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PROTOCOL TITLE

A Phase I/II Study of the Safety and Feasibility of Administering T-Cells Expressing Anti-EGFRvIII Chimeric Antigen Receptor to Patients with Malignant Gliomas Expressing EGFRvIII

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Investigational Agent:

Drug Name:	Anti-EGFRvIII CAR (PG13-139-CD8-CD28BBZ (F10) transduced PBL
IND Number:	14767
Sponsor:	Center for Cancer Research
Manufacturer:	Surgery Branch Cell Production Facility

Commercial Agents: Cyclophosphamide, Fludarabine, and Aldesleukin

PRÉCIS

Background:

- Patients with recurrent gliomas have very limited treatment options. EGFR variant III (EGFRvIII) is the most common mutant variant of EGFR and is present in 24-67% of patients with glioblastoma.
- EGFRvIII expression promotes oncogenesis and is associated with poor prognosis.
- EGFRvIII is not expressed in normal tissue and is an attractive target for immunotherapy.
- We have constructed a retroviral vector that contains a chimeric antigen receptor (CAR) that recognizes the EGFRvIII tumor antigen, which can be used to mediate genetic transfer of this CAR with high efficiency without the need to perform any selection.

Objectives:

- Primary objectives:
  - To evaluate the safety of the administration of anti-EGFRvIII CAR engineered peripheral blood lymphocytes in patients receiving the non-myeloablative, lymphodepleting preparative regimen and aldesleukin.
  - Determine the six-month progression free survival of patients receiving anti-EGFRvIII CAR-engineered peripheral blood lymphocytes and aldesleukin following a non-myeloablative, lymphodepleting preparative regimen.

Eligibility:

- Histologically proven glioblastoma or gliosarcoma expressing EGFRvIII as determined by IHC or RT-PCR
- Failed prior standard treatment with radiotherapy with or without chemotherapy
- Karnofsky performance score (KPS) ≥ 60
- Cardiac, pulmonary and laboratory parameters within acceptable limits

Design:

- The study will be conducted using a Phase I/II design.
- Patients will receive a non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed by intravenous infusion of *ex vivo* tumor-reactive, CAR gene-transduced PBMC, plus IV aldesleukin.
- Once the maximum tolerated cell dose (MTD) has been determined, the study will proceed to the phase II portion.
- In the Phase II portion of the trial, patients will be accrued to two cohorts:
  - Patients with recurrent malignant glioma receiving steroids at the time of treatment.
  - Patients with recurrent malignant glioma not receiving steroids at the time of treatment.
- A total of 107 patients may be enrolled over a period of 7 years.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives

- To evaluate the safety of the administration of anti-EGFRvIII CAR engineered peripheral blood lymphocytes in patients receiving the non-myeloablative, lymphodepleting preparative regimen and aldesleukin
- Determine the six-month progression free survival of patients receiving anti-EGFRvIII CAR-engineered peripheral blood lymphocytes and aldesleukin following a non-myeloablative, lymphodepleting preparative regimen.

1.1.2 Secondary Objective

- Determine the *in vivo* survival of CAR gene-engineered cells.
- Evaluate radiographic changes after treatment

1.2 BACKGROUND AND RATIONALE

1.2.1 Gliomas

The American Cancer Society estimates that approximately 20,500 new cases of primary brain and nervous system tumors will develop and approximately 12,740 patients will die in the U.S. each year[1]. Brain tumors account for approximately 85 to 90% of all central nervous system malignancies. Glioblastoma is the most aggressive and most common glioma accounting for 51% of all gliomas[2]. Once the primary modalities of treatment, surgery, radiation, and chemotherapy have failed the treatment options are limited. With the current standard of care including resection, radiation and chemotherapy, the median overall survival is 14.6 months[3]. There are no good options for patients with recurrent brain tumors and participation in clinical trials is warranted.

Despite recent advances in neuro-imaging, neuro-anesthesia, and neuro-surgical techniques, the prognosis of patients with malignant gliomas treated by surgical resection alone remains dismal with a median survival of 4 to 6 months[4]. The low survival rates reflect the unique infiltrative growth characteristics of malignant gliomas, which make true "total resection" impossible without causing unacceptable neurological damage to the patient. To date, radiotherapy has proven to be the most effective treatment for malignant gliomas extending median survival to 8 to 12 months[5-9]. Adjuvant chemotherapy with temozolomide prolongs median survival to 14 months, however tumor progression is the rule and few patients survive beyond 18 months. Furthermore, once these tumors have progressed, conventional chemotherapy has not been shown to prolong survival[10]. There are multiple reasons why gliomas are relatively resistant to standard chemotherapy including diminished drug delivery to the tumor as a consequence of the blood-brain barrier, tumor hypoxia, and their relatively low growth fraction. Most importantly is the fact that gliomas tend to have significant intrinsic resistance to most standard cytotoxic agents. Clearly, novel therapies are needed, and in recent years' immunotherapeutic approaches for gliomas have emerged as a strategy of interest.

Animal studies have demonstrated that the cellular rather than the humoral arm of the immune response plays the major role in the elimination of murine tumors[11]. Much of this evidence was derived from studies in which the adoptive transfer of T lymphocytes from immune animals

could transfer resistance to tumor challenge or in some experiments, the elimination of established cancer. Thus, most strategies for the immunotherapy of patients with cancer have been directed at stimulating strong T-cell immune reactions against tumor-associated antigens. In contrast to antibodies that recognize epitopes on intact proteins, T-cells recognize short peptide fragments (8-18 amino acids) that are presented on surface class I or II major histocompatibility (MHC) molecules, and it has been shown that tumor antigens are presented and recognized by T-cells in this fashion. The molecule that recognizes these peptide fragments is the T-cell receptor (TCR). The TCR is analogous to the antibody immunoglobulin molecule in that two separate proteins (the TCR alpha and beta chains) are brought together to form the functional TCR molecule.

An alternate approach to enable T-cells to recognize targets is to utilize a CAR which is constructed by using the variable regions of the heavy and light chains of an antibody connected by a linker sequence and fused to T-cell signaling protein domains from CD28, CD3 zeta, and 41BB. When a CAR is introduced into a T-cell, the T-cell can recognize and signal based on the non-MHC restricted recognition of the target. The goal of this protocol is to transfer peripheral blood lymphocytes (PBL) transduced with genes encoding a CAR that recognizes the mutant protein EGFRvIII that is expressed on a subset of glioma patients into autologous PBL. Normal PBL derived from glioma patients will be genetically engineered with a viral vector encoding the CAR and infused into patients with the aim of mediating regression of their tumors. This trial is similar to previous National Cancer Institute Surgery Branch (NCI-SB) TCR gene transfer adoptive immunotherapy protocols except that we will use a receptor that recognizes targets based on the recognition of an antibody reactive with the EGFRvIII molecule expressed on the tumor surface.

1.2.2 EGFRvIII and Glioblastomas

The epidermal growth factor receptor (EGFR) is a 170-kDa transmembrane tyrosine kinase. Activation of EGFR launches a host of downstream signaling cascades leading to cell proliferation, invasion, motility and adhesion. EGFR overexpression leads to unregulated cell growth and malignant transformation, and is frequently implicated in a wide range of malignancies.

Increased EGFR signaling via overexpression or mutation is the most frequent genetic alteration associated with GBMs, occurring in 40-50% of these tumors[12, 13]. The most common EGFR mutant is the EGF receptor variant III (EGFRvIII) which harbors an in-frame deletion of exons 2-7. This results in a truncated extracellular ligand binding domain, but renders the protein constitutively active in a ligand-independent fashion[14]. EGFRvIII expression has been shown to enhance tumorigenicity[15, 16], promote cellular motility[17], and confer resistance to radiation and chemotherapy[18-20]. EGFRvIII is present in 24-67% of GBMs but not in normal tissue[21, 22], making it an attractive target for therapy.

Preclinical studies using monoclonal antibodies (mAbs), and small-molecule inhibitors against EGFRvIII have provided modest results, however only peptide vaccines against EGFRvIII are currently offered in experimental clinical trials.

Preclinical experience with peptide vaccines involving mice that were pre-immunized with a peptide vaccine against EGFRvIII[23-25] led to significant inhibition of tumor formation by cells expressing EGFRvIII. Moreover, immunization beginning within 4 days of tumor injection of tumors expressing EGFRvIII led to modest prolongation of survival. Other strategies for

EGFRvIII antigen presentation have included the use of dendritic cells (DCs) [26, 27]. Again, preimmunization with DCs pulsed with peptide vaccine increased median survival times in mice compared with controls.

Based on these preclinical data, several early clinical trials have evaluated immunization with EGFRvIII peptide vaccines[28, 29]. A phase I clinical trial in patients with newly diagnosed GBM demonstrated the safety of vaccination in this population using DCs loaded with an EGFRvIII peptide vaccine[28]. A phase II trial evaluating EGFRvIII peptide vaccine administered with GM-CSF again showed minimal toxicity, and a favorable median time to tumor progression of 64.5 weeks compared with 28.5 weeks in historical controls. The safety of EGFRvIII peptide vaccines in conjunction with standard care using temozolomide chemotherapy has also been established. These various trials have demonstrated tolerability of peptide vaccines and documented their ability to elicit antigen specific cellular and humoral responses however the effectiveness of any of these approaches has yet to be proven.

There has also been preclinical work in the arena of adoptive immunotherapy using genetically modified T-cells. Bullian, et al. created an EGFRvIII specific CAR, MR1-ζ. They went on to demonstrate that human T-cells genetically modified with MR1-ζ specifically recognized EGFRvIII expressing tumor cells and inhibited growth in vitro[30]. Similarly, Ohno, et al. engineered T-cells expressing a CAR against EGFRvIII based on a mouse monoclonal antibody, 3C10. They demonstrated *in vivo* inhibition of intracranial tumor growth in mice[31].

1.2.3 Preclinical Studies to Provide the Rationale for this Clinical Protocol

Based on a review of scientific literature and publicly available databases, the amino acid sequences for seven monoclonal antibodies (4 murine and 3 human) specific for EGFRvIII were obtained and used to assemble single-chain variable fragment (scFv) genes. These scFv genes were inserted into gamma-retroviral vector MSGV1 using T-cell signaling domains from CD28-CD3zeta (28Z) or, CD8-CD28-41BB-CD3zeta (28BBZ) to produce CAR expression vectors (**Figure 1**). Gamma-retroviral vectors were used to transduce human PBL and tested for reactivity by co-culture assays using as targets a variety of cell lines engineered to express EGFRvIII (there are no established cell lines that express EGFRvIII). Initial characterization of the seven different anti-EGFRvIII CARs suggested that a CAR based on human mAb 139 yielded high specific reactivity against EGFRvIII expressing targets, and was thus chosen for development as a clinical reagent. Human mAb-based CARs may also have an advantage that they may not elicit an anti-CAR immune response as has been seen in some patients being treated with murine-based CARs. Anti-EGFRvIII mAb 139-CAR vectors were used to transduce PBL and CAR expression determined by FACS (**Figure 1**) and biological activity tested by co-culture (**Table 1**).

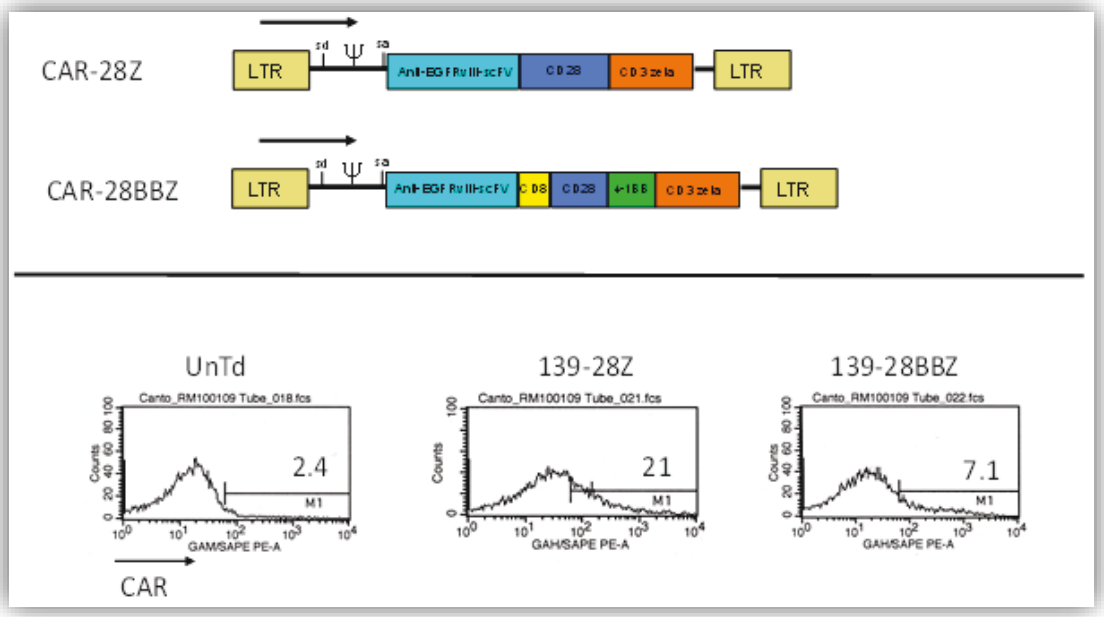


Figure 1. Construction and analysis of anti-EGRFvIII CAR vectors. *Shown on the top of the figure is a diagram of the two CAR vector designs showing the relative locations of the scFv and T-cell signaling domains. In the lower half of the figure, CAR vectors based on human mAb 139 were used to engineer human T-cells. One week post-transduction, cells were stained with goat anti-human Fab and analyzed by FACS. The shift in the histogram versus untransduced cells (UnTd) demonstrated successful gene transfer and expression of the CD28-CD3zeta (28Z) and CD8-CD28-41BB-CD3zeta (28BBZ) based vectors.*

Effector I	**U251**	**U251-EGFR**	**U251-vIII**	
UnTd	0	0	0	
GFP	0	0	0	
Anti-ERBB2	**1195**	**2201**	**2692**	
139-28Z	0	0	**2743**	
139-28BBZ	0	0	**1820**	
Effector II	**U87**	**U87-GFP**	**U87-EGFR**	**U87-vIII**
GFP	203	389	236	339
Anti-ERBB2	**1959**	**1061**	**671**	**932**
139-28Z	759	451	561	**1797**
139-28BBZ	605	460	499	**2217**

Table 1. Test of CARs targeting EGFR-vIII. *Two donor PBL (Effector I and II) were transduced with anti-EGFRvIII CAR vectors containing CD28-CD3zeta (139-28Z) or CD8-*

CD28-41BB-CD3zeta (139-28BBZ) signaling domains. 5-6 post transduction PBL were co-cultured with glioma cell lines U251 and U87 that had been engineered to express wild type EGFR, EGFRvIII, or GFP. An anti-ERBB2 CAR served as a positive control in all co-cultures. Shown is resultant IFN-g production (pg/mL) following 18-hour incubation.

While detection of the 28BBZ construction by FACS was less than the 28Z construct, the transduced T-cells were equally reactive against EGFRvIII expressing targets. We next determined the ability of EGFRvIII CAR engineered T-cells to lyse target cells in a standard 51Cr-release assay (**Figure 2** and **Figure 3**). As shown in **Figure 2** and **Figure 3**, both vectors specifically lysed only cell lines engineered to express the mutant EGFRvIII and not control or wild-type EGFR engineered cell lines. Based on experience in the NCI-SB and in publications reported by others, the presence of signaling domains from the 4-1BB protein is associated with a better survival of CAR engineered cells in animal models. Thus, the construct containing the 4-1BB signaling elements was chosen for clinical production.

