Repeated peripheral infusions of anti-EGFRvIII CAR T cells in combination with pembrolizumab show no efficacy in glioblastoma: a phase 1 trial

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nature cancer

Article

https://doi.org/10.1038/s43018-023-00709-6

Repeated peripheral infusions of anti-EGFRvIII CAR T cells in combination with pembrolizumab show no efficacy in glioblastoma: a phase 1 trial

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Phase I Study of EGFRvIII-Directed CAR T cells Combined with PD-1 Inhibition in Patients with Newly Diagnosed, *MGMT*-Unmethylated Glioblastoma

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Regulatory Sponsor	University of Pennsylvania
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Funding Sponsor	Novartis
Protocol Numbers	UPCC# 13318; Penn IRB# 831706
Main Investigational Products	EGFRvIII redirected autologous T cells (CART-EGFRvIII cells); Pembrolizumab
IND Number	15968
Sponsor Cell Manufacturing-University of Pennsylvania	Clinical Cell and Vaccine Production Facility (CVPF)
Correlative Laboratory Team:	Translational and Correlative Studies Laboratory (TCSL) University of Pennsylvania
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Date:	V1.08-31-2018 V2.11-01-2018 V3.03-05-2019 V4.08-01-2019 V5.01-02-2020

STUDY SUMMARY

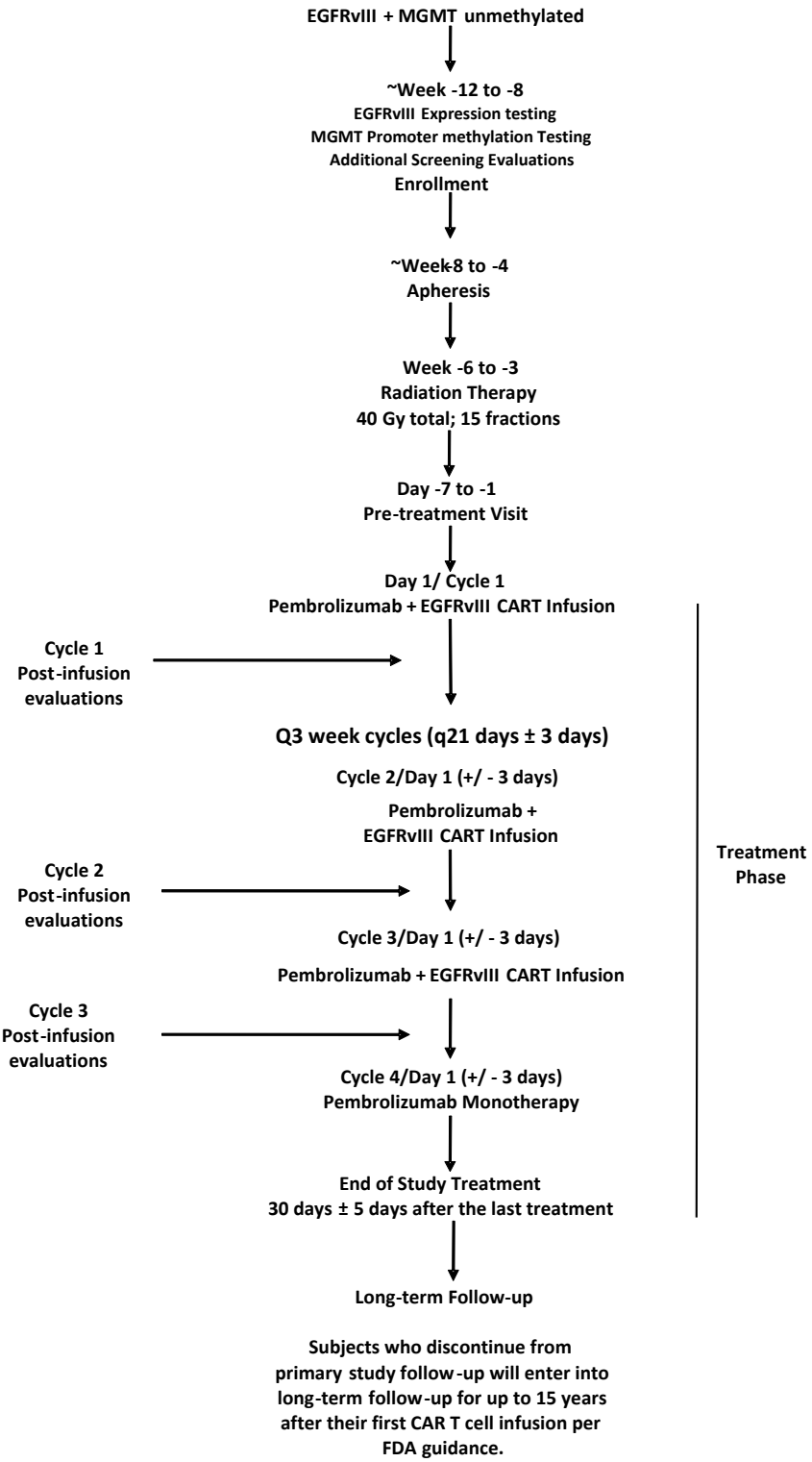
Title	Phase 1 Study of EGFRvIII-Directed CAR T cells Combined with PD-1 Inhibition in Patients with Newly Diagnosed, *MGMT*-Unmethylated Glioblastoma
Short Title	CART-EGFRvIII + Pembrolizumab in GBM
Protocol Numbers	UPCC#13318; Penn IRB#831706; IND#15968
Phase	Phase 1
Methodology	This is an open-label, phase 1 study to assess the safety and tolerability of EGFRvIII T cells in combination with pembrolizumab (PD-1 Inhibitor) in patients with newly diagnosed, EGFRvIII+, *MGMT*-unmethylated GBM. This population also includes patients with diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV.
Study Duration	The protocol will require approximately 12 months to complete enrollment. Each subject will be followed for up to 15 years post-infusion as part of this study.
Study Center(s)	Single-center at the University of Pennsylvania
Number of Subjects	Up to 7 evaluable subjects
Study Design	This is a single-center, single-arm, open-label phase 1 study to determine the safety and tolerability of CART-EGFRvIII cells in combination with pembrolizumab in patients with newly diagnosed, EGFRvIII+, *MGMT*-unmethylated glioblastoma/diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV (GBM), post-surgical resection. All patients with GBM tumors resected at the Hospital of the University of Pennsylvania undergo EGFRvIII expression testing and *MGMT* promoter methylation testing as part of the standard neuropathology work flow for newly diagnosed gliomas. This testing is performed at either the Center for Personalized Diagnostics in the Department of Pathology at the University of Pennsylvania or by NeoGenomics Laboratories, which are both CLIA certified laboratories. In order to participate in this study, the subject's tumor must be EGFRvIII positive and negative for *MGMT* promoter methylation according to tests performed by one of these laboratory facilities. Subjects will receive a short course of adjuvant radiation to the brain with a total dose of 40 Gy, administered over 3 weeks (15 fractions). Study treatment (CART-EGFRvIII cells and pembrolizumab) will begin 2-3 weeks after completing a short course regimen of adjuvant radiation therapy (Cycle 1/Day 1). Thereafter, subjects will receive CART-EGFRvIII cells + pembrolizumab in 3 week cycles. Up to 3 infusions of CART-EGFRvIII cells may be received depending on product availability. Up to 4 infusions of

	Pembrolizumab may be received as long as the subject continues to derive clinical benefit. For the first three subjects enrolled, infusions will be staggered such that a new subject is not infused until 21 days after the 1st infusions of CART-EGFRvIII cells + pembrolizumab for the previous subject, to allow for DLTs to be evaluated. A safety pause will occur until all three subjects have completed 21 days of follow-up and a formal DLT assessment is performed by the Medical Director and Principal Investigator, protocol stopping rules have been assessed (per **Section 8.4.1**), and any recommended protocol changes have been implemented and approved by appropriate oversight bodies. If no DLTs were identified per Medical Director and PI assessment, subsequent subjects may be enrolled without timing restrictions. Subjects will be followed for up to 15 years after initiation of study treatment to assess long-term adverse effects of the CAR T cells.
Objectives	Primary Objective: 1) Determine the safety and tolerability of administering multiple infusions of CART-EGFRvIII cells in combination with a PD-1 inhibitor (pembrolizumab) in the treatment of newly diagnosed, *MGMT*-unmethylated GBM. Secondary Objectives: 1) Describe overall survival 2) Describe progression-free survival (PFS) based on standard MRI evaluation using modified RANO criteria. 3) Describe objective response rate (ORR) Exploratory/Correlative Objectives: 1) Determine the persistence of modified CART-EGFRvIII cells in the peripheral blood and tumor. 2) Determine bioactivity of modified CART-EGFRvIII cells in the peripheral blood by modulation of systemic soluble factors (cytokines, chemokines, growth factors). 3) Evaluate development of secondary anti-tumor responses as a consequence of CART-EGFRvIII cells induced epitope spreading (if feasible) by: a. Using high throughput antibody screening. b. Performing exome and transcriptome gene analyses of the resected tumor by next generation sequencing (NGS) and evaluating whether cellular (i.e., T-cell) and/or humoral (i.e., B-cell) responses are elicited against neo-antigens that are found by the NGS (i.e., detection of epitope spreading against neo-antigens).

	4) Use plasma cell-free DNA (cfDNA) and RNA (cfRNA) as correlative measures of EGFRvIII-directed activity. 5) Where post-treatment tumor material or CSF is obtained as part of routine care, a portion of the sample will be collected for research purposes for the following testing: a. Measure trafficking of EGFRvIII-transduced cells to tumor by Q-PCR. b. Measure EGFRvIII expression to determine escape variants by next generation sequencing, immunohistochemistry, and/or fluorescent in situ hybridization (FISH). c. Determine evidence of anti-tumor immune activity using high throughput assays. d. Use advanced magnetic resonance imaging sequences and analysis to assess relative tumor blood volume (which has been shown to correlate with expression of EGFRvIII) by perfusion imaging; assess markers of pseudoprogression by MR spectroscopy; and assess axonal pathway integrity by diffusion tensor imaging. e. Use a newly developed plasma cell-free DNA assay as a correlative measure of disease response and EGFRvIII-directed activity.
Diagnosis and Main Inclusion Criteria	Adult patients with newly diagnosed, *MGMT*-unmethylated, EGFRvIII positive GBM who have undergone tumor resection. This population also includes patients with diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV.
Investigational Products, Dose, and Regimen	Investigational Agent(s): • Pembrolizumab: programmed death receptor-1 (PD-1)-blocking antibody • CART-EGFRvIII cells: autologous T cells transduced with a lentiviral vector to express anti-EGFRvIII scFv 41BB:TCRζ. Dose and Route of Administration: • Pembrolizumab: 200mg by intravenous infusion over 30 minutes • CART-EGFRvIII cells: $2x10^8$ cells by intravenous infusion; Minimum acceptable dose for infusion is $2x10^7$. ○ Dosing plan includes a de-escalation scheme to be considered in the event of dose-limiting toxicity (See **Section 8.4.1**). The de-escalated dose is $2x10^7$. Regimen: • Pembrolizumab: q3 week cycles for up to four total infusions • CART-EGFRvIII cells: q3 week cycles for up to three total infusions
Reference therapy	None
Statistical Methodology	The statistical analysis will be primarily descriptive in keeping with the phase I nature of the study. The primary objective for this study will be safety and tolerability. Frequency and severity of adverse events (AEs) will be

	summarized and tabulated by organ system for both overall and within major categories and by grade. Exact 90% confidence intervals (CI) will be produced for adverse event rates and DLT rates. For tolerability, the proportion of subjects that completed the scheduled infusions will be computed. Changes or abnormal laboratory values and vital signs from time of infusion will be summarized descriptively including mean, medians, standard deviation, and inter-quartile range. Secondary endpoints include overall survival (OS), progression-free survival (PFS) and objective response rate (ORR). The survival function of OS and PFS will be estimated by Kaplan-Meier method with 90% confidence interval if appropriate. Median survival time if reached will be presented. ORR will be computed as a proportion along with the associated exact 90% CI. Descriptive statistics will be calculated for correlative endpoints including mean, median, standard deviation, inter-quartile range for continuous variables (e.g., number of percent of EGFRvIII-transduced cells) and frequency and proportions for discrete variables). 90% confidence interval appropriate for each statistic will be used. The change of CART-EGFRvIII cells or other anti-tumor measurements and biomarkers over time will be examined graphically. Up to 7 evaluable patients will be enrolled. All subjects infused with at least one infusion of EGFRvIII-transduced cells (minimum acceptable dose for infusion is $2x10^7$) will be considered evaluable and included in analysis of primary, secondary, and exploratory/correlative endpoints. Infusions of the first three subjects will be staggered to allow for DLTs to be evaluated. A safety pause will occur until the first three subjects have completed 21 days of follow-up and a formal DLT assessment is performed by the Medical Director and Principal Investigator, in which protocol stopping rules are assessed (per **Section 8.4.1**) before proceeding with enrollment of additional subjects.

Figure 1: Study Schema



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LIST OF ABBREVIATIONS

AE, adverse event

ALL, acute lymphoblastic leukemia

AML, acute myeloid leukemia

CAR, chimeric antigen receptor

CART, T cells expressing a CAR construct

CART19, T cells expressing a CD19 specific CAR construct

CCI, Center for Cellular Immunotherapies

CD3ζ, signaling domain found in the intracellular region of the TCR/CD3 zeta, gamma and epsilon chains,

CD3z, identical to CD3ζ

CD137, 4-1BB costimulatory molecule

CFR, code of federal regulations

CLL, chronic lymphocytic leukemia

CRF, case report form

CRM, Continual Reassessment Method

CRS, Cytokine Release Syndrome

CTL, cytotoxic T lymphocyte

CTX, cyclophosphamide

CVPF, Clinical Cell and Vaccine Production Facility at the University of Pennsylvania

DSMB, Data and Safety Monitoring Board

DSMC, Data and Safety Monitoring Committee

DLT, dose-limiting toxicity

EDC, electronic data capture

EGFR, Epidermal growth factor receptor

FDA, food and drug administration

GBM, glioblastoma; for the purposes of this study this also includes diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV

GCP, good clinical practices

GMP, good manufacturing practices

HACA, human anti-CAR antibody

HAMA, human anti-mouse antibody

HLH, hemophagocytic lymphohistiocytosis

IBC, Institutional Biosafety Committee

IDH, Isocitrate dehydrogenase

IFN, interferon

IL, interleukin

IMiD, Immunomodulatory drug

ION, inferior olivary nucleus

IRB, Institutional Review Board

irRC, immune related response criteria

ISH, in situ hybridization

IVIG, intravenous immunoglobulin

MABEL, Minimum Anticipated Biological Effect Level

MAS, Macrophage Activation Syndrome

MFC, multiparameter flow cytometry

MRD, minimal residual disease

OBD, optimal biologic dose

OS, overall survival

PBMC, peripheral blood mononuclear cells

PCAM, Perelman Center for Advance Medicine at the University of Pennsylvania

PDAE, protocol-defined adverse event

PDSAE, protocol-defined serious adverse event

PFS, progression free survival

RCL, replication competent lentivirus

ROA, route of administration

SAE, serious adverse event

scFv, single chain fragment variable, the antigen specific portion of an antibody

SUSAR, suspected unexpected serious adverse reaction

TCR, T cell receptor

TCR-ζ or CD3ζ signaling domain found in the intracellular region of the TCR/CD3 zeta, gamma and epsilon chains,

TCRz or CD3z, identical to TCR-ζ or CD3ζ

TCSL, Translational and Correlative Studies Laboratory at the University of Pennsylvania

TERT, Telomerase reverse transcriptase

TNF, tumor necrosis factor

UPENN, University of Pennsylvania

1. INTRODUCTION

This study is to be conducted according to Good Clinical Practice as implemented by the FDA (FDA Title 21 part 312, International Conference on Harmonization guidelines, and other applicable government regulations and Institutional research policies and procedures.

This is a phase I study to evaluate the safety and tolerability of a combination immunotherapy using CART cells targeting the tumor antigen EGFRvIII and an immune checkpoint inhibitor that inhibits the interaction off programmed cell death protein 1 (PD-1) and its ligand PD-L1, in patients diagnosed with GBM. This study builds on the results of the University of Pennsylvania sponsored phase I study of the safety of CART-EGFRvIII cells administered in one dose in glioblastoma patients (NCT02209376/UPCC#35313/IND#15968/ IRB#820381). Infusion of $5x10^8$ CART-EGFRvIII cells was safe, the cells were able to expand in the host and were able to reach the glioblastoma tumor in the brain. In addition, there was no cross-reactivity of CART-EGFRvIII cells with the wild-type EGFR normally expressed by human tissues. Some patients required surgical resection of tumor after they had received CART-EGFRvIII cells. In situ evaluation of the tumor environment demonstrated increased and robust expression of inhibitory molecules, such as PD-L1, after CART-EGFRvIII infusion, compared to pre–CART-EGFRvIII infusion tumor specimens. Therefore, we hypothesized that using a combination therapy of CART-EGFRvIII cells and an immune checkpoint inhibitor administered at least twice, will improve the outcome of the treatment.

Safety, tolerability and immunologic effects of PD-1 blockade are currently clinically evaluated in patients with malignant gliomas in several clinical trials (NCT02017717, NCT02617589). So far, anti PD-1 inhibitor Nivolumab proved to be well tolerated, with treatment related adverse events occurring in 18% of patients, with no additional reported immune-related adverse events (**Simonelli et al., 2018**). However, no clinical trials have addressed so far the safety and tolerability of combination immunotherapy of CART cells and immune checkpoint inhibitors.

Compared to our previous study using CART cell in glioblastoma (NCT02209376), the one we propose here is designed to test multiple infusions of CART-EGFRvIII cells in combination with the PD-1 inhibitor pembrolizumab (obtained commercially for research purposes). Since CART-EGFRvIII cells will be infused at least twice, the dose tested in this study will be $2x10^8$ cells. The dose of the PD-1 checkpoint inhibitor pembrolizumab administered per infusion will be 200mg administered q3 weeks (safety information regarding this dose is presented in the pembrolizumab package insert).

As a phase I clinical trial, the primary objective is to determine the safety and tolerability of administering multiple infusions of CART-EGFRvIII cells in combination with a PD-1 inhibitor (pembrolizumab). The secondary objectives will be to describe the overall survival, the progression-free survival and the objective response rate to the treatment.

The sections below provide detailed rationale and supporting data for investigating the safety and tolerability of combining two immunotherapies, one targeting the EGFRvIII tumor antigen and one targeting the PD-1 immune checkpoint, for treatment of GBM, which currently has no curative treatment and a dismal survival prognosis after tumor resection.

1.1 Background

1.1.1 GBM

GBM is a malignant, often rapidly progressive primary brain tumor derived from astrocytic cells. GBM represents ~15% of all primary brain tumors and ~55% of all gliomas (**Ostrom et al., 2016)**. As a result, GBM is the most common primary malignant brain tumor in adults, with an estimated 12,390 new cases predicted in the United States in 2017 (**Ostrom et al., 2016**). The disease is approximately 5 times more likely to occur in elderly patients (65+ years of age) than in younger patients (**Chakrabarti et al., 2005**), and has a slight predilection towards males (**Ohgaki and Kleihues, 2005**). Common clinical presentations of GBM include headache, seizures, memory loss, motor weakness, and other neurological signs and symptoms (**Chang et al., 2005**). Typical radiographic findings on magnetic resonance imaging (MRI) include a necrotic mass that is hypointense on T1-weighted images and enhances heterogeneously following contrast infusion (**Scott et al., 2002**).

Histopathologically, GBM is characterized by poorly differentiated neoplastic astrocytes, cellular polymorphism, nuclear atypia, mitotic activity, vascular thrombosis, microvascular proliferation, and necrosis (most commonly "pseudo-palisading" necrosis) (**Reni et al., 2017)**. In the 2016 World Health Organization (WHO) classification of Central Nervous System (CNS) tumors, GBMs are divided in to "GBM, Isocitrate Dehydrogenase (IDH)-wildtype (about 90% of cases, also referred to as "primary" or *de novo* GBM) and "GBM, IDH-mutant" (about 10% of cases, also referred to as "secondary" GBM due to the evolution of these tumors from lower grade precursor gliomas) (**Louis et al., 2016**). IDH mutation status is significantly associated with clinical outcomes, with IDH-mutant tumors being independently associated with improved prognosis. The other major molecular marker with clinical significance in GBM is the methylation status of the promoter of the O6-methylguanine-DNA-methyltransferase (*MGMT)* gene. MGMT is a DNA damage repair protein that removes the guanine-alkyl group and prevents apoptosis (**Ludwig and Kornblum, 2017**). Thus, MGMT mediates resistance to alkylating chemotherapy, and its loss makes tumors more sensitive to alkylating agents. Expression of *MGMT* is tightly regulated by methylation of its promoter, which leads to decreased expression of this protein and ultimately increased response to alkylating chemotherapy treatment (**Ludwig and Kornblum, 2017**). *MGMT* promoter methylation is found in about 40% of GBMs and, importantly, is associated with improved prognosis even without regard to whether alkylating chemotherapy is administered (**Ludwig and Kornblum, 2017**).

A working group of the Consortium to inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) made recommendations for a new integrated diagnosis for a subset of IDH-wildtype astrocytomas that exhibit an aggressive clinical course similar to GBM but do not meet histopathologic criteria for GBM. Based on expert opinion and an extensive literature review, cIMPACT-NOW established that histologic IDH-wildtype astrocytoma of WHO grade II or III can be considered "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV", if one of the following is present: a). high-level amplification of EGFR; or b). combined whole chromosome 7 gain and whole chromosome 10 loss (+7/-10); or c). *TERT* promoter mutation (**Brat et al., 2018**).

The treatment standard for newly diagnosed GBM since 2005 has been maximal safe surgical resection, followed by adjuvant radiation therapy (RT) with the oral alkylating agent temozolomide (TMZ) administered once daily concurrently (**Stupp et al., 2005**). After completion of RT/TMZ and a ~4-week treatment break, TMZ is continued alone as a maintenance therapy for a minimum of 6 months with the option of adding tumor-treating fields (TTFields) therapy (see next paragraph for full explanation; (**Stupp**

et al., 2017). No treatment has been shown to improve overall survival once the tumor has recurred after standard first-line treatment, although bevacizumab is approved by the US Food and Drug Administration (FDA) in this setting due to its impact on progression-free survival (PFS) (**Lai et al., 2011**). Despite recent advances in surgery and RT, the prognosis for patients with GBM treated with this regimen is dismal, with a median overall survival (OS) of just 14-17 months (**Herrlinger et al., 2016**). Furthermore, due to the high degree of tumor heterogeneity in GBM and the presence of the blood-brain barrier (**Patel et al., 2014**), the use of targeted therapies across hundreds of trials has yet to result in an improvement in outcomes in GBM. Thus, this disease remains incurable in 2017. Furthermore, the ~60% of GBM patients whose tumors harbor an unmethylated *MGMT* promoter gain very little, if any, benefit from TMZ (**Hegi et al., 2005; Hegi and Stupp, 2015**). Effective treatment for *MGMT*-unmethylated GBM therefore represents a significant unmet medical need, and multiple past and current trials in this population have omitted TMZ altogether in favor of alternative investigational agents (**Raizer et al., 2016; Wick et al., 2016**)(NCT02617589; NCT03050736).

1.1.2 Immunology of CNS Tumors

Several recent discoveries have prompted enthusiasm for immunotherapy in GBM. First, lymphatic vessels in the brain were recently discovered, and there is now clear evidence that T cells can traffic across the blood-brain barrier (**Louveau et al., 2015**). Second, it has been shown that an intrinsic tumor-associated T cell response exists in human GBM (**Sims et al., 2016**). Third, and perhaps most importantly, immunotherapy for GBM may overcome the challenges posed by its extensive molecular heterogeneity, as a robust anti-tumor immune response may include T cell clones specific to multiple different tumor antigens (**Blank et al., 2016**).

Programmed Death-1 (PD-1) is a critical immune checkpoint receptor that is expressed on CD4 and CD8 T cells upon activation (**Freeman, 2008**). Engagement of PD-1 by its ligands, PD-L1 and PD-L2, transduces a signal that inhibits T-cell proliferation, cytokine production, and cytolytic function (**Riley, 2009**). During tumorigenesis, cancer cells from a wide range of tumor types exploit immune checkpoint pathways, such as PD-1/PD-L1, to avoid detection by the adaptive immune system (**Murphy, 2011**). Monoclonal antibody (mAb) inhibitors of immunological checkpoints, including PD-1 and PD-L1, have demonstrated significant antitumor activity in patients with various solid tumors with less toxicity than broad immune activators, such as interleukin-2 (IL-2) and Interferon-alpha (IFN-α) (**Hamid and Carvajal, 2013; Seiwert et al., 2014; Topalian et al., 2012; Topalian et al., 2014**).

1.1.3 Engineered T cells with Redirected Specificity: Chimeric Antigen Receptors

The daunting task of breaking tolerance to self-antigens is the major obstacle facing the field of cancer immunotherapy. This can be difficult or impossible if the TCR repertoire has been deleted or rendered nonfunctional by various post thymic tolerance mechanisms (**Molldrem et al., 2003; Theobald et al., 1997**). One strategy is the chimeric antigen receptor (CAR) approach (**Figure 2**), which uses genetically programmed, patient-derived lymphocytes transfected with chimeric receptor genes to combine the effector functions of T lymphocytes with the ability of antibodies to recognize predefined surface antigens with high specificity in a non-MHC restricted manner (**Gross et al., 1989; Pinthus et al., 2003**).

These receptors have the ability to recognize intact membrane proteins independent of antigen processing. CARs typically encode an extracellular domain to bind tumor or virus linked to an intracellular signaling domain that mediates T cell activation (reviewed in (**Brocker and Karjalainen, 1998; Sadelain et**

al., 2003). In principle, universal targeting vectors can be constructed because the scFv bind to native cell surface epitopes and bypass the requirement for MHC restriction. The tumor binding function of CAR is usually accomplished by the inclusion of a single chain variable fragment (scFv) antibody, containing the V_H and V_L chains joined by a peptide linker of about 15 residues in length (**Mullaney and Pallavicini, 2001**). Upon CAR engagement of its associated antigen, primary T cell activation occurs and leads to cytokine release, cytolytic degranulation, and T cell proliferation and migration (**Hombach et al., 2001; van der Stegen et al., 2015**). Additional T cell effector mechanisms and memory responses also occur in a manner dependent on the mechanism of co-stimulation (41BB or CD28 in the case of "second generation CARs", or both of these signaling domains for "third generation CARs") (**Jensen and Riddell, 2015**).

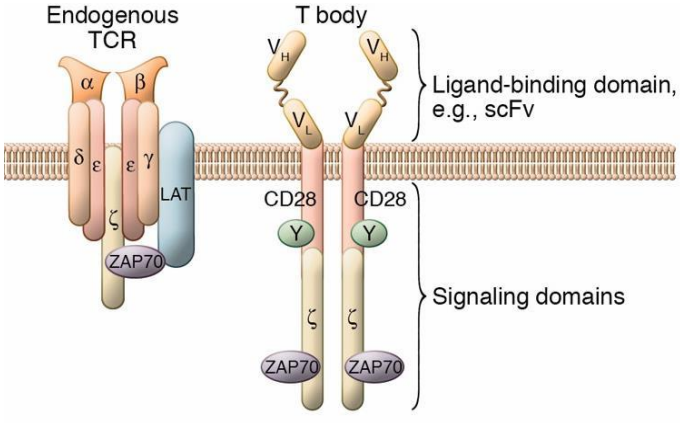


Fig. 2. T cells can be engineered to have retargeted specificity for tumors using 'T body' or Chimeric Immune Receptor (CAR) approach. T bodies express an extracellular ligand generally derived from an antibody and intracellular signaling modules derived from T cell–signaling proteins. LAT, linker for activation of T cells; ScFv, single chain variable fragment; ZAP70, ζ-chain– associated protein kinase 70 kDa.

The greatest clinical advances for CAR T cells to date have occurred in the treatment of hematologic malignancies, with the United States Food and Drug Administration (FDA) now having approved two CAR T cell therapies. Kymriah (Tisagenlecleucel), a CD19-targeted CAR T cell therapy formerly known as CTL019 and developed at Penn, was approved for the treatment of patients up to 25 years of age with B-cell precursor acute lymphoblastic leukemia (ALL) that is refractory or in second or later relapse (**Buechner J, 2018**). Subsequently, Yescarta (axicabtagene ciloleucel), another anti-CD19 CAR T cell treatment, was approved for large B-cell lymphoma patients who have failed at least two prior therapies (**Neelapu et al., 2017**). Remarkably, both of these CAR T products led to durable remissions in patients refractory to standard salvage therapies.

1.1.4 Immunogenicity of EGFRvIII

Epidermal growth factor receptor (EGFR) variant III (EGFRvIII), resulting from an in-frame deletion of exons 2 to 7, is the most common variant of this receptor observed in human tumors (**Li and Wong, 2008**). Approximately 40% of all newly diagnosed GBMs carry amplification of the EGFR gene, and about 50% of EGFR-amplified GBMs contain the constitutively active and oncogenic EGFRvIII variant (**Ekstrand et al., 1992; Sugawa et al., 1990**). Prior studies have found that the EGFRvIII alteration is associated with shorter survival in GBM, although recent data suggests that prognosis for these EGFRvIII+ patients may not differ from those with EGFR gene amplification (**Felsberg et al., 2017**). The amino acid sequence resulting from the EGFRvIII alteration yields a novel glycine residue at the junction of exons 1 and 8, generating a tumor-specific and immunogenic epitope within the extracellular domain of EGFR.

The Penn Brain Tumor Center previously participated in a clinical trial of a peptide vaccine strategy (rindopepimut) against EGFRvIII sponsored by *Celldex Therapeutics*. Rindopepimut consists of the EGFRvIII specific peptide sequence conjugated to the carrier protein Keyhole Limpet Hemocyanin (KLH).

Penn investigators completed the Ph II/III study referred to as "Act III" and accrued the highest number of patients nationally in this multi-center trial. Penn also accrued patients to the Re-Act study for recurrent GBMs expressing the EGFRvIII antigen.

Three Phase 2 trials of rindopepimut have been completed in newly diagnosed EGFRvIII-positive glioblastoma patients with consistent results – ACTIVATE, ACT II, ACT III. Across all studies, rindopepimut has been generally well tolerated with generation of robust, specific, and durable immune responses. In the phase III clinical trial (ACT IV, 745 patients), the most common grade 3-4 adverse event was thrombocytopenia. Other adverse events reported were injection site reactions (rash, pruritus), fatigue, nausea, and headache. However, progression-free survival was similar between treatment groups within the minimal residual disease (MRD) and significant residual diseases (SRD) populations. The proportion of patients who achieved an objective tumor response in the response-evaluable population irrespective of MRD or SRD status, did not differ between treatment groups either. Therefore the trial was stopped for futility (**Weller et al., 2017**).

Adoptive immunotherapy with re-directed T cells obviates the need for antigen presentation and stimulation of a primary immune response; therefore, direct transfer of EGFRvIII-directed T cells is potentially more effective and has favorable kinetics compared to cell-based vaccines.

1.1.5 EGFRvIII CAR Constructs

Although many of the monoclonal and polyclonal Abs directed against EGFRvIII have cross reactivity to wild type EGFR or other non-specific proteins, a monoclonal antibody (mAb) 3C10, which was originally developed by immunization of mice with a 14 amino acid peptide including the EGFRvIII-specific fusion junction, demonstrated highly specific recognition of EGFRvIII without any detectable binding to wild-type EGFR (**Okamoto et al., 1996**). Subsequently, a single-chain variable fragment (scFv) of mAb 3C10 was produced and cDNA for the 3C10 scFv was obtained (**Nakayashiki et al., 2000**). While avidity and/or antigen-specificity of the original mAbs can be often lost in scFV forms, the 3C10 scFv retained its selective reactivity with the EGFRvIII-specific epitope (**Nakayashiki et al., 2000**), which is an essential prerequisite for its use in the current CAR project. We have obtained the 3C10 scFv from Dr. Atsushi Natume (Nagoya University, Japan) and constructed a novel CAR using the 3C10 scFv into the lentiviral backbone used in Penn's previous clinical trials of CART directed to CD19. Initial testing conducted at University of Pennsylvania demonstrated robust and specific activity of 3C10-based CARs encompassing costimulatory domains from 4-1BB and TCRζ (BBz) or in tandem with CD28 costimulatory domains (28BBz). T cells transduced with these CARs demonstrate specific and potent lysis of EGFRvIII-expressing U87 human GBM cells *in vitro* and *in vivo* (data summarized in the EGFRvIII Investigator's Brochure). The results of the *in vivo* testing of various CAR redirected T cells indicated that all EGFRvIII CAR exerted potent anti-tumor efficacy by day 21; however, the CAR comprised of the mouse 3C10 scFv linked to 4-1BB and TCRζ (BBz) signaling domains cleared the tumors by day 7 post T cells infusion and it was chosen to advance for clinical testing (see EGFRvIII Investigator's Brochure)

Because of the immunogenicity of murine-derived scFv chains, we proceeded to humanize the 3C10 scFv fragment in collaboration with our collaborators at the Novartis Institute for Biomedical Research. A panel of 8 humanized constructs was screened and characterized extensively *in vitro*, both as soluble chains for affinity and binding studies and in functional evaluation of the corresponding T cells transduced with each scFv fused to the 4-1BB and TCRζ domains. The humanized CART-EGFRvIII construct selected for further clinical development (denoted 2173) presented a high affinity binding to the EGFRvIII target and an efficient cytolytic effect against EGFRvIII expressing targets (**Johnson et al., 2015**). The selected CAR

construct was used to generate a lentiviral vector under clinical grade conditions, and this accessory product is used to transduce autologous T cells to generate the clinical-grade CART-EGFRvIII cells investigational agent in this study.

1.1.6 Insertion-Mediated Oncogenesis

Retroviral vectors are useful gene delivery vehicles because they insert a deoxyribonucleic acid (DNA) copy of their genome into the host cell. However, insertion of vector sequences is distributed throughout the cellular genome and insertion has the potential to upregulate, dysregulate, or knockout local gene expression. Thus, there has always been a theoretical risk of insertional oncogenesis resulting from disruption of normal function of genes that control cell growth. This risk has now been realized in a clinical trial utilizing murine-derived retroviral vectors to transfer the γ signaling chain to stem cells of infants with X-linked severe combined immunodeficiency (SCID) (**Hacein-Bey-Abina et al., 2003a**).

Today a total of 4 of 9 children in that trial have developed integration site-induced cancers, and one of those children died after relapse. Of note, in a parallel study to the French study where the same disease, payload and target cell were used, no tumorigenesis was observed at first, suggesting that minor differences, such as vector envelope and stem cell growth factors, might be relevant in long-term safety of integrating vectors (**Gaspar et al., 2004**). However, in December of 2007, one of the 10 children treated in that study also developed insertion-mediated oncogenesis (www.asgt.org). Today, the causes of insertional oncogenesis remain poorly understood, but in the X-linked SCID studies, a common integration site, LMO-2, is observed in at least 3 of the 5 patients. In general, the target cell, vector, and disease payload are considered major factors contributing to the risks of vector-induced tumors. However, there is insufficient data to date to support the relative contribution of each of these factors in assuring safety of retroviral gene transfer.

Lentiviral vectors are an important subset of retroviral vectors, but demonstrate distinct integration patterns to oncoretroviral vectors which have been the predominant vector to date for gene transfer studies. The integration pattern of lentiviral vectors tends to be inside active transcription units as opposed to upstream in the locus control region where the insertion would have a greater chance of upregulating gene expression. In addition, lentiviral vectors have no enhancer activity in their long terminal repeat (LTR) and have lower levels of poly-A read-through, all factors which may improve gene transfer safety **(Zaiss et al., 2002**). Thus, it may be that lentiviral vectors are a safer alternative to oncoretroviral vectors for gene transfer, and animal models have provided supporting evidence for this (**Montini et al., 2006**). As lentiviral vectors are also superior for gene transfer, it is possible that these vectors will ultimately replace oncoretroviral vectors for stable gene transfer.

To date, malignant cell transformation after vector-mediated insertional mutagenesis has only been observed in three clinical entities (X-linked Severe Combined Immunodeficiency [SCID-X1], Chronic granulomatous disease [CGD], and Wiskott-Aldrich syndrome [WAS]) involving the use of first-generation gammaretroviral vectors harboring LTRs with strong enhancer/promoter sequences **(Boztug et al., 2010; Hacein-Bey-Abina et al., 2003b; Persons and Baum, 2011; Stein et al., 2010**). In contrast, trials with lentiviral vectors have demonstrated more polyclonal patterns (**Biffi et al., 2013; Cartier et al., 2009**) with the exception of the first patient reported from β-thalassemia trial (**Cavazzana-Calvo et al., 2010**). However, despite high transduction efficiency achieved by lentiviral vectors, molecular clonality studies in published clinical trials have not indicated any reasons for concern (**Schambach et al., 2013**).

In direct contrast to oncoretroviral vectors, lentiviral vectors have not been shown to be oncogenic in nature except for a single study in neonatal mice where direct injection for liver delivery was performed (**Themis et al., 2005**). The oncogenesis in this study may be associated with a vector element that can be modified to reduce such a risk (**Schambach et al., 2013**), and also it may be unique to the animal model used in that study. A newer study in tumor prone mice comparing the tumorigenicity of retroviral vectors to lentiviral vectors demonstrated that lentiviral vector gene transfer into hematopoietic stem cells of up to an average of 6 copies per cell was not tumorigenic in contrast to retroviral vectors at an average copy number of 3 per cell (**Montini et al., 2006**). It is notable that T cell leukemia is not a recognized side effect of HIV infection, although a high proportion of proviruses are defective and therefore would not mask a leukemic event.

Retroviral and lentiviral safety has been demonstrated in modified T cell gene therapy trials. Recently, a long-term retrospective study of >500 years of collective patient samples tested for at least 11 years after infusion from three clinical trials using gammaretroviral modified T cells to express CD4+ CAR did not show evidence of transgene silencing, atypical gammaretroviral integration patterns or clonal expansion (**Scholler et al., 2012**). A favorable safety profile was also determined for a conditionally replicating HIV-derived lentivirus that delivered HIV envelope antisense to patient T cells. In two separate treatment cohort analyses, no evidence for insertional mutagenesis or enrichment of vector copies near proto-oncogenes was observed (**Levine et al., 2006; Wang et al., 2009a**). These data represent follow-up after 21-36 months (**Levine et al., 2006**) and 28-32 weeks (**Wang et al., 2009a**). Another group reported no apparent risk of vector related AEs following 263 infusions of autologous, lentiviral transduced T cells with a long ribonucleic acid (RNA) antisense to HIV-1 envelope (**McGarrity et al., 2013**). More recently *ex vivo* lentiviral-transduced hematopoietic stem cells were used to correct an inherited storage disease in three children and an inherited disease in 3 children with WAS with follow-up for up to 24 months and 20-32 months, respectively. Lentiviral integrations showed sustained gene marking with polyclonal engraftment of transduced cells with no evidence of aberrant clonal expansion (**Biffi et al., 2013**), no evidence of *in vivo* selection of clones carrying integrations near oncogenes and therefore no evidence of vector-induced genotoxicity.

In light of the potential serious adverse events associated with retroviral gene transfer, the FDA has attempted since 2001 to arrive at a consensus recommendation for long-term follow-up of patients for delayed adverse events associated with these studies. Follow-up as long as the patient's lifetime has been proposed, but a compromise was reached that combined feasibility with safety concerns. Please refer to the current FDA guidance documents for follow-up requirements.

The frequency of follow-up visits was increased in the first 5 years post treatment to no more than every 6 months. This is primarily because in the X-SCID case, cancer was observed within 3 years post treatment, and an increase in monitoring would help to provide advance notice to a clinical event. Monitoring after year 5 is annual and the nature of the monitoring is dependent upon whether or not vector sequences are still detectable in the subject.

Recently, at the University of Pennsylvania a chronic lymphocytic leukemia (CLL) patient was treated with CAR T-cells targeting the CD19 protein, under NCT01029366. Following CAR T-cell infusion, anti-tumor activity was evident in the peripheral blood, lymph nodes and bone marrow, and was accompanied by complete remission. Unexpectedly, at the peak of the response, 94% of CAR T-cells originated from a single clone in which lentiviral vector–mediated insertion of the CAR transgene disrupted both alleles of the methylcytosine dioxygenase TET2 gene. Thus, leukemia was eliminated in this patient primarily by the

progeny of a single CAR T-cell that demonstrated massive *in vivo* expansion. In addition, it was determined that at the peak of the *in vivo* expansion, 65% of the CAR T cells presented a central memory phenotype, and possessed high levels of granzyme B expression (**Fraietta et al., 2018**). This is the first example of insertional mutagenesis involving a lentivirus vector with a beneficial outcome in producing functionally potent CAR T cells.

1.1.7 Monitoring for Replication Competent Retrovirus/Lentivirus (RCR/L)

The overriding safety issues associated with generation of a replication competent retrovirus (RCR) associated with the use of retroviral vectors are exemplified by the findings of an experiment involving administration of ex vivo transduced bone marrow progenitor cells that had been inadvertently exposed to high titer RCR contained in the retroviral vector material to severely immunosuppressed Rhesus monkeys. In this setting 3 of 10 animals developed lymphomas and died within 200 days (**Donahue et al., 1992**).

It is theoretically possible that RCL may be generated during the CAR T cell manufacturing phase or subsequently after infusion into the patient. However, an RCL resulting from the production phase is highly unlikely since elements are incorporated in the design of the vector system that minimize vector recombination and generation of RCL. Furthermore, the vector used to transduce the product undergoes sensitive assays for detection of RCL before it can be used in the manufacturing process. A recent analysis of 26 clinical trials of lentivirus-transduced cellular therapies was recently reported; in this analysis, no RCL was identified in 460 lentivirus-transduced cell products nor in 296 subjects monitored for at least one month post-infusion (**Marcucci et al., 2018**) (**Cornetta et al., 2018**). Nevertheless, generation of an RCL following infusion remains a theoretical possibility. The consequences of such recombination events in subjects without a known lentiviral infection are unknown, and therefore subjects with coexistent HIV infection are excluded from participation in this study in order to minimize this possibility. The development of RCL could pose a risk to both the subject and their close contact(s), and therefore, monitoring for RCL will be conducted during the course of the trial. RCL assessment (Q-PCR for VSV-G) in subjects' samples will be performed according to the FDA guidance- prior to treatment (as a baseline) and after T cell infusion at Months 3, 6, and 12. If the tests are negative during the first year, the subsequent samples will be archived in a temperature monitored and alarmed freezer at -80°C at the Translational and Correlative Studies Laboratory (TCSL).

1.2 Investigational Agents

1.2.1 CART-EGFRvIII cells

Autologous T cells will be engineered to express an extracellular single chain antibody (scFv) with specificity for EGFRvIII. This is expected to redirect specificity of the transduced T cells towards cells that express EGFRvIII molecule which is restricted in expression to the surface of GBM cells. Based on previous experience using CART-EGFRvIII cells in patients (NCT02209376), infusion of these cells was safe without evidence of off-tumor toxicity or cytokine release syndrome (CRS). All infused subjects had detectable engraftment of EGFRvIII CAR T cells in the peripheral blood. In addition, 7 of the 11 subjects in this study had post-CAR T cell surgical intervention, allowing for tissue-specific analysis of CAR T cell trafficking and other pharmacodynamic endpoints. In 2 of these subjects, both of whom had their tumors resected within 2 weeks of CAR T cell infusion, CART-EGFRvIII cells were found at higher concentrations in the brain than in the peripheral blood at the same time point, suggesting that the CAR T cells had effectively trafficked and expanded in situ within active regions of GBM (**O'Rourke et al., 2017**).

One of the main differences between this protocol and the one used in NCT02209376 is that CART-EGFRvIII cells will be infused up to three times in 3 week intervals, depending on the availability of CAR T cell manufactured for the subject and resolution of prior toxicity. Therefore a lower number of cells will be administered at each infusion ($2x10^8$ cells) versus $5x10^8$ cells infused only once.

1.2.2 Pembrolizumab (Keytruda®)

Pembrolizumab (or Keytruda®) is a humanized monoclonal immunoglobulin (Ig) G4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1) with potential immune checkpoint inhibitory and antineoplastic activities. Upon administration, pembrolizumab binds to PD-1, an inhibitory signaling receptor expressed on the surface of activated T cells, and blocks the binding to and activation of PD-1 by its ligands, which results in the activation of T-cell-mediated immune responses against tumor cells. The ligands for PD-1 include programmed cell death ligand 1 (PD-L1), overexpressed on certain cancer cells, and programmed cell death ligand 2 (PD-L2), which is primarily expressed on APCs. Activated PD-1 negatively regulates T-cell activation and plays a key role in in tumor evasion from host immunity. Therefore, blocking the interaction of PD-L1 with PD1 using pembrolizumab has the potential to relieve the immunosuppression of CART-EGFRvIII cells once they reach the tumor.

Pembrolizumab is an FDA approved agent for use in the following malignancies: melanoma, non-small cell lung cancer (NSCLC), head and neck squamous cell cancer (HNSCC), classical Hodgkin lymphoma (cHL), primary mediastinal large B-cell lymphoma (PMBCL), urothelial carcinoma, microsatellite instability-high cancer, gastric cancer, and cervical cancer. It is not approved for use in GBM, therefore its use in this study is considered investigational. The dose proposed for use in this protocol will be in line with the FDA approved package insert; 200mg every 3 weeks. Please refer to the approved package insert for additional details.

In a preclinical study using murine models of sarcoma and breast cancer, administration of syngeneic CAR T cells in combination with pembrolizumab did not cause autoimmunity (**John et al., 2013**). Recently, several clinical trials attempting to evaluate the safety and efficacy of CART treatment in combination with anti-PD1 antibodies in solid tumors, have started and they are in the recruiting phase. One human study examined the effect of pembrolizumab combined with CAR T cells targeting another type of cancer (neuroblastoma). In that trial, the combination of CAR T cells and pembrolizumab did not cause any extra toxicity beyond what would be expected from either treatment by itself (**Heczey et al., 2017**) . However, this study included only 3 subjects, and in general we still do not know a lot about the safety of combining CAR T cells with pembrolizumab. Currently, no clinical trials are specifically evaluating the combination therapy anti-PD1 antibodies + EGFRvIII CART cells in GBM (www.clinicaltrials.gov, search term: "PD1 and CART").

1.3 Radiation Therapy

Short course radiation therapy is considered standard of care (**Perry et al., 2017**). Because immunotherapy has led to unprecedented extent and duration of responses in previously refractory patients with a variety of solid tumors (**Marin-Acevedo et al., 2018**), and in light of recent data suggesting this possibility with CAR T cells and immune checkpoint inhibitor therapies in GBM (**Brown et al., 2016; Omuro et al., 2018**), researchers across neuro-oncology are actively investigating alterations to the current standard of care that may be more amenable to the inclusion of systemic immune-based

therapies. One approach that has emerged is the use of short course radiation (40 Gy over 3 weeks) in front-line immunotherapy protocols (for example, see NCT03367715 at New York University) as opposed to the standard dose and duration of radiation therapy for GBM following initial maximal safe resection which is 60Gy administered Monday-Friday over 6 weeks (2Gy x 30 fractions) (**Stupp et al., 2005**). The primary reason to use a lower dose/duration of radiation in an immunotherapeutic setting is to minimize the degree of immunosuppression that occurs as a result of radiation. Prior research has demonstrated that standard radiation treatment plans for GBM deliver lymphocytotoxic radiation doses to the entire circulating blood pool (**Yovino et al., 2013**). As a result, many patients receiving standard radiation for first-line GBM treatment experience profound lymphopenia, which can be long-lasting (**Campian et al., 2017**).

For these reasons, we have elected to administer short course adjuvant radiation in this protocol (40 Gy administered in 15 fractions). By taking this approach, subjects will be exposed to less lymphocytotoxicity, thus preserving the very cells that will be critical in maintaining an immune response via epitope spreading if the CAR T cell therapy is effective. In addition, because it is known that EGFRvIII expression can be lost during the course of front-line standard therapy for GBM, we aim to minimize the chance of this happening by halving the time course of radiation and thus initiating CART-EGFRvIII therapy as soon as possible in relationship to the time point when EGFRvIII expression is confirmed to be positive (i.e. initial surgical resection).

1.4 In-Vitro Diagnostics

All patients with GBM tumors resected at the Hospital of the University of Pennsylvania undergo EGFRvIII expression testing and *MGMT* promoter methylation testing as part of the standard neuropathology work flow for newly diagnosed gliomas. This testing is performed prior to study participation as part of routine care practice, at either the Center for Personalized Diagnostics in the Department of Pathology at the University of Pennsylvania or by NeoGenomics Laboratories, which are both CLIA certified laboratories. In order to participate in this study, the subject's tumor must be EGFRvIII positive and negative for *MGMT* promoter methylation according to tests performed by one of these laboratory facilities. While this testing is being performed per routine care practice and the results will be known prior to study consent, this testing has not yet been FDA approved, thus its use in this study as an eligibility parameter is considered investigational.

Patients with diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV, will also be considered for the study. To be clinically diagnosed as such, patients must have one of the following: a). high-level amplification of EGFR; or b). combined whole chromosome 7 gain and whole chromosome 10 loss (+7/-10); or c). *TERT* promoter mutation. This testing is performed prior to study participation as part of routine care practice, at either the Center for Personalized Diagnostics in the Department of Pathology at the University of Pennsylvania or by NeoGenomics Laboratories, which are both CLIA certified laboratories. While the *TERT* promoter mutation testing is being performed per routine care practice and the results will be known prior to study consent, this testing has not yet been FDA approved, thus its use in this study as an eligibility parameter is considered investigational. In order to participate in this study, the subject's tumor must also be EGFRvIII positive and negative for *MGMT* promoter methylation according to tests performed by one of the laboratory facilities (as defined above).

1.5 Preclinical Data: CART-EGFRvIII

An extensive amount of literature supports the use of engineered T cells for tumor immunotherapy in rodent tumor models, reviewed in **(Calogero et al., 2000; Clay et al., 2002; Hombach et al., 2002; Pule et al., 2003; Sadelain et al., 2003**). Others have used electroporation or retroviral vectors to create CAR-transduced T cells, and have shown *in vivo* safety and efficacy of adoptively transferred T cells in immunodeficient mouse models (**Brentjens et al., 2003; Cooper et al., 2003; Roessig et al., 2002; Serrano et al., 2006; Willemsen et al., 2000**). The incorporation of signaling modules such as 4-1BB in CAR constructs increases potency of the engineered T cells in pre-clinical studies (**Eshhar et al., 2001; Finney et al., 2004; Finney et al., 1998; Friedmann-Morvinski et al., 2005; Imai et al., 2004; Krause et al., 1998; Maher et al., 2002**).

Over the past decade, we have developed improved T cell culture systems and T cell transduction conditions. The T cell culture systems have been tested in phase I/II trials in patients with HIV infection and hematologic malignancies. The culture systems use anti-CD3 and CD28 costimulation and have proven to be efficient and feasible for large scale manufacturing, thereby overcoming a major barrier to adoptive immunotherapy. No significant safety concerns have emerged with more than 200 patients treated to date with CD4 and CD8 T cells, with and without genetic engineering with retroviral vectors (**Deeks et al., 2002; Laport et al., 2003; Levine et al., 2002; Lum et al., 2001; Mitsuyasu et al., 2000; Porter et al., 2011b; Rapoport et al., 2004; Rapoport et al., 2005; Thompson et al., 2003**). The advantage of HIV-based lentiviral vectors is efficiency and safety with respect to insertional mutagenesis for adoptive immunotherapy (**Dropulic and June, 2006; Naldini et al., 1996**). We originally created a novel CAR (3C10 CAR) using the lentiviral platform and incorporating a scFv derived from anti-EGFRvIII monoclonal antibody 3C10. This CAR has been tested *in vitro* and in xenogeneic mouse models. NOD/scid/γc(-/-) (NSG) mouse models have been widely used for pre-clinical assessments of CAR therapy, including evaluation of long-term persistence of infused human T-cells (**Carpenito et al., 2009; Paulos et al., 2010; Wang et al., 2011; Zhao et al., 2010**).

In summary, our preclinical results found that EGFRvIII specific CARs recognize the EGFRvIII antigen presented on target cells (including gliomas). Data summarized in the Investigator's brochure (IB Fig. 3 and 4) show that EGFRvIII CAR T cells respond in an antigen-specific manner to EGFRvIII expressing targets (as compared to wild type EGFR) by:

i. effectively proliferating
ii. signaling intracellularly via the NFAT T-cell activation pathway,
iii. producing type 1 anti-tumor cytokines,
iv. surviving multiple consecutive antigen-specific stimulation cycles, resulting in increased proportions and numbers of CAR+ T cells,
v. specifically lysing target cells.

In addition, the humanized version of the EGFRvIII CAR demonstrated increased target affinity, specific activity towards EGFRvIII expressing targets and non-crossreactivity with EGFR wild-type (Johnson et al., 2015).

1.6 Previous Clinical Data

1.6.1 CART-EGFRvIII

Please refer to the CART-EGFRvIII Investigator Brochure for detailed information about safety. A summary is provided below.

We conducted the first-in-human and only published study of CART-EGFRvIII cells in human glioblastoma at Penn (NCT02209376)(**O'Rourke et al., 2017**). This publication presents data on the first 10 subjects with recurrent GBM as part of this study. One additional subject was treated after this publication.

As a phase 1 trial, the primary end point of this study was safety. The individual and significant post-CART infusion adverse events are described in the CART-EGFRvIII Investigator's Brochure.

Detailed description of the adverse effects that occurred during this trial are presented in the Investigator Brochure and in the publication: O'Rourke et al, *Sci Transl Med* 2017 (**O'Rourke et al., 2017**).

1.6.2 Pembrolizumab (Keytruda®)

Please refer to the pembrolizumab package insert for complete information.

1.7 Dose Rationale and Risks/Benefits

The primary objective of this protocol is to test the safety and tolerability of administering multiple CART-EGFRvIII cells in combination with pembrolizumab in patients with newly diagnosed, EGFRvIII+, *MGMT*-unmethylated GBM.

1.7.1 Dose Rationale: CART-EGFRvIII cells

In the phase I study of CART-EGFRvIII cells for GBM (NCT02209376), subjects received a single dose of CART-EGFRvIII cells at a median dose of $5x10^8$ (range $1.75 \times 10^8 – 5 \times 10^8$). The target dose was $1 \times 10^8 – 5 \times 10^8$. The basis for setting that target dose was based on 1) Guidance for Industry: S6 Preclinical Safety Evaluation of Biotechnology-derived Pharmaceuticals; 2) Joint taskforce report from the BioIndustry Association (BIA) and the Association of the British Pharmaceutical Industry (ABPI) and 3) (Draft) Guidance for Industry: Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products; 4) the available literature on phase I trials to date conducted with CAR T cells, and 5) our institutional experience in treating patients with CAR T cells for other indications.

Unlike standard drugs that are metabolized, CAR T cells are able to proliferate in the patients, and thus the actual *in vivo* amount of CAR T cells after engraftment and expansion vary from patient to patient. Thus, the administered dose may underestimate the *in vivo* amount of CAR T cells. Based on these observations, as well as our recent clinical experience using CART-EGFRvIII cells in human GBM (NCT02209376), we have chosen to use a dose of $2x10^8$ EGFRvIII CART cells for each infusion, which has been identified as safe in patients with CLL and ALL as well as in our first CAR T cell trial of patients with GBM.

One key difference for this protocol compared to the first cohort of CART-EGFRvIII treated patients (NCT02209376) is the plan for multiple CAR T cell infusions. In hematologic malignancies, for example, a single dose of CAR T cells is sufficient to induce sustained antitumor response (**Porter et al., 2011b**). In

hematologic tumors, T cell amplification in the peripheral blood seems to be required to achieve an effective T cell-to-tumor cell ratio and predicts clinical efficacy (**Abken, 2017**). In solid tumors, however, the peripheral blood is not the compartment of therapeutic action, and the effective CAR T cell dose and frequency/schedule of administration are elusive. In our phase I study of CART-EGFRvIII cells in GBM, maximal detectable trafficking of CART-EGFRvIII cells to the brain coincided with peak engraftment in the peripheral blood, around 1-2 weeks after infusion. However, in some patients CART-EGFRvIII was not detectable in the tumor at more distant time points after infusion, such as 2-3 months. Based on this data, as well as a recent report of a durable complete response in a patient with GBM treated with multiple infusions of IL13 R2 – targeted CAR T cells (**Brown et al., 2016**), we hypothesize that increased persistence of the CART-EGFRvIII cells and hence tumor cell killing will occur with multiple infusions. We therefore plan to administer up to three infusions of CART-EGFRvIII cells depending on the availability of CAR T cell doses manufactured for the subject. Each infusion will include a target CART-EGFRvIII cell dose of $2x10^8$ cells. CART-EGFRvII cell doses will be formulated to obtain the maximum number of doses at the target dose level.

1.7.2 Dose Rationale: Pembrolizumab

Pembrolizumab will be administered at a dose of 200mg IV once every 3 weeks as per the standard dosing described in the package insert. This is also the dose previously demonstrated to be safe in GBM (**Omuro et al., 2018**) (**David A. Reardon, 2018**).

1.7.3 Dose Rationale: Radiation Therapy

Please refer to **Section 1.3** for complete details.

1.7.4 Study Population Rationale

Full inclusion and exclusion criteria are described in detail in ***Section 4.1* and *Section 4.2* respectively.**

Briefly, the current study population is similar to that of the original Penn-Sponsored CART-EGFRvIII study (UPCC#35313; Penn IRB#820381; NCT02209376) (**O'Rourke et al, 2017**) in that patients must have a histopathologically proven diagnosis of GBM and must have tumors that are EGFRvIII positive.

However, the targeted patient population for this combination study differs from this first-in-human study in the following key ways:

- Patients will have newly diagnosed and previously untreated, rather than recurrent GBM. There are two primary reasons for this. First, due to increased neurologic impairments, poorer performance status, higher tumor burden and steroid requirements, and more treatment-induced mutations that increase the invasiveness and treatment refractoriness of tumors, no therapy has ever been shown to improve overall survival when GBM has recurred following standard first-line radiation and chemotherapy. Second, EGFRvIII expression can vary temporally, for example being present at original diagnosis but then absent or significantly decreased at time of first tumor recurrence. Thus, treating a population of newly diagnosed patients provides the best opportunity to mount a successful immune response against the tumor when tumor burden is low, and also assures that the target of the CAR T cells (EGFRvIII) will actually be present.
- Patients with diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV, will also be considered for the study.

- Patients will have undergone tumor resection prior to enrolling on study. This will allow for decreased mass effect and steroid requirements, which translates to increased safety and efficacy for an immunotherapeutic approach.
- Patients must have a tumor that is negative for methylation of the O6-methylguanine-DNA-methyltransferase *(MGMT)* promoter. GBM patients with tumors negative for *MGMT* methylation (i.e. "unmethylated") gain little to no benefit from standard first-line temozolomide treatment. Novel therapies are urgently needed for this subgroup of GBM patients, and this population offers a unique opportunity to study new treatments in lieu of temozolomide.

1.7.5 Risks/Benefits

1.7.5.1 CART-EGFRvIII cells

A summary of risk information is presented below. Please refer to the CART-EGFRvIII Investigator's Brochure for complete information. The risks of administering CART-EGFRvIII cells in combination with pembrolizumab are not known at this time.

On-Target Off-Tumor Toxicity

Unanticipated cross-reactivity (off tumor) with wild-type EGFR, which is expressed on most epithelial tissues, particularly in lung epithelium and skin; the expected toxicity is development of skin rash and diarrhea, as is seen when wild type EGFR is targeted with other agents such as cetuximab.

We have performed extensive testing in vitro and *in vivo* on the EGFRvIII CAR construct to verify specific recognition of the mutated, oncogenic EGFRvIII over the wild-type EGFR. In vitro studies included binding assays of a soluble version of the antigen-recognition portion of the CAR (the scFv) against wild-type EGFR and EGFRvIII, and *in vitro* cytotoxicity and proliferation assays of CAR-modified T cells in response to cell lines expressing wild-type EGFR and/or EGFRvIII (details in the Investigator's Brochure). Evaluation of cross-reactivity of EGFRvIII CART with human skin *in vivo* revealed mild infiltration of the dermis and no infiltration in the basal cell layer or of the epidermis; in contrast, injection of cetuximab based CART in human skin grafted NSG mice revealed prominent lymphocytic infiltrate (**Johnson et al., 2015**). Moreover, in our first in man clinical trial using CART-EGFRvIII (NCT02209376), none of the above potential toxicities were observed.

Bystander inflammation

Bystander inflammatory responses at the site of tumor (intracranial) leading to swelling of brain areas surrounding the tumor (pseudo-progression) or generalized brain edema which could potentially occur as a cytokine-mediated effect; this is one of the safety endpoints of the study and will be managed by standard clinical interventions if it occurs. Because of the poor tolerance for pressure changes, edema, and fluid shifts in the closed intracranial space, even a moderate inflammatory response could cause significant toxicity when T cells are directed to an intracranial lesion. Symptoms and signs of brain edema will be closely monitored and managed in this protocol, as is standard in immunotherapy-based protocols aimed at this high-risk population.

Although we cannot completely rule out the possibility of bystander inflammation, which could cause substantial neurologic toxicity, no T cell activation or cytokine release syndrome (CRS) were observed in the 11 subjects enrolled in clinical trial (NCT 02209376).

Febrile reaction
In the unlikely event that the subject develops sepsis or systemic bacteremia following T cell infusion, appropriate cultures and medical management should be initiated. If a contaminated CART-EGFRvIII T cell product is suspected, the product can be retested for sterility using archived samples that are stored in the CVPF.

Infusion reactions
Several reactions may develop during and immediately after T cell infusions such as flu-like symptoms, hemodynamic effects, and dermatologic reactions. A summary description of these reactions and the medical management is provided in **Section 8.4.2**.

Risk of EGFRvIII Expression and *MGMT* Promoter Methylation Test Failure
All patients with GBM tumors resected at the Hospital of the University of Pennsylvania undergo EGFRvIII expression testing and *MGMT* promoter methylation testing as part of the standard neuropathology work flow for newly diagnosed gliomas. This testing is performed prior to study participation as per routine care practice, at either the Center for Personalized Diagnostics in the Department of Pathology at the University of Pennsylvania or by NeoGenomics Laboratories, which are both CLIA certified laboratories. Therefore the results of this testing will be known prior to study consent. However this testing has not yet been FDA approved, thus its use in this study as an eligibility parameter is considered investigational.

In order to participate in this study, the subject's tumor must be EGFRvIII positive and negative for *MGMT* promoter methylation according to tests performed by this laboratory facility. The risk of a failed EGFRvIII expression (i.e. false-positive) and *MGMT* promoter methylation testing (i.e. false-negative) are the same as the risks listed above for On-target, Off-Tumor Toxicity.

1.7.5.2 Pembrolizumab
Please refer to the pembrolizumab package insert for completion information. As pembrolizumab will be administered after brain irradiation and in combination with CAR T cell therapy, this might potentially increase the frequency and severity of known side effects. Unknown side effects may also occur.

The combination of CART-EGFRvIII cells and pembrolizumab has not previously been evaluated in clinical trials. However, pembrolizumab has been tested in combination with CAR T cells targeting another antigen in relapsed or refractory neuroblastoma (GD2 CAR T cells), and this combination was found to be feasible and