB, the UW and SCCA IBC's, the FDA, and the RAC (NIH/OBA).

D. Plans for assuring that any action resulting in a temporary or permanent suspension of NCI funded clinical trial is reported to the NCI grant program director responsible for the grant

Any temporary or permanent suspension for any reason including adverse events, IRB mandate, or FDA mandate will be reported by the investigator within 72 hours to the National Cancer Institute Program Director responsible for this grant. In addition, any communication from the FDA to the awardee institution regarding continuation or changes in the protocol will be forwarded within 72 hours to the NCI Program Director responsible for administration of the grant.

The Cancer Consortium Monitoring Plan requires that the Research Trials Office notify the Grants and Contracts Administration (GCA) when a suspension or closure has occurred. GCA will contact the investigator, confirm which if any federal funding agencies are supporting the project, and then notify those agencies if the investigator has not yet done so.

E. Plans for Assuring Data Accuracy and Protocol Compliance

Dr. Kiem is responsible as sponsor of the IND for this study to follow the specific guidelines set forth by the FDA for assuring data accuracy and protocol compliance. A report is submitted to the FDA annually detailing adverse events, patient status, and significant findings to date. In addition, he is responsible for

compliance with the FHCRC/UW Cancer Consortium Data and Safety Monitoring Plan mentioned above. Details of the Consortium Monitoring Plan are as follows:

INTRODUCTION

Protocol monitoring activities at FHCRC occur under the jurisdiction of the Research Trials Office (RTO) of the Fred Hutchinson Cancer Research Center/University of Washington Cancer Consortium. Monitoring is conducted by employees, contractors, or consultants of the CTSO in accordance with the Standard Operating Procedures (SOPs) of the CTSO. The CTSO will assure that each monitor is qualified both by training and experience to carry out these tasks. The CTSO will maintain a file of the résumés of all monitors and will add training records and updates as appropriate.

Scope

The monitoring activities will include:

- Brief orientation to the IND and specific protocol and to IND- and study-specific documents, and orientation to FHCRC, Seattle Cancer Care Alliance (SCCA), and University of Washington (UW) medical records and facilities associated with the study.
- Review of current protocol document and consent form.
- Thorough source document (informed consent documents, hospital charts, outpatient charts, physician and research notes, correspondence, and other appropriate documentation) review of select subjects, including:
    - Verify that appropriate written informed consent was obtained, current IRB-approved consent forms were utilized, that consent was obtained prior to initiating study procedures, and that the process was documented.
    - Verify that the subject was eligible based on the protocol inclusion/exclusion criteria.
    - Verify adherence to the protocol-specified treatment and evaluation plan.
    - Verify the accuracy and completeness of data reported on case report forms, other data collection instruments, and/or of data directly downloaded into a study database or electronic application.
    - Verify receipt, storage, preparation, use or dispensing of logs/documents and tracking forms for investigational products or devices.
    - Verify that the investigational products(s) are supplied only to enrolled subjects.
- Communicate to study personnel all discrepancies noted between source documents and CRFs, data collection instruments, and downloaded data.
- The monitor will keep a list of all needed changes and additions for review and verification at the next visit.
- Review of critical (regulatory) documents, review of all correspondence to and from the IRB and FDA, if applicable, including review of FDA Form 1572 for accuracy and completeness.
- For IND/IDE studies, verify annual reports were submitted to the FDA.
- Determine if serious adverse events, unanticipated problems posing risks to subjects and IND safety reports (if applicable), Unanticipated adverse event device effects (if applicable) as defined in the protocol, were accurate, complete and reported per IRB policy and 21 CFR, if applicable.

- Determine if Serious Non-compliance events were reported to the IRB and the FDA, if applicable.
- Determine adequate principal investigator oversight.
- Review of qualifications of study personnel via curricula vitae (CVs) and résumés at initial study visits and as new staff are assigned to the study.
- Periodic review of a Study Personnel Signature/Delegation of Authority Log for completeness.
- Periodic review of Study Training logs for Human Subjects, GCP and any protocol-specific training if applicable.
- Review selected subject records to determine that subjects are provided with necessary instruction on using and returning the investigational product(s).
- Review study SOPs, if applicable, for completeness, investigator sign off, and compliance.
- Identify any additional documentation needs and suggest tools (i.e.; memos to study file, logs, etc.) or systems to assure that protocol, GCP and current Good Manufacturing Practice (GMP) compliance are fully demonstrated.
- Promptly after each monitoring visit, complete a detailed written report of all activities and significant findings such as discrepancies in study eligibility or data reporting/recording, deviations from the protocol-specified treatment plan, incomplete or missing documentation of informed consent, or evidence of failure to secure consent according to GCP regulations and questions/comments about the appropriateness of study personnel for specific functions. Submit the visit reports within four weeks of the study visit to the CTSO Manager. The CTSO Manager will distribute the reports to the sponsor-investigator and the Regulatory Affairs Director.
- Report any urgent findings by telephone as specified in the applicable SOPs. The CTSO Manager will be the contact for such reports.
- Upon request of a sponsor-investigator, the monitor may also assist with selected or complete verification of electronic data via direct comparison of data listings to fully monitored CRFs, other data collection instruments, or source documents.