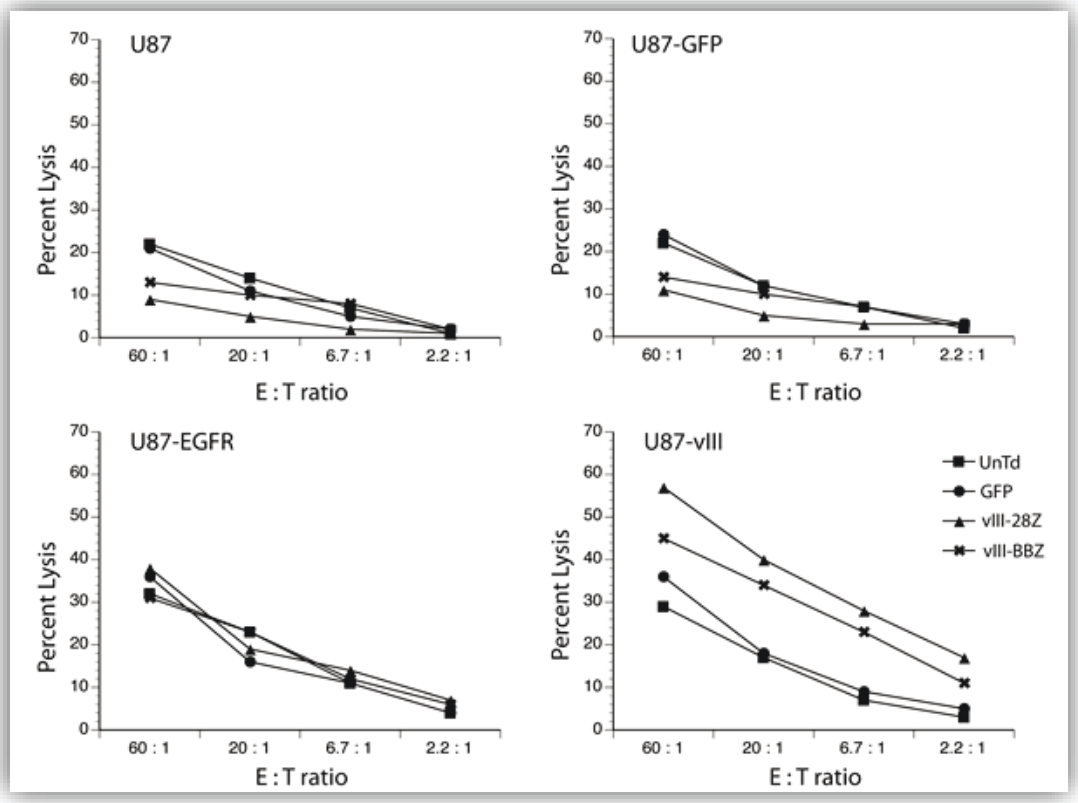


Figure 2. Specific lysis of U87 EGFRvIII by anti-EGFRvIII CAR transduced T-Cells. *Anti-EGFRvIII CAR transduced human PBL were co-cultured for 4 hours with indicated ^{51}Cr labeled target tumor cell lines (parent U87, GFR, wild type EGFR, or EGFRvIII engineered). Specific lysis of tumor cells was measured at the given E:T ratio using the formula: [(specific release-spontaneous release)/(total release-spontaneous release)]. Specific lysis of untransduced (UnTd), control GFR vector (GFP), 139-28Z CAR (vIII-28Z), and 139-28BBZ (vIII-BBZ) human PBL are plotted on the graph as indicated.*

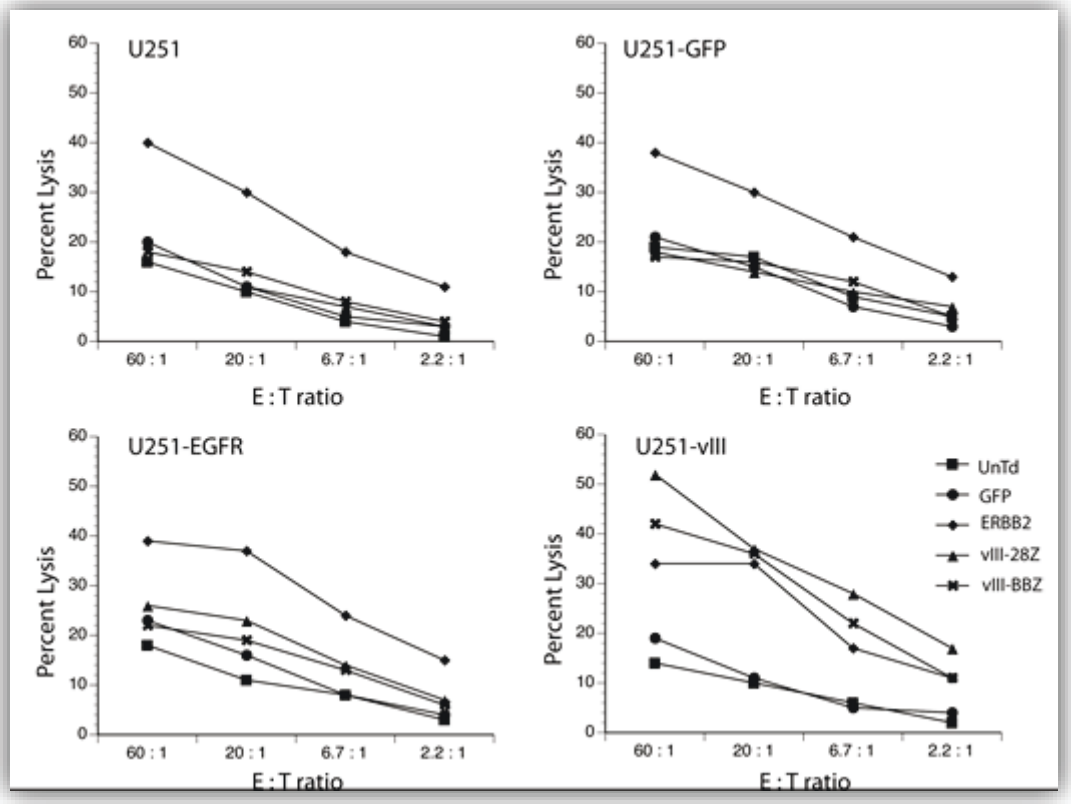


Figure 3. Specific lysis of U251 EGFRvIII by anti-EGFRvIII CAR trasduced T-cells. *Anti-EGFRvIII CAR transduced human PBL were co-cultured for 4 hours with indicated ^{51}Cr labeled target tumor cell lines (parent U251, GFR, wild type EGFR, or EGFRvIII engineered). Specific lysis of tumor cells was measured at the given E:T ratio using the formula: [(specific release-spontaneous release)/(total release-spontaneous release)]. Specific lysis of untransduced (UnTd), control GFR vector (GFP), anti-ERBB2 CAR (ERBB2), 139-28Z CAR (vIII-28Z), and 139-28BBZ (vIII-BBZ) human PBL are plotted on the graph as indicated.*

By detailed molecular analysis of many different classes of cancer cell lines, it has now been demonstrated that established cancer cell lines often do not mirror the molecular characteristics of primary human cancers and this is the case for glioma lines. An alternative to the use of establish glioma cell lines is the analysis of tumor stem cell (TSC) lines. The TSC paradigm proposes that a subpopulation of cells exist in cancer that give rise to all the cells in a differentiated tumor. The Neuro-Oncology Branch NCI has demonstrated that in situ glioma cells share properties not found in glioma cell lines, and harbor features consistent with tumor stem cells. It was further demonstrated that marked phenotypic and genotypic differences exist between primary human tumor-derived TSCs and their matched glioma cell lines. TSCs derived directly from primary glioblastomas harbor extensive similarities to normal neural stem cells and recapitulate the genotype, gene expression patterns, and *in vivo* biology of human glioblastomas. These findings suggest that glioma-derived TSCs may be a more reliable model than many

commonly utilized glioma cell lines for understanding the biology of primary human tumors. We therefore analyzed three TSC lines for the presence of EGFRvIII and demonstrated by RT-PCR that EGFRvIII is expressed in these lines (**Figure 4**). PBL from two donors were then engineered with the EGFRvIII CAR (28BBZ) and co-culture with glioma TSC lines and control EGFRvIII expressing cell lines. Data shown in **Table 2** demonstrate that EGFRvIII CAR engineered cells from both donors produced IFN-g following co-culture with the TSC lines. These results further support the use of EGFRvIII CAR engineered T-cells as a potential immunotherapy for glioma patients.

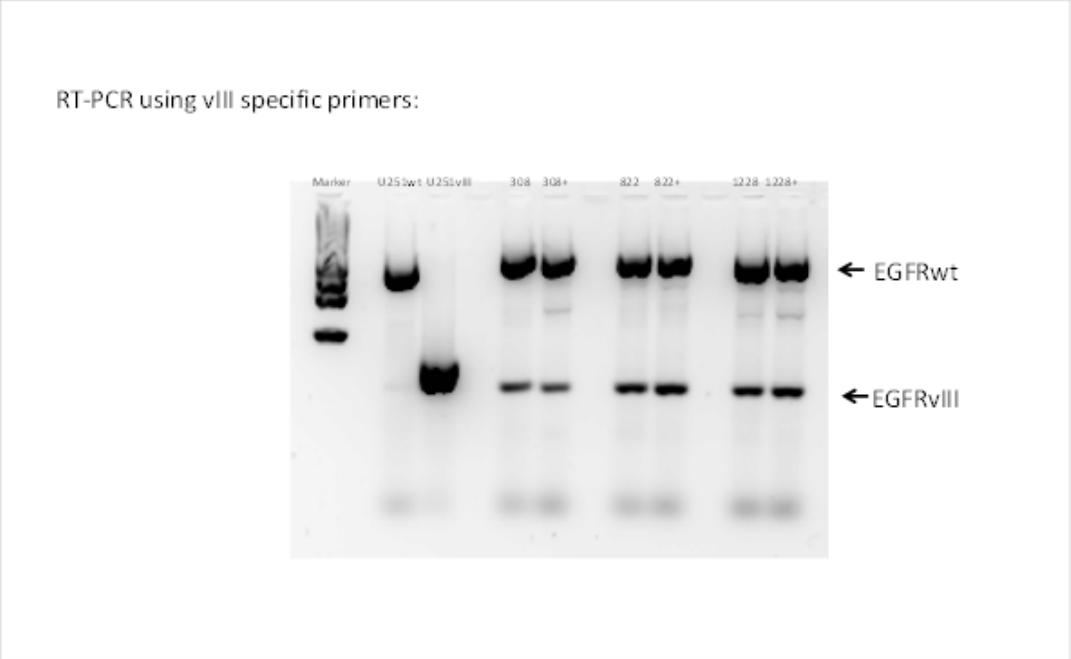


Figure 4. Expression of EGFRvIII in glioma tumor stem cell lines. *RNA from control U251 cells (wild type EGFR or vIII engineered), TSC lines 308, 822, 1228, or these lines grown for 3 days in serum (+symbol) were isolated and subject to RT-PCR using vIII specific primers. RT-PCR products were subject to gel electrophoresis, and visualized with cyber green staining. The location of the appropriate size product bands is as indicated on the right.*

Effector I	Media	U251-EGFR	U251-vIII	TSC 1228	TSC 308	308+serum	TSC 822	822+serum
UnTd	0	0	0	47	34	0	100	0
GFP	0	0	0	28	34	33	30	2
ERBB2	0	**2186**	**2014**	**1170**	**1836**	**2369**	187	42
EGFRvIII	37	16	**1821**	**729**	**473**	86	**918**	**471**

Effector II	Media	U251-EGFR	U251-vIII	TSC 1228	TSC 308	308+serum	TSC 822	822+serum
UnTd	0	0	0	0	147	0	80	80
GFP	0	0	0	0	0	0	180	0
ERBB2	0	**4317**	**4128**	**2573**	**3308**	**4651**	**1067**	**259**
EGFRvIII	384	331	**4523**	**3306**	**3351**	**680**	**4406**	**3153**

Table 2. Test of EGFR-vIII CAR recognition of glioma TSCs. *Two donor PBL (Effector I and II) were transduced with anti-EGFRvIII CAR vector containing CD8-CD28-41BB-CD3zeta (EGFR-vIII) signaling domains. 5 post-transduction PBL were co-cultured with glioma TSC lines or cell line U251 that had been engineered to express wild type EGFR, or EGFRvIII. TSC lines 308 and 822 were also grown in serum for 3 days to induced differentiation (+ serum). GFP transduced cells served as negative control and an anti-ERBB2 CAR served as a positive control in all co-cultures (untransduced cells, UnTd). Shown is resultant IFN-g production (pg/mL) following 18-hour incubation.*

1.2.4 Prior NCI-SB Trials of Cell Transfer Therapy Using Tumor-Infiltrating Lymphocytes (TIL) in Patients with Metastatic Melanoma

In the great majority of murine models demonstrating the therapeutic effectiveness of the adoptive transfer of lymphocytes mediating tumor regression, immunosuppression of the host prior to the adoptive transfer of lymphocytes was required. We thus incorporated a lymphodepleting chemotherapy into our human cell transfer studies. In the NCI-SB we developed a protocol to rapidly expand heterogeneous TIL for adoptive transfer. TIL were expanded using the rapid expansion protocol (REP) in the presence of OKT3, irradiated allogeneic feeder cells and IL-2. These REPed TIL retained highly specific in vitro anti-tumor activity, often contained reactivities against several antigenic epitopes and contained both CD8+ and CD4+ lymphocytes. These autologous bulk TIL were re-infused to patients following a nonmyeloablative chemotherapy with cyclophosphamide and fludarabine. These patients subsequently received high-dose IL-2 (protocol 99-C-0158). Forty-three patients received this treatment. This regimen resulted in objective cancer regressions in 49% of patients (21 of 43) with metastatic melanoma (**Table 3**)[32]. Grade 3 and 4 toxicities are shown in **Table 4**. Murine models predicted that increasing the extent of lymphodepletion could increase the effectiveness of the cell transfer therapy. Thus, we performed two additional sequential trials of ACT with autologous TIL in patients with metastatic melanoma. Increasing intensity of host preparative lymphodepletion consisting of cyclophosphamide and fludarabine with either 200cGy (25 patients) or 1200 cGy (25 patients) total body irradiation (TBI) was administered prior to cell transfer. While non-myeloablative chemotherapy alone showed an objective response rate of 49%, when 200cGy or 1200cGy TBI was added the response rates were 52% and 72% respectively (**Table 3**). Complete response rates for the three trials were 13%, 20% and 40%. TBI appeared to result in increased patient survival. Nineteen of the 20 patients that experienced a complete response are ongoing from 3 to 5 years. Responses were seen in all visceral sites including brain.

Host lymphodepletion was associated with increased serum levels of the lymphocyte homeostatic cytokines IL-7 and IL-15. Objective responses were correlated with the telomere length of the transferred cells. Patients exhibited the expected hematological toxicities associated with the cyclophosphamide, fludarabine and TBI preparative regimens. Patients recovered marrow function rapidly after cell infusion with absolute neutrophil counts greater than 500 per mm^3 by day 12 and sustained platelet counts above 20,000 per mm3 by day 14 (except 4 patients on the TBI 1200 protocol with platelet recovery on days 16, 17, 20, and 22).

Table 3. *Cell Transfer Therapy (8/1/10)*

Treatment	**Total**	**PR**	**CR**	**OR (%)**
		number of patients (duration in months)		
No TBI	43	16 (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (82+, 81+, 79+, 78+, 64+)	21 (49%)
200 TBI	25	8 (14, 9, 6, 6, 5, 4, 3, 3)	5 (68+, 64+, 60+, 57+, 54+)	13 (52%)
1200 TBI	25	8 (21, 13, 7, 6, 6, 5, 3, 2)	10 (48+, 45+, 44+, 44+, 39+, 38+, 38+, 38+, 37+, 19)	18 (72%)

(52 responding patients: 42 had prior IL-2; 22 had prior IL-2 + chemotherapy)
(20 complete responses: 19 ongoing at 37 to 82 months)

Table 4. Time in Hospital and Non-Hematological Grade 3 and 4 Toxicities Related to Lymphodepleting Chemotherapy and Cell Transfer

Attribute Measured	**Duration, Number or Type**	**Number of Patients (%)**
Days in Hospital[1]	6-10	6 (17%)
	11-15	18 (51%)
	16-20	4 (11%)
	21-25	7 (20%)

pRBC Transfusions	0	2 (6%)
	1-5	18 (51%)
	6-10	13 (37%)
	11-15	2 (6%)
Platelet Transfusions	0	6 (17%)
	1-5	21 (60%)
	6-10	5 (14%)
	11-15	2 (6%)
	16-20	1 (3%)
Autoimmunity	Uveitis	5 (14%)
	Vitiligo	13 (37%)
Opportunistic Infections	Herpes zoster	3 (9%)
	Pneumocystis pneumonia	2 (6%)
	EBV-B cell lymphoma	1 (3%)
	RSV pneumonia	1 (3%)
Other	Febrile neutropenia	13 (37%)
	Intubated for dyspnea	3 (9%)
	Cortical blindness	1 (3%)

[1]Measured from the day of cell administration to discharge

1.2.5 NCI-SB Trials of Cell Transfer Therapy Using Transduction of Anti-TAA TCR Genes into Non-reactive TIL or PBL

It is often not possible to isolate sufficient tumor samples from melanoma patients and even when tumor is available, only about 60 to 70% generate melanoma reactive TIL cultures. As a potential alternative to the requirement to establish TIL cultures from melanoma patients, we sought methods that could be used to easily obtain a polyclonal population of T-cells with anti-TAA properties. Transfer of antigen specific TCR genes to PBL is a potential method generating large numbers of reactive anti-cancer T-cells. In a murine model of this approach, TCR gene transfer into murine splenocyte T-cells was performed using a retroviral vector. The engineered T-cells were shown to expand *in vivo* upon viral challenge and efficiently homed to effector sites. In addition, small numbers of TCR-transduced T-cells promoted the inhibition of antigen-expressing tumors in the mice. Retroviral vector mediated gene transfer can be used to engineer human T-cells with high efficiency.

Studies in the NCI-SB identified genes that encode melanoma tumor associated antigens (TAA) recognized by TIL in the context of multiple MHC class I molecules [33-35]. These TAA appeared to be clinically relevant antigens responsible for mediating tumor regression in patients with advanced melanoma since the TIL used to identify these antigens were often capable of mediating *in vivo* anti-tumor regression. Two antigens, which were present in virtually all fresh

and cultured melanomas, were called MART-1 (Melanoma Antigen Recognized by T-Cells - 1), and gp100. The genes encoding these two antigens have been cloned and sequenced. The MART-1 gene encodes a 118 amino acid protein of 13 kd. The gp100 gene encodes a protein identical to that recognized by monoclonal antibody HMB-45. These antigens were thus the original targets of our gene therapy cell transfer protocols in patients with metastatic melanoma.

We thus isolated TCR genes from both gp100 and MART-1 reactive T-cells. These studies have recently been published and are presented briefly here[36, 37]. To test the *in vivo* efficacy of these MART-1 TCR engineered T-cells, 31 HLA-A*0201 patients with progressive metastatic melanoma were treated. Results in the first 17 patients were published in[36]. All patients were refractory to prior therapy with IL-2. T-cell cultures from all patients were biologically reactive, with specific secretion of interferon-γ following co-culture with either MART-1 peptide pulsed T2 cells and or melanoma cell lines expressing the MART-1 antigen. Gene transfer efficiencies measured by staining for V 12 expression in these lymphocytes ranged from 17% to 67% (mean value 42%). Four of the 31 patients demonstrated a sustained objective regression of their metastatic melanoma assessed by standard RECIST criteria. There were no toxicities in any patient attributed to the gene-marked cells. We thus demonstrated for the first time in humans, that normal autologous T lymphocytes, transduced *ex vivo* with anti-TAA TCR genes and reinfused in cancer patients can persist and express the transgene long-term *in vivo* and mediate the durable regression of large established tumors. A similar study was conducted using gp100 TCR gene marked cells, however this retroviral vector had a low titer when produced under GMP conditions. Fourteen patients were treated on this study. No antitumor responses have been seen. There have been no grade 5 toxicities observed on this study, and all grade 3 and 4 toxicities observed were expected toxicities associated with the non-myeloablative chemotherapy regimen or IL-2.

The low response rate in our prior MART-1 TCR gene transfer protocol led us to identify MART-1 reactive TCR with higher avidity than the MART-1 F4 TCR used in the prior gene therapy clinical trial[37]. We have now treated 24 patients with metastatic melanoma using autologous PBL transduced with an improved MART-1 F5 TCR following a non-myeloablative chemotherapy. Six patients (25%) have achieved an objective partial response. Toxicities were similar to those seen in the prior TCR gene therapy trial except that 15 patients developed a transient mild anterior uveitis easily reversed by steroid eye drops and ten patients developed decreased hearing reversed by middle ear steroid injections. Transient rashes have also been seen. There were no treatment related deaths. We have also conducted a clinical trial with a TCR that recognizes the gp100:154-162 melanoma peptide. This TCR was raised in an HLA-A2 transgenic mouse immunized with this peptide[37]. We have now treated 21 patients with metastatic melanoma using autologous PBL transduced with this improved gp100 TCR following a non-myeloablative chemotherapy. Four patients (19%), have achieved an objective partial response. Seven patients developed a transient mild anterior uveitis reversed by steroid eye drops and ten patients developed decreased hearing reversed by middle ear steroid injections. There were no treatment related deaths.

In addition to the studies listed above in patients with metastatic melanoma, we have recently initiated several studies for patients with other metastatic cancers using PBL transduced with TCR genes or CAR genes. We have studied PBL transduced with TCR genes targeting p53, CEA, NY-ESO-1 and TRAIL bound to the DR4 Receptor, and PBL transduced with CAR targeting Her-2 and CD19. Patients on these studies also received a non-myeloablative

chemotherapy regimen consisting of cyclophosphamide and fludarabine, and high dose IL-2. In two studies, 08-C-0121 (anti-ESO-1 TCR) and 09-C-0082 (anti-CD19 CAR), we have seen impressive clinical responses. The clinical responses observed in seven of the fifteen evaluable patient receiving anti-ESO TCR-engineered PBL have been very encouraging. Of the six synovial cell sarcoma, four patients have confirmed PRs. Of the nine patients with highly ESO expressing melanoma, there have been 2 confirmed complete responses and 2 confirmed partial responses. In study 09-C-0082, an impressive ongoing partial remission at 7 months post treatment of the lymphoma occurred in the first patient treated on this study[38]. This patient had heavily pre-treated, progressive follicular lymphoma that involved all major lymph node areas. Only three small lesions that were consistent with lymphoma persisted on positron emission tomography (PET) imaging post-treatment. Most toxicities observed in these studies were expected toxicities of the chemotherapy and aldesleukin administration. However, in 2 studies, we have observed serious adverse events related to the transduced cells. In 09-C-0051 (anti-Her2 CAR transduced PBL), the first patient, with Her-2 expressing metastatic colorectal cancer, was treated with 10^{10} autologous T-cells transduced with the retrovirus encoding an anti-Her-2 CAR. This patient developed respiratory distress and died four days later[39]. This toxicity was apparently due to a previously unrecognized ability of this CAR to recognize Her-2 expressed on lung epithelial cells although the exact explanation for the toxicity is not clear. In 09-C-0047 (anti-CEA TCR transduced PBL), all three patients treated experienced a variety of gastrointestinal events which were attributed to the gene/cell therapy including diarrhea, and colitis. All gastrointestinal events have since resolved in these patients, and all of the patient's colonic mucosa has returned to normal, and the patients have normal bowel function. Grade 3 diarrhea lasting longer than 72 hours is considered a DLT per protocol and this event was observed in two of three patients enrolled in protocol 09-C-0047, meeting the criteria for stopping protocol accrual. One of the patients experienced an objective regression of liver metastases.

1.2.6 Safety Considerations

Several safety concerns regarding the infusion of large numbers of retrovirally modified tumor-reactive T-cells have been addressed in our previous clinical studies. The non-myeloablative chemotherapy and the administration of high-dose IL-2 have expected toxicities discussed earlier. The non-myeloablative chemotherapy used in this protocol has been administered to over 200 patients and all have reconstituted their hematopoietic systems. Since IL-2 can induce a capillary leak syndrome, patients with malignant gliomas may be at risk of increased intracerebral edema following IL-2 administration. Since the capillary leak syndrome occurs within hours of drug administration and all IL-2 administration will be done as an inpatient, any increases in cerebral edema will be followed closely and treated aggressively (i.e. mannitol) should it be clinically indicated. Due to the risks of thrombocytopenia in this patient population, once the platelet count drops below 30,000/mm^3 platelets will be monitored and transfusions given as clinically indicated until the platelet count is greater than 30,000/mm^3 for 24 hours without transfusion support.

Although we recognize the dangers of giving a non-myeloablative chemotherapy and IL-2 to patients with recurrent brain tumors, and often after prior surgery, we are encouraged by our experience treating metastatic tumors to the brain in patients with melanoma using TIL along with IL-2 following the same preparative regimen used in the current protocol. Seven of 17 patients (41%) achieved a complete response in the brain. Two of 9 patients treated using TCR

gene transduced cells (anti-MART-1 or anti-gp100) achieved a complete response. One of the 26 patients experienced a tumor- associated subarachnoid hemorrhage during the thrombocytopenic phase of therapy and had an uneventful metastasectomy.

CAR transduced human T-cells have been administered to humans in several clinical trials. Pule, et al. engineered human T-cells to express a CAR directed to the diasialoganglioside GD2, a tumor associated antigen expressed by human neuroblastoma cells[40]. These CAR transduced cells were administered to eleven children between the ages of 3 and 10, all of whom had metastatic neuroblastoma. There were no adverse effects attributable to the genetically modified CAR T-cells in the eleven subjects that were followed for up to two years following cell infusion. Two patients exhibited an objective response. Persistence of the transduced cells was greater when the CAR were inserted into EBV specific cytolytic T lymphocytes than when the CAR were inserted into T-cells that were activated by an anti-CD3 monoclonal antibody.

In the NCI-SB we have an approved clinical protocol to treat patients with B cell lymphomas utilizing lymphocytes transduced with a retrovirus encoding a CAR that recognizes the CD19 molecule. Thus far six patients have been treated with between $2x10^8$ to $4x10^9$ cells. To date, three of the patients treated have exhibited partial responses and one complete response (ongoing at over 6 months) has been observed. As expected by the targeting of CD19, the protocol was associated with elimination of normal B cells. One patient required ICU support for toxicity similar to tumor lysis syndrome and a second patient treated on this study died prior to response evaluation from H1N1 influenza infection. This death is possibly related to the research but is not related to the anti-CD19 CAR transduced cells.

In a second CAR at the NCI-SB a patient with metastatic colorectal cancer was treated with 10^{10} autologous T-cells transduced with the retrovirus encoding an anti-Her-2 CAR. This patient developed respiratory distress and died four days later. This toxicity was apparently due to a previously unrecognized ability of this CAR to recognize Her-2 expressed on lung epithelial cells although the exact explanation for the toxicity is not clear. As the EGFRvIII protein is tumor specific, we do not expect any recognition of normal cells. In addition, as previously described, multiple EGFRvIII vaccine trials have been reported and no significant vaccine-related toxicities have been observed.

In other protocols we have administered over $3x10^{11}$ TIL with widely heterogeneous reactivity including CD4, CD8, and NK cells without difficulty. The expansion of tumor-reactive cells is a desirable outcome following the infusion of antigen reactive T-cells and this expansion could mimic the clonal expansion of a transformed lymphocyte. We do not believe the transfer of these gene modified cells has a significant risk for malignant transformation in this patient population. While the risk of insertional mutagenesis is a known possibility using retroviral vectors, this has only been observed in the setting of patients with primary immunodeficiencies using retroviral vector-mediated gene transfer into CD34+ bone marrow cells. In the case of retroviral vector-mediated gene transfer into mature T-cells, there has been no evidence of long-term toxicities associated with these procedures since the first NCI sponsored gene transfer study in 1989. Although continued follow-up of all gene therapy patients will be required, data suggest that the introduction of retroviral vectors transduced into mature T-cells is a safe procedure. While we believe the risk of insertional mutagenesis is extremely low, the proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients with histologically proven glioblastomas or gliosarcomas that express EGFRvIII as assessed by IHC or PCR confirmed by the NCI Laboratory of Pathology.

2.1.1.2 Patients must have progression of disease after radiotherapy (including patients that undergo surgery for recurrent disease and are rendered NED). This includes recurrent GBM after receiving all standard first-line treatment, including surgery (if feasible due to neurosurgical and neuro-anatomical considerations) and adjuvant radiotherapy ± chemotherapy.

2.1.1.3 Patients must either not be receiving steroids, or be on a stable dose of steroids for at least five days prior to registration.

2.1.1.4 Age ≥ 18 years and ≤ 70 years.

2.1.1.5 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.1.6 Willing to sign a durable power of attorney.

2.1.1.7 KPS ≥ 60 (see **Appendix F**).

2.1.1.8 Patients of both genders must be willing to practice birth control from the time of enrollment on this study and for four months after treatment.

2.1.1.9 Women of child-bearing potential must have a negative pregnancy test because of the potentially dangerous effects of the treatment on the fetus.

2.1.1.10 Serology

- Seronegative for HIV antibody. (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who are HIV seropositive may have decreased immune-competence and thus be less responsive to the experimental treatment and more susceptible to its toxicities.)
- Seronegative for hepatitis B antigen, and seronegative for hepatitis C antibody. If hepatitis C antibody test is positive, then patients must be tested for the presence of antigen by RT-PCR and be HCV RNA negative.

2.1.1.11 Hematology

- WBC ≥ 3000/mm^3
- ANC ≥ 1000/mm^3 without the support of filgrastim
- Platelet count ≥ 100,000/mm^3
- Hemoglobin ≥ 8.0 g/dL. Subjects may be transfused to reach this cut-off.

2.1.1.12 Chemistry

- Serum ALT/AST ≤ 2.5 x ULN

- Serum creatinine ≤ 1.6 mg/dL
- Total bilirubin ≤ 1.5 mg/dL, except in patients with Gilbert's Syndrome, who must have a total bilirubin < 3.0 mg/dL.

2.1.1.13 Patients must be at least 4 weeks from radiation therapy. Additionally, patients must be at least 6 weeks from nitrosoureas, 4 weeks from temozolomide, 3 weeks from procarbazine, 2 weeks from vincristine and 4 weeks from last bevacizumab administration. Patients must be at least 4 weeks from other cytotoxic therapies not listed above and 2 weeks for non-cytotoxic agents (e.g., interferon, tamoxifen) including investigative agents. All toxicities from prior therapies should be resolved to CTCAE ≤ grade 1 (except for toxicities such as alopecia, or vitiligo).

2.1.1.14 Subjects must be co-enrolled on protocol 03-C-0277.

2.1.2 Exclusion Criteria

2.1.2.1 A prior history of gliadel implantation in the past six months.

2.1.2.2 Women of child-bearing potential who are pregnant or breastfeeding because of the potentially dangerous effects of the treatment on the fetus or infant.

2.1.2.3 Active systemic infections requiring anti-infective treatment, coagulation disorders, or any other active or uncompensated major medical illnesses.

2.1.2.4 Any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease).

2.1.2.5 Concurrent opportunistic infections (The experimental treatment being evaluated in this protocol depends on an intact immune system. Patients who have decreased immune-competence may be less responsive to the experimental treatment and more susceptible to its toxicities.)

2.1.2.6 History of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, or aldesleukin.

2.1.2.7 History of coronary revascularization or ischemic symptoms.

2.1.2.8 Clinically significant hemorrhagic or ischemic stroke, including transient ischemic attacks and other central nervous system bleeding in the preceding 6 months that were not related to glioma surgery. History of prior intratumoral bleeding is not an exclusion criterion; patients who with history of prior intratumoral bleeding, however, need to undergo a non-contrast head CT to exclude acute bleeding.

2.1.2.9 Other concomitant anti-cancer therapy except corticosteroids.

2.1.2.10 Any patient known to have LVEF ≤ 45%.

2.1.2.11 Documented FEV1 ≤ 60% predicted tested in patients with:

- A prolonged history of cigarette smoking (≥ 20 pack-year smoking history, with cessation within the past two years).
- Symptoms of respiratory dysfunction.

2.1.2.12 Patients who are receiving any other investigational agents.

2.1.2.13 Documented LVEF ≤ 45% tested in patients:

- Age ≥ 65 years
- With clinically significant atrial and/or ventricular arrhythmias, including but not limited to: atrial fibrillation, ventricular tachycardia, second- or third-degree heart block or have a history of ischemic heart disease and/or chest pain.

2.2 SCREENING EVALUATION

Note: Testing for screening evaluation is conducted under the NCI-SB companion protocol, 99-C-0128 (Evaluation for NCI Surgery Branch Clinical Research Protocols).

2.2.1 Within 3 Months Prior to Starting the Preparative Regimen

- HIV antibody titer, HBsAg determination, and anti HCV
- Immunohistochemistry or PCR confirmation of tumor tissue for expression of EGFRvIII for histologically proven glioblastomas or gliosarcomas confirmed by the NCI Laboratory of Pathology. (Note: Testing is permitted to be conducted at any time prior to starting the preparative regimen.)

2.2.2 Within 8 Weeks Prior to Starting the Preparative Regimen

- Pulmonary function testing for patients with a prolonged history of cigarette smoking (≥ 20 pack-year smoking history, with cessation within the past two years), symptoms of respiratory dysfunction, or other clinical indications which may include thoracic surgeries.
- Cardiac evaluation commensurate to patients' history and clinical presentation (e.g., stress thallium, echocardiogram, MUGA) for patients who are ≥ 65 years of age, or who have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias, including but not limited to: atrial fibrillation, ventricular tachycardia, heart block. Patients with a LVEF of ≤ 45% will not be eligible. Patients < 65 years of age with cardiac risk factors (e.g., diabetes, hypertension, obesity) may undergo cardiac evaluations as noted above.

2.2.3 Within 4 Weeks Prior to Starting the Preparative Regimen

- Complete history and physical examination, including weight and vital signs, noting any organ system involvement and any allergies/sensitivities to antibiotics. (Note: Patient history may be obtained within 8 weeks prior to starting the preparative regimen).

2.2.4 Within 14 Days Prior to Starting the Preparative Regimen

- Screening blood tests:
  - CBC w/differential
  - Chemistries: Creatinine, ALT/GPT, AST/GOT, Total bilirubin
- Urinalysis, with culture if indicated

2.2.5 Within 7 Days Prior to Starting the Preparative Regimen

- β-HCG pregnancy test (serum or urine) on all women of child-bearing potential.

- Physical examination, including neurological examination
- KPS ≥ 60 (see **Appendix F**).

2.2.6 Within 1-4 Days Prior to Starting the Preparative Regimen

- Dynamic contrast-enhanced MRI scan with perfusion of the head. For patients on steroids, the dose must be fixed for at least 5 days prior to imaging. If the steroid dose is increased between the date of imaging and registration, a new baseline MRI is required.

2.3 REGISTRATION AND TREATMENT ASSIGNMENT PROCEDURES

2.3.1 Prior to Registration for this Protocol

Patients will sign the consent for and enroll on the NCI-SB companion protocol 03-C-0277 (Cell Harvest and Preparation for Surgery Branch Adoptive Cell Therapy Protocols), prior to transduction of PBL cells (either fresh or cryopreserved samples). Once cells exceed the potency requirement and are projected to exceed the minimum number specified in the Certificate of Analysis (CoA), patients will sign the consent document for this protocol.

2.3.2 Registration Procedure

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to the NCI-CRO at ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at the NCI-CRO, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol. Verification of registration will be forwarded electronically via email to the research team. A recorder is available during non-working hours.

2.3.3 Treatment Assignment Procedures

2.3.3.1 Cohorts

Cohort	Name	Description
1A	*Steroids (Phase I)*	Patients receiving steroids at the time of treatment.
1B	*No Steroids (Phase I)*	Patients not receiving steroids at the time of treatment.
2A	*Steroids (Phase II)*	Patients receiving steroids at the time of treatment.
2B	*No Steroids (Phase II)*	Patients not receiving steroids at the time of treatment.

2.3.3.2 Arms

Number	Name	Description
1	*Phase I Arm*	Non-myeloablative, lymphodepleting preparative regimen of cyclophosphamide and fludarabine + escalating doses of anti-EGFRvIII CAR transduced PBL + aldesleukin
2	*Phase II Arm*	Non-myeloablative, lymphodepleting preparative regimen of cyclophosphamide and fludarabine + MTD of anti-EGFRvIII CAR transduced PBL established in Phase I + aldesleukin

2.3.3.3 Randomization and Arm Assignment

This is a non-randomized study. All patients will be directly assigned based on cohort as follows:

- Patients in Cohorts 1A and 1B will be directly assigned to Arm 1.
- Patients in Cohorts 2A and 2B will be directly assigned to Arm 2.

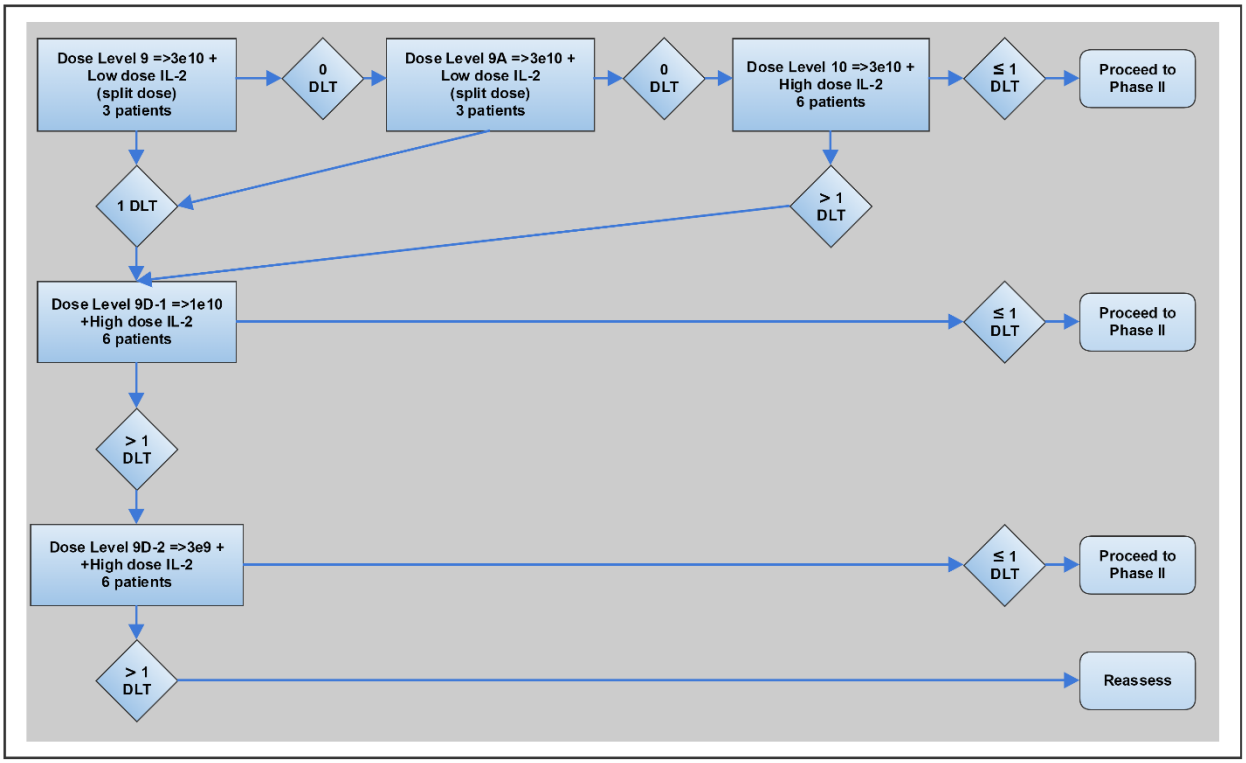
3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

The study will be conducted using a Phase I/II design. With the approval of Amendment K, additional dose levels were added: dose levels 9-10. Prior to receiving the engineered PBL cells, patients will receive a non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed in 2-4 days by intravenous infusion of *ex vivo* tumor-reactive, EGFRvIII CAR gene-transduced PBMC, plus IV high- or low-dose aldesleukin (720,000 or 72,000 IU/kg every 8 hours for a maximum of 15 doses).

Patients will undergo complete evaluation of tumor with physical and neurological examination, MRI of the brain with and without gadolinium, and clinical laboratory evaluation four weeks (± 7 days) after completion of treatment. If the patient has stable disease or tumor shrinkage, repeat complete evaluations will be performed every 1 month (± 7 days). After the first year, patients continuing to respond will continue to be followed with this evaluation every 2 months (± 7 days) until off-study criteria are met. All patients will receive one course of treatment. The start date of the course will be the start date of the chemotherapy; the end date will be the day of the first post-treatment evaluation.

3.1.1 Schema – Beginning with Amendment K



3.1.2 Pre-Treatment Phase: Cell Preparation Performed on 03-C-0277

PBMC will be obtained by leukapheresis (approximately $1x10^{10}$ cells). PBMC will be obtained while enrolled on protocol 03-C-0277. PBMC will be grown, transduced and expanded for this trial according to standard operating procedures submitted in the IND. Whole PBMC will be cultured in the presence of anti-CD3 (OKT3) and aldesleukin in order to stimulate T-cell growth. Transduction is initiated by exposure of approximately $1x10^7$ to $5x10^8$ cells to supernatant containing the anti-EGFRvIII CAR retroviral vector. These transduced cells will be expanded and tested for their anti-tumor activity. Successful CAR gene transfer will be determined by FACS analysis for the CAR protein and anti-tumor reactivity will be tested by cytokine release as measured on EGFRvIII expressing cells. Successful CAR gene transfer for each transduced PBL population will be defined as > 10% CAR positive cells and for biological activity, gamma-interferon secretion must be ≥ 200 pg/mL and twice the background level as tested against the positive control and < 400 pg/mL against the negative control including wt-EGFRvIII. If the gamma-interferon secretion against the negative control is greater than 400 pg/mL the cells will remain in culture and tested daily until the target level is reached.

3.1.3 Treatment Phase

Note: Paragraphs highlighted in grey below are no longer applicable after the approval of amendment K.

3.1.3.1 Phase I – Dose Escalation: Closed with Amendment H

The protocol will begin with a Phase I dose escalation design, with eight dose levels and with two different cohorts; one for patients receiving steroids at the time of treatment (Cohort 1A) and one for patients not receiving steroids at the time of treatment (Cohort 1B). Each cohort in Phase I will be escalated independently of the other, and different MTDs may be identified.

Initially, the protocol will enroll one patient in each of the first 4 dose levels unless that patient experiences a dose-limiting toxicity (DLT). Following Dose Level 4, all subsequent dose levels will proceed in a Phase I dose escalation design, with 4 dose levels of n = 3.

The total number of EGFRvIII engineered cells transferred for each dose level will be:

Dose Escalation Schedule	
Dose Level	**Dose of Anti-EGFRvIII CAR T-Cells**
Level 1 (Cohorts 1A & 1B)	10^7
Level 2 (Cohorts 1A & 1B)	$3x10^7$
Level 3 (Cohorts 1A & 1B)	10^8
Level 4 (Cohorts 1A & 1B)	$3x10^8$
Level 5 (Cohorts 1A & 1B)	10^9
Level 6 (Cohorts 1A & 1B)	$3x10^9$
Level 7 (Cohorts 1A & 1B)	10^{10}
Level 8 (Cohorts 1A & 1B)	$3 – 6x10^{10}$ (closed following patient 17)

Patients will be enrolled sequentially, therefore enrollment will not proceed to a higher dose level until patients have been treated in the prior dose level. Patients, however, will be dose-escalated to the next dose level within a given cohort independent on what is occurring in the other strata. If sufficient cells cannot be grown to meet the criteria for the assigned dose level, the patient will be enrolled in the appropriate dose level for the number of cells infused.

In dose levels 1-4, if the patient experiences a DLT, five more patients would be treated at that dose level to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If a level with 2 or more DLTs in 3-6 patients has been identified, five additional patients will be accrued at the next-lowest dose level, for a total of 6, in order to further characterize the safety of the MTD prior to starting the phase II portion. If there are 1 or fewer DLTs in the first dose level, the study will proceed to the second dose level. If a DLT occurs in the first dose level, that dose level will be expanded to n = 6 patients. If two DLTs occur in the first dose level, the study will be terminated.

In dose levels 5-8, should a single patient experience a DLT due to the cell infusion at a particular dose level, three more patients would be treated at that dose level to confirm that no greater than 1/6 patients have a DLT prior to proceeding to the next higher level. If a level with 2 or more DLTs in 3-6 patients has been identified, three additional patients will be accrued at the next-lowest dose level, for a total of 6, in order to further characterize the safety of the MTD prior to starting the phase II portion.

The MTD is the highest dose at which $\leq$ 1 of 6 patients experienced a DLT or the highest dose level studied if DLTs are not observed at any of the dose levels.

Prior to receiving the engineered PBL cells, all patients will receive a non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed in 1-4 days by intravenous infusion of in vitro tumor-reactive, EGFRvIII CAR gene-transduced PBL plus IV aldesleukin (72,000 IU/kg every 8 hours for a maximum of 15 doses).

A two-week safety assessment period following regimen completion (defined as the last dose of IL-2) will be conducted at the following times for each cohort:

- For the first patient in each dose level before a second patient will be accrued in the dose level.
- After the last patient is treated in each dose level prior to enrollment into the next dose level.
- For the second and third patients in dose levels 1-4 (if the first patient treated in dose levels 1-4 experiences a DLT and the dose level is expanded).

3.1.3.2 Phase I – Dose Escalation: Beginning with Amendment K

Beginning with Amendment K, patients enrolled in dose levels 9-10 will receive a non-myeloablative, lymphodepleting preparative regimen followed by intravenous infusion of EGFRvIII CAR gene-transduced PBMC, plus high- or low-dose aldesleukin.

The total number of EGFRvIII engineered cells transferred for each dose level will be:

Dose Escalation Schedule		
Dose Level	**Dose of Anti-EGFRvIII CAR T-Cells**	**Aldesleukin (IL-2)**
Level 6 (Completed)	$3x10^9$	Low-Dose
Level 7 (Completed)	10^{10}	Low-Dose
Level 8 (Closed)	$3 – 6x10^{10}$	Low-Dose
Level 9	$3x10^{10}$ given as a split dose, 2 hours apart	Low-Dose
Level 9A	$3x10^{10}$ given as a single dose	Low-Dose
Level 9D-1	10^{10}	High-Dose
Level 9D-2	$3x10^9$	High-Dose
Level 10	$3x10^{10}$	High-Dose

If one DLT is observed in Dose Level 9 or 9A, the dose will be de-escalated (Dose Level 9D-1) and high-dose IL-2 will be added. If no more than one DLT is seen, a total of 6 patients will be enrolled in Dose Level 9D-1 to further characterize the safety of the MTD.

If more than one DLT is observed in Dose Level 9D-1, then the dose will be de-escalated (Dose Level 9D-2) and high-dose IL-2 will be added. If no more than one DLT is seen, a total of 6 patients will be enrolled in Dose Level 9D-2 to further characterize the safety of the MTD. If more than one DLT is observed in Dose Level 9D-2, we will re-evaluate our strategy.

If no DLTs are observed in Dose Level 9 or 9A, the dose will be escalated to include high-dose IL-2 (Dose Level 10). If no more than one DLT is seen in Dose Level 10, a total of 6 patients will be enrolled in Dose Level 10 to further characterize the safety of the MTD. If more than one DLT is observed in Dose Level 10, a total of 6 patients will be enrolled in Dose Level 9D-1.

If sufficient cells cannot be grown to meet the criteria for the assigned dose level, the patient will be enrolled in the appropriate dose level for the number of cells infused.

The MTD is the highest dose at which < 1 of 6 patients experienced a DLT or the highest dose level studied if DLTs are not observed at any of the dose levels.

A two-week safety assessment period following regimen completion (defined as the last dose of IL-2) will be conducted at the following times for each dose level:

- After the first patient in each dose level before a second patient will be accrued in the dose level.
- After any patient experiences a DLT.

3.1.4 Dose-Limiting Toxicity

A DLT is defined as follows:

- Grade 2 or greater allergic reaction or reaction that involves bronchospasm or generalized urticaria.
- All grade 3 and greater toxicities with the exception of:

- Myelosuppression, defined as lymphopenia, neutropenia, decreased hemoglobin, and thrombocytopenia, due to the chemotherapy preparative regimen.
- IL-2 expected toxicities as defined in **Appendix B** and **Appendix C**.
- Expected chemotherapy toxicities as defined in Section **11**.
- Immediate hypersensitivity reactions occurring within 2 hours of cell infusion (related to cell infusion) that are reversible to a grade 2 or less within 24 hours of cell administration with standard therapy.
- Grade 3 fever
- Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 within 7 days.
- Grade 3 autoimmunity that resolves to less than or equal to a grade 2 autoimmune toxicity within 10 days.

3.1.5 Phase II Portion

Once the MTD has been determined, the study will then proceed to the Phase II single stage portion utilizing the MTD of anti-EGFRvIII engineered cells as determined in the Phase I portion. Patients will be accrued into two cohorts: those patients with recurrent malignant glioma receiving steroids at the time of treatment (Cohort 2A), and those patients with recurrent malignant glioma not receiving steroids at the time of treatment (Cohort 2B).

Similar to the Phase I portion, prior to receiving the engineered PBL cells, patients in the Phase II portion will receive a non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed in 1-4 days by intravenous infusion of *ex vivo* tumor-reactive, anti-EGFRvIII CAR gene-transduced PBL plus IV high-dose aldesleukin.

Note: If the MTD is not established in Dose Level 9D, we will re-evaluate our strategy and amend the protocol.

3.2 PROTOCOL STOPPING RULES

New subject enrollment to the protocol will be temporarily halted if any of the following conditions are met in each cohort separately, and discussions with the FDA or NIH IRB regarding safety and the need for protocol revisions if applicable:

- During the Phase I portion of the study – If two or more patients develop a grade 3 or greater toxicity related to the cell product, with the exception of:
  - Grade 3 metabolic laboratory abnormalities without significant clinical sequela that resolve to grade 2 or less within 7 days
  - Grade 3 fever
- If 1 of the first 3 patients (OR 2 of the first 6 patients, OR 3 of the first 9 patients, OR 4 of the first 12 patients) develop grade 3 autoimmunity, that cannot be resolved to less than or equal to a grade 2 autoimmune toxicity within 10 days, or any grade 4 or greater autoimmune toxicity.
- Two or more patients remain neutropenic (grade 3 or 4 ANC) 4 weeks following treatment.

- Two or more patients remain thrombocytopenic (grade 3 or 4 platelets) 8 weeks following treatment.
- Two or more patients develop a major neurological event defined as:
  - Significant grade 3 toxicity that does not resolve to ≤ grade 1 with best medical management and supportive care within 3 weeks
  - Significant grade 4 toxicity
- During the Phase II portion of the study – Once five or more patients have been enrolled, if grade 4 non-reversible non-hematological toxicities exceed 20% or more in patients cumulatively enrolled in this phase. Grade 4 non-reversible toxicities are defined as those grade 4 toxicities that are not reversible over a 7-day period.
- During the Phase I and II portions of the study – Any death that is at least possibly attributed to the investigational agent and which occurs within 30 days of receiving the investigational agent.

The rationale for these neurological guidelines is based on this patient population's inherent increased risk of cerebral edema. In particular, aldesleukin is known to cause capillary leak syndrome which can lead to cerebral edema and its ensuing symptoms including the following: seizures, depressed level of consciousness, encephalopathy, headache, clinically evident herniation syndromes, and worsening of baseline focal neurological symptoms.

These symptoms are generally transient, directly related to cerebral edema, and reversible with supportive care. Therefore, any grade 3 neurological toxicity should first be treated with best medical management and supportive care (see table below) until resolution of the toxicity to ≤ grade 1. If the neurological toxicity does not resolve to grade 1 with best medical management and supportive care, it will qualify as a major neurological event as defined above.

3.2.1.1 Management of Expected Significant Neurological Toxicities ≥ Grade 3

Expected Toxicity	Medical Management/Supportive Care
Cerebral Edema	IV Steroids Mannitol, per protocol
Seizures	Institute AEDs, up to a maximum of 3 agents
Encephalopathy	Treat metabolic abnormalities IV Steroids
Headache	IV Steroids Analgesics, including at least 1 narcotic agent

3.3 DRUG ADMINISTRATION

3.3.1 Preparative Regimen with Cyclophosphamide and Fludarabine

Treatment will be according to the schedule described below and in Section **3.3.4**. Starting on Day -6, study medication start times for drugs given once daily should be given within 2 hours of the scheduled time. Chemotherapy infusions may be slowed or delayed as medically indicated.

Administration of diuretics, electrolyte replacement, and hydration and monitoring of electrolytes should all be performed as clinically indicated.

Days -7 and -6

Approximately 6 Hours Prior to Cyclophosphamide

Hydrate: Begin hydration with 0.9% Sodium Chloride Injection containing 10 meq/L of potassium chloride at 1.5-2.6 mL/kg/hour (starting approximately 6 hours pre-cyclophosphamide and continuing until 24 hours after last cyclophosphamide infusion). The hydration rate will be capped at 250 mL/hour. At any time during the preparative regimen, if urine output is <1.0 mL/kg/hour or if body weight is >2 kg over pre-cyclophosphamide value, furosemide 10-20 mg IV may be administered.

Approximately 1 Hour Prior to Cyclophosphamide

Ondansetron (0.15 mg/kg/dose [rounded to the nearest even mg dose between 8 mg and 16 mg based on patient weight] IV every 8 hours x 3 days) will be given for nausea.

Cyclophosphamide 60 mg/kg/day x 2 days IV in 250 mL D5W infused simultaneously with mesna 15 mg/kg/day over 1 hour x 2 days. If patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix A**.

A decreased dose of cyclophosphamide at 30 mg/kg/day (x 2 days) will be considered for patients who have a history of prolonged hematologic recovery from prior chemotherapy treatments.

Immediately following the end of Cyclophosphamide

Begin mesna infusion at 3 mg/kg/hour intravenously diluted in a suitable diluent (see Section **11.2.1**) over 23 hours after each cyclophosphamide dose. If patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix A**.

Days -7 to -3

Fludarabine 25 mg/m2/day IVPB daily over 30 minutes for 5 days. If patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix A**. (Fludarabine will be started approximately 1-2 hours after the cyclophosphamide and mesna on Days -7 and -6).

3.3.2 Cell Infusion

Day 0 (2-4 Days After the Last Dose of Fludarabine)

Cells are delivered to the Patient Care Unit by a staff member from the Surgery Branch Cell Production Facility (SB-CPF). Prior to infusion, the cell product identity label is double-checked by two authorized staff (MD or RN), and an identification of the product and documentation of administration are entered in the patient's chart. The cells are to be infused intravenously on the Patient Care Unit over 20-30 minutes or as clinically determined by an investigator for patient safety via non-filtered tubing, gently agitating the bag during infusion to prevent cell clumping.

Note: For patients enrolled in Dose Level 9, the cell dose will be given in two doses, 2 hours apart. If the patient experiences a grade 1 toxicity that does not resolve within one hour or a grade 2 or greater toxicity, the second half of the dose will be withheld.

3.3.3 Aldesleukin Administration

Days 0-5 (Day 0 = Day of Cell Infusion)

- Beginning on Day 1 or 2, filgrastim will be administered subcutaneously at a dose of 300 mcg/day (dose may be adjusted as clinically indicated). Filgrastim administration will continue daily until neutrophil count > $1x10^9$/L x 3 days or > $5x10^9$/L.
- Aldesleukin 72,000 IU/kg IV (Dose Levels 9 & 9A) or 720,000 IU/kg IV (Dose Levels 9D & 10) (based on total body weight) over 15 minutes approximately every eight hours beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum of 15 doses).
- For Phase II portion: Aldesleukin 720,000 IU/kg IV (based on total body weight) over 15 minutes approximately every eight hours beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum of 15 doses). If high-dose aldesleukin is not tolerated in Dose Levels 9D or 10, then we will re-evaluate our strategy and amend the protocol.

Aldesleukin (based on total body weight) will be administered as an intravenous bolus over a 15-minute period approximately every 8 hours beginning within 24 hours of cell infusion and continuing for up to 5 days (maximum 15 doses). Doses will be preferentially administered every eight hours; however, up to 24 hours may elapse between doses depending on patient tolerance. Aldesleukin dosing will be stopped if toxicities are not sufficiently recovered by supportive measures within 24 hours of the last dose of aldesleukin. Dosing will be delayed or stopped if patients reach grade 3 or 4 toxicity due to aldesleukin, except for the reversible grade 3 toxicities common to aldesleukin such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in **Appendix B**. Toxicities will be managed as outlined in **Appendix C**. Dosing may be held or stopped at the discretion of the treating investigator. (**Appendix D** lists the toxicities seen in patients treated with aldesleukin at the NIH Clinical Center).

Because confusion is a possible side effect of aldesleukin administration, a Durable Power of Attorney will be signed by the patient to identify a surrogate to make decisions about their medical care if the patient becomes incapacitated or cognitively impaired.

3.3.4 Treatment Schedule

Day	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5
Therapy													
Cyclophosphamide (60 mg/kg)	X	X											
Fludarabine (25 mg/m^2)	X	X	X	X	X								
Anti-EGFRvIII PBL								X[1]					
Aldesleukin								X[2]	X	X	X	X	X
Filgrastim[3] (5 mcg/kg)									X	X	X	X	X
TMP/SMX[4] 160 mg/800 mg (example)								X		X		X	

Fluconazole[5] (400 mg PO)								X	X	X	X	X	X
Valacyclovir PO or Acyclovir IV[6]								X	X	X	X	X	X

[1]Two to four days after the last dose of fludarabine. The cells will be split into two doses, two hours apart for patients in Dose Level 9.

[2]Initiate within 24 hours after cell infusion.

[3]Continue until neutrophil count > $1x10^9$/L for 3 consecutive days or > $5x10^9$/L.

[4]The TMP/SMX schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six months and until CD4 > 200 x2, starting Day 0 or within one week of anticipated lymphopenia.

[5]Continue until ANC > 1000/mm^3

[6]In patients positive for HSV or VZV, continue for at least 6 months and until CD4 > 200 x2.

3.4 BASELINE EVALUATION

3.4.1 Within 14 Days Prior to Starting the Preparative Regimen

- Apheresis, as indicated
- Baseline blood tests:
  - CBC w/differential
  - Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
  - PT-INR/PTT
  - TBNK
  - Thyroid Panel
  - Anti-CMV antibody titer, HSV and VZV serology, and EBV panel (may be performed within 3 months prior to starting the preparative regimen)
- Urinalysis, with culture if indicated
- Chest x-ray
- EKG

3.5 ON-STUDY EVALUATION

3.5.1 During the Preparative Regimen (Daily)

- CBC w/differential
  - Note: Once the platelet count drops below 80,000/mm^3, platelets will be monitored and transfusions given as clinically indicated until the platelet count is greater than 30,000/mm^3 for 24 hours without transfusion support.

- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
- PT-INR/PTT (every 3 days)
- Urinalysis, as needed
- Weight, as indicated

3.5.2 Post-Cell Infusion

- Vital signs, including neurological examinations, will be monitored hourly (± 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.

3.5.3 During Aldesleukin Administration (Daily)

Note: Until values return to normal limits/baseline.

- CBC (except platelets as noted in Section **3.5.1**)
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein

3.5.4 During Hospitalization (Every 1-2 Days)

Note: Once electrolytes are stable and platelet count is above 30,000/mm^3 for 24 hours without support.

- Physical examination, including weight and vital signs, as clinically indicated
- Toxicity assessment, including a review of systems, as clinically indicated
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
- Once total lymphocyte count is > 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized). See Section **5.5** for post-cell infusion evaluations.
- Other tests will be performed as clinically indicated.

3.6 POST-TREATMENT (FOLLOW-UP) EVALUATION

- All patients will return to the NIH Clinical Center for their first follow-up evaluation for response 4 weeks (± 7 days) following the administration of the cell product.
- Patients who have received multiple transfusions during the treatment phase or have been discharged with grade 3 or greater significant adverse events should be evaluated by the

referring physician within two weeks of discharge and repeat labs as appropriate to be faxed to the research team. Patients will receive appropriate treatment as determined by their treating physician

- Patients who are unable or unwilling to return for follow-up evaluations may be followed via phone or email contact. A request will be made to send laboratory, imaging, and physician exam reports performed by their treating physician. Any outstanding toxicities will be reviewed with the patient.

3.6.1 Time-Period of Evaluations

Patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities after their first follow-up evaluation will return to the NIH Clinical Center as noted below:

- Monthly (± 7 days) for 12 months
- Every 1-2 months (± 7 days) as feasible until off-study criterion is met

Note: Patients may be seen more frequently as clinically indicated.

3.6.2 Scheduled Evaluations

At each scheduled evaluation for response, patients will undergo:

- Physical examination, including neurological examination
- KPS
- Weight and vital signs
- Toxicity assessment, including a review of systems
- CBC w/differential
- Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein
- PT-INR/PTT
- Urinalysis, as needed
- Thyroid Panel, as clinically indicated
- TBNK
- MRI of the brain with and without gadolinium
- A 5-liter apheresis may be performed at the first follow-up visit. If the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Subsequently, approximately 60 mL of blood will be obtained at follow-up visits for at least 3 months. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed. This will be performed on protocol 03-C-0277.

- Detection of RCR and persistence of CAR gene transduced cells (see Section **5.8**). This will be performed on the NCI-SB long-term follow-up protocol 09-C-0161 (Follow up Protocol for Subjects Previously Enrolled in NCI Surgery Branch Studies).
- Long-term follow-up of patients receiving gene transfer: Physical examinations will be performed and documented annually for 5 years following cell infusion to evaluate long-term safety. After 5 years, health status data will be obtained from surviving patients via telephone contact or mailed questionnaires. The long-term follow-up period for retroviral vectors is 15 years. This will be performed on protocol 09-C-0161.

3.7 STUDY ASSESSMENT CALENDAR

Assessments	Prior to Starting Preparative Regimen						Prior to Preparative Regimen	During Preparative Regimen *(Daily)*	Prior to Cell Infusion	Post-Cell Infusion	During IL-2 Admin.[1]	During Hospitalization *(Every 1-2 Days)*[2]	Post-Treatment Follow-up[3]
	Within 3 Months	Within 8 Weeks	Within 4 Weeks	Within 14 Days	Within 7 Days	Within 1-4 Days							
IHC or PCR confirmation of tumor tissue[4]	X												
Medical history[5]		X	X										
Physical exam			X		X							X	X
Neurological exam					X					X[6]			X
Performance score (KPS)[7]					X								X
Weight			X					X				X	X
Vital signs			X							X[8]		X	X
β-HCG pregnancy test[9]					X								

1 Until values return to normal limits/baseline.
2 Once electrolytes are stable and platelet count is above 30,000/mm^3 for 24 hours without support.
3 All patients will return to the NIH Clinical Center for their first follow-up evaluation for response 4 weeks (± 7 days) following the administration of the cell product. Patients who experience stable disease, a partial response, or a complete response or have unresolved toxicities after their first follow-up evaluation will return to the NIH Clinical Center monthly (± 7 days) for 12 months and then every 1-2 months (± 7 days) as feasible until off-study criterion is met. See Section **3.6**.
4 Immunohistochemistry or PCR confirmation of tumor tissue for expression of EGFRvIII for histologically proven glioblastomas or gliosarcomas confirmed by the NCI Laboratory of Pathology. Testing is permitted to be conducted at any time prior to starting the preparative regimen.
5 Note organ system involvement and any allergies/sensitivities to antibiotics. Patient history may be obtained within 8 weeks prior to starting the preparative regimen.
6 Neurological examinations will be monitored hourly (± 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.
7 KPS ≥ 60 (see **Appendix F**).
8 Vital signs will be monitored hourly (± 15 minutes) for four hours and then routinely (every 4-6 hours) unless otherwise clinically indicated.
9 Serum or urine; on all women of child-bearing potential.

Assessments	Prior to Starting Preparative Regimen						Prior to Preparative Regimen	During Preparative Regimen *(Daily)*	Prior to Cell Infusion	Post-Cell Infusion	During IL-2 Admin.[1]	During Hospitalization *(Every 1-2 Days)*[2]	Post-Treatment Follow-up[3]
	Within 3 Months	Within 8 Weeks	Within 4 Weeks	Within 14 Days	Within 7 Days	Within 1-4 Days							
Urinalysis[10]				X				X					X
Pulmonary function test[11]		X											
Cardiac evaluation[12]		X											
EKG				X									
Toxicity assessment[13]												X	X
Serology													
HIV antibody, HBsAg, anti-HCV	X												
Anti-CMV, HSV and VZV serology, EBV panel	X			X[14]									
Laboratory Procedures													
CBC w/differential				X				X[15]			X[16]	X	X

10 With culture if indicated.
11 For patients with a prolonged history of cigarette smoking (≥ 20 pack-year smoking history, with cessation within the past two years), symptoms of respiratory dysfunction, or other clinical indications which may include thoracic surgeries.
12 Commensurate to patients' history and clinical presentation (e.g., stress thallium, echocardiogram, MUGA) for patients who are ≥ 65 years of age, or who have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias, including but not limited to: atrial fibrillation, ventricular tachycardia, heart block. Patients with a LVEF ≤ 45% will not be eligible. Patients < 65 years of age with cardiac risk factors (e.g., diabetes, hypertension, obesity) may undergo cardiac evaluation as noted above.
13 Including a review of systems.
14 Anti-CMV antibody titer, HSV and VZV serology, and EBV panel may be performed within 3 months prior to starting the preparative regimen.
15 Once the platelet count drops below 80,000/mm^3, platelets will be monitored and transfusions given as clinically indicated until the platelet count is greater than 30,000/mm^3 for 24 hours without transfusion support.
16 Except platelets as noted in Section **3.5.1**.

Assessments	Prior to Starting Preparative Regimen						Prior to Preparative Regimen	During Preparative Regimen *(Daily)*	Prior to Cell Infusion	Post-Cell Infusion	During IL-2 Admin.[1]	During Hospitalization *(Every 1-2 Days)*[2]	Post-Treatment Follow-up[3]
	Within 3 Months	Within 8 Weeks	Within 4 Weeks	Within 14 Days	Within 7 Days	Within 1-4 Days							
Blood chemistries[17]				X				X			X	X	X
PT-INR/PTT				X				X[18]					X
TBNK				X								X[19]	X
Thyroid Panel				X									X
Additional apheresis[20]				X									X
Correlatives[21]													
CPT tubes (SB-CPF)							X			X			
SST tube (Figg Lab)							X		X	X			
Imaging													
MRI scan						X[22]							X[23]
Chest x-ray				X									

17 **Screening:** Creatinine, ALT/GPT, AST/GOT, Total bilirubin. **All other times:** Acute Care Panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN), Hepatic Panel (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral Panel (albumin, calcium, magnesium, phosphorus), Uric acid, Creatinine kinase, Lactate dehydrogenase, Total protein.
18 Every 3 days.
19 Once total lymphocyte count is > 200/mm^3, TBNK for peripheral blood CD4 count will be drawn weekly (while the patient is hospitalized).
20 Apheresis may be performed prior to and 6 weeks (± 2 weeks) following the administration of the cell product. If the patient is unable to undergo apheresis, approximately 96 mL of blood may be obtained. Subsequently, approximately 60 mL of blood will be obtained at follow-up visits for at least 3 months. Peripheral blood mononuclear cells will be cryopreserved so that immunologic testing may be performed. This will be performed on protocol 03-C-0277.
21 Research samples, as described in Section **5**.
22 Dynamic contrast-enhanced MRI scan with perfusion of the head. For patients on steroids, the dose must be fixed for at least 5 days prior to imaging. If the steroid dose is increased between the date of imaging and registration, a new baseline MRI is required.
23 MRI of the brain with and without gadolinium.

3.8 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects compete an evaluation safety visit approximately 4 weeks (± 7 days) following administration of the cell product (at the first follow-up evaluation).

3.8.1 Criteria for Removal from Protocol Therapy

Patients will be taken off treatment for the following:

- Completion of first follow-up evaluation
- Progression of disease
- Investigator discretion
- Patient requests to be withdrawn from protocol therapy
- Positive pregnancy test

3.8.2 Off-Study Criteria

Patients will be taken off-study for the following:

- Completion of study follow-up period
- Progression of disease
- Patient begins a new therapy for their cancer
- Investigator discretion
- Patient requests to be withdrawn from the study
- Significant noncompliance
- Patient lost to follow-up
- Death

All patients will be co-enrolled on protocol 09-C-0161. Patients who are taken off-study for progressive disease or study closure on this treatment protocol may be followed on protocol 09-C-0161.

Once a subject is taken off-study, no further data can be collected on this treatment protocol.

3.8.3 Off Protocol Therapy and Off-Study Procedure

Authorized staff must notify the NCI-CRO when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Update Form from the website (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to the NCI-CRO at ncicentralregistration-l@mail.nih.gov.

4 CONCOMITANT MEDICATIONS/MEASURES

Concomitant medications to control side effects of therapy will be given. Meperidine (25-50 mg) will be given intravenously if severe chilling develops. Other supportive therapy will be given as required and may include acetaminophen (650 mg every 4 hours), indomethacin (50-75 mg every 6 hours) and ranitidine (150 mg every 12 hours). Ondansetron 0.15 mg/kg/dose IV every 8 hours

will be administered for nausea and vomiting. Additional antiemetics will be administered as needed for nausea and vomiting uncontrolled by ondansetron.

4.1 MANNITOL

Mannitol will be preferentially used in the acute hospital setting to control the signs and symptoms of increased intracranial pressure. If necessary, corticosteroids may then be used in the subacute and outpatient setting for the control of the signs and symptoms of increased intracranial pressure. However, all attempts will be made to withhold steroids in the non-steroid strata unless clearly medically indicated. Increases in steroid dose should be discussed with the Principal Investigator (PI). Changes in the steroid dose should be carefully documented on the flow sheets.

4.2 NON-ENZYME INDUCING ANTI-EPILEPTIC DRUGS (NON-EIAED)

- Non-EIAEDs are permitted but should be recorded in the patient's chart. If anti-seizure medications are required during study, every effort should be made to choose non-EIAEDs (**Appendix G**).
- Changes in the doses of dexamethasone and anti-epileptics will be allowed, but must be clearly documented in the patient's chart. It is strongly recommended that if a patient needs to be taken off a particular anti-epileptic agent (i.e. allergic reaction, ineffective), that it be replaced with another agent that is a non-inducer of the cytochrome P450 system.

4.3 INFECTION PROPHYLAXIS

Note: Other anti-infective agents may be substituted at the discretion of the treating investigator.

4.3.1 Pneumocystis Jirovecii Pneumonia

All patients will receive the fixed combination of trimethoprim and sulfamethoxazole (TMP/SMX) as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) PO daily three times a week on non-consecutive days, beginning Day 0 or within one week of anticipated lymphopenia.

Dapsone (in G6PD sufficient patient), atovaquone, or pentamidine may be substituted for TMP/SMX-DS in patients with sulfa allergies.

4.3.2 Herpes Simplex or Varicella Zoster Virus Prophylaxis

Patients with positive HSV or VZV serology will be given valacyclovir orally at a dose of 500 mg daily starting on the day of cell infusion, or acyclovir, 250 mg/m^2 IV every 12 hours if the patient is not able to take medication by mouth. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Prophylaxis for pneumocystis, HSV, and VZV will continue for 6 months post-chemotherapy. If the CD4 count is < 200 at 6 months post-chemotherapy, prophylaxis will continue for at least 6 months and until CD4 count is > 200 for two consecutive measures.

Note: A missed prophylactic dose will not be considered a protocol deviation, and thus a deviation will not be reported to the NIH-IRB (see sections **7.1.8** and **7.2**), if the patient is compliant with taking at least 75 percent of their required dose.

4.3.3 Fungal Prophylaxis (Fluconazole)

Patients will start fluconazole 400 mg PO starting on the day of cell infusion and continue until the absolute neutrophil count is > 1000/mm^3. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

4.3.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, in accordance with the current institutional guidelines, for fever of 38.3°C once or two temperatures ≥ 38.0°C at least one hour apart, AND an ANC < 500/mm^3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

4.4 BLOOD PRODUCT SUPPORT

Using daily CBCs as a guide, the patient will receive platelets and packed red blood cells (PRBCs) as needed. As a general guideline, patients may be transfused for:

- Hemoglobin < 8 gm/dL
- Platelets < 10,000/mm^3

All blood products will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBCs and decrease the risk of CMV infection.

5 BIOSPECIMEN COLLECTION

Blood and tissue are tracked at the patient level and can be linked to all protocols on which the patient has been enrolled. Samples will be used to support the specific objectives listed in the treatment protocol(s), e.g., immunologic monitoring, cytokine levels, persistence, as well as to support long-term research efforts within the NCI-SB and with collaborators as specified in protocol 03-C-0277.

The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

5.1 SAMPLES SENT TO FIGG LAB

- Venous blood samples will be collected in either a 4-mL or an 8-mL SST tube to be processed for serum and stored for future research. Record the date and exact time of draw on the tube. Blood tubes may be kept in the refrigerator until pick-up.
- For sample pick-up, page 102-11964.
- For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

- For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.
- The samples will be processed, barcoded, and stored in the Figg lab until requested by the investigator.

5.2 SAMPLES SENT TO SURGERY BRANCH CELL PRODUCTION FACILITY

- Venous blood samples will be collected in 8-mL CPT tubes to be processed and stored for future research. Record the date and exact time of draw on the tube. Blood tubes are kept at room temperature until pick-up.
- Samples will be picked up by the research nurse or designee and transported to the SB-CPF within 24 hours of blood draw.
- The samples will be processed, barcoded, and stored in SB-CPF.

5.3 PRIOR TO CHEMOTHERAPY ADMINISTRATION

- 5 CPT tubes (8 mL each): SB-CPF
- 1 SST tube (8 mL): Figg Lab
- 1 SST tube (4 mL): Daily, starting day of chemotherapy through day of discharge. Send to Figg Lab.

5.4 PRIOR TO CELL INFUSION (BASELINE SAMPLE FOR CYTOKINE ANALYSIS)

- 1 SST tube (8 mL): Figg Lab

5.5 POST-CELL INFUSION EVALUATIONS

Once total lymphocyte count is > 200/mm^3, the following samples will be drawn and sent to the SB-CPF on Monday, Wednesday, and Friday x5 days, then weekly (while the patient is hospitalized):

- 5 CPT tubes (8 mL each): SB-CPF
- 1 SST tube (8 mL): Figg Lab

5.6 SAMPLE COLLECTION SCHEDULE

Test	Volume Blood	Type of tube	Collection Point	Disposition
Research blood	52 mL	CPT and SST	Prior to chemo	Deliver SST tubes to Figg Lab. Deliver CPT tubes to SB-CPF.
Research blood	8 mL	SST	Prior to cell infusion	Deliver to Figg Lab.
Research blood	48 mL	CPT and SST	Post-cell infusion and at follow-up visits[1]	Deliver SST tubes to Figg Lab. Deliver CPT tubes to SB-CPF.

[1] Research blood will be obtained at follow-up visits for at least 3 months.

5.7 IMMUNOLOGICAL TESTING

- Apheresis may be performed prior to and 6 weeks (± 2 weeks) following the administration of the cell product. At other time points, patient PBL will be obtained from whole blood by purification using centrifugation on a Ficoll cushion. Aliquots of these PBMC will be cryopreserved for immunological monitoring of cell function.
- Lymphocytes will be tested directly and following in vitro culture using some or all of the following tests. Direct immunological monitoring will consist of quantifying T-cells reactive with EGFRvIII by FACS analysis using CAR-specific staining. *Ex vivo* immunological assays will consist of cytokine release by bulk PBL (± antigen stimulation) and by other experimental studies such as cytolysis if sufficient cells are available. If cell numbers are limiting, preference will be given to the direct analysis of immunological activity. Immunological assays will be standardized by the inclusion of 1) pre-infusion PBMC and 2) an aliquot of the engineered PBL cryopreserved at the time of infusion. In general, differences of 2- to 3-fold in these assays are indicative of true biologic differences.

5.8 MONITORING GENE THERAPY TRIALS: PERSISTENCE AND RCR

- Engineered cell survival. CAR and vector presence will be quantitated in PBMC samples using established PCR techniques. Immunological monitoring using CAR-specific staining will be used to augment PCR-based analysis. This will provide data to estimate the *in vivo* survival of lymphocytes derived from the infused cells. In addition, measurement of CD4 and CD8 T-cells will be conducted and studies of these T-cell subsets in the circulation will be determined by using specific PCR assays capable of detecting the unique DNA sequence for each retroviral vector engineered T-cell. Note: samples will be batched and assayed at the conclusion of the study.
- Patients will be co-enrolled on protocol 09-C-0161 and will adhere to the follow-up schedule described in that protocol. Patients' blood samples will be obtained and undergo analysis for detection of RCR by PCR prior to cell infusion and RCR PCR will be performed at 3 and 6 months, and at one-year post cell administration. Blood samples will be archived annually thereafter if all previous testing has been negative with a brief clinical history. If a patient dies or develops neoplasms during this trial, efforts will be made to assay a biopsy sample for RCR. If any post-treatment samples are positive, further analysis of the RCR and more extensive patient follow-up will be undertaken, in consultation with the FDA. RCR PCR assays detect the GaLV envelop gene and are performed under contract by the Indiana University Vector Production Facility. The results of these tests are maintained by the contractor performing the RCR tests and by the NCI-SB research team.

5.9 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR SURGERY BRANCH CELL PRODUCTION FACILITY

Blood and tissue collected during the course of this study will follow the Cell Tracking and Labeling System established by the SB-CPF. The Cell Tracking and Labeling System is designed to unambiguously ensure that patient/data verification is consistent. The patients' cell samples (blood or tissue) are tracked by distinct identification labels that include a unique patient identifier and date of specimen collection. Cryopreserved blood and tissue samples also bear the

date the sample was frozen. All cryopreserved samples are tracked for freezer location and storage criteria. All samples are stored in monitored freezers/refrigerators in 3NW NCI-SB laboratories at specified temperatures with alarm systems in place. Serum samples will be sent to the Blood Processing Core (BPC) for storage. Samples will be barcoded and stored on site or offsite at NCI Frederick Central Repository Services in Frederick, MD. All samples collected (blood or tissue) are entered into a central computer database with identification and storage location, and this database is backed up every night.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Blood and tissue collected during the course of this study will be stored, tracked, and disposed of as specified in protocol 03-C-0277.

5.10 SAMPLE STORAGE, TRACKING AND DISPOSITION FOR FIGG LAB

5.10.1 Sample Data Collection

All samples sent to the BPC will be barcoded, with data entered and stored in the LABrador (aka LabSamples) utilized by the BPC, and data will be updated to the NCI-SB central computer database weekly. This is a secure program, with access to LABrador limited to defined Figg Lab personnel, who are issued individual user accounts. Installation of LABrador is limited to computers specified by Dr. Figg. These computers all have a password-restricted login screen. All Figg Lab personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

LABrador creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without LABrador access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.10.2 Sample Storage and Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in LABrador. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI PI to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed, and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e. broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the LABrador. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password-protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The PI, associate investigators (AI), research nurses, and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant. Data will be entered into the NCI CCR C3D database.

All adverse events (AEs), including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for AEs until their first follow-up evaluation (4 weeks (± 7 days) following administration of the cell product) or until off-study, whichever comes first.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

All AEs must be recorded on the AE case report form unless otherwise noted below in Section **6.1.1**.

End of study procedures: Data will be stored according to HHS and FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.1.1 Exclusions to Routine Adverse Event Recording

Patients will be receiving multiple agents, which include commercially available agents (fludarabine, cyclophosphamide, aldesleukin, and supportive medications) in combination with the investigational agents; therefore, grade 1 events not related to the cell product will not be reported/recorded.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

De-identified human data generated for use in future and ongoing research will be shared through a NIH-funded or approved repository (ClinicalTrials.gov) and BTRIS. At the completion of data analysis, data will be submitted to ClinicalTrials.gov either before publication or at the time of publication or shortly thereafter. Data may also be used to support long-term research efforts within the NCI-SB, and de-identified data may also be shared with collaborators as specified in protocol 03-C-0277.

6.2.2 Genomic Data Sharing Plan

The NIH Genomic Data Sharing Policy does not apply to this study.

6.3 RESPONSE CRITERIA

For the purpose of this study, patients will be evaluated for response 4 weeks (± 7 days) after cell infusion. If the patient has stable disease or tumor shrinkage, repeat complete evaluations will be performed monthly (± 7 days) as feasible for 12 months, and then every 1-2 months (± 7 days). In addition to a baseline scan, confirmatory scans should also be obtained at least 4 weeks (not less than 4) following initial documentation of objective response.

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.0).

As part of this trial, as well as to assist in the determination of tumor progression, all efforts will be made to observe radiographic changes in the patient's tumors over time.

6.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with Cyclophosphamide.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of treatment will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle

of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.1.1 Objective Criteria for Measurable Lesions[41, 42]:

If there are too many measurable lesions to measure at each evaluation, choose the largest two to be followed before a patient is entered on study. The remaining lesions will be considered evaluable for the purpose of objective status determination.

6.3.2 Disease Parameters

6.3.2.1 Measurable Criteria

Measurable disease: Bi-dimensionally contrast-enhancing lesions with clearly defined margins by MRI scan, with two perpendicular diameters of at least 10 mm, visible on two or more axial slices. Measurement of tumor around a cyst or surgical cavity represents a particularly difficult challenge. In general, such lesions should be considered nonmeasurable unless there is a nodular component measuring ≥ 10 mm in diameter. The cystic or surgical cavity should not be measured in determining response.

Note: Measurable criteria always take precedence over evaluable criteria. For enhancing tumors, contrast-enhancement measurable or evaluable disease will be recorded. For non-enhancing tumors, which are a small percentage of high-grade gliomas, the T2/FLAIR measurable or evaluable disease will be recorded.

Non-measurable but evaluable disease: Unidimensionally measurable lesions, masses with margins not clearly defined, or lesions with a multiple cystic component.

Non-evaluable disease: No definitive, measurable or evaluable tumor.

6.3.3 Evaluation of Measurable Lesions

6.3.3.1 Definition of Response

Complete Response (CR): Complete response requires all of the following: complete disappearance of all enhancing measurable and nonmeasurable disease sustained for at least 4 weeks; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions; and patient must be off corticosteroids or on physiologic replacement doses only, and stable or improved clinically. In the absence of a confirming scan 4 weeks later, this response will be considered only stable disease.

Partial Response (PR): Partial response 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 nonmeasurable disease; no new lesions; stable or improved nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan; and patient must be on a corticosteroid dose not greater than the dose at time of baseline scan and is stable or improved clinically. In the absence of a confirming scan 4 weeks later, this response will be considered only stable disease.

Stable: Stable disease occurs if the patient does not qualify for complete response, partial response, or progression and requires the following: stable nonenhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan and clinically stable status. 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.

Progression: Progression is defined by any of the following: ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions (compared to best response or with baseline if no decrease) on stable or increasing doses of corticosteroids; a significant increase in T2/FLAIR nonenhancing lesions on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy, not due to comorbid events; the appearance of any new lesions; clear progression of nonmeasurable lesions; or definite clinical deterioration not attributable to other causes apart from the tumor, or to decrease in corticosteroid dose. Failure to return for evaluation as a result of death or deteriorating condition should also be considered as progression. Patients with nonmeasurable enhancing disease whose lesions have significantly increased in size and become measurable (minimal bidirectional diameter of ≥10 mm and visible on at least two axial slices) will also be considered to have experienced progression. The transition from a nonmeasurable lesion to a measurable lesion resulting in progression can theoretically occur with relatively small increases in tumor size (e.g., a 9 x 9 mm lesion [nonmeasurable] increasing to a 10 x 11 mm lesion [measurable]). Ideally, the change should be significant (> 5 mm increase in maximal diameter or ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions). In general, if there is doubt about whether the lesion has progressed, continued treatment and close follow-up evaluation will help clarify whether there is true progression.

6.3.3.2 Evaluable Lesions

Contrast enhancing evaluable lesions will be recorded at each evaluation. FLAIR or T2-weighted images should also be assessed as evaluable disease if appropriate.

The following scale will be used to designate relative changes in MRI scans:

+3	disappearance of tumor (CR)
+2	definitely better (PR)
+1	possibly better
0	unchanged
-1	possibly worse
-2	definitely worse (PD)
-3	development of a new lesion (PD)

6.3.4 Definition of Response for Evaluable Lesions

Complete Response (CR): Defined as the circumstance when the MRI scan is ranked +3 and the tumor is no longer seen by neuroimaging, and the patient no longer requires steroids for control of tumor-induced cerebral edema.

Partial Response (PR): Defined as a MRI scan ranked +2 provided that the patient has not had his/her dose of steroids increased since the last evaluation period.

Progression (PD): Defined as the circumstance when the MRI scan is ranked -2 or -3, or the presence of a new lesion.

Stable disease (SD): Defined as the circumstance when the MRI scan shows no change or possible (-1 or +1) changes. Patients should be receiving stable or decreasing doses of steroids.

6.3.5 Best Response

Two assessments at least 4 weeks apart are required for a description of complete response and partial response.

6.3.6 Central Radiology Review

We will centrally (NCI) review all MRI scans that have been designated as a partial or complete response. Dr. John Butman of NIH Neuro-Radiology or his designee will assist in the review of all MRI scans.

6.3.7 Neurological Examination

Since it is not used for determining response, an objective assessment of the neurological exam will not be required data. Nevertheless, the neurological exam could be useful as a corollary piece of data to support or refute the validity of subtle MRI changes. Thus, we will request (but not require) that the following information will be recorded at each follow-up visit:

- Normal versus abnormal neurologic exam
- Status of neurologic exam compared to last exam
  - Definitely better
  - Possibly better
  - Stable
  - Possibly worse
  - Definitely worse
- BARS assessment and score

6.3.8 Progression-Free Survival

Defined as the time from the date of registration to the date of first observation of progressive disease. If a patient was removed from a study for a reason other than progression, the patient will be censored for further evaluation of progression in that study as of the date of starting other therapy, if that was known. Otherwise, the date the patient was removed from the study will be used. If the patient was followed routinely for progression after being removed from the study and had progression without further therapy, that progression date will be used.

6.3.9 Overall Survival

Defined as the time from date of registration to date of death due to any cause. Patients not known to have died will be censored for survival as of the last date known alive.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI

Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

7.1.2 Suspected Adverse Reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected Adverse Reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed; or, if an Investigator's Brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the Investigator's Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-Threatening Adverse Drug Experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB approved research protocol.

7.1.9 Non-Compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

1. Is unexpected in terms of nature, severity, or frequency in relation to:
    a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
    b. the characteristics of the subject population being studied; **AND**
2. Is related or possibly related to participation in the research; **AND**
3. Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease
- All protocol deviations
- All unanticipated problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance.
3. A tabular summary of the following adverse events:
    - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research
    - All Grade 5 events regardless of attribution
    - All serious events regardless of attribution.

Note: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI-IRB.

7.3 IND SPONSOR REPORTING CRITERIA

From the time the subject receives the investigational agent/intervention to the time of the first follow-up evaluation (4 weeks (± 7 days) following the administration of the cell product), the investigator must immediately report to the sponsor, using the mandatory MedWatch Form FDA 3500A or equivalent, any serious adverse event, whether or not considered drug-related, including those listed in the protocol or Investigator's Brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur after the first follow-up evaluation, only those events that have an attribution of at least possibly related to the agent/intervention will be reported.

Required timing for reporting per the above guidelines:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Waiver of Expedited Reporting to CCR

The investigators are requesting a waiver from reporting specific events in an expedited manner to the CCR. Patients will be receiving commercially available agents, such as fludarabine, cyclophosphamide, and aldesleukin. The majority of toxicities observed on NCI-SB adoptive cell therapy (ACT) protocols are expected toxicities of the non-myeloablative, lymphodepleting preparative regimen or IL-2 and occur in approximately 95% of the patients enrolled, therefore,

we are requesting a waiver from reporting the following events in an expedited manner to the CCR.

- Grade 3 or greater myelosuppression, defined as lymphopenia, neutropenia, decreased hemoglobin, and thrombocytopenia.
- Grade 3 or greater nausea, vomiting, mucositis - oral, anorexia, diarrhea, fever, chills, fatigue, and rash maculo-papular.
- Grade 3 hypoxia, dyspnea, hematuria, hypotension, sinus tachycardia, urine output decreased, confusion, infections, and febrile neutropenia.

The PI will submit a summary table of all grade 3-5 events, whether or not considered related to the product, every 6 months. The report shall include the number of patients treated in the timeframe, the number of events per AE term per grade which occurred in the 6-month timeframe and in total since the start of the study, attribution, and type/category of serious.

Reports will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov.

7.3.2 Reporting Pregnancy

7.3.2.1 Maternal Exposure

If a patient becomes pregnant during treatment and for the first four months following treatment, the study treatment should be discontinued immediately and the pregnancy should be reported to the Sponsor. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agents(s) should be documented in box B5 of the Medwatch form "Describe Event or Problem".

Pregnancy itself is not regarded as an SAE. However, as patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, the CCR is requesting that pregnancy should be reported in an expedited manner as grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the Pregnancy, puerperium and perinatal conditions SOC.

Congenital abnormalities or birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

If any pregnancy occurs in the course of the study, then the investigator should inform the Sponsor within one day, i.e., immediately, but **no later than 24 hours** of when he or she becomes aware of it.

The designated Sponsor representative will work with the investigator to ensure that all relevant information is provided to the Sponsor within 1 to 5 calendar days for SAEs and within 30 days for all other pregnancies.

The same timelines apply when outcome information is available.

7.3.2.2 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 120 days after the last dose of aldesleukin.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 120 days after the last dose should, if possible, be followed up and documented.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The PI (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of the cell product as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the cell product, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form FDA 3500A or equivalent.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the PI (or delegate) shall submit the information described below. Alternatively, the IRB continuing review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- Title and purpose of the trial
- Clinical site
- Principal Investigator
- Clinical protocol identifiers
- Participant population (such as disease indication and general age group, e.g., adult or pediatric);
- Total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- Status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed
- If the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- Narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- Summary of all serious adverse events submitted during the past year
- Summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- If any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- Brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 DATA AND SAFETY MONITORING PLAN

Careful evaluation to ascertain the toxicity, immunologic effects and anti-tumor efficacy of cell infusions will be performed. Due to the nature of these studies, it is possible that expansion of specific T-cell clones will be observed as tumor-reactive T-cell proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly according to plan specified in sections **5.7** and **5.8**.

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about enrollment will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by PI or AI. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be reported to the IRB using iRIS.

The PI will review adverse event and response data on each patient to ensure safety and data accuracy. The PI will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subject's protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring

- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

7.5.3 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NCI-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the PI and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 STATISTICAL CONSIDERATIONS

The primary objectives of this study are to determine the safety and six-month progression free survival of patients receiving anti-EGFRvIII CAR-engineered PBL and aldesleukin following a non-myeloablative, lymphodepleting preparative regimen. The secondary objectives are to determine the *in vivo* survival of CAR gene-engineered cells, and to evaluate radiographic changes after treatment.

The protocol will begin with a Phase I dose escalation design, with eight dose levels and with two different cohorts; one for patients receiving steroids at the time of treatment (Cohort 1A) and one for patients not receiving steroids at the time of treatment (Cohort 1B). Each cohort in Phase I will be escalated independently of the other, and different MTDs may be identified. With Amendment H, patients were no longer divided among two separate cohorts for the Phase I dose escalation portion of the trial. Thus, dose levels 6-8 included all patients (those who required steroids and those who did not). In addition, the safety assessment period was revised as described in Section **3.1.3.2**. With Amendment K, three dose levels (9, 9A, 10) and two de-escalation levels (9D-1 and 9D-2) were added to the Phase I dose escalation portion. Prior to receiving the engineered PBL cells, patients will receive a non-myeloablative, lymphodepleting preparative regimen consisting of cyclophosphamide and fludarabine followed in 2-4 days by intravenous infusion of *ex vivo* tumor-reactive, EGFRvIII CAR gene-transduced PBMC, plus IV high- or low-dose aldesleukin (720,000 or 72,000 IU/kg every 8 hours for a maximum of 15 doses).

Refer to Section **3.1** for additional details.

In the Phase II portion of the trial, patients treated with (Cohort 2A) or without (Cohort 2B) steroids will be evaluated separately based on the dose found appropriate in Phase I, using the same single-stage Phase II trial design. The objective of this part of the trial is to determine if the protocol treatment is able to result in a 6-month progression-free survival of 30% rather than the historical value for recurrent glioblastoma of 10% based on a recent study[43]. In this portion of the trial, we will plan to accrue 32 patients in each cohort. With 32 patients, an exact binomial

test with a 0.10 one-sided significance level will have 94% power to detect the difference between 10% as the 6 month PFS probability and 30% as the 6 month PFS probability.

In addition to determining the 6-month PFS probability, a Kaplan-Meier curve of PFS will be constructed for each cohort.

In order to ensure that patients are not receiving a treatment which may be associated with worse results than those normally considered to be available, an early stopping rule will be imposed independently for each cohort. If after 16 patients have been enrolled and potentially followed for 6 months in either cohort, fewer than 10% of patients have been progression-free for 6 months, then no further patients will be enrolled in that particular cohort as soon as this has been determined.

As of Amendment Q, 18 patients have been accrued. The last patient treated on study at Dose Level 9 (the first patient treated at this dose level) experienced a DLT. Thus, the next patient will be treated at Dose Level 9D-1.

Enrollment Summary per Dose Level		
Dose Level	**Dose of Anti-EGFRvIII CAR T-Cells**	**Total Patients Enrolled**
Level 1 (Cohort 1A) – Completed	10^7	1
Level 1 (Cohort 1B) – Completed	10^7	1
Level 2 (Cohort 1A) – Completed	$3x10^7$	1
Level 2 (Cohort 1B) – Completed	$3x10^7$	1
Level 3 (Cohort 1A) – Completed	10^8	1
Level 3 (Cohort 1B) – Completed	10^8	1
Level 4 (Cohort 1A) – Eliminated	$3x10^8$	0
Level 4 (Cohort 1B) – Completed	$3x10^8$	1
Level 5 (Cohort 1A) – Eliminated	10^9	0
Level 5 (Cohort 1B) – Completed	10^9	3
Level 6 (All patients) – Completed	$3x10^9$	3
Level 7 (All patients) – Completed	10^{10}	3
Level 8 (All patients) – Closed	$3 – 6x10^{10}$	1
Level 9 (All patients)	$3x10^{10}$ given as a split dose, 2 hours apart	1
Level 9A (All patients)	$3x10^{10}$ given as a single dose	0
Level 9D-1 (All patients)*	10^{10}	0
Level 9D-2 (All patients)	$3x10^9$	0
Level 10 (All patients)	$3x10^{10}$	0

*Next enrolled patient will be treated at this dose level

To complete the Phase I dose-escalation portion, and both cohorts in the Phase II portion of the study, a total of up to 107 patients may be required (including up to 17 patients already accrued)—approximately 9 to 12 patients for dose levels 9 and 10 (6 patients will be the maximum for dose level 9D or 10), 2 additional patients in case patients need to be replaced for any reason, and two Phase II cohorts with a maximum of 32 each. Provided that about 1-2 patients per month will be able to be enrolled onto this trial, approximately 7 years may be required to enroll up to 107 patients onto this trial.

9 COLLABORATIVE AGREEMENTS

We have established a Cooperative Research and Development Agreement (CRADAs #02716 and #03168) with Kite Pharma, Inc., and will be sharing data with them.

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

The patients to be entered in this protocol have malignant gliomas which are refractory to standard therapy, and have limited life expectancies.

Subjects from both genders and all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared to another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on the one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to gender or to ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

10.2 PARTICIPATION OF CHILDREN

The use of the non-myeloablative, lymphodepleting preparative regimen in this protocol is a major procedure which entails serious discomforts and hazards for the patient, such that fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life threatening malignant gliomas. Since the efficacy of this experimental procedure is unknown, it does not seem reasonable to expose children to this risk without further evidence of benefit.

Should results of this study indicate efficacy in treating malignant gliomas, which is not responsive to other standard forms of therapy, future research can be conducted in the pediatric population to evaluate potential benefit in that patient population.

10.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (Section **10.5**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. The PI or AI will contact the NIH Ability

to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 Evaluation of Benefits and Risks/Discomforts

The experimental treatment has a chance to provide clinical benefit though this is unknown. The NCI-SB has extensive experience with ACT following treatment with high-dose aldesleukin, however, this experimental treatment is only available at a very few centers throughout the country. Although we have seen responses to this treatment, we do not know if this change in our process will improve patient outcome. The risks associated with ACT are substantial, including a delay in treatment due to the need to harvest and grow the cells; a surgical procedure (possibly major) to obtain tumor for the cell product; the possibility that a cell product cannot be generated; infection and sepsis due to the non-myeloablative, lymphodepleting preparative regimen; intubation; renal toxicities due to aldesleukin; and death. The risks in this treatment are detailed in Section **11**.

10.5 Risk/Benefit Analysis

Because all patients in this protocol have malignant glioma and limited life expectancies, the potential benefit is thought to outweigh the potential risks. The risk/benefit analysis for adults with the capacity to consent, as well as for adults who may become unable to provide consent, is greater than minimal risk but presenting the prospect of direct benefit to individual subjects based on the risks and potential benefits described in Section **10.4**.

10.6 Consent Process and Documentation

Patients are initially consented on protocols 99-C-0128 and 03-C-0277. If the lymphocytes can be generated for infusion and the patient meets the thorough screening for eligibility, the patient, with family members or friends at the request of the patient, will be presented with a detailed description of the protocol treatment. The procedures and tests involved in this study and the associated risks, discomforts and benefits of these processes, will be carefully explained to the patient and a signed informed consent document will be obtained prior to entry onto the study. The informed consent document is given to the patient, who is requested to review it and to ask questions prior to agreeing to participate. The patient is reassured that participation on trial is entirely voluntary and that he/she can withdraw or decide against treatment at any time without adverse consequences. The PI, AI, or clinical fellow is responsible for obtaining written consent from the patient.

10.6.1 Informed Consent of Non-English Speaking Subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB-approved consent document, the PI and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both

the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long-translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

11 PHARMACEUTICAL INFORMATION

Cyclophosphamide, fludarabine, and aldesleukin, the commercial drugs used in this study, will not alter labeling of the FDA-approved drugs. The investigation is not intended to support a new indication for use or any other significant changes to labeling or advertising in cyclophosphamide, fludarabine, or aldesleukin. The investigation does not involve a route of administration or dosage level in use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug products.

11.1 INVESTIGATIONAL REGIMEN

11.1.1 Anti-EGFRvIII CAR Transduced PBL

The procedure for expanding the human PBL and the CoA are similar to those approved by the Food and Drug Administration, and used at the NCI in ongoing protocols evaluating cell therapy in the NCI-SB. The CoA is included in **Appendix E** and in the IND submission for these cells. The PBL will be transduced with retroviral supernatant containing the chimeric anti-EGFRvIII CAR.

11.1.2 Retroviral Vector Containing the anti-EGFRvIII CAR Gene

The retroviral vector supernatant (PG13-139-CD8-CD28BBZ (F10) encoding a CAR directed against the antigen, EGFRvIII, was prepared and preserved following cGMP conditions in the SB-CPF. The retroviral vector utilizes the MSGV1 retroviral vector backbone and consists of 4,032 bps including the 5' LTR from the murine stem cell virus (promoter), packaging signal including the splicing donor (SD) and splicing acceptor sites, human anti-EGFRvIII scFv-based (mAb 139) CAR protein containing a signal peptide signal (human GM-CSFR), 139 light chain variable region, linker peptide, 139 heavy chain variable region, CD8 (hinge, transmembrane), CD28 (cytoplasmic region), 4-1BB (cytoplasmic region) and TCR zeta (cytoplasmic region), followed by the murine stem cell virus 3'LTR. The physical titer will be determined by RNA dot blot according to sponsor certificate. The supernate will be stored at SBVPF upon the completion of production at –80° C with around-the-clock temperature monitoring. Upon request, supernatant will be delivered on dry ice to be used in in vitro transduction. There will be no re-use of the same unit of supernate for different patients. Retroviral titer has been shown to be stable after immediate thawing and immediate administration (coating the tissue culture wells previously coated with Retronectin). Handling of the vector should follow the guidelines of

Biosafety Level-2 (BSL-2). The specific guidelines for Biosafety Level-2 (BSL-2) can be viewed at http://bmbl.od.nih.gov/sect3bsl2.htm.

Note: Penicillin, streptomycin, and gentamycin will not be used in the manufacture of products for patients with documented allergies to these drugs.

11.1.3 Interleukin-2 (Aldesleukin, Proleukin, Recombinant Human Interleukin 2)

How Supplied: Interleukin-2 (aldesleukin) will be provided by the NIH Clinical Pharmacy Department from commercial sources.

Formulation/Reconstitution: Aldesleukin, NSC #373364, is provided as single-use vials containing 22 million IU (~1.3 mg) IL-2 as a sterile, white to off-white lyophilized cake plus 50 mg mannitol and 0.18 mg sodium dodecyl sulfate, buttered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The vial is reconstituted with 1.2 mL of Sterile Water for Injection, USP, and the resultant concentration is 18 million IU/mL or 1.1 mg/mL. Diluent should be directed against the side of the vial to avoid excess foaming. Swirl contents gently until completely dissolved. Do not shake. Since vials contain no preservative, reconstituted solution should be used with 24 hours.

Storage: Intact vials are stored in the refrigerator (2-8°C) protected from light. Each vial bears an expiration date.

Dilution/Stability: Reconstituted aldesleukin should be further diluted with 50 mL of 5% Human Serum Albumin (HSA). The HSA should be added to the diluent prior to the addition of RIL-2. Dilutions of the reconstituted solution over a 1000-fold range (i.e., 1 mg/mL to 1 mcg/mL) are acceptable in either glass bottles or polyvinyl chloride bags. Aldesleukin is chemically stable for 48 hours at refrigerated and room temperatures, 2-30°C.

Administration: The dosage will be calculated based on total body weight. The final dilution of aldesleukin will be infused over 15 minutes. Aldesleukin will be administered as an inpatient.

Toxicities: Expected toxicities of aldesleukin are listed in the product label and in **Appendix B** and **Appendix C**. Grade 3 toxicities common to aldesleukin include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in **Appendix B**. Additional grade 3 and 4 toxicities seen with aldesleukin are detailed in **Appendix C**.

11.1.4 Fludarabine

(Please refer to the FDA-approved package insert for complete product information)

Description: Fludarabine phosphate is a synthetic purine nucleoside that differs from physiologic nucleosides in that the sugar moiety is arabinose instead of ribose or deoxyribose. Fludarabine is a purine antagonist antimetabolite.

How Supplied: It will be purchased by the NIH Clinical Pharmacy Department from commercial sources. Fludarabine is supplied in a 50 mg vial as a fludarabine phosphate powder in the form of a white, lyophilized solid cake.

Stability: Following reconstitution with 2 mL of sterile water for injection to a concentration of 25 mg/mL, the solution has a pH of 7.7. The fludarabine powder is stable for at least 18 months at 2-8°C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Because no preservative is present, reconstituted fludarabine will typically be administered

within 8 hours. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribnucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

Storage: Intact vials should be stored refrigerated (2-8°C).

Administration: Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP over 15-30 minutes. The doses will be based on body surface area (BSA). If patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix A**.

Toxicities: At doses of 25 mg/m2/day for 5 days, the primary side effect is myelosuppression; however, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fever, chills, fatigue, anorexia, nausea and vomiting, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is only rarely observed at the currently administered doses of fludarabine. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesia, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of CLL. Treatment on previous ACT protocols in the NCI-SB have caused persistently low (below 200) CD4 counts, and one patient developed polyneuropathy manifested by vision blindness, and motor and sensory defects.

11.1.5 Cyclophosphamide

(Please refer to FDA-approved package insert for complete product information)

Description: Cyclophosphamide is a nitrogen mustard-derivative alkylating agent. Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkyating agent; the drug also possesses potent immunosuppressive activity. The serum half-life after IV administration ranges from 3-12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

How Supplied: Cyclophosphamide will be obtained from commercially available sources by the Clinical Center Pharmacy Department.

Stability: Following reconstitution as directed with sterile water for injection, cyclophosphamide is stable for 24 hours at room temperature or 6 days when kept at 2-8°C.

Administration: It will be diluted in 250 mL D5W and infused over one hour. The dose will be based on the patient's body weight. If patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix A**.

Toxicities: Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea and vomiting, rash and alopecia occur, especially after

high-dose cyclophosphamide; diarrhea, hemorrhagic colitis, infertility, and mucosal and oral ulceration have been reported. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as a uroprotective agent in patients receiving cyclophosphamide. Prophylactic mesna is not effective in preventing hemorrhagic cystitis in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity (due to allopurinol induction of hepatic microsomal enzymes). At high doses, cyclophosphamide can result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain without edema occurs. At high doses, cyclophosphamide can result in cardiotoxicity. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from a syndrome of acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis have occurred following IV administration. Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

11.2 SUPPORT MEDICATIONS

11.2.1 Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891)

(Please refer to the FDA-approved package insert for complete product information)

Description: Mesna will be obtained commercially by the Clinical Center Pharmacy Department and is supplied as a 100 mg/mL solution.

Storage: Intact ampoules are stored at room temperature.

Stability: Diluted solutions (1-20 mg/mL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% NaCl, or 24 hours in 0.9% NaCl.

Administration: Dilute to concentrations ≤ 20 mg mesna/mL fluid in D5W or 0.9% NaCl and to be administered intravenously as a continuous infusion. If patient is obese (BMI > 35), drug dosage will be calculated using practical weight as described in **Appendix A**. Toxicities include nausea, vomiting and diarrhea.

11.2.2 Filgrastim (Granulocyte Colony-Stimulating Factor, G-CSF, Filgrastim, Neupogen)

Filgrastim will be obtained commercially by the Clinical Center Pharmacy Department and is supplied in 300 µg/mL and 480 µg/1.6 mL vials. Filgrastim should be refrigerated and not allowed to freeze. The product bears the expiration date. The product should not be shaken. It is

generally stable for at least 10 months when refrigerated. The appropriate dose is drawn up into a syringe.

Filgrastim will be given as a daily subcutaneous injection. The side effects of filgrastim are skin rash, myalgia and bone pain, an increase of preexisting inflammatory conditions, enlarged spleen with occasional associated low platelet counts, alopecia (with prolonged use) elevated blood chemistry levels.

11.2.3 Trimethoprim and Sulfamethoxazole Double Strength (TMP/SMX DS)

TMP/SMX DS will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of PCP pneumonia. The oral dose is 1 tablet PO daily three times a week (MUST be on non-consecutive days) beginning on Day 0 or within one week of anticipated lymphopenia and continuing for at least 6 months and until the CD4 count is > 200 on two consecutive lab studies. Like other sulfa drugs, TMP/SMX DS can cause allergies, fever, photosensitivity, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever 8-14 days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

Dapsone (in G6PD sufficient patient), atovaquone, or pentamidine will may be substituted for TMP/SMX-DS in patients with sulfa allergies.

11.2.3.1 Dapsone

Dapsone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of Pneumocystis pneumonia. The dose is 100 mg by mouth daily, starting on Day 0 (± 7 days) and continuing at least 6 months and until the CD4+ count is > 200 on two consecutive lab studies. It is supplied as 25 mg and 100 mg tablets. Dapsone contains a sulfa group, although the cross reactivity in patients with sulfa allergies is quite low. Dapsone may be considered in patients with mild to moderate sulfa allergies. Dapsone should be avoided in patients with severe (i.e., a history of anaphylaxis or other equally serious reaction) reactions to sulfa drugs. Additionally, dapsone has been reported to cause hemolytic anemia is patients with G6PD deficiency. It is recommended that patients be tested for G6PD deficiency prior to the initiation of dapsone therapy. Dapsone is generally well tolerated, but may cause a number of hematologic adverse reactions, including increased reticulocyte counts, hemolysis, decreased hemoglobin, methemoglobinemia, agranulocytosis, anemia, and leukopenia. Other rare but serious adverse reactions include bullous exfoliative dermatitis, Stevens-Johnson syndrome, toxic epidermal necrolysis, pancreatitis, interstitial pneumonitis, and pulmonary eosinophilia. For more detailed information about adverse reactions, consult the package insert.

11.2.3.2 Atovaquone

Atovaquone will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used for the prevention of Pneumocystis pneumonia in patients who cannot tolerate or are allergic to sulfamethoxazole/trimethoprim, dapsone, or pentamidine. Atovaquone may be given as a single daily dose of 1500 mg orally or the dose may be split into 750 mg given orally twice daily. Atovaquone will be started on Day 0 (± 7 days), and will continue for at least 6 months and until the CD4+ count is > 200 on two consecutive lab studies. Atovaquone is supplied as an oral suspension containing 150 mg/mL. Common adverse reactions to atovaquone include: headache, rash, diarrhea, nausea, vomiting, abdominal pain, cough, and fever. Rare but

serious adverse reactions include acute renal failure, hepatitis and hepatic failure, angioedema, pancreatitis, and Stevens-Johnson syndrome. For more detailed information about adverse reactions, consult the package insert.

11.2.3.3 Aerosolized Pentamidine

Patients with sulfa allergies will receive aerosolized pentamidine 300 mg per nebulizer within one week prior to admission and continued monthly until the CD4 count is above 200 on two consecutive follow up lab studies and for at least 6-months post chemotherapy. Pentamidine Isethionate will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of PCP infections. It is supplied in 300 mg vials of lyophilized powder and will be administered via nebulizer. Toxicities reported with the use of Pentamidine include metallic taste, coughing, bronchospasm in heavy smokers and asthmatics; increased incidence of spontaneous pneumothorax in patients with previous PCP infection or pneumatoceles, or hypoglycemia.

11.2.4 Herpes Simplex and Varicella Zoster Virus Prophylaxis

11.2.4.1 Valacyclovir (Valtrex)

Valacyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used orally to prevent the occurrence of herpes virus infections in patients with positive HSV serology. It is supplied in 500 mg tablets. Valacyclovir will be started at a dose of 500 mg orally daily if the patient is able to tolerate oral intake. See package insert for dosing adjustments in patients with renal impairment. Common side effects include headache, upset stomach, nausea, vomiting, diarrhea or constipation. Rare serious side effects include hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

11.2.4.2 Acyclovir

Acyclovir will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prevent the occurrence of herpes virus infections in patients who cannot take oral medications. It is supplied as powder for injection in 500 mg/vials. Reconstitute in 10 mL of sterile water for injection to a concentration of 50 mg/mL. Reconstituted solutions should be used within 12 hours. IV solutions should be diluted to a concentration of 7 mg/mL or less and infused over 1 hour to avoid renal damage. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs have been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Stomach upset, headache or nausea, rash or hives; peripheral edema; pain, elevated liver function tests; and leukopenia, diarrhea, lymphadenopathy, myalgias, visual abnormalities and elevated creatinine have been reported. Hair loss from prolonged use has been reported. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

11.2.5 Fluconazole

Fluconazole will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to prophylax against fungal infections. It is available in 200 mg tablets. It can cause headache, nausea, vomiting, diarrhea or abdominal pain, and liver damage which may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson

Syndrome. It has several significant drug interactions. The package insert should be consulted prior to prescribing. For IV administration in patients who cannot tolerate the oral preparation, Fluconazole comes in 2 mg/mL solution for injection, and prepared according to Clinical Center Pharmacy standard procedures. It should be administered at a maximum IV rate of 200 mg/hour.

11.2.6 Ondansetron Hydrochloride

Ondansetron hydrochloride will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritis, constipation and urinary retention. Consult the package insert for specific dosing instructions.

11.2.7 Furosemide

Furosemide will be obtained by the Clinical Center Pharmacy Department from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash and pruritis. Consult the package insert for a complete list of all side effects.

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13 APPENDICES

13.1 APPENDIX A: MODIFICATION OF DOSE CALCULATIONS* IN PATIENTS WHOSE BMI IS GREATER THAN 35

Unless otherwise specified in this protocol, actual body weight is used for dose calculations of treatment agents. In patients who are determined to be obese (BMI > 35), the **practical weight** (see 3 below) will be used.

1. BMI Determination:

   BMI = weight (kg) / [height (m)]2

2. Calculation of ideal body weight:

   Male = 50 kg + 2.3 (number of inches over 60 inches)

   Example: Ideal body weight of 5'10" male

   50 + 2.3 (10) = 73 kg

   Female = 45.5 kg + 2.3 (number of inches over 60 inches)

   Example: Ideal body weight of 5'3" female

   45.5 + 2.3 (3) = 57 kg

3. Calculation of "practical weight":

   Calculate the average of the actual and the ideal body weights. This is the practical weight to be used in calculating the doses of chemotherapy and associated agents designated in the protocol.

*Practical weight will NOT be used in the calculation of dose for aldesleukin.

13.2 APPENDIX B: ADVERSE EVENTS OCCURRING IN ≥10% OF PATIENTS TREATED WITH ALDESLEUKIN (N=525)[1]

Body System	% Patients
Body as a Whole	
Chills	52
Fever	29
Malaise	27
Asthenia	23
Infection	13
Pain	12
Abdominal pain	11
Abdomen enlarged	10
Cardiovascular	
Hypotension	71
Tachycardia	23
Vasodilation	13
Supraventricular tachycardia	12
Cardiovascular disorder[a]	11
Arrhythmia	10
Digestive	
Diarrhea	67
Vomiting	50
Nausea	35
Stomatitis	22
Anorexia	20
Nausea and vomiting	19
Hemic and Lymphatic	
Thrombocytopenia	37
Anemia	29
Leukopenia	16

Body System	% Patients
Metabolic and Nutritional Disorders	
Bilirubinemia	40
Creatinine increase	33
Peripheral edema	28
SGOT increase	23
Weight gain	16
Edema	15
Acidosis	12
Hypomagnesemia	12
Hypocalcemia	11
Alkaline phosphatase incr	10
Nervous	
Confusion	34
Somnolence	22
Anxiety	12
Dizziness	11
Respiratory	
Dyspnea	43
Lung disorder[b]	24
Respiratory disorder[c]	11
Cough increase	11
Rhinitis	10
Skin and Appendages	
Rash	42
Pruritus	24
Exfoliative dermatitis	18
Urogenital	
Oliguria	63

Legend:

a. Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.

b. Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.

c. Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.

[1]Source: Proleukin® Prescribing Information – June 2007

13.3 APPENDIX C: EXPECTED IL-2 TOXICITIES AND THEIR MANAGEMENT

Expected Toxicity	Expected Grade	Supportive Measures	Stop Cycle*	Stop Treatment **
Chills	3	IV Meperidine 25-50 mg, IV q1h, prn	No	No
Fever	3	Acetaminophen 650 mg, po, q4h; Indomethicin 50-75 mg, po, q8h	No	No
Pruritis	3	Hydroxyzine HCL 10-20 mg po q6h, prn; Diphenhydramine HCL25-50 mg, po, q4h, prn	No	No
Nausea/Vomiting/ Anorexia	3	Ondansetron 10 mg, IV, q8h, prn; Granisetron 0.01 mg/kg IV daily prn; Droperidol 1 mg, IV q4-6h, prn; Prochlorperazine 25 mg q4h PO, prn or 10 mg IV q6h prn	No	No
Diarrhea	3	Loperamide 2mg, po, q3h, prn; Diphenoxylate HCl 2.5 mg and atropine sulfate 25 mcg, po, q3h, prn; codeine sulfate 30-60 mg, po, q4h, prn	If uncontrolled after 24 hours despite all supportive measures	No
Malaise	3 or 4	Bedrest interspersed with activity	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No

Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No
Edema/Weight gain	3	Diuretics prn	No	No
Hypotension	3	Fluid resuscitation Vasopressor support	If uncontrolled despite all supportive measures	No
Dyspnea	3 or 4	Oxygen or ventilatory support	If requires ventilatory support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased creatinine	3 or 4	Observation	Yes (grade 4)	No
Renal failure	3 or 4	Dialysis	Yes	Yes
Pleural effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion	If uncontrolled despite all supportive measures	No

		or electrical conversion therapy		
Elevated troponin levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose
Myocardial infarction	4	Supportive care	Yes	Yes
Elevated transaminases	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Hyperbilirubinemia	3 or 4	Observation	For grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours.

** Unless the toxicity is not reversed to grade 2 or less by next treatment.

13.4 APPENDIX D: INTERLEUKIN-2 TOXICITIES OBSERVED IN PATIENTS TREATED AT THE NIH CLINICAL CENTER

TABLE 8. *Toxicity of Treatment with Interleukin-2*

Interleukin-2 Plus	Alone	TNF	a-IFN	MoAB	CYT	LAK	TIL	Total
Number of Patients	155	38	128	32	19	214	66	652*
Number of Courses	236	85	210	35	30	348	95	1039
Chills	75	16	68	8	8	191	33	399
Pruritus	53	9	26	2	2	82	6	180
Necrosis	3	—	2	—	—	—	—	5
Anaphylaxis	—	—	—	1	—	—	—	1
Mucositis (requiring liquid diet)	6	1	7	—	2	12	2	30
Alimentation not possible	1	—	1	—	—	2	—	4
Nausea and vomiting	162	42	117	14	20	263	48	666
Diarrhea	144	38	98	15	13	250	38	596
Hyperbilirubinemia (maximum/mg %)								
2.1–6.0	126	49	97	21	18	190	46	547
6.1–10.0	49	3	12	8	9	72	26	179
10.1+	26	1	4	3	1	40	8	83
Oliguria								
<80 ml/8 hours	81	37	67	14	9	114	25	347
<240 ml/24 hours	19		2	3	1	12	5	42
Weight gain (% body weight)								
0.0–5.0	106	23	65	8	9	117	49	377
5.1–10.0	78	41	111	22	10	148	26	436
10.1–15.0	43	17	26	3	9	62	15	175
15.1–20.0	7	3	8	1	1	15	3	38
20.1+	2	1		1	1	6	2	13
Elevated creatinine (maximum/mg %)								
2.1–6.0	148	43	121	20	14	237	54	637
6.1–10.0	21	1	14	3	—	34	12	85
10.1+	5	—	1	1	—	2	1	10
Hematuria (gross)	—	—	—	—	—	2	—	2
Edema (symptomatic nerve or vessel compression)	4	—	6	—	—	7	—	17
Tissue ischemia	—	—	—	—	1	1	—	2
Resp. distress:								
not intubated	17	1	9	4	1	28	7	67
intubated	15	—	6	3	—	12	5	41
Bronchospasm	2	—	2	—	1	4	—	9
Pleural effusion (requiring thoracentesis)	4	1	—	1	2	8	1	17
Somnolence	29	2	22	6	2	45	8	114
Coma	9	1	8	—	2	8	5	33
Disorientation	52	3	50	7	4	89	10	215
Hypotension (requiring pressors)	119	16	40	17	12	259	45	508
Angina	5	1	8	—	—	8	—	22
Myocardial infarction	4	—	1	—	—	1	—	6
Arrythmias	15	2	13	3	—	39	6	78
Anemia requiring transfusion (number units transfused)								
1–15	77	16	53	9	6	176	40	377
6–10	22	1	5	3	2	53	9	95
11–15	4	—	1	—	—	15	4	24
16+	1	—	1	—	—	11	1	14
Thrombocytopenia (minimum/mm^3)								
<20,000	28	1	2	4	6	71	19	131
20,001–60,000	82	11	62	14	12	150	30	361
60,001–100,000	53	36	76	11	8	79	22	285
Central line sepsis	13	—	7	1	4	36	2	63
Death	4	—	1	—	—	3	2	10

* Eleven patients are in two protocols.

13.5 APPENDIX E: CERTIFICATE OF ANALYSIS – ANTI-EGFRVIII CAR TRANSDUCED T-CELLS

Date of preparation of final product:

Patient:

Tests performed on final product:

Test	*Method*	*Limits*	*Result*	*Tests Performed by*	*Initials /Date*
Cell viability[1]	trypan blue exclusion	>70%			
Total viable cell number[1]	visual microscopic count	>1 x10^7			
Tumor reactivity[2]	γ-IFN release vs. positive control γ-IFN release vs. negative control including wt-EGFRvIII	>200 pg/mL, and > 2 times background <400 pg/mL			
CAR expression[2]	FACS analysis of the transduced cells	PBL, >10%			
Microbiological studies	gram stain[1,3,]	no micro-organisms seen			
	aerobic culture[3,4]	no growth			
	fungal culture[3,4]	no growth			
	anaerobic culture[3,4]	no growth			
	mycoplasma test[5]	negative			
Endotoxin	limulus assay[1]	≤ 5 E.U./kg			
RCR	S+L- Assay[4] RCR-PCR[6]	 negative			

[1] Performed on sample of the final product immediately prior to infusion. Results are available at the time of infusion.

[2] Performed 2-10 post transduction. Results are available at the time of infusion.

[3] Performed 2-4 days prior to infusion. Results are available at the time of infusion but may not be definitive.

[4] Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the patient.

[5] Performed 2-10 days prior to infusion. Results are available at the time of infusion.

[6] Performed on sample approximately 1-4 days prior to infusion. Results are available at the time of infusion.

Prepared by: _____ Date: _____

QC sign-off: _____ Date: _____

Qualified Laboratory or Clinical Supervisor

13.6 APPENDIX F: KARNOFSKY PERFORMANCE SCORE (KPS)

KPS	Description
100	Normal, no complaints, no signs of disease
90	Capable of normal activity, few symptoms or signs of disease
80	Normal activity with some difficulty, some symptoms or signs
70	Caring for self, not capable of normal activity or work
60	Requiring some help, can take care of most personal requirements
50	Requires help often, requires frequent medical care
40	Disabled, requires special care and help
30	Severely disabled, hospital admission indicated but no risk of death
20	Very ill, urgently requiring admission, requires supportive measures or treatment
10	Moribund, rapidly progressive fatal disease processes
0	Death

13.7 APPENDIX G: ANTI-EPILEPTIC MEDICATIONS

Non-EIAEDs	EIAEDs
Valproic acid	Carbamazapine
Gabapentin	Oxcarbazepine*
Lamotrigine	Phenytoin
Topriamate	Fosphenytoin
Levetiracetam	Phenobarbital
Tiagabine	Primidone
Zonisamide	
Clonazepam	

* enzyme induction limited and less potent than carbamazapine