A Phase I Study

ExCeL: EGFRvIII Chimeric Antigen Receptor (CAR) Gene-modified T cells for Patients with Newly-Diagnosed GBM during Lymphopenia

DUKE CANCER INSTITUTE

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		(Section 9.1.2); Added language on CRS (Sections 4, 9.1, & 12); Clarified micro curie for Indium (Sections 9.1.1 & 10.4.3); Clarified storage of CARs & dosage concentration of labeled versus unlabeled CARs (Section 10.4.1); Inserted positive test result management plan (Section 10.4.2); Clarified BactAlert testing for a total of 14 days on CARs (Section 10.4.2); Clarified age range & removed reference to Gilbert's Syndrome in Inclusion Criteria (Section 11); Clarified SOC clinic visit will occur during the pause between TMZ cycles 3 & 4 for restaging (Section 12); Added measurements of blood results to withdrawal criteria (Section 12.6); Included a maximum follow up time period for subjects treated with CARs (Section 12.4); Increased the monitoring time from 2 to 4 weeks for safety and DLT evaluation between cohort enrollment (Sections 9.1.2 & 15.1); Study assessments clarified to occur with each study/clinic visit (Section 12.7); Efficacy analysis with and without subjects whose products failed release criteria included (Sections 12.7.2, 15.1, & 15.6); AE language updated to match 21CFR312 (Section 13).
Amended version for missing FDA mod:	01/27/2016	Clarified that dosage of EGFRvIII CARs will be based on transduced cells/kg (Sections 9.1.1 & 15.4).
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		tracking timeframe (Section 9.1.4). Added "radiographic response" to the End of Study requirements (Section 12.6). Changed RT and TMZ completion requirement to 5 weeks (Section 12.7.1). Added collection of survival status, treatment regimens, dose, dates, and radiographic responses to Follow-up Requirements for Early Withdrawal (Section 12.7.2). Modified the AE and SAE collection windows (Section 13 and 13.1). Added Section 13.2: Adverse Events of Special Interest. Removed redundant withdrawal criteria and updated the criteria (Section 12.7.1).
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Amended version for protocol changes	4/2/18	Increased immune monitoring blood draws from 6 yellow top tubes to 9 yellow top tubes (Sections 9.1, 12.2, 12.5, and Table 6).

1 TABLE OF CONTENTS

1 TABLE OF CONTENTS 4
2 LIST OF FIGURES 7
3 LIST OF TABLES 7
4 LIST OF ABBREVIATIONS 8
5 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY 11
6 STUDY SCHEMA 12
7 BACKGROUND AND SIGNIFICANCE 13
7.1 Study Disease 13
7.1.1 Disease and Current Therapy 13
7.2 Study Agent 13
7.2.1 Rationale for Immunotherapy 13
7.2.2 EGFRvIII and GBM 14
7.2.3 Preclinical Studies to Provide the Rationale for this Clinical Protocol 15
7.2.4 Prior Trials of Cell Transfer Therapy Using Tumor Infiltrating Lymphocytes in Patients with Metastatic Melanoma 16
7.2.5 Trials of Cell Transfer Therapy Using Transduction of Anti-TAATCR Genes into Non-reactive PBL 17
7.2.6 ^{111}Indium-labeling of Cells for in vivo Trafficking Studies 18
7.2.7 Safety Considerations 18
7.3 Study Purpose/Rationale 19
8 OBJECTIVES AND ENDPOINTS 19
9 INVESTIGATIONAL PLAN 20
9.1 Study Design 20
9.1.1 Dose Escalation and Expansion 21
9.1.2 Definition of Dose-Limiting Toxicity (DLT) 22
9.1.3 Safety Considerations 22
9.1.4 Concomitant Medications 25
9.2 Rationale for Selection of Dose, Regimen, and Treatment Duration 25
9.3 Rationale for Correlative Studies 25
9.4 Definition of Evaluable Subjects, On Study, and End of Study 25
9.5 Early Study Termination 26
10 STUDY DRUG 26
10.1 Names, Classification, and Mechanism of Action 26
10.2 Packaging and Labeling 26

10.3 Supply, Receipt, and Storage 26
10.4 Dispensing and Preparation 26
10.4.1 EGFRvIII CARs 26
10.4.2 Release Criteria for EGFRvIII CARs 26
10.4.3 ^{111}In-labeled T cells 27
10.5 Compliance and Accountability 28
10.6 Disposal and Destruction 28
11 SUBJECT ELIGIBILITY 28
12 SCREENING AND ON-STUDY TESTS AND PROCEDURES 29
12.1 Screening Examination 32
12.2 Treatment Period 32
12.3 CRS Management Plan 33
12.4 End of Treatment 33
12.5 Follow-up Period 33
12.6 End of Study 34
12.7 Early Withdrawal of Subject(s) 34
12.7.1 Criteria for Early Withdrawal 34
12.7.2 Follow-up Requirements for Early Withdrawal 34
12.7.3 Replacement of Early Withdrawal(s) 34
12.8 Study Assessments 34
12.8.1 Medical History 34
12.8.2 Physical Exam 35
12.8.3 Correlative Assessments 35
13 SAFETY MONITORING AND REPORTING 35
13.1 Adverse Events 36
13.1.1 Reporting of AEs 36
13.2 Adverse Events of Special Interest 36
13.3 Serious Adverse Events 36
13.3.1 Reporting of SAEs 37
13.4 Safety Oversight Committee (SOCom) 37
14 QUALITY CONTROL AND QUALITY ASSURANCE 37
14.1 Monitoring 37
14.2 Audits 38
14.3 Data Management and Processing 38
14.3.1 Study Documentation 38

14.3.2 Case Report Forms (CRFs) 38
14.3.3 Data Management Procedures and Data Verification 39
14.3.4 Coding 39
14.3.5 Study Closure 39
15 STATISTICAL METHODS AND DATA ANALYSIS 40
15.1 Analysis Sets 40
15.2 Patient Demographics and Other Baseline Characteristics 40
15.3 Treatments 40
15.4 Primary Objective 40
15.5 Secondary Objectives 40
15.6 Exploratory Objectives 40
15.7 Sample Size Calculation 41
16 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS 41
16.1 Regulatory and Ethical Compliance 41
16.2 DUHS Institutional Review Board and DCI Cancer Protocol Committee 41
16.3 Informed Consent 42
16.4 Privacy, Confidentiality, and Data Storage 42
16.5 Data and Safety Monitoring 42
16.6 Protocol Amendments 43
16.7 Records Retention 43
16.8 Conflict of Interest 43
16.9 Registration Procedure 43
17 REFERENCES 44
18 APPENDICES 50
18.1 Research Summary 50
18.2 Figures and Schemas (in order of reference in protocol) 51

2 LIST OF FIGURES

Figure 1. Study Schema 12
Figure 2. Highly Reactive Transferred Cells Traffic to Brain and Destroy Tumor Deposit in Melanoma Patients. 51
Figure 3. Design of 3rd Generation EGFRvIII CAR. 51
Figure 4. EGFR Mutation 52
Figure 5. CAR Transduced Murine T-Cells Specifically Recognize EGFRvIII Expressing GBM. 52
Figure 6. Construction and Analysis of Anti- EGFRvIII CAR Vectors. 53
Figure 7. Specific Lysis of U87 EGFRvIII by Anti-EGFRvIII CAR Transduced T Cells. 54
Figure 8. Specific Lysis of U251 EGFRvIII by Anti-EGFRvIII CAR Transduced T Cells. 55
Figure 9. Expression of EGFRvIII in Glioma Tumor Stem Cell Lines. 56
Figure 10. Correlative Studies Schema 57

3 LIST OF TABLES

Table 1. Test of CARs Targeting EGFRvIII 16
Table 2. Objectives and Endpoints 19
Table 3. Dose Escalation and Expansion 21
Table 4. Dose Escalation Rules 21
Table 5. Release Criteria for EGFRvIII CARs 26
Table 6. Screening and On-Study Tests and Procedures 31
Table 7. Half Width of 95% Confidence Interval as a Function of the DLT Rate 41
Table 8. Frequency and Duration of Objective Responses 58

4 LIST OF ABBREVIATIONS

Ab	Antibody
ABC	Automated Blood Count
ADP	Adenosine 5'-diphosphate
AE	Adverse Event
ALT	Autologous Lymphocyte Transfer
AST	Aspartate Aminotransferase
BBB	Blood Brain Barrier
β-HCG	Beta-Human Chorionic Gonadotropin
BMP	Basic Metabolic Panel
BMT	Bone Marrow Transplant
BTC	Brain Tumor Center
Ca^{++}	Calcium
CAR	Chimeric Antigen Receptor
CBC	Complete Blood Count
CLIA	Clinical Laboratory Improvement Act
CMP	Comprehensive Metabolic Panel
CNC	Clinical Neurologic Change
CNS	Central Nervous System
CPC	Cancer Protocol Committee
CRP	C-Reactive Protein
CRS	Cytokine Release Syndrome
CSF	Cerebral Spinal Fluid
CT	Computed Tomography
CTLs	Cytotoxic T lymphocytes
DCI	Duke Cancer Institute
DI	Dose Intensified
DLT	Dose Limiting Toxicity
DUHS	Duke University Health System
ELISA	Enzyme-Linked ImmunoSorbent Assay
ELISPOT	Enzyme-linked Immunospot
EGFR	Epidermal Growth Factor Receptor
EGFRvIII	EGFR variant III
FACS	Fluorescence Activated Cell Sorting
Fc	Fragment Crystallizable
FDA	Federal Drug Administration
GBM	Glioblastoma Multiforme
GCP	Good Clinical Practice
Gd-DTPA	Gadolinium-Diethylene Triamine Pentaacetic Acid
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HPLC	High Pressure Liquid Chromatography
IC	Intracerebral
ICH	International Conference on Harmonisation

ICP	Intracranial Pressure
IFNγ	Interferon-gamma
IHC	Immunohistochemistry
^{124}I-HSA	^{124}I-labeled Human Serum Albumin
IL-2	Interleukin-2
IL-4	Interleukin-4
IM	Immune Monitoring
IND	Investigational New Drug
IRB	Institutional Review Board
KPS	Karnofsky Performance Status
LTR	Long Terminal Repeat
MAb	Monoclonal Antibody
MART-1	Melanoma Antigen Recognized by T Cells - 1
MG	Malignant Glioma
MHC	Major Histocompatibility Complex
MRI	Magnetic Resonance Imaging
MTD	Maximally Tolerated Dose
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NICU	Neuroscience Intensive Care Unit
NIH	National Institutes of Health
OARC	Office of Audit, Risk and Compliance
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
PBLs	Peripheral Blood Lymphocytes
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PE-conjugated	Phycoerythrin-conjugated
PET	Positron Emission Tomography
PFS	Progression Free Survival
PI	Principle Investigator
PT	Prothrombin Time
PTT	Partial Thromboblastin Time
RCR	Replication Competent Retrovirus
RDSP	Research Data Security Plan
RECIST	Response Evaluation Criteria in Solid Tumors
REP	Rapid Expansion Protocol
RIO	Research Integrity Office
RT	Radiation Therapy
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Severe Adverse Event
ScFv	Single-chain Variable Fragment
SOC	Standard of Care
SOCom	Safety Oversight Committee
SPORE	Specialized Program of Research Excellence

TAA	Tumor Associated Antigens
TBI	Total Body Irradiation
TCR	T cell Receptor
TIL	Tumor Infiltrating Lymphocyte
TMZ	Temozolomide
TNFα	Tumor Necrosis Factor alpha
TSC	Tumor Stem Cell
WHO	World Health Organization
XRT/TMZ	Temozolomide During Concomitant Radiation Therapy

5 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

Please see separate document (available upon request).

6 STUDY SCHEMA

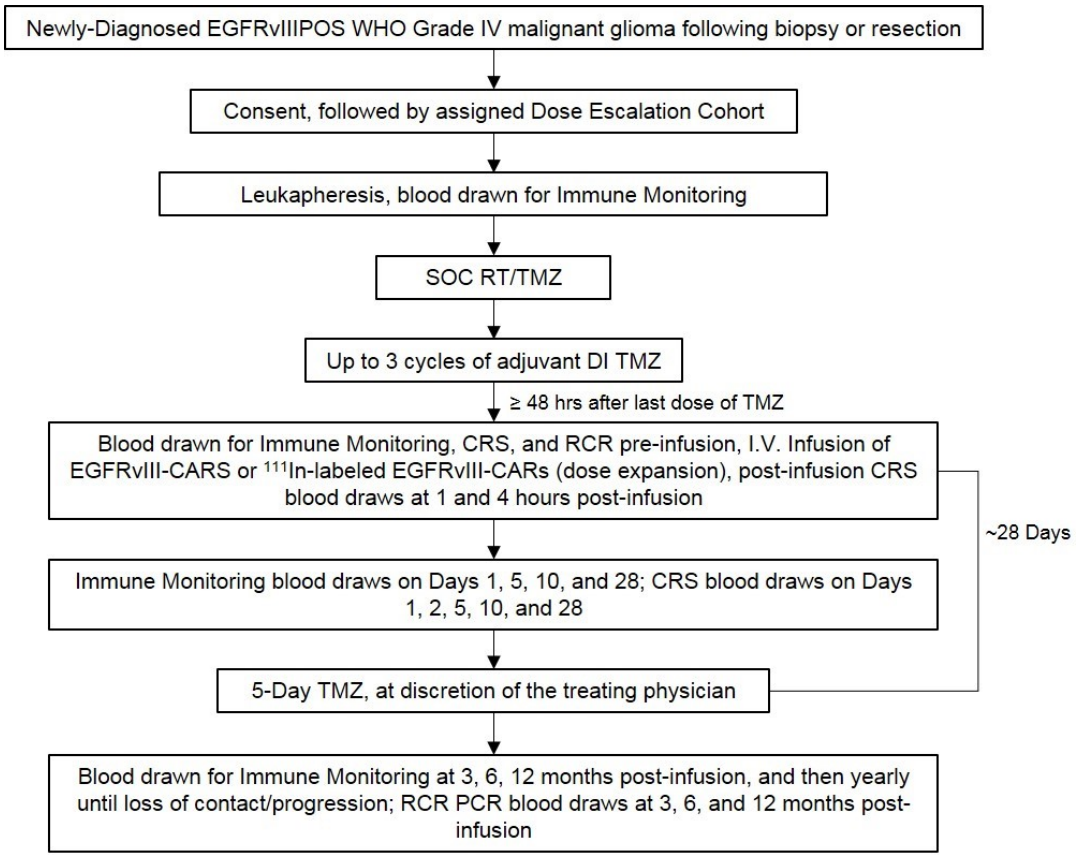

Figure 1. Study Schema

7 BACKGROUND AND SIGNIFICANCE

7.1 Study Disease

7.1.1 Disease and Current Therapy

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 from those tumors in the U.S. each year. 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, and it is uniformly lethal. Once the primary modalities of treatment, surgery, radiation, and chemotherapy have failed, the treatment options for patients with GBM are limited and median overall survival from diagnosis is only 14.6 months.