Extent

Monitoring visits will begin no later than 6 months after the first subject has completed study treatment and should occur approximately every six months for high risk trials when subjects are enrolling and receiving study treatment. Due to the classification of this trial as “high risk” by the Consortium Monitoring Plan, and the frequency may be increased at the discretion of the PDMC. Investigators, the SRC, and the IRB may direct requests for increased monitoring to the PDMC or CTSO manager. Monitoring visits will continue throughout the accrual and treatment periods. If the study closes to accrual and no subjects enroll or receive study treatment after the last monitoring visit and there were no major deviations or follow-up items noted in the previous monitoring visit, then no further monitoring is required.

At the first visit, the monitor will review the informed consent forms (ICFs) of all patients enrolled at consortium sites. At each subsequent visit, the monitor will review the ICFs and pertinent notes of all patients enrolled at Consortium sites since the last monitoring visit. Data verification/reconciliation including eligibility, exposure to investigational agent or device, safety data, and other CRF or data collection instrument entries will be conducted on specified portion

of the cases enrolled and/or treated since the last visit. If any review reveals major deviations from the protocol in terms of eligibility or protocol-specified treatment, or if there is evidence of failure or significant delay in reporting safety data, the review may be expanded to include more cases, and the CTSO manager will be notified immediately. The CTSO manager or designee will choose cases to review, unless advised by the PDMC of specific cases to review. Critical documents, including IRB correspondence and IND safety reports, will be reviewed at every visit. Review of receipt, storage, preparation and use or dispensing logs for the investigational agent will be done at every visit while subjects are being treated. Personnel qualifications will be reviewed at the initial monitoring visit and as new staff are assigned to the study.

XVIII. APPENDIX B – STERILITY: VALIDITY OF METHOD FOR MEMBRANE FILTRATION OF O^6-BENZYLGUANINE (BG)

A. Purpose/Summary

To demonstrate that filtration of the investigational drug O^6-benzylguanine using a Millex 0.2 µm GV filter results in a sterile solution. BG is received from the NCI for the purpose of treating patients on Case 6307.

B. Type

Validation of filtration process, verification of sterility.

C. Acceptance criteria

1. No organisms seen in 14 day BacT Alert culture bottles (aerobic and anaerobic cultures);
2. LAL test results using Endosafe PTS: sample reaction time < 25%; Spike reaction time CV: <25%; % Spike recovery 50%-200%; EU/ml/kg ≤ 5.00EU/kg (use estimated pt weight of 80kg and maximum infusion volume of 90 ml).

D. Sample Size

10 vials of BG will be reconstituted, filtered and tested

E. Personnel responsibilities

Investigational pharmacy personnel will suspend the lyophilized drug in diluent, filter, repackage and remove a sample for inoculation into BacT/Alert SA and SN bottles provided by the NCRM/CTIS Cellular Therapy Laboratory ; the NCRM/CTIS Cellular Therapy Laboratory will perform endotoxin testing. The CTIS Operations Director will compile culture and endotoxin data and prepare validation results and conclusion. The IND Investigator will sign off on validation report.

F. Testing Conditions

Per normal laboratory operating conditions.

G. Timeline

Filtration and testing will take place during the week of March 17, 2014. Results will be compiled and approved by April 4, 2014.

H. Equipment and supplies

1. O^6-BG (BG) Injection (NSC# 637037),100mg vial
2. 40% PEG 400 with phosphate buffer (NSC 659805), 30 ml
3. Millex GV 0.2 micron filter (Millipore, Cat #SLGUM33RS)
4. Viaflex container (BAXTER, Item #2B8012 or equivalent)
5. Endosafe PTS device, Charles River Laboratories

6. LAL test cartridges PTS20F, 5-0.5 EU/ml, Charles River Laboratories, LAL reagent water, pyrogen-free tubes
7. BacT/Alert SA and SN bottles

I. Procedure

1. Remove BG from Investigational Pharmacy refrigerator RF5-A4.
2. Reconstitute a 100 mg vial of with 30 ml of PEG DILUENT provided with the BG.
3. Shake well until complete solution is observed. Final concentration will be 3.3mg BG/1ml solution.
4. Do NOT further dilute BG with normal saline or D5W due to risk of product precipitation.
5. After reconstitution, filter the 30 ml suspension of BG through a sterile MILLEX GV 0.22 micron filter into an empty Viaflex container.
6. Label prepared (filtered) BG solution Case 6307.01 through Case 6307-10.
7. Remove 1 ml of filtered solution using a syringe and inoculate an anaerobic BacTAlert bottle. Repeat inoculation of the aerobic bottle. Label bottles with sample ID. Using a Z-requisition, submit samples to Microbiology (Z-req to be provided by J. Reese).
8. Send Viaflex container to the NCRM CTIS Cellular Therapy lab for endotoxin testing using the Limulus Amebocyte Lysate assay using internal SOPs.
9. The Viaflex container will be stored in the refrigerator for 14 days until microbiology results are final.

J. Records/Worksheets

Pharmacy record of supplies, Microbiology report from Soft Lab, Endotoxin worksheet.

K. Additional SOPs

BG Filtration SOP: Case 6307O6bG, Endtoxin SOP CTIS: WKSCase6307-08.

L. Results

1. **Sterility Testing**

   Reconstituted filtered BG (1 ml) was inoculated into an anaerobic and aerobic BacT/Alert culture bottle and incubated for 14 days in a BacT/Alert Microbial Detection System instrument. All test samples (n=10) demonstrated “no growth” in both bottles, demonstrating the filtered BG was microbe-free.

2. **Endotoxin**

   Endotoxin was performed using the Endosafe PTS device and LAL test cartridges (Charles River Laboratories) per manufacturer’s directions. FDA-licensed LAL test cartridges with a sensitivity of 0.05-5.0 EU/ml (Cat # PTS2005F) were used. The final product sample was diluted 1:80 in endotoxin free water due to interference, presumably from the PEG diluent, however final results are adjusted for this dilution. The sample value (EU/ml) was valid if the Spike Recovery was in the range of 50-200% and the sample CV and spike CV was <25%. Results were converted to EU/kg, using a standard weight of 80kg and a maximum potential dose volume of 90 ml. The standard endotoxin limit is 5 EU/kg per dose. All samples evaluated were valid, within linearity

of the assay cartridge, and had an endotoxin level below 5EU/kg per maximum potential dose (n=10). See attached endotoxin results, including QC of the test cartridges.

3. **Conclusion**

   These data demonstrate that clinical grade BG prepared from the NCI inventory is sterile after filtration through a 0.2 micron membrane and is safe for infusion into patients.

Study Calendar	Study Screen	Radiation Therapy	Transplant work-up	Mobilization & Transduction					Conditioning		Transplant & Engraftment‡			Chemotherapy					End of Treatment
		Weeks -7 to -1		Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Each week	Every 2 weeks	Day 1	Day 8	Day 15	Day 21	Every other cycle	Follow Up
Review Inclusion/Exclusion Criteria	X		X																
Sign Informed Consent*	X		X																
Surgery or biopsy**	X																		
MGMT promoter methylation**	X																		
History & Physical	X		X																
Physical exam	X		X								X		X	X					X
Laboratory:																			
CBC w/differential	X	Every 2 weeks	X	X	X	X	X	X			X	X		X	X	X	X		X
Comprehensive Metabolic Panel	X		X								X			X		X			X
Virologies per SPG			X																
Level of gene modified cells***												X		X					X
Anticonvulsant level†											X†			X†					
UA	X		X								X			X					
Pregnancy Test†			X†																
Pulmonary Function Test			X															X†	
EKG			X																
MUGA or Echocardiogram			X																
MRI	X		X															X	
Radiation Therapy	X	X																	
G-CSF for mobilization				X	X	X	X	X											
Apheresis							X	X	(X)										
BCNU§									X	(X)§									
Transduction									X	X	X								
Infusion of transduced stem cells											X								
O6-BG (120 mg/m^2) & Temozolomide														X					
Broad spectrum antibiotic per SPG·												X		X	X	X	X		

*Informed consent for the gene therapy portion of the study will be obtained after MGMT promoter methylation status is determined. **Surgery or biopsy and MGMT promoter methylation analysis will be done before subjects are enrolled on the gene therapy portion this study. ***RCR testing will be done at 3, 6 and 12 months after transplant, then annually. †For female patients of child-bearing potential. ‡Cycle length is approximately 6 weeks after re-infusion of genetically modified cells. § BCNU will be administered on Day -1 if third apheresis is scheduled on Day -2.