Patients with GBM have no curative and limited therapeutic options. As mentioned, median OS for patients with newly diagnosed GBM is 14.6 months despite maximal resection, incapacitating radiation and systemic chemotherapy, which are inherently non-specific and damaging to surrounding eloquent normal tissue.[1,2] Immunotherapeutic treatments serve as promising new alternatives due to the inherent specificity of the immune system.

7.2 Study Agent

7.2.1 Rationale for Immunotherapy

One emerging immunotherapeutic platform that shows potential success is ACT, the infusion of tumor reactive immune cells into a patient with cancer. The infusion of antitumor CTLs derived from the tumor itself (TILs) or T cells with TCRs of endogenous or genetically engineered anti-tumor specificity can cause regression in established human tumors such as lymphoma, nasopharyngeal cancer, synovial sarcoma and metastatic melanoma and has shown efficacy against lesions in the ”immune-privileged” brain in the case of melanoma Figure 2).[3-6] While ACT with TILs in the treatment of metastatic melanoma has shown objective response rates of 49% to 72%,[7] it has been limited by difficulties in obtaining tumor-reactive cells from patients, excluding many patients from treatment. Additionally, such therapies based on TCRs are reliant upon engagement of the endogenous or engineered TCR with the tumor-associated peptide as presented by HLA; however, a common mechanism of tumor escape is HLA down regulation[8-10] which renders these malignant T cells invisible to TCR-based immunotherapy. Clearly, more efficacious methodologies that can be applied to a larger pool of patients are still required.

CARs are cell surface receptors engineered to be expressed on T cells and generated through combining the antibody Fc variable region of an antibody with intracellular T cell signaling moieties (Figure 2)[11]. Engagement of the incredibly high avidity CAR Fc variable region with antigen triggers T cell activation similar to that observed through the native TCR. Furthermore, as the Fc variable region is antibody-based, it is not only higher avidity than a TCR but it binds antigen directly rather than in the context of a peptide-HLA complex as a TCR does. Therefore, a single CAR can be used with any patient regardless of HLA type, making it more widely applicable than other HLA dependent platforms such as TCR-based ACT or active immunotherapeutic vaccination with tumor-associated peptide that can only be bound in the context of a specific HLA.[12,13] As CAR-based therapy does not rely on HLA presentation of antigen, it can additionally subvert the common immune-evasion of HLA down-regulation by malignant cells,[14,15] rendering CARs a potentially more efficacious therapy than TCR-based modalities.

One of the critical barriers to CAR-based ACT has been the physical and functional persistence of transduced T cells in the host.[16-18] Initial 1st generation CAR vectors generally included a single CD3ζ intracellular domain and successfully redirected cellular cytotoxicity but could not persist *in vivo*. Second generation CARs included an additional intracellular signaling domain (frequently CD28) to promote survival and proliferation after repeated antigen exposure;[19-21] however, 3rd generation CARs incorporating OX40 or 41BB domains further enhanced survival and showed greater anti-tumor efficacy than 2nd generation CARs,[22-24] bringing the total intracellular signaling moieties to three. Both the humanized EGFRvIII-targeted CAR vector for clinical studies

and the preclinical murine vector outlined in this proposal are 3rd generation constructs with CD3ζ, CD28 and 41BB intracellular signaling domains; these domains in total have been shown to be superior in inducing anti-tumor efficacy in comparison to vectors containing any combination of the two.[24] In recent murine preclinical models, newer generation CARs have shown tremendous anti-tumor efficacy when redirected against ovarian cancer,[25] lymphoma,[26] breast cancer,[23] and as an anti-angiogenic agent against vascular endothelial growth factor receptor 2 (VEGF-R2). [27] Clinical trials utilizing CARs have targeted a variety of antigens and malignancies, including GD2 in neuroblastoma,[16] carbonic anhydrase IX in renal cell carcinoma,[28] ERBB2+ metastatic cancer,[29] and CD19 in B cell malignancies.[30] Targeting GD2 showed promising tumor necrosis or regression in half of the tested subjects and targeting CD19 resulted in 6 of 8 patients with progressive malignancy obtaining remission; however; in both of the other aforementioned trials, severe adverse events were reported, including the death of a patient in the trial targeting ERBB2. These events were due to the expression of the targeted antigen on normal tissues and the subsequent potent cytotoxicity of the CAR bearing T cells at these off-target sites. This is another critical barrier to CAR-based immunotherapy, the target antigen must be carefully selected to reduce the risk of life-threatening toxicity from activated CAR-transduced T cells.

An ideal target for a CAR must be tumor-specific to prevent the redirected cytotoxicity from engendering life-threatening on-target toxicity and should be located on the cell surface to enable binding of the Fc variable region. Additionally, the length of the antigen must be optimized with the vector design to facilitate binding between the tumor epitope and the CAR for productive signaling. Variations on CAR design to include or exclude extracellular spacer regions have shown that the cytotoxicity of the CAR can vary based upon how well the CAR can access the targeted epitope[31,32].

7.2.2 EGFRvIII and GBM

The 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.

The EGFRvIII mutation (Figure 3) consists of an in-frame deletion of 801 base pairs from the extracellular domain of the EGFR that splits a codon and produces a novel glycine at the fusion junction[33] and is most frequently seen in patients with GBM, but has been found in a broad array of other common cancers including breast, lung, head and neck.[34-37] EGFRvIII is an absolutely tumor-specific antigen not expressed on normal tissues and present on the cellular surface of one-third of GBMs[38] with a largely truncated extracellular motif leaving the tumor-specific epitope easily accessible.[39]

As previous CAR clinical trials have engendered severe adverse events due to specific off-site but on-target toxicity, EGFRvIII as the targeted antigen overcomes a critical barrier in current CAR-mediated immunotherapy as it is both tumor-specific and physically well-suited for CAR-based therapy. EGFRvIII is also a validated tumor antigen as recent work from Dr. Sampson, lead PI on this grant, demonstrates that humoral responses induced by peptide vaccination with EGFRvIII in patients with GBM leads to significant improvement in both PFS and OS.[38] In collaboration with Dr. Rosenberg at the NCI, we have developed 3rd generation human and murine (CD3ζ/CD28/4-1BB) EGFRvIII-targeted CAR vectors that specifically recognize the EGFRvIII tumor mutation as demonstrated by *in vitro* IFNγ secretion **(**Figure 4**).** It is also worth noting that EGFRvIII promotes tumor growth through the secretion of cytokines[40] and exosomes,[41] is expressed on CD133+ brain tumor cells,[42] and serves as a negative prognostic indicator.[43] Therefore, EGFRvIII is not only a tumor-specific target, but inhibition of this protein should also be efficacious in a mixed setting of EGFRvIII+ and EGFRvIII- tumor cells. Importantly, it has also been shown that the “immunologically privileged” location of brain tumors behind the BBB is not an impediment to ACT as activated T cells have been shown to enter the CNS[24,44] and furthermore, that patients with melanoma brain metastases showed regression to immunotherapy with T cells bearing TCRs of engineered specificity to the melanoma antigens gp100 or DMF5 [5] as well as the necrosis and regression showed by CAR-based therapy targeting GD2 in neuroblastoma.[16] The ongoing collaboration between Drs. Johnson and Sampson represents a unique opportunity to combine expertise in the fields of genetically engineered tumor reactive T cells and the immunotherapeutic targeting of

EGFRvIII. Furthermore, examining the use of EGFRvIII-specific CARs will serve as an important proof-of-concept by testing CARs against a validated target and will furthermore overcome any limitations inherent to a humoral-biased anti-tumor response through incorporating the T cell arm of adaptive immunity as well as any subsequent bystander effects[45-47] of CTL function. Translation of these findings to clinical practice as outlined in AIM 3 will be broadly applicable to cancer immunotherapy as EGFRvIII is present in numerous malignancies. Furthermore, successful EGFRvIII-targeted CAR based therapy could significantly alter critical barriers in clinical practice by replacing non-specific and collaterally damaging current standard-of-care treatments with a tumor-specific modality possessing dramatically improved therapeutic efficacy.

7.2.3 Preclinical Studies to Provide the Rationale for this Clinical Protocol

Based on a review of scientific literature and publically available databases, the amino acid sequences for seven monoclonal antibodies (4 murine and 3 human) specific for EGFRvIII were obtained and used to assemble 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 5). 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 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 5) and biological activity tested by co-culture **(**Table 1). 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 and others[48] have previously determined the ability of EGFRvIII CAR engineered T cells to lyse target cells in a standard 51Cr-release assay (

Figure 6 and Figure 7) and mediate effective regression of intracranial established high grade gliomas. As shown in figures 6 and 7, 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 our experience and in publications reported by others[22,48], 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.

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 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. They therefore analyzed three TSC lines for the presence of EGFRvIII and demonstrated by RT-PCR that EGFRvIII is expressed in these lines (Figure 8). 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[48]. Previous published studies demonstrate that EGFRvIII CAR engineered cells from both donors produced IFN-g following co-culture with the TSC lines[48]. These results further support the use of EGFRvIII CAR engineered T cells as a potential immunotherapy for patients with GBM.

Table 1. Test of CARs Targeting EGFRvIII

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 18hr incubation.

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**

7.2.4 Prior Trials of Cell Transfer Therapy Using Tumor Infiltrating Lymphocytes 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[49-52], thus NCI incorporated a lymphodepleting chemotherapy into their human cell transfer studies. A protocol was developed to rapidly expand heterogeneous TILs for adoptive transfer. TILs were expanded using the REP in the presence of OKT3, irradiated allogeneic feeder cells and IL-2. These REP'ed TILs 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 TILs were re-infused to patients following a non-myeloablative 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 8)[53].

Murine models predicted that increasing the extent of lymphodepletion could increase the effectiveness of the cell transfer therapy. Thus, they performed two additional sequential trials of ACT with autologous anti-TILs 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) 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 8). 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 mm3 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).

7.2.5 Trials of Cell Transfer Therapy Using Transduction of Anti-TAATCR Genes into Non-reactive 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 obtain tumor reactive T cells, TCR gene transfer with anti-TAA properties have been developed and utilized in clinical studies[52,54,55]. Retroviral vector mediated gene transfer have been used to engineer human T cells with high efficiency and mediate regression of melanoma metastasis in patients with disseminated disease [5,52].

Studies in the NCI have identified genes that encode melanoma TAA recognized by TILs in the context of multiple MHC class I molecules 32-34. 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 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 their gene therapy cell transfer protocols in patients with metastatic melanoma.

They then isolated TCR genes from both gp100 and MART-1 reactive T cells. These studies have recently been published and are presented briefly here[52,5]. To test the *in vivo* efficacy of these F4 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. The NCI then 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[5,52]. 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-myeloabalative chemotherapy regimen or IL-2.

The low response rate in the prior MART-1 TCR gene transfer protocol led to identification of F5 MART-1 reactive TCR with higher avidity than the MART-1 F4 TCR used in the prior gene therapy clinical trial[5], and additionally to generate a TCR that recognizes the gp100:154-162 This TCR was raised in an HLA-A2 transgenic mouse immunized with this peptide37. NCI has now treated 24 patients with metastatic melanoma using autologous PBL transduced with an improved MART-1 F5 TCR following a non- myeolablative chemotherapy. Six patients (25%) have achieved an objective partial response. Toxicities observed were expected toxicities associated with the non-myeloabalative chemotherapy regimen or IL-2, and 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. They 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, the NCI has recently initiated several studies for patients with other metastatic cancers using PBL transduced

with TCR genes or CAR genes. They 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), they have seen impressive clinical responses. The clinical responses observed in seven of the fifteen evaluable patient receiving anti-ESO TCR-engineered peripheral blood lymphocytes 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[56]. 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 PET imaging post-treatment. Most toxicities observed in these studies were expected toxicities of the chemotherapy and aldesleukin administration. However, in 2 studies, they 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 1010 autologous T cells transduced with the retrovirus encoding an anti-Her-2 CAR. This patient developed respiratory distress and died four days later[29]. 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.

7.2.6 ^{111}Indium-labeling of Cells for in vivo Trafficking Studies

The *in vivo* distribution of adoptively transferred CAR cells will be evaluated using ^{111}In-labeled autologous lymphocytes. ^{111}In-labeled leukocytes have been used extensively for evaluation of adoptively transferred tumor infiltrating lymphocytes, natural killer cells, granulocytes, and whole blood leukocytes, for *in vivo* localization studies in humans[57-62]. EGFRvIII CARs will be labeled at the Duke Radiopharmacy Lab according to standardized protocols. Briefly, CARs will be counted and re-suspended in PBS. 4-6x10^8 cells will be labeled with 500 µCi of ^{111}In. The cells will be washed and mixed with cold CARs to achieve the desired cell dose with a total of 500 µCi of ^{111}In. The labeled CARs will be infused into patients through intravenous catheter within the Ambulatory Bone Marrow Transplant Unit. Distribution of ^{111}In-labeled EGFRvIII CARs will be evaluated at 1, 2 and 3 days post-infusion using SPECT/CT.

7.2.7 Safety Considerations

Several safety concerns regarding the infusion of large numbers of retrovirally-modified tumor reactive T cells have been addressed in previous clinical studies.

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 disialoganglioside GD2, a tumor associated antigen expressed by human neuroblastoma cells[16]. 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.

The NCI treated patients with B cell lymphomas utilizing lymphocytes transduced with a retrovirus encoding a CAR that recognizes the CD19 molecule[30,63-65]. 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.

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[29]. 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 normal cell recognition is not expected. In addition, as previously described, multiple EGFRvIII vaccine trials have been reported and no significant vaccine-related toxicities have been observed[66-68].

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. The transfer of 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. The NCI risk of insertional mutagenesis is extremely low, and the proposed protocol follows all current FDA guidelines regarding testing and follow up of patients receiving gene transduced cells.

7.3 Study Purpose/Rationale

The goal of this protocol is to transfer PBL transduced with genes encoding a CAR that recognizes an EGFRvIII, tumor-specific antigen into patients with WHO Grade IV malignant glioma. The CAR used will be targeted to a tumor-specific mutation, EGFRvIII, which is expressed on a subset of patients. Normal PBLs derived from patients with WHO Grade IV malignant glioma will be genetically engineered with a viral vector encoding the CAR and infused into newly diagnosed malignant glioma patients with the aim of mediating regression of their tumors. In a recent trial, EGFRvIII CARs were administered to recurrent malignant glioma patients. The starting EGFRvIII CAR dose for this study (ExCeL) was shown to be safe in that recent study in patients with recurrent malignant glioma.

Correlative studies are also proposed to document the expansion, functional persistence,[19,69] and trafficking[70-72] to tumor of adoptively transferred EGFRvIII CARs as these have been the correlates of successful therapy for other investigators.

8 OBJECTIVES AND ENDPOINTS

Table 2. Objectives and Endpoints

	Objective	Endpoint	Analysis
Primary	Determine the MTD of a single IV infusion of EGFRvIII CAR T cells in patients with newly-diagnosed WHO Grade IV malignant glioma.	MTD	See Section 15.4
Key Secondary	To determine the DLT of a single IV infusion EGFRvIII CAR T cells in patients with newly-diagnosed WHO Grade IV malignant glioma.	DLT	See Section 15.5
Exploratory	To determine if EGFRvIII CARs transferred into patients with WHO Grade IV malignant glioma expand.	Median number of EGFRvIII CARs in peripheral blood and KI67	See Section 15.6
Exploratory	To determine if EGFRvIII CARS persist and remain functional in the periphery.	Median number of EGFRVIII CARs by tetramer and functional assays	See Section 15.6

Exploratory	To assess whether EGFRvIII CARs traffic to tumor site.	Median proportion of injected ^{111}In-labeled EGFRvIII CARs present in tumor	See Section 15.6
Exploratory	To describe the survival experience of patients treated with EGFRvIII CAR T cells.	Median survival	See Section 15.6
Exploratory	To describe the progression-free survival experience of newly-diagnosed, EGFRvIII-expressing WHO Grade IV malignant glioma patients treated with EGFRvIII CAR T cells.	Proportion of patients alive without disease progression 6 months after EGFRvIII CAR treatment	See Section 15.6

9 INVESTIGATIONAL PLAN

9.1 Study Design

Treatment Plan

Following consent, subjects who are part of the dose escalation cohort will be assigned into the appropriate dose-escalation cohorts. Patients will undergo a 2-4 hour leukapheresis to harvest PBMCs for the generation of EGFRvIII CAR T cells prior to beginning RT and concurrent TMZ. T cells will be isolated from the patient's PBMCs and transduced to express CARs. Briefly, PBMC will be stimulated with OKT3 (anti-CD3 mAb) and transduced on RetroNectin® coated plates. Transduced cells will be expanded in IL-2 for 14 days.

Patients will then complete approximately 6 weeks of standard of care RT and concurrent TMZ at a targeted dose of 75 mg/m^2/d. Patients whose blood counts do not meet eligibility within 6 weeks of completing standard of care RT and concurrent TMZ will be withdrawn from the study before initiation of TMZ treatment and replaced. Patients who appear to have progressive disease following radiation may actually have pseudoprogression due to the radiation and will be allowed to continue to cycle 1 of (dose intensified) DI TMZ in order to not be excluded from the study prematurely, at the discretion of the treating physician. If the patient is determined to have progressive disease, the patient may be eligible for the INTERCEPT study (INTracerebral EGFR-vIII Chimeric Antigen Receptor Gene-Modified T Cells for PaTients with Recurrent GBM, Pro00083828). If the patient is determined to be eligible for the INTERCEPT study (Pro00083828), the cells extracted from leukapheresis while they were on this study, ExCeL, will be used to create the CAR T cells for administration in the INTERCEPT study.

Patients without definitive disease progression who remain eligible will then receive up to 3 cycles of dose-intensified (DI) TMZ at 50-100 mg/m^2/day. Each full cycle of DI TMZ is 50-100 mg/m^2/day for 21 days every 28 days, at the discretion of the treating physician. The purpose of the DI TMZ cycles is to achieve grade 3 lymphopenia. If the patient is able to tolerate the TMZ, they will receive 3 full cycles of DI TMZ prior to receiving the CAR T cells. If the patient is unable to complete the 3 cycles of DI TMZ, but has achieved grade 3 lymphopenia, they may receive CAR treatment at the discretion of the study PI and/or treating oncologist. If the patient's grade 3 lymphopenia subsides during the ~2 weeks it takes to produce the CAR T cells and before the scheduled receipt of CAR T cells, the treating physician has the discretion to re-initiate TMZ to re-establish grade 3 lymphopenia. If the patient is unable to tolerate the 3 full cycles of TMZ and does not reach grade 3 lymphopenia, or if the CAR-specific T cells do not meet release criteria, the patient will be withdrawn before CAR treatment and replaced.

Two weeks prior to CAR T cell administration, the patient must be on stable or decreasing steroids (≤ 4 mg/day). If patients are decreasing steroid use, once they are at 2 mg/day, they may be supplemented with hydrocortisone, at the discretion of the treating oncologist.

At least 48 hours after the patient's last dose of TMZ, the total dose of EGFRvIII CAR T cells will be delivered intravenously. If sufficient CAR-specific T cells cannot be generated to meet the targeted assigned dose within the dose-escalation portion of the study, the patient will be treated at a lower pre-defined dose level using available CAR-specific T cells and replaced in the assigned higher dose. The administered dose will be the highest defined dose level for which there are sufficient CAR-specific T cells available. Within the expanded cohort, if sufficient CAR-specific T cells can't be generated to meet the MTD dose, all available T cells will be administered.

Blood samples for immune monitoring (9 yellow, 1 red) will be drawn prior to EGFRvIII CAR infusion, 1, 5, 10, and 28 days after the infusion, then 3 and 6 months, then yearly until progression (or death or lost to contact). The return visits for immune monitoring at the 3 months, ~6 months, and ~yearly will

coincide with standard of care clinic visits. A portion of the immune monitoring blood sample will be used for RCR PCR per the FDA prior to EGFRvIII CAR infusion, and at 3, 6 and 12 months during standard of care clinic visits. Lastly, blood for evaluation of CRS (1 red, 1 purple top – 3mLs each) will be drawn prior to cell infusion, 1 and 4 hours after infusion, and on days 1, 2, 5, 10, and at approximately 28 days. Measurements for CRS include IL-2, IL-6, TNFα, IFNγ, GM-CSF, and C-reactive protein (CRP).

Patients will return to clinic approximately 28 days following the EGFRvIII CAR T cell infusion to be evaluated for cycles of standard of care 5-day TMZ at 150-200 mg/m^2/day for the first 5-day cycle, followed by 200 mg/m^2/day (if tolerated by the patient) for 5-days every 28 days at the discretion of the treating oncologist. This will result in an approximately > 30-day delay between the last TMZ administration prior to EGFRvIII CAR T cell infusion and the TMZ cycle initiated after the study drug infusion. This delay in the TMZ cycle after the CAR T cell infusion is done in an attempt to decrease the chance of study drug washout by TMZ.

Tumor progression will be documented histologically, unless there are clinical contraindications, to exclude inflammatory responses presenting as radiographic or clinical changes, which could indicate a potentially toxic or therapeutic responses and not tumor progression. If tissue is obtained through the Duke Brain Tumor Center Biorepository IRB# Pro00007434, it will be used to confirm tumor progression histologically, and to assess immunologic cell infiltration and EGFRvIII antigen escape at the tumor site. Patients will be eligible for additional adjuvant therapy at the time of tumor progression.

9.1.1 Dose Escalation and Expansion

A classical “3+3” study design will be used to estimate the MTD for CAR-specific T cells treatment among patients with newly-diagnosed WHO grade IV malignant glioma. As shown below in Table 3, 4 dose levels will be considered based on transduced cells/kg: #1: 4.5 x 10^6/kg, #2: 1.5 x 10^7/kg, #3: 4.5 x 10^7/kg, and #4: 1.5 x 10^8/kg.

Table 3. Dose Escalation and Expansion

	Dose Escalation and Expansion		
Cohort	**Dose of CAR-specific T cells**	**# of Subjects**	**Schema**
1	4.5 x 10^6/kg	3-6	Dose escalation: “Standard 3+3”
2	1.5 x 10^7/kg	3-6	
3	4.5 x 10^7/kg	3-6	
4	1.5 x 10^8/kg *	3-6	
* If the patient’s targeted dose is 1.5 x 10^8/kg (dose level #4), the calculated dose will be capped at 1.0 x 10^{10} total cells.			

Starting with the lowest dose level, cohorts of 3-6 subjects will be accrued at each dose level according to the following dose escalation rules:

Table 4. Dose Escalation Rules

Number of patients with DLT observed during the first 4 weeks after CAR treatment	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level
1 out of 3	Enter 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level • If 1 or more of this group suffer DLT, then dose escalation is stopped. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.

≥ 2	Dose escalation will be stopped. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
DLT = dose limiting toxicity; MTD = maximum tolerated dose	

If a patient terminates the infusion of EGFRvIII CAR T cells prematurely or is lost to follow-up during the first 4 weeks after CAR treatment without experiencing a DLT, then the patient will not be evaluable for the determination of the MTD and will be replaced. The MTD is the highest dose level at which ≤1 of 6 patients experiences DLT during the 4 week observation period after CAR treatment. The last patient in a given cohort, receiving that dose level, should be observed for DLTs for at least 4 weeks prior to moving on to the next cohort/higher dose. Section 9.1.2 provides information about what constitutes a DLT.

An expanded cohort of a total of 12 patients will be enrolled at the MTD of EGFRvIII CAR T cells in order to obtain a more precise estimate of the probability of unacceptable toxicity. This cohort will also have the cells radiolabeled with ^{111}In to track their distribution. Briefly, CARs will be counted and re-suspended in PBS. 4-6x10^8 cells will be labeled with 500 micro Ci of ^{111}In. The cells will be washed and mixed with cold CARs to achieve the desired cell dose with a total of 500 µCi of ^{111}In. The labeled CARs will be infused into the patient through an intravenous catheter within the Ambulatory Bone Marrow Transplant (BMT) Unit. Distribution of ^{111}In-labeled EGFRvIII CARs will be evaluated at 1, 2, and 3 days post-infusion using whole body planar imaging followed by SPECT/CT, when indicated.

In the rare event that a sufficient amount of CAR-specific T cells cannot be generated for a patient's intended dose within the dose-escalation portion of this study, the patient will be treated at a lower pre-defined dose level using available CAR-specific T cells. If this patient experiences a DLT, data will be carefully reviewed to determine its impact upon dose escalation and future patient treatment. If this situation occurs with the expanded cohort, all available cells will be administered.

9.1.2 Definition of Dose-Limiting Toxicity (DLT)

The DLT observation period is the 4 weeks after EGFRvIII CAR T cell infusion. Toxicities will be graded according to the NCI CTCAE version 4 criteria. A DLT will be defined as any Grade IV event of any duration that is at least (possibly, probably, or definitely) attributable to EGFRvIII CARs, or any Grade III toxicity that is at least (possibly, probably, or definitely) attributable to EGFRvIII CARs that is not reversible within 4 weeks. DLT definition includes and will be reported to FDA all of the following:

a. Any treatment-emergent CTCAE Grade 4 or 5 CRS.
b. Any treatment-emergent CTCAE Grade 3 CRS that do not resolve to ≤ Grade 2 within 7 days.
c. Any treatment-emergent autoimmune toxicity ≥ Grade 3.
d. CTCAE grade 3-5 allergic reactions related to the study cell infusion.
e. CTCAE grades 3 and greater organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) not pre-existing or due to the underlying malignancy and occurring within 30 days of study product infusion.

9.1.3 Safety Considerations

Potential Benefits

Based on experience in our previous EGFRvIII vaccine trials, immunotherapy may be of benefit to patients with MGs. Of course, because individuals respond differently to therapy, no one can know in advance if it will be beneficial in an individual case. The potential benefits may include reduction and/or remission of the patient's brain cancer. Because this procedure is experimental, it cannot be guaranteed that patients will receive any benefit as a result of participating in this research study. The information collected in this research may help scientists better understand the mechanisms involved in the immune system's ability to fight cancer. If such an understanding comes from this research, then it may benefit society by furthering the development of improved treatment methods for human malignant brain tumors in the future.

Potential Risks

Cytokine Release Syndrome

Cytokine release syndrome (CRS) is a potentially life-threatening toxicity that has been observed following administration of natural and bispecific antibodies and, more recently, following adoptive T cell therapies for cancer[73]. CRS is associated with elevated circulating levels of cytokines including IL-6 and IFNγ. Commonly referred to as an infusion reaction, it results from the release of cytokines from cells targeted by the antibody as well as immune effector cells recruited to the area. When cytokines are released into the circulation, systemic symptoms such as fever, nausea, chills, hypotension, tachycardia, asthenia, headache, rash, scratchy throat, and dyspnea can result. In most patients, the symptoms are mild to moderate in severity and are managed easily. However, some patients may experience severe, life-threatening reactions that result from massive release of cytokines. Severe reactions occur more commonly during the first infusion in patients with hematologic malignancies who have not received prior chemotherapy; severe reactions are marked by their rapid onset and the acuity of associated symptoms. Massive cytokine release is an oncologic emergency, and special precautions must be taken to prevent life-threatening complications (please see CRS Management Plan).

Tumor Lysis Syndrome

The infusion of antigen reactive T cells may cause tumor lysis syndrome, a group of complications from release of large amounts of potassium, phosphate, and nucleic acid caused by the breakdown of tumor cells after cancer treatment. Tumor lysis syndrome may cause kidney failure, abnormal heart rhythm, and may even lead to death.

Cerebral Edema

Cerebral edema may be secondary to the disease process itself, the surgical procedure, necrosis from previous radiation, or inflammation due to immune infiltration of the brain or destruction of tumor cells. Symptoms may include, but are not limited to, severe headache, confusion, lethargy, unresponsiveness, coma, or focal neurological deficits. Patients will be monitored throughout the course of the study and those patients with any signs or symptoms of cerebral edema may need their steroid doses increased, treatment with an osmotic diuretic, or surgical decompression. Edema that fails to respond to aggressive therapy may lead to permanent neurological impairment. The probability of this risk can be predicted to some degree based upon tumor size, location, pre-operative neurological impairment, and post-operative course prior to EGFRvIII CAR T cell infusion. Patients will be monitored throughout the course of the study.

Infection

The EGFRvIII CAR T cells may include the risk of infection due to potential contamination of the cells in the laboratory. This may result in localized redness, swelling, or induration at the infusion site. In the most extreme situation, this may lead to systemic bacterial/fungal sepsis and possibly death. The probability of this risk is relatively low, given the fact that the cells will be strictly tested for sterility prior to each infusion. The risk of infection due to potential contamination of the cells in the laboratory will be minimized by biosafety quality assurance and testing. All cell cultures will be handled under sterile conditions in a core tissue culture facility dedicated to the processing of human cells. Prior to infusion into patients, EGFRvIII CAR T cells must pass sterility tests. Following infusions, patients will be monitored throughout the course of the study for any signs and symptoms of infection. If an active infection is suspected, patients will be cultured and treated with appropriate antibiotics.

RCR

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.

Delayed Autoimmune Diseases

It is possible that delayed autoimmune disease(s) may develop as a result of infusion with EGFRvIII CAR T cells. This means that the immune system may be stimulated to attack natural tissue in the body. Animal studies have reported the development of autoimmunity in the context of T cell infusion and recovery from lymphopenia. However, our current experience with autologous lymphocyte transfer in patients with GBM has not demonstrated evidence of autoimmunity in treated patients. Furthermore, the doses of TMZ used in this study for induction of lymphopenia are standard doses administered to patients with GBM. It therefore, is unknown what the risk of delayed autoimmune disease is for this study.

Phlebotomy

Drawing blood or inserting an intravenous catheter into an arm vein may result in bruising or swelling in the area of the insertion, bleeding at the site of the needle puncture, light headedness, fainting and very rarely, local infection, which may be severe. These risks are reduced by the fact that the blood will be drawn by a qualified physician, nurse or phlebotomist (a professional trained to draw blood).

MRI

The risks and/or discomforts associated with the performance of MRI include the anxiety produced from being in a tight, enclosed space (claustrophobia). In addition, the machine operates using a large and powerful magnet. The magnetism of the machine attracts certain metals: therefore, people with these metals in their bodies (specifically pacemakers, infusion pumps, metal aneurysm clips, metal prostheses, joints, rods or plates) will be excluded from the study. Patients will also be checked to make sure that they do not bring any metal objects into the MRI facility. Dental fillings are less affected by the magnetic fields generated and are therefore permitted. It will be asked that patients let the physicians conducting this study know of any metal in their bodies other than dental fillings.

Allergic Reactions to Contrast Agents

During the MRI, patients will be given a contrast agent. The agent is given routinely to obtain enhanced MRI scans of the brain. The agent is administered through the vein and requires the placement of an IV catheter. The catheter placement is similar to drawing blood except that the catheter remains in the vein during the time the agent is actively delivered. The risks of a blood draw and insertion of a catheter are similar. There have been a few, rare cases of allergies to the agent used in MRI contrast enhanced scans. Patients with any known severe allergies to contrast agents will be excluded from the study. Patients with mild allergies (i.e., rash only) will be pretreated with Tylenol and Benadryl prior to injection of the contrast agent.

Temozolomide

TMZ has been well tolerated by both adults and children with the most common toxicity being mild myelosuppression. Other, less likely, potential toxicities include nausea and vomiting, constipation, headache, alopecia, rash, burning sensation of skin, esophagitis, pain, diarrhea, lethargy, hepatotoxicity, anorexia, fatigue and hyperglycemia. Hypersensitivity reactions have not yet been noted with TMZ. As in the case with many anti-cancer drugs, TMZ may be carcinogenic. Rats given TMZ have developed breast cancer. The significance of this finding for human is not presently known. TMZ therapy will be followed but given as standard of care. If toxicities occur, the Principle investigator and primary physician will titrate therapy based on standard clinical guidelines as outlined above.

^{111}Indium Labeled EGFRvIII CARs

Patients' CARs will be radiolabeled with ^{111}In for correlative studies in the expanded cohort. The radiation exposure to the patient who receives an ^{111}In-labeled EGFRvIII CARs will be minimal at proposed doses and is roughly equivalent to natural radiation that people experience living in higher altitudes. This can be compared to the same amount of radiation one would get from living in a high altitude city such as Denver for 48 weeks or taking 110 airplane flights from New York to Los Angeles. The tests or treatments will include a whole body planar imaging scan and a white cell localization scan.

Unknown Risks

The overall risk classification of this research is unknown. Clinical trials using immunotherapy on brain tumor patients only recently published in the literature. From our experience in ongoing and previous trials we have not seen any toxicities or serious unexpected adverse events.

Confidentiality

Participation in research investigations may result in a loss of confidentiality. However, all data from preoperative and postoperative evaluations will be coded to protect the patient's identity. The coding, and the results of these studies will be available only to the individuals involved with the study, the clinical staff administering the study, representatives of the National Institutes of Health, and representatives of the U.S. Food and Drug Administration. Any publications resulting from this study will not use patient identifying data.

Treatment Alternatives & Financial Reimbursement

Alternative treatments for newly-diagnosed malignant brain tumors include additional surgery, radiation, and/or chemotherapy. If the patient chooses not to participate in this trial, they certainly may seek alternative treatment. If the patient fails treatment through this trial, these alternatives may still be available to the patient. There will be no financial reimbursement to patients for study participation.

9.1.4 Concomitant Medications

Concomitant medications will be managed by the treating neuro-oncologist and recorded at each study visit, beginning at pre-CAR T cell infusion visit, with the exception of steroids, which may be tracked earlier by the study coordinator/research nurse.

9.2 Rationale for Selection of Dose, Regimen, and Treatment Duration

In a recent trial, EGFRvIII CARs were administered to recurrent malignant glioma patients. The starting EGFRvIII CAR dose for this study (ExCeL) was shown to be safe in that recent study in patients with recurrent malignant glioma. The EGFRvIII CAR infusion will be given after chemoradiation and up to 3 cycles of dose-intensified (DI) post-radiation temozolomide to leverage the homeostatic effects of chemotherapy-induced lymphodepletion.

9.3 Rationale for Correlative Studies

Correlative studies are also proposed to document the expansion, functional persistence,[19,69] and trafficking[70-72] to tumor of adoptively transferred CARs as these have been the correlates of successful therapy for other investigators.

Peripheral blood for IM will be drawn leukapheresis, pre-infusion, 1, 5, 10, and 28 days after CARs treatment, then 3 and 6 months post-infusion, and then yearly (until progression, death, or lost to contact). Whole body planar imaging and SPECT/CT on the expanded cohort of 12 patients enrolled at the MTD on days 1, 2, and 3 post-infusion will be used to determine the IC localization of indium-labeled T cells. If there is no signal on whole body planar imaging, the radiologist may decide not to perform SPECT/CT.

9.4 Definition of Evaluable Subjects, On Study, and End of Study

While all patients who received any EGFRvIII CAR T cells will be included in safety summaries for the purposes of monitoring and reporting of adverse events to appropriate agencies, patients evaluable for determination of the MTD will include:

- Patients who receive the entire CARs infusion (i.e. the infusion is not stopped prematurely), and 2) have 4 weeks of follow-up for adverse events after the infusion
- Patients who receive the entire CARS infusion (i.e. the infusion is not stopped prematurely) and terminated follow-up prematurely during the 4 week follow-up period after having experienced a DLT.
- Patients who terminate infusion before completion due to a DLT

For the efficacy analyses, evaluable patients will include two groups of patients: all patients, and the subgroup of patients who receive EGFRvIII CAR. Once a patient's eligibility is confirmed after completion of XRT/TMZ, that subject will be considered "on study". The rationale for taking a patient off study will be documented. Please see Section 12.7 for information on patient withdrawals.

9.5 Early Study Termination

This study can be terminated at any time for any reason by the PI-sponsor. If this occurs, all subjects on study will be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 12.7, which describes procedures and processes for prematurely withdrawn patients.

10STUDY DRUG

10.1 Names, Classification, and Mechanism of Action

The name of the drug is CAR gene-modified T cells or abbreviated as EGFRvIII CARs. The class of action is a biological and the mechanism of action is cytotoxicity. The drug substance is autologous T cells transduced with a retroviral vector encoding for a chimeric antigen receptor (CAR) directed against the tumor specific antigen, EGFRvIII. EGFRvIII CARs are genetically engineered T cells that have been taken from patients with WHO Grade IV malignant glioma *ex vivo* to express a CAR recognizing the GBM tumor-specific antigen EGFRvIII, which is expressed on a subset of GBMs but not in normal human tissues with the aim of mediating regression of their tumors.

10.2 Packaging and Labeling

Pt. Name/MRN
EGFRvIII CAR T Cells
Dose
Administration Date
For Autologous use only
Not evaluated for infectious substances
Caution New Drug Limited by Federal Law to Investigational Use

10.3 Supply, Receipt, and Storage

The drug will be manufactured in the Molecular Products and Cellular Therapies (MPACT) cGMP facility. All drug accountability, transfers, receipts, and disposals are recorded in the Duke Nautilus system.

10.4 Dispensing and Preparation

10.4.1 EGFRvIII CARs

The CAR product will be held at room temperature (21-25^{O}C) until the gram stain and endotoxin tests are completed. The cells are delivered from the MPACT facility directly to the bedside under the supervision of the research nurse or her designee. EGFRvIII CARs will be administered according to protocol. The patient's name, Study ID, DOB, and Duke history number will be verified by two separate people prior to the CAR administration as is standard Duke BMT transfusion procedure. For ^{111}In labeling a portion of the cells will be labeled with ^{111}In and then mixed with unlabeled cells to achieve the final dose. The unlabeled cells will be held at 37^{O}C until final mixing. The radiolabeled EGFRvIII CARs will be dispensed from the Duke Radiopharmacy.

10.4.2 Release Criteria for EGFRvIII CARs

Tests performed on the final product:

Table 5. Release Criteria for EGFRvIII CARs

Test	*Method*	*Limits*	*Result*	*Initials/Date*

Cell viability[1]	Trypan blue exclusion	>70%		
Total viable cell number[1]	Visual microscopic count	>1 x 10^7		
Tumor reactivity[2]	γ-IFN release vs. EGFRvIII antigen	>200 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		

In the event of a positive sterility test, after the product has been infused, the Principal Investigator or his or her designate will notify the treating physician and the patient. The FDA and IRB will be notified within 15 calendar days. The patient will be asked to be evaluated by a physician within 24 hours. If the patient has or develops a temperature >38.5 °C or clinical evidence of infection at the injection site (erythema or edema) or systemically, the patient will have samples of from the injection sites, along with blood, urine and sputum (if possible) sent for bacterial, fungal, culture and sensitivity testing and will be treated expectantly with antibiotics based on the sensitivities of the organisms identified from the immunization product and an independent infectious disease consultation will be obtained to guide further therapy. Any remaining lymphocytes will be sent for bacterial, and fungal, culture and sensitivity testing and endotoxin testing.

10.4.3 ^{111}In-labeled T cells

CARs are washed three times with PBS, assessed for viability, and counted. CARs are re-suspended in PBS. 4-6x10^8 cells will be labeled with 500 micro Ci of ^{111}In. The cells will be washed and mixed with cold CARs to achieve the desired cell dose containing 500 micro Ci of ^{111}In. The cell concentration is adjusted to 2.5 x10^7 cells per mL and resuspended in preservative free saline with 1% human serum albumin (Baxter Healthcare Corp., US License No 140 NDC0944-0490-0L) and placed into the appropriate size syringe for infusion.

From this final preparation, a sample of cells will be sent for Gram stain and endotoxin testing prior to administration. ^{111}In-labeled T cells will not be given until endotoxin testing has been passed (< 5.0 E.U./Kg and the Gram stain has been found to be negative. An aliquot of cells will also be sent for aerobic and anaerobic bacterial cultures (1 x 10^6 PBLs) and fungal cultures (1 x 10^6 PBLs). The final formulated

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

[2] Performed 2-10 days 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. BactAlert bottles will be incubated for 14 days.

[4] Sample collected from the final product prior to infusion. Results will not be available before cells are infused into the patient. BactAlert bottles will be incubated for 14 days.

[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.

T cells will be stored at 2-8°C for up to 6 hours. After this time, the material will be disposed of if not given to the patient.

In the event of a positive sterility test, the Principal Investigator or his or her designate will notify the treating physician and the patient. The FDA and IRB will be notified within 15 calendar days. The patient will be asked to be evaluated by a physician within 24 hours. If the patient has or develops a temperature >38.5 ^{o}C or clinical evidence of infection at the injection site (erythema or edema) or systemically, the patient will have samples of from the injection sites, along with blood, urine and sputum (if possible) sent for bacterial, fungal, culture and sensitivity testing and will be treated expectantly with antibiotics based on the sensitivities of the organisms identified from the immunization product and an independent infectious disease consultation will be obtained to guide further therapy. Any remaining lymphocytes will be sent for bacterial, and fungal, culture and sensitivity testing and endotoxin testing.

10.5 Compliance and Accountability

The EGFRvIII CAR will be signed out and distributed by the BTIP laboratory manager. The Duke BTIP personnel use safe medication practices to reduce the risk of medication errors and adverse events when setting up study drug procedures. Investigational drugs are stored separately from other drugs in an area of limited access and in accordance with special storage requirements. They are clearly labeled with the identity of the study drug and other control numbers. Investigational drugs are accounted for using the Duke Nautilus System. All drug transfers, receipts, and disposal are recorded in Nautilus.

10.6 Disposal and Destruction

Radioactively labeled drug will be disposed of according to standard radiation safety practices. Patient rooms and the patient themselves will be monitored before discharge by a member of the Duke Radiation safety committee. Un-labeled cells will be autoclaved.

11 SUBJECT ELIGIBILITY

Inclusion Criteria

1. Age 18-80 years of age
2. Histopathologically proven newly-diagnosed, supratentorial glioblastoma or gliosarcoma (WHO Grade IV).
3. KPS score ≥ 70.
4. The presence of the target antigen, EGFRvIII, must be identified on tumor tissue by IHC or PCR.
5. Hematology:
    - ANC ≥ 1000/mm^3 without the support of filgrastim
    - Platelet count ≥ 100,000/mm^3
    - Hemoglobin ≥ 8.0 g/dl (eligibility level for hemoglobin may be reached by transfusion).
6. Chemistry:
    - ALT/AST ≤ 2.5 times the upper limit of normal
    - Creatinine ≤ 1.6 mg/dl
    - Total bilirubin ≤ 1.5 mg/dl.

Exclusion Criteria

1. Patients who are pregnant, breast-feeding, or unwilling to practice an effective method of birth control.
2. Patients with known potentially anaphylactic allergic reactions to gadolinium-DTPA.
3. Patients who cannot undergo MRI or SPECT for any reason including due to obesity or to having certain metal in their bodies (specifically pacemakers, infusion pumps, metal aneurysm clips, metal prostheses, joints, rods, or plates).
4. Patients with evidence of tumor in the brainstem, cerebellum, or spinal cord, or with evidence of leptomeningeal disease.
5. Active infection requiring treatment or an unexplained febrile (> 101.5^{o} F) illness.
6. Known autoimmune disease, immunosuppressive disease or human immunodeficiency virus infection (i.e., known HIV or Hepatitis C).
7. Patients with unstable or severe intercurrent medical conditions such as severe heart or lung disease.

8. Patients with previous history of radiosurgery, brachytherapy, gliadel implantation, or radiolabeled monoclonal antibodies.
9. Prior antitumor therapy for glioma (other than steroids).
10. Allergic to TMZ.

12 SCREENING AND ON-STUDY TESTS AND PROCEDURES

In brief, patients will be screened and evaluated for study entry (please see Table 6). The screening examination must include a pathology report confirming the EGFRvIII$^+$ WHO grade IV malignant glioma diagnosis. Blood tests per Table 6 will be drawn as well as the post-surgical MRI. The baseline physical and neurologic examination with KPS score will be performed and documented by the neuro-oncology team and verified by the study team during the Duke Brain Tumor Center clinic visit (of note, the standard of care clinic visit may be used to fulfill the initial screening visit requirement). Patients will be offered treatment options during this BTC clinic visit.

Once the consent has been signed, patients will be scheduled to undergo a leukapheresis in order to obtain T cells for infusion (please see Table 6). Patients will then receive approximately 6 weeks of standard of care RT and concurrent TMZ at a standard targeted dose of 75 mg/m^2/d. Patients whose blood counts do not meet eligibility within 6 weeks of completing standard of care RT and concurrent TMZ will be considered a screen failure. Patients who appear to have progressive disease following radiation may actually have pseudoprogression due to the radiation and will be allowed to continue to cycle 1 of DI TMZ in order to not be excluded from the study prematurely, at the discretion of the treating physician. If the patient is determined to have progressive disease, the patient may be eligible for the INTERCEPT study (INTracerebral EGFR-vIII Chimeric Antigen Receptor Gene-Modified T Cells for PaTients with Recurrent GBM, Pro00083828). If the patient is determined to be eligible for the INTERCEPT study (Pro00083828), the cells extracted from leukapheresis while they were on this study, ExCeL, will be used to create the CAR T cells for administration in the INTERCEPT study.

Patients without definitive disease progression who remain eligible will then receive up to 3 cycles of DI TMZ at 50-100 mg/m^2/day. Each full cycle of DI TMZ is 50-100 mg/m^2/day for 21 days every 28 days, at the discretion of the treating physician. The purpose of the DI TMZ cycles is to achieve grade 3 lymphopenia. If the patient is able to tolerate the TMZ, they will receive 3 full cycles of DI TMZ prior to receiving the CAR T cells. If the patient is unable to complete the 3 cycles of DI TMZ, but has achieved grade 3 lymphopenia, they may receive CAR treatment at the discretion of the study PI and/or treating oncologist. If the patient's grade 3 lymphopenia subsides during the ~2 weeks it takes to produce the CAR T cells and before their scheduled receipt of CAR T cells, the treating physician has the discretion to re-initiate TMZ to re-establish grade 3 lymphopenia, at the discretion of the treating physician. If the patient is unable to tolerate the 3 full cycles of TMZ and does not reach grade 3 lymphopenia, or if the CAR-specific T cells do not meet release criteria, the patient will be withdrawn and replaced.

Two weeks prior to CAR T cell administration, the patient must be on a stable or decreasing steroid dose (≤ 4 mg/day). If patients are decreasing steroid use, once they are at 2 mg/day, they may be supplemented with hydrocortisone, at the discretion of the treating physician.

The patient will be scheduled for CAR T cell (or CAR T cell labeled with ^{111}In) infusion at least 48 hours (+72 hours) after their last dose of DI TMZ. In the expanded cohort group, 3 days of whole body planar imaging and SPECT/CT imaging will be done following infusion on days 1, 2, and 3. Note, if there is no signal on whole body planar imaging, the radiologist may decide not to perform SPECT/CT. Blood for immune monitoring will be taken on all patients at leukapheresis, pre-infusion, 1, 5, 10, and 28 days following infusion, and at 3 and 6 months post-infusion, and then yearly (until progression, death, or lost to contact). The return visits for immune monitoring at the 3, 6, and yearly will coincide with standard of care clinic visits. Blood for RCR PCR will be taken prior to cell infusion and at 3 and 6 months, and at one year as required by the FDA during routine clinic visits. Lastly, blood for evaluation of CRS will be drawn prior to cell infusion, 1 and 4 hours after infusion, and on days 1, 2, 5, 10, and at 28 days. Measurements for CRS include IL-2, IL-6, TNFα, IFNγ, GM-CSF, and CRP.

Blood samples will be archived annually thereafter if all previous testing has been negative.

Patients will return to clinic, as per standard of care, approximately 28 days after EGFRvIII CAR T cell infusion to be evaluated radiographically and clinically for cycles of standard of care 5-day TMZ at 150-200 mg/m^2/day per the treating oncologist. This will result in a 28-day pause between the last cycle of TMZ

administered prior to EGFRvIII CARs and the first cycle administered after study drug infusion in order to decrease the chance of study drug washout from TMZ.

Table 6. Screening and On-Study Tests and Procedures

	Pre-XRT/TMZ Screening	Leukapheresis	Post-XRT/TMZ screening	DI TMZ Cycle 1	Post DI TMZ Cycle 1	DI TMZ Cycle 2[1]	DI TMZ Cycle 3[1]	Pre-CAR T Cell Infusion	CAR T Cell Infusion [2]	5-day TMZ Cycles 4-12	Progression
Main study consent	X										
History and Physical	X		X		X[3]			X[4]		X[5]	
Neurological Exam	X		X		X[3]			X[4]		X[5]	
KPS	X		X		X[3]			X[4]		X[5]	
Curran			X								
CBC w/ differential	X	X	X	X[6]	X[3]	X[6,3]	X[6]	X[4]		X[7]	
CMP	X	X	X		X[3]	X[3]		X[4]		X[8]	
Beta HCG	X	X									
CRS									X[9]	X[10]	
Immune Monitoring		X[11]							X[12]	X[13]	X[13]
CAR T Cells									X[14]		
DI Temodar				X[15]		X[15]	X[15]				
5-day Temodar										X[16]	
Vitals	X		X		X[3]			X[3]	X[17]	X[5]	
MRI	X		X		X[3]			X[3]		X[5]	
Pathology testing	X										X
Adverse Events								Ongoing			
Con Meds								Ongoing			

[1] Some patients may not receive DI TMZ cycle 3 or cycles 2 and 3, please see Section 9.1 for details about DI TMZ requirements.
[2] 48 hours (+72 hours) after last dose of DI TMZ
[3] ± 7 days from the last dose of TMZ
[4] Within 2 days prior to CAR T Cell infusion.
[5] Prior to Cycle 4 and then prior to every other Cycle (Cycle 6, 8, etc), at the discretion of the treating physician.
[6] Day 14 (± 3 Days) of the TMZ cycle.
[7] Day 21 (± 3 days) and day 28 (± 7 days) of each 5-day TMZ cycle
[8] Day 28 (± 7 days) of each 5-day TMZ cycle
[9] Prior to CAR T Cell Infusion, and at 1 and 4 hours and days 1, 2, 5, and 10 post CAR T Cell infusion. Draw 1 red top and 1 purple top (3 mL each) and 1 gold top at each time point.
[10] 28 days post CAR T Cell Infusion. Draw 1 red top and 1 purple top (3mL each) and 1 gold top.
[11] 2 red tops (10 mL each).
[12] Prior to CAR T Cell Infusion on day 22 (+3 day window), and at 1, 5, and 10 days after the infusion (9 yellow tops (8.5mL each) and 1 red top (10mL) at each time point.
[13] 1, 3, 6, and 12 months post CAR T Cell infusion and then yearly and at progression. Draw 9 yellow tops (8.5mL each) and 1 red top (10mL) at each time point. Draws will coincide with standard of care return clinic visits.
[14] At least 48 hours after last dose of DI TMZ. Patients will be pre-medicated 30-60 minutes prior to infusion with acetaminophen (650 mg po) and Benadryl (25 mg by mouth) to reduce infusion-related reactions. Patients must be on stable of decreasing steroid dose (≤ 4 mg/day) 2 weeks prior to CAR T cell infusion.
[15] Days 1-21 of a 28-day cycle, at the discretion of the treating physician.
[16] 5 day TMZ to be taken on days 1-5 of a 28 day cycle. The first cycle of 5-day TMZ will be initiated approximately 1 month after CAR T Cell infusion.
[17] Vital signs will be measured prior to the CAR T cell infusion and every 15 minutes for 1 hour post-infusion. Measurements to be reported include temperature in Celsius or Fahrenheit, pulse (beats per minute), respiratory rate (breaths per minute) and blood pressure.

12.1 Screening Examination

A preliminary screening visit will take place prior to the patient undergoing leukapheresis and radiation. In order to not have to repeat clinic visits on back to back days, the standard of care clinic visit can be used to fulfil the initial screening visit requirement. Subject data to be collected at the initial screening examination includes a pathology report, MRI report, CMP and CBC with Differential (just prior to leukapheresis only), and a physical and neurologic examination with KPS score. The patient will then return for the final screening visit after radiation has been completed. Subject data to be collected at the Screening Examination includes CMP and CBC with Differential, a physical and neurologic examination with KPS score, and baseline Curran Status (Curran only performed at post XRT screening). The blood test results from the post radiation screening visit will be the results used to confirm final eligibility. If these test results do not meet the eligibility criteria, the patient can recheck these tests at a local lab up until 6 weeks post radiation in order to be considered eligible for the study. Patients whose lab results do not meet the eligibility criteria by 6 weeks post radiation will be considered a screen failure.

Patients with newly-diagnosed EGFRvIII-positive WHO grade IV malignant glioma will be imaged by MRI for baseline measurements and to assess progression prior to receiving EGFRvIII CARs; although the purpose of this study is not to detect tumor responses, any evidence of tumor response will be determined by serial measures of the product of the 2 largest cross-sectional diameters.[74,75] For patients with ≥ 25% increase in the product of the largest perpendicular diameters of any enhancing lesion or any new enhancing tumor on MRI scans or unequivocal and significant worsening of non-measurable tumor that is not attributable to co-morbid event or concurrent medication, progression will be defined. Patients may also be classified as progressive disease with significant neurologic decline felt to be due to underlying tumor and not attributable to a co-morbid event or concurrent medication regardless of MRI findings. Note – If there has been a major reduction in steroid dosage in the interval and the patient is felt to be clinically stable or improved, the proper assessment may be “indeterminate” and the therapy could be continued pending the next evaluation. The RANO criteria[76] will be used for overall assessment of tumor response and pseudoprogression (PRTBTC Imaging SOP and FORM is available upon request). Tumor progression will need to be documented histologically, unless there are clinical contraindications, to exclude inflammatory responses presenting as radiographic or clinical changes, which could indicate potentially toxic or therapeutic responses and not tumor progression. If tissue is obtained, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and antigen escape. Upon progression, patients may be treated on other therapies as directed by the treating neuro-oncologist.

12.2 Treatment Period

Patients that have consented onto this study must first undergo a 2 to 4-hour leukapheresis to obtain T cells. After completion of SOC chemoradiotherapy, the treatment period will commence with patients receiving the single infusion of EGFRvIII CARs. The expanded cohort will receive the ^{111}In-labeled EGFRvIII CARs.

Infusions will be administered in the BMT Clinic, monitored by an RN during and for 1 hour following the procedure. If a patient develops signs of infection or is febrile prior to the infusion, treatment will be postponed pending clinical work-up. The T cell product will be delivered from the MPACT facility directly to the bedside under the supervision of the research nurse or her designate. For subjects receiving CAR T cells in the expanded cohort, the T cell product that will be labeled with ^{111}In will be delivered from the Radiopharmacy. T cells will be administered according to protocol. The patient’s name, Study ID, DOB, and MRN will be double verified prior to infusion as is standard Duke BMT transfusion procedure.

The patient will be pre-medicated 30-60 minutes prior to infusion with acetaminophen (650 mg po) and Benadryl (25 mg by mouth) to reduce infusion-related reactions. An intravenous catheter (18-20 G) will be placed for the procedure. The EGFRvIII CARs will be delivered to the patient’s bedside in a syringe and applied according to SOP BTC-00069444.01, or the most recent version of the SOP. Vital signs will be assessed prior to and every 15 minutes for 1 hour following the infusion. The infusion

will be documented as well as any adverse experiences with infusion. All Duke transfusion and cell infusion policies will be followed.

Prior to infusion, approximately 65 mLs of blood for IM, RCR PCR assay, and CRS will be drawn (9 yellow top, 1 (10 mL) red top, 1 purple top, and 1 (3mL) red top).

12.3 CRS Management Plan

Grade 1 toxicity

1. Will implement symptomatic management only.

Grade 2 toxicity

1. Monitoring hypotension: we will have clearly established a baseline blood pressure, pre-infusion of CAR T cells, for each patient; if hypotension develops give fluids or 1 low-dose vasopressor.

2. Respiratory symptoms: will be managed by oxygen.

   Care for the patients with grade 2 toxicity will be individually tailored, depending on the patient age and medical co-morbidity, and therefore clinical judgement will be crucial prior to use of immunosuppression for Grade 2 toxicity. Patients with Grade 2 toxicity would be monitored in step-down units, including very close cardiac monitoring.

Grade 3 toxicity

1. If grade 3 toxicity develops where fluid resuscitation and low dose vasopressor(s) are not sufficient to reverse the hypotension, or patients require increasing oxygen support or develop grade 3 organ toxicity, the patients will be transferred and monitored closely in intensive care unit. All patients with Grade 3 will receive immunosuppressive agents. In severe cases Tocilizumab, antihuman IL-6R mAb, will be administered. This drug is stocked in the Duke pharmacy.

Grade 4

1. All patients with immediate life threatening toxicity will be treated with full support including mechanical ventilation, immunosuppressive agents, Tocilizumab to prevent inflammatory cascade and with close comprehensive organ monitoring.

12.4 End of Treatment

Once the EGFRvIII CARS infusion is complete, the treatment phase of the study will be over and the follow-up period will begin.

12.5 Follow-up Period

Following the infusion, blood samples for IM (9 yellow, 1 red) will be drawn 1, 5, 10, and 28 days after the infusion, then 3 and 6 months, then yearly until progression (or death or lost to contact). Lymphocytes in patients' peripheral blood will be analyzed by FACS and quantified for persistence. Isolated PBMCs will be stained with fluorescence-labeled antibodies against cell surface and intracellular cytokine markers to determine function including staining with our novel EGFRvIII tetramer reagent. Whole planar imaging, followed by SPECT/CT, if appropriate, will be done on days 1, 2, and 3 following infusions to determine the IC localization of ^{111}In-labeled EGFRvIII CARs. Quantification of radioactivity will be assessed by previously published methods that calculate counts from reconstructed images based on the percentage of injected dose. Blood will be taken for RCR PCR at 3, 6 and 12 months and blood for evaluation of CRS (1 red, 1 purple top – 3mLs each) will be drawn pre-infusion, 1 and 4 hours after CARs infusion, and on days 1, 2, 5, 10, and 28.

Patients treated with EGFRvIII CARs will be followed for survival and data recorded by the study coordinator/research nurse until death or for 15 years (whichever comes first).

12.6 End of Study

Rationale for taking a patient off study will be documented (see section 12.7.1). All patients will be followed for survival, subsequent therapies, and radiographic response and this information will be recorded by the study team.

12.7 Early Withdrawal of Subject(s)

12.7.1 Criteria for Early Withdrawal

Subjects may voluntarily withdraw from the study at any time.
Subjects may be withdrawn by the PI prior to infusion of EGFRvIII CARS for the following:

- Progressive disease following any of the cycles of DI TMZ, at the discretion of the treating physician. *Note that patients who are removed from study due to progressive disease may be eligible for the INTERCEPT study (Pro00083828), this would result in the autologous T cells from the leukapheresis in this study being transferred to the INTERCEPT study (Pro00083828).

The PI may also withdraw a subject from the study at any time based on his/her discretion. Reasons for PI-initiated withdrawal may include, but are not limited to the following:

- Pregnancy.
- Upon request of the subject.
- If, in the investigator's medical judgment, further participation would be injurious to the subject's health or wellbeing.
- Protocol deviation.
- Administrative reasons, such as a major violation of the clinical trial protocol.
- Non-compliance of the subject.

12.7.2 Follow-up Requirements for Early Withdrawal

Subjects who have received EGFRvIII CAR T cell infusion on this study that are withdrawn by the PI for any of the aforementioned reasons will continue to be followed for survival by the study coordinator/research nurse until death or are lost to follow up. This follow-up may include the collection of survival status and doses, dates, and final radiographic responses associated with future treatment regimens.

12.7.3 Replacement of Early Withdrawal(s)

Subjects who do not receive EGFRvIII CAR treatment will be replaced. Subjects who terminate infusion prematurely or are lost to follow-up before the completion of 4 weeks of follow-up observation and do not experience dose-limiting toxicity will also be replaced.

12.8 Study Assessments

12.8.1 Medical History

Medical history will be obtained from the Duke EPIC system and from the subject and/or family at the screening visit and reviewed at **each** study/clinic visit. This data will include the following:

- All past medical and surgical history
- Current medications
- Changes in physical or neurologic symptoms
- Any adverse events

12.8.2 Physical Exam

Vital signs and physical and neurologic examinations will be assessed and recorded along with a KPS score prior to enrollment and at each study/clinic visit.

12.8.3 Correlative Assessments

To assess if EGFRvIII CARS transferred into patients with WHO Grade IV malignant glioma expand and remain functional in the periphery, 2 red top tubes will be drawn at the leukapheresis visit for immune monitoring. Fifty mLs of whole blood in 6 ACD yellow top tubes and 1 red top tube (9 mLs) for immune monitoring will be drawn pre-infusion and 1, 5, 10, and 28 days after CARs treatment, then 3 and 6 months post-infusion, then yearly until progression, death or lost to contact. Patient PBMCs will be obtained from whole blood by purification using centrifugation on a Ficoll cushion within 8 hours of draw using a standard SOP. Cells will be frozen to -70 ˚C at -1 ˚C/min and stored in liquid nitrogen. To quantify the kinetics of CARs in blood, the expression of EGFRvIII CARs in PBMCs recovered from patients will be determined using the novel EGFRvIII peptide tetramer reagent developed by our laboratory. The number of CARs will be compared to total numbers of $CD4^+$ and $CD8^+$ lymphocytes using FACs; Ki67 staining will be used to assess cell proliferation. Cell phenotype and function will be assessed using a validated polyfunctional T cell assay in collaboration with Kent Weinhold, Ph.D., using the following markers: anchor gates (CD3 AmCyan, CD4 PerCP-Cy5.5 & CD8 APC-Cy7), a multiplexed exclusion channel (CD14 Cascade Blue+ CD19 Cascade Blue+ vAmine all collected in a single channel), maturational markers (CD27 APC, CD57 FITC & CD45RO ECD) to identify naïve (CD45RO-CD27+), central memory (CD45RO+CD27+), effector memory (CD45RO+CD27-), effector (CD45RO+CD57+), and terminal effector (CD45RO-CD57+) $CD4^+$ and $CD8^+$ lymphocytes, and functional markers (IL-2 PE, TNF-a Alexa700, CD107a PE-Cy5.5 & IFN-g PE-Cy7). We will include the EGFRvIII peptide tetramer reagent in this assay to evaluate CAR-specific polyfunctionality using a fluorochrome not already in the panel. Samples from the expanded cohort will be analyzed after radioactivity has decayed. Polyfunctional analysis will also be done on the cells transferred to the patients.

Also following infusion of ^{111}In-labeled CARs in the expanded cohort, imaging on days 1, 2, and 3 by SPECT/CT will be used to determine the IC localization of ^{111}In-labeled EGFRvIII CARs to assess if EGFRvIII CARS traffic to the tumor site. Quantification of radioactivity will be assessed by previously published methods that calculate counts from reconstructed images based on the percentage of injected dose.

For monitoring the RCR PCR assays to detect the GaLV envelop gene, the RCR will be performed using a validated assay prior to cell infusion and at 3 and 6 months, and at one year as required by the FDA. Blood samples will be archived annually thereafter if all previous testing has been negative. If a patient dies or develops neoplasms during this trial, efforts will be made to obtain 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.

Please see Figure 10. Correlative Studies Schemain Appendices.

Lastly, blood for evaluation of CRS will be drawn prior to cell infusion, 1 and 4 hours after infusion, and on days 1, 2, 5, 10, and at 28 days. Measurements for CRS include IL-2, IL-6, TNFα, IFNγ, GM-CSF, and CRP.

13 SAFETY MONITORING AND REPORTING

The PI is responsible for the identification and documentation of AEs and SAEs that may occur following treatment with EGFRvIII CARs, as defined below. At each study visit, the PI or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred. AEs and/or SAEs will be collected starting at the CAR T cell infusion visit and will be recorded for baseline assessments. Only Special Interest AEs related to leukapheresis, as described in Section 13.2 will be recorded prior to the CAR T cell infusion. Following EGFRvIII CAR infusion, all AEs and SAEs will be recorded by the study team(see Section 13.1.1 below).

13.1 Adverse Events

An AE is any untoward medical occurrence in a subject receiving EGFRvIII CARs and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of EGFRvIII CAR T cells, whether or not related to use of the EGFRvIII CAR T cells. All CTCAE-defined AEs, including grade 1 laboratory events, are recorded and reported as required in 21CFR312.

From the time of CAR T cell infusion through the End of Study visit (as defined in Section 12.6), as well as any Special Interest AEs related to leukapheresis (as defined in Section 13.2), all AEs must be recorded in the subject medical record and adverse events case report form. However, for reporting purposes, only AEs that occur at the time of the EGFRvIII CAR T cell infusion or following the infusion will be submitted to the appropriate agencies as indicated.

AEs will be assessed according to the CTCAE version 4.0. If CTCAE grading does not exist for an AE, the severity of the AE will be graded as mild (1), moderate (2), severe (3), life-threatening (4), or fatal (5).

Attribution of AEs will be indicated as follows:

- Definite: The AE is clearly related to the study drug
- Probably: The AE is likely related to the study drug
- Possible: The AE may be related to the study drug
- Unlikely: The AE is doubtfully related to the study drug
- Unrelated: The AE is clearly NOT related to the study drug

13.1.1 Reporting of AEs

A summary of all adverse events (not just those considered related to EGFRvIII CARs) that occur following treatment with EGFRvIII CARs will be kept which will categorize the event by organ system, relationship to treatment, its grade of severity, and resolution. The PI will periodically review the collective adverse events with the intention of identifying any trends or patterns in toxicity. If any such trends are identified, depending on their severity and frequency, a protocol amendment will be considered.

13.2 Adverse Events of Special Interest

Adverse events will not be collected until the patient has received CAR T cells, unless they are considered an Adverse Event of Special Interest related to the leukapheresis procedure. Only these special interest adverse events will be collected prior to CAR T cell infusion, all other events occurring prior to CAR T cell administration will not be recorded or monitored.

Special Interest Adverse Events that may occur during the leukapheresis procedure include:

- Allergic Reaction
- Anaphylaxis
- Presyncope
- Syncope
- Vasovagal reaction

Special Interest Adverse Events that may occur after the procedure, but may still be related to leukapheresis include:

- Vascular Access Complications
- Venous Injury

13.3 Serious Adverse Events

An AE is considered “serious” if in the view of either the investigator or sponsor it leads to one of five specific sequelae:

- Death
- Life-threatening adverse event
- Inpatient hospitalization of 24 hours or longer or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Constitutes a congenital anomaly or birth defect

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious 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.

13.3.1 Reporting of SAEs

All SAEs that occur following treatment with EGFRvIII CARs should be reported immediately to Dr. David Ashley (Pager: 919-206-3433), Dr. Dan Landi (Pager: 919-206-3405), or their designee (919-684-5301) and to the FDA and Duke University Medical Center IRB. Fatal or life-threatening, unexpected adverse events will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the sponsor followed by as complete a report as possible within 8 additional calendar days. Serious, unexpected adverse events that are not fatal or life-threatening will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the sponsor.

All adverse events that are considered serious, unanticipated, and related or possibly related to EGRvIII CARs (as defined by 21CRF312.32[a]) will be reported to the Duke University Medical Center IRB and the FDA using the appropriate SAE report form. At the time of the annual progress report to the Duke University Medical Center IRB and the FDA, a summary of the overall toxicity experience will be provided.

13.4 Safety Oversight Committee (SOCom)

The Duke Cancer Institute SOCom is responsible for annual data and safety monitoring of DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent DSMB. The primary focus of the SOCom is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The SOCom in concert with the DCI Monitoring Team (see Monitoring section for Monitoring Team description) oversees the conduct of DUHS cancer-related, sponsor-investigator therapeutic intervention and prevention intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, SOPs, GCP, and applicable regulatory requirements.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring

The DCI Monitoring Team will conduct monitoring visits to ensure subject safety and to ensure that the protocol is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, good clinical practice, and applicable regulatory requirements. As specified in the DCI Data and Safety Monitoring Plan, the DCI Monitoring Team will conduct routine monitoring after the third subject is enrolled, followed by annual monitoring of 1 – 3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of DUHS

and DCI leadership, the DCI Cancer Protocol Committee, the SOCom, the sponsor, the Principal Investigator, or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, including but not limited to the National Institute of Health, National Cancer Institute, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

14.2 Audits

The Duke School of Medicine OARC office may conduct audits to evaluate compliance with the protocol and the principles of GCP. The PI agrees to allow the OARC auditor(s) direct access to all relevant documents and to allocate his/her time and the time of the study team to the OARC auditor(s) in order to discuss findings and any relevant issues.

OARC audits are designed to protect the rights and well-being of human research subjects. OARC audits may be routine or directed (for cause). Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, Investigator initiated Investigational Drugs or Devices, federally-funded studies, high degree of risk (based upon adverse events, type of study, or vulnerable populations), Phase I studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized Institutional Official.

OARC audits examine research studies/clinical trials methodology, processes and systems to assess whether the research is conducted according to the protocol approved by the DUHS IRB. The primary purpose of the audit/review is to verify that the standards for safety of human subjects in clinical trials and the quality of data produced by the clinical trial research are met. The audit/review will serve as a quality assurance measure, internal to the institution. Additional goals of such audits are to detect both random and systemic errors occurring during the conduct of clinical research and to emphasize “best practices” in the research/clinical trials environment.

14.3 Data Management and Processing

14.3.1 Study Documentation

Study documentation includes but is not limited to source documents, case report forms, monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the DCI-mandated “Regulatory Binder”, which includes but is not limited to signed protocol and amendments, approved and signed informed consent forms, FDA Form 1572, CAP and CLIA laboratory certifications, and clinical supplies receipts and distribution records.

Source documents are original records that contain source data, which is all information in original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documents include but are not limited to hospital records, clinical and office charts, laboratory notes, memoranda, subjects’ diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

14.3.2 Case Report Forms (CRFs)

The subject’s medical records and CRFs will be the primary source document for the study. This data will be transferred to the electronic database. Only the PI, key personnel, the Radiolabeled Pharmacy personnel, and the PRTBTC data manager are permitted to make entries, changes, or corrections in the source documents or database.

Errors on the source documents will be crossed out with a single line, and this line will not obscure the original entry. Changes or corrections will be dated, initialed, and explained (if necessary). Electronic database changes will be tracked automatically. The PI or authorized key personnel will maintain a record of the changes and corrections.

For electronic CRFs, an audit trail will be maintained by the electronic CRF management system.

14.3.3 Data Management Procedures and Data Verification

The DCI IS Shared Resource has a track record of developing Title 21 CFR Part 11 compliant databases for cancer clinical trials. IS also has extensive expertise in database quality assurance, data standards, and use of caBIG tools to support cancer researchers.

Data queries will be generated automatically by the eCRF system. These data queries signify the presence of data inconsistencies. The study and data management team will cross-reference the data to verify accuracy. Missing or implausible data will be highlighted for the PI requiring appropriate responses (i.e., confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

14.3.4 Coding

All medical terms will be coded using CTCAE (version 4).

14.3.5 Study Closure

Following completion of the studies, the PI will be responsible for ensuring the following activities:

- Data clarification and/or resolution
- Accounting, reconciliation, and destruction/return of used and unused study drugs
- Review of site study records for completeness
- Shipment of all remaining laboratory samples to the designated laboratories

15 STATISTICAL METHODS AND DATA ANALYSIS

All statistical analysis will be performed under the direction of the statistician designated in key personnel. Any data analysis carried out independently by the investigator must be approved by the statistician before publication or presentation.

15.1 Analysis Sets

EGFRvIII CAR Efficacy Analysis Set: Efficacy analyses will focus on two groups of patients: all patients, and the subgroup of patients who receive EGFRvIII CAR.

Overall EGFRvIII CAR Safety Analysis Set: For monitoring purposes and for reporting to appropriate agencies, safety analyses will include all patients who receive any EGFRvIII CAR treatment.

MTD Analysis Set: For the determination of MTD, patients without DLT who do not complete 4 weeks of follow-up after CAR infusion or do not complete the intended infusion will be excluded from the Overall EGFRvIII CAR Safety Analysis Set to create the MTD Analysis Set.

15.2 Patient Demographics and Other Baseline Characteristics

Patient clinical and socio-demographic characteristics will be summarized for all patients enrolled on this study, as well as the subgroup of patients who receive EGFRvIII CAR treatment. Categorical descriptors will be summarized using frequency distributions; whereas, interval variables will be summarized using percentiles, as well as means and standard deviations.

15.3 Treatments

The number of patients who receive EGFRvIII CAR treatment will be summarized by dose level.

15.4 Primary Objective

Within this “3+3” phase I study, the primary objective is to determine the MTD of a single IV infusion of EGFRvIII CAR T cells in patients with newly-diagnosed WHO grade IV malignant glioma. Four dose levels will be considered based on transduced cells/kg: #1: 4.5×10^6/kg, #2: 1.5×10^7/kg, #3: 4.5×10^7/kg, and #4: 1.5×10^8/kg. The MTD is the highest dose level at which ≤1 or 6 patients experience dose-limiting toxicity during the 4 weeks after CAR infusion. Section 9.1.2 provides information about what constitutes a dose-limiting toxicity. Section 9.1.1 provides details concerning the determination of the MTD.

15.5 Secondary Objectives

To determine the DLT of a single IV infusion EGFRvIII CAR T cells in patients with newly-diagnosed WHO grade IV malignant glioma. The proportion of patients who experience DLT within each dose level will be tabulated.

15.6 Exploratory Objectives

1. To determine if CARs transferred into patients with WHO grade IV malignant glioma being treated with TMZ expand.
2. To determine if EGFRvIII CARS persist and remain functional in the periphery.
3. To assess whether EGFRvIII CARs traffic to the site of EGFRvIII expressing WHO grade IV malignant glioma.
4. To describe the survival experience of patients with newly diagnosed, EGFRvIII-expressing WHO grade IV malignant glioma treated with EGFRvIII CAR T cells.
5. To describe the progression free survival experience of EGFRvIII-expressing newly-diagnosed WHO grade IV malignant glioma patients treated with EGFRvIII CAR T cells.

The analyses that address the exploratory correlative study objectives will be exploratory and descriptive. Based upon PBMCs collected from the peripheral blood, the median number of EGFRvIII CARs, the median percentage of $CD4^+$ and $CD8^+$ lymphocytes that are CARs, and the median level of KI67 will be estimated at each assessment time. Assuming laboratory analyses demonstrate

CARs are present in the periphery, functional analyses may be conducted focusing on specific functions or time points. Generalized linear models may be used to explore how these phenotypes change over time as a function of dose.

Among patients within the expansion cohort who have been treated with ^{111}In-labeled EGFRvIII CARs, the median proportion of injected ^{111}In-labeled EGFRvIII CARs present in tumors will be estimated at each assessment point. If the planar image provides no signal and the SPECT/CT is not conducted, we will impute the number of ^{111}In-labeled EGFRvIII CARs present in tumors as 0.

Two sets of efficacy analyses will be conducted. One set of analysis will focus on all patients who enroll in the study whether or not EGFRvIII CAR treatment is administered, and the other set of analyses will focus on patients who receive EGFRvIII CAR treatment. Among all patients, Kaplan-Meier methods will graphically describe their survival from the time of study enrollment or histologic diagnosis. Among patients who receive CAR treatment, Kaplan-Meier methods will also be used to describe the distribution of overall survival and progression-free survival. In these analyses, OS and PFS will be computed from the date that CAR treatment was initiated. OS is defined as the time between the landmark (e.g. histologic diagnosis or initiation of CAR treatment) and death or date of last follow-up. PFS is defined as the time between the landmark and first progression, death, or last follow-up if the patient remains alive without disease progression.

15.7 Sample Size Calculation

After completion of the expansion cohort, we anticipate that 18 patients (6 without ^{111}In-labeled EGFRvIII CARs and 12 with) will have been treated at the MTD. One motivation for treating additional patients at the MTD is to more precisely estimate the rate of DLT at the MTD. Tabulated below as a function of the DLT rate is the half width of the associated 95% confidence interval:

Table 7. Half Width of 95% Confidence Interval as a Function of the DLT Rate

DLT Rate	Half Width of 95% Confidence Interval
0.05	0.025
0.1	0.032
0.15	0.039
0.2	0.044
0.25	0.047

16 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

16.1 Regulatory and Ethical Compliance

This protocol was designed and will be conducted and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable federal, state, and local regulations.

16.2 DUHS Institutional Review Board and DCI Cancer Protocol Committee

The protocol, informed consent form, advertising material, and additional protocol-related documents must be submitted to the DUHS IRB and DCI CPC for review. The study may be initiated only after the Principal Investigator has received written and dated approval from the CPC and IRB.

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form. The CPC should be informed about

any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, statistical analysis, etc.).

The Principal Investigator must obtain protocol re-approval from the IRB within 1 year of the most recent IRB approval. The Principal Investigator must also obtain protocol re-approval from the CPC within 1 year of the most recent IRB approval, for as long as the protocol remains open to subject enrollment.

16.3 Informed Consent

The informed consent form must be written in a manner that is understandable to the subject population. Prior to its use, the informed consent form must be approved by the IRB.

The Principal Investigator or authorized key personnel will discuss with the potential subject the purpose of the research, methods, potential risks and benefits, subject concerns, and other study-related matters. This discussion will occur in a location that ensures subject privacy and in a manner that minimizes the possibility of coercion. Appropriate accommodations will be made available for potential subjects who cannot read or understand English or are visually impaired. Potential subjects will have the opportunity to contact the Principal investigator or authorized key personnel with questions, and will be given as much time as needed to make an informed decision about participation in the study.

Before conducting any study-specific procedures, the Principal Investigator must obtain written informed consent from the subject. The original informed consent form will be stored with the subject's study records, and a copy of the informed consent form will be provided to the subject. The Principal Investigator is responsible for asking the subject whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Principal Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

16.4 Privacy, Confidentiality, and Data Storage

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained. RDSPs will be approved by the appropriate institutional Site Based Research group.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his family, if desired. For all future visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private exam room. All research related interactions with the participant will be conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using an Oracle Clinical database, which is housed by the DCI. Access to electronic databases will be limited to the Principal Investigator, key personnel, statisticians, the Radiolabeled Pharmacy personnel, and the BTC data manager. Data stored on portable memory devices will be de-identified. The security and viability of the IT infrastructure will be managed by the DCI and/or Duke Medicine.

Upon completion of the study, research records will be archived and handled per DUHS HRPP policy.

Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

16.5 Data and Safety Monitoring

Data and Safety Monitoring will be performed in accordance with the DCI Data and Safety Monitoring Plan. For a more detailed description of the DSMP for this protocol, refer to Sections 11 and 12 and to Section 12.1 in eIRB for the Research Data Security Plan (available upon request).

16.6 Protocol Amendments

All protocol amendments must be initiated by the Principal Investigator and approved by the IRB prior to implementation. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an immediate hazard. However, the Principal Investigator must inform the IRB and all other applicable regulatory agencies of such action immediately.

Though not yet required, the CPC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, etc.).

16.7 Records Retention

The Principal Investigator will maintain study-related records for the longer of a period of:

- at least two years after the date on which a New Drug Application is approved by the FDA
- at least two years after formal withdrawal of the IND associated with this protocol
- at least six years after study completion (Duke policy).

16.8 Conflict of Interest

The Principal Investigator and Sub-Investigators must comply with applicable federal, state, and local regulations regarding reporting and disclosure of conflict of interest. Conflicts of interest may arise from situations in which financial or other personal considerations have the potential to compromise or bias professional judgment and objectivity. Conflicts of interest include but are not limited to royalty or consulting fees, speaking honoraria, advisory board appointments, publicly-traded or privately-held equities, stock options, intellectual property, and gifts.

The Duke University School of Medicine's RIO reviews and manages research-related conflicts of interest. The Principal Investigator and Sub-Investigators must report conflicts of interest annually and within 10 days of a change in status, and when applicable, must have a documented management plan that is developed in conjunction with the Duke RIO and approved by the IRB/IEC.

16.9 Registration Procedure

After patients have been enrolled, protocol-specific information and subject registration will be entered into the Velos e-Research software system. This system is managed by the School of Medicine.

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18 APPENDICES

18.1 Research Summary

Please see this Duke-specific document as a separate upload in the Duke eIRB (available upon request).

18.2 Figures and Schemas (in order of reference in protocol)

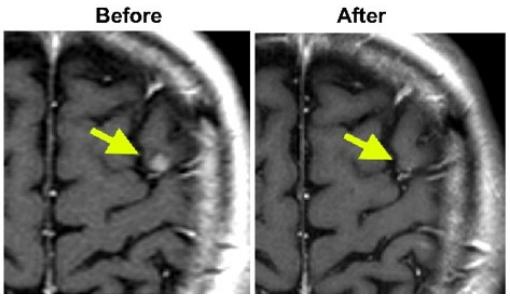

Figure 2. Highly Reactive Transferred Cells Traffic to Brain and Destroy Tumor Deposit in Melanoma Patients. Highly reactive transferred cells traffic to and destroy brain metastases in melanoma patients. Arrows in melanoma patient represent location of melanoma metastases.

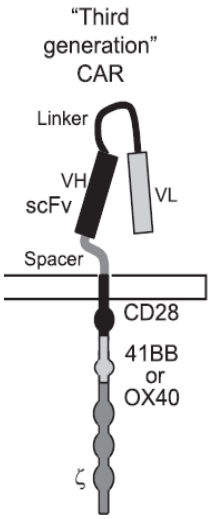

Figure 3. Design of 3rd Generation EGFRvIII CAR. Third-generation CARs incorporate at least two co-stimulatory molecule endodomains, such as the endodomains of CD28 and 41BB. VH and VL were derived from the anti-EGFRvIII-specific clone 139.

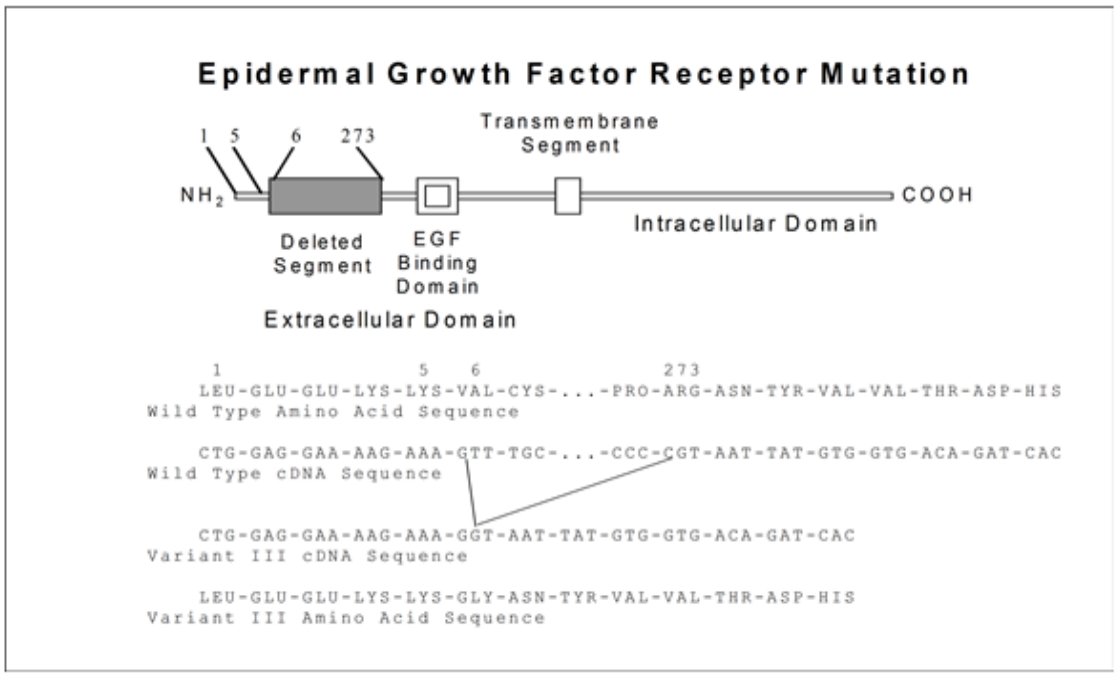

Figure 4. EGFR Mutation

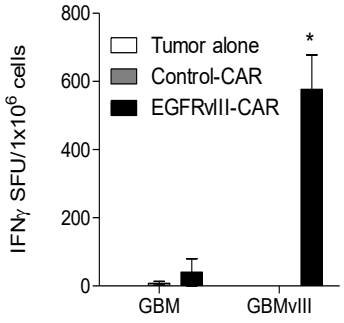

Figure 5. CAR Transduced Murine T-Cells Specifically Recognize EGFRvIII Expressing GBM. EGFRvIII CARs were incubated with EGFRvIII-positive and negative GBM and submitted to IFNγ ELISpot assay. (*P<0.0022, Two-way ANOVA)

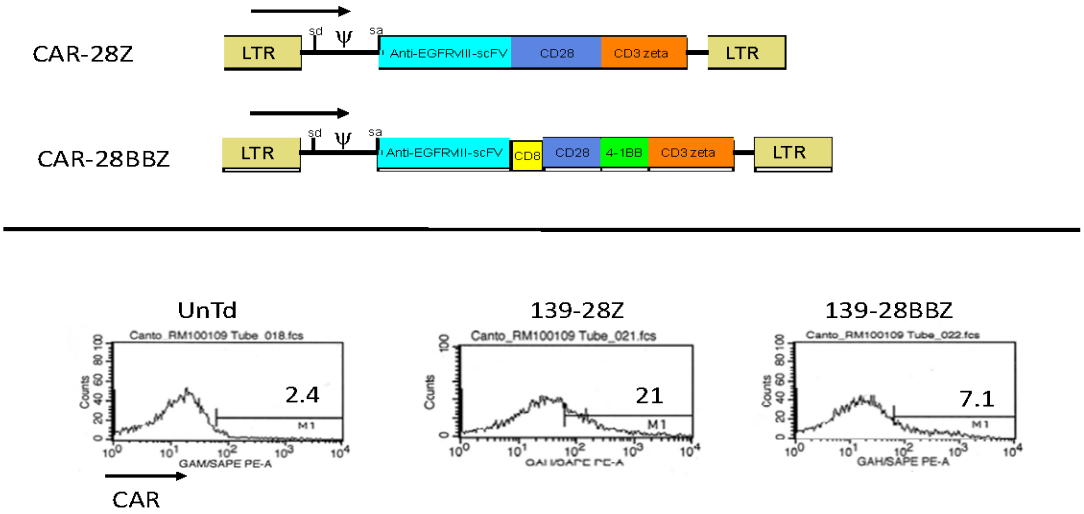

Figure 6. Construction and Analysis of Anti- EGFRvIII CAR Vectors. Shown on the top of the figure is a diagram of the two CAR vector designs showing the relative locations of the scFv and T cell signaling domains. In the lower half of the figure, CAR vectors based on human mAb 139 were used to engineer 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.

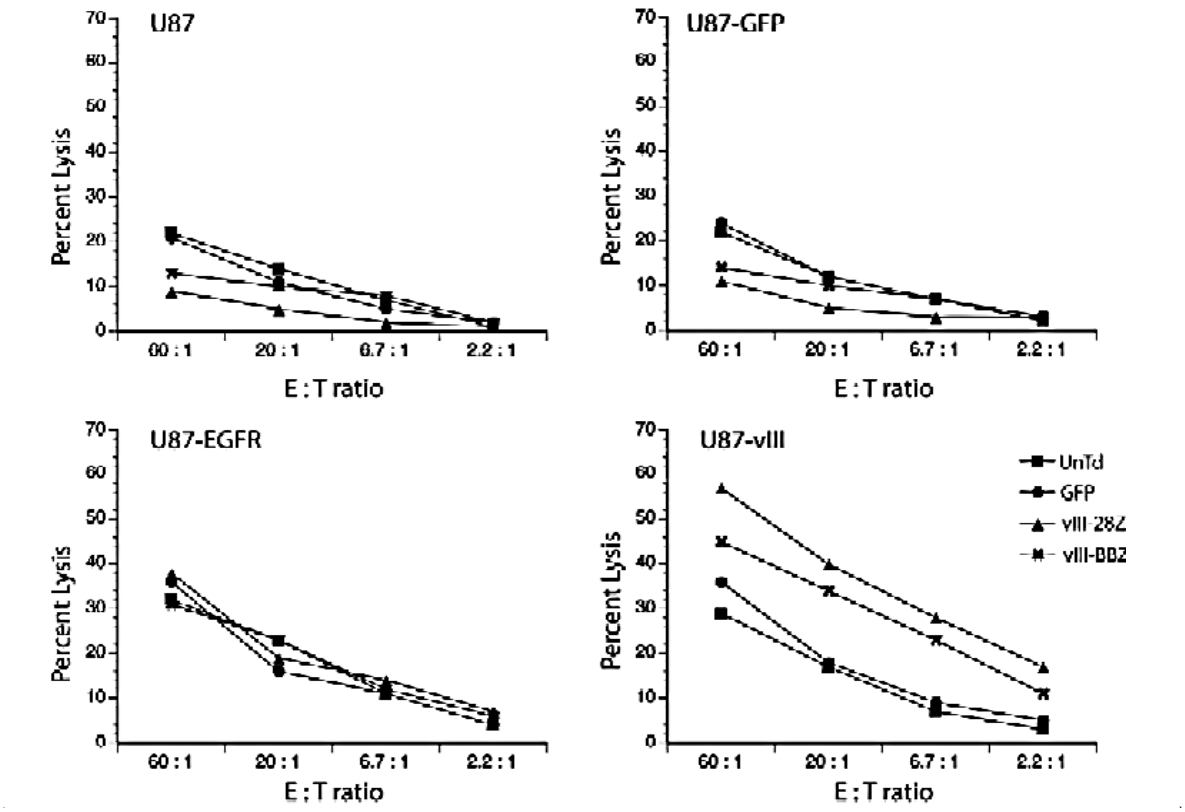

Figure 7. Specific Lysis of U87 EGFRvIII by Anti-EGFRvIII CAR Transduced T Cells.

Anti-EGFRvIII CAR transduced human PBL were co- cultured for 4h with indicated 51Cr labeled target tumor cell lines (parent U87, GFP, 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 GFP vector (GFP), 139-28Z CAR (vIII-28Z), and 139-28BBZ (vIII-BBZ) human PBL are plotted on the graph as indicated.

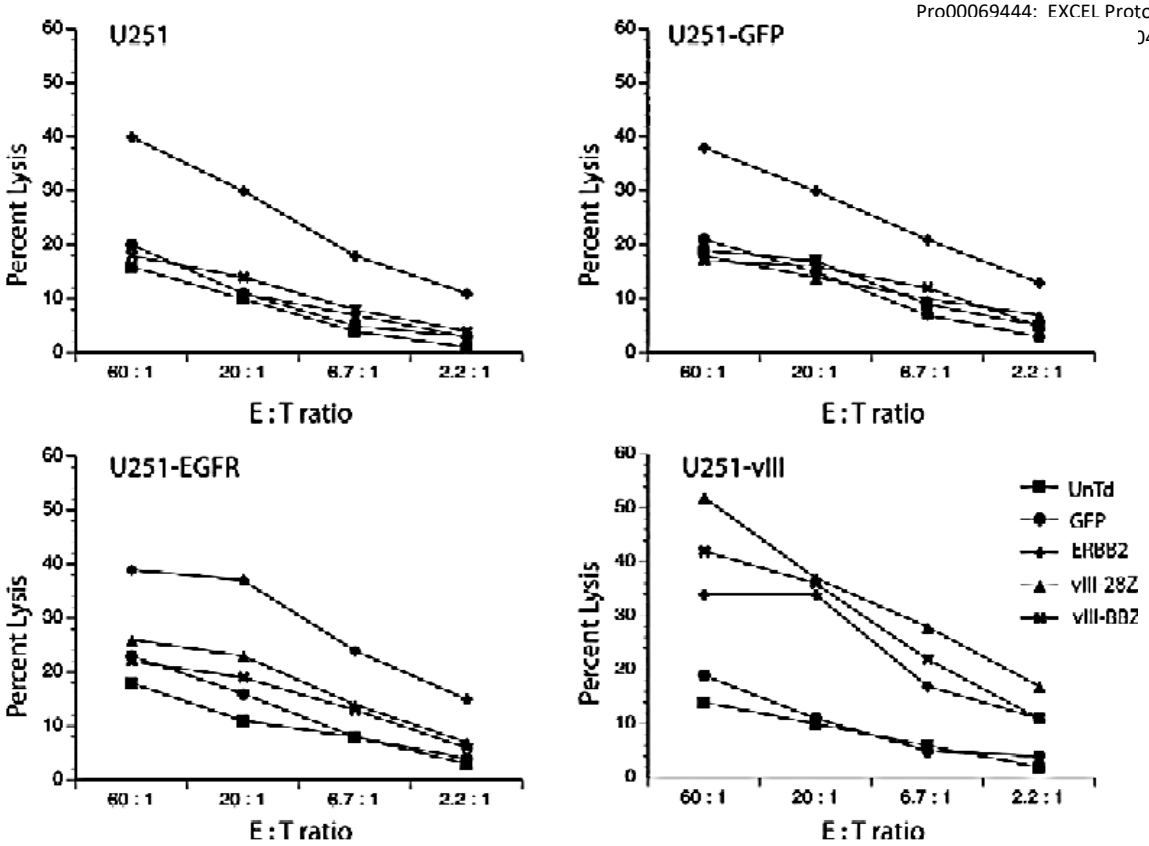

Figure 8. Specific Lysis of U251 EGFRvIII by Anti-EGFRvIII CAR Transduced T Cells.

Anti-EGFRvIII CAR transduced human PBL were co-cultured for 4h with indicated 51Cr labeled target tumor cell lines (parent U251, GFP, 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 GFP 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.

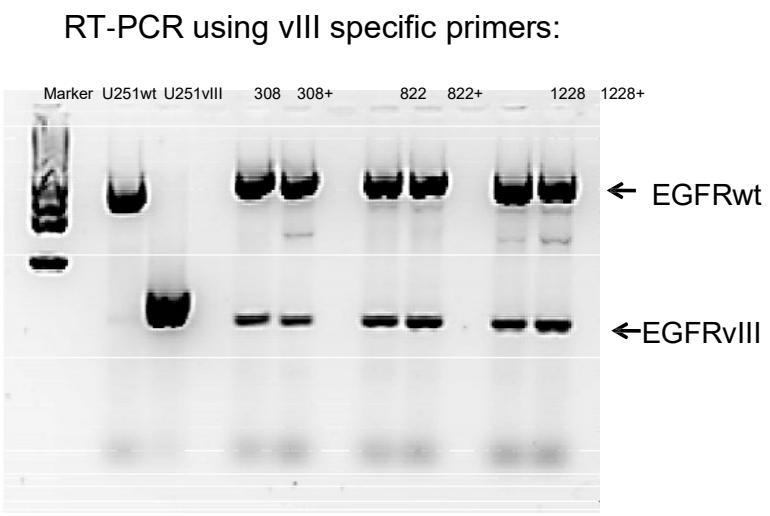

Figure 9. Expression of EGFRvIII in Glioma Tumor Stem Cell Lines.

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.

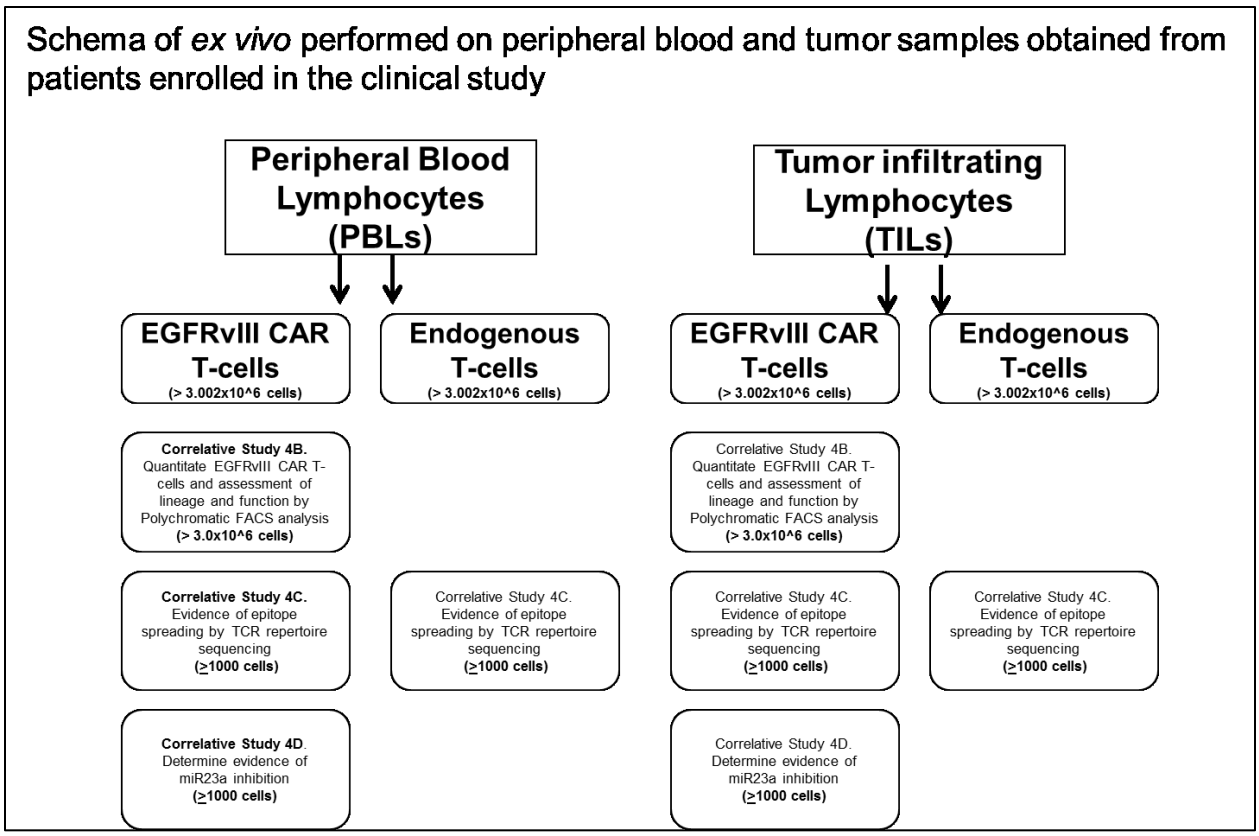

Figure 10. Correlative Studies Schema

Table 8. Frequency and Duration of Objective Responses

Frequency and Duration of Objective Responses									
		PR			CR			OR	
TBI	Total No. of Patients	No.	%	Duration (months)	No.	%	Duration (months)	No.	%
None*	43	17	39.5	64+, 32+, 20+, 29, 28, 14, 13, 11, 8, 8, 7, 4, 3, 3, 2, 2, 2	4	9.3	63+, 58+, 48+, 47+	21	48.8
2 Gy	25	11	44.0	33+, 29+, 23+, 14, 10, 6, 5, 5, 4, 3, 3	2	8.0	37+, 25+	13	52.0
12 Gy	25	14	56.0	14+, 13+, 10+, 7+, 7+, 7+, 6+, 6+, 4+, 7, 6, 6, 4, 3	4	16.0	17+, 15+, 13+, 8+	18	72.0

NOTE. All patients received cyclophosphamide 60 mg/kg X 2 days + fludarabine 25 mg/m^2 X 5 days.
Abbreviations: TBI, total-body irradiation; PR, partial response; CR, complete response; OR, objective response; TIL, tumor-infiltrating lymphocytes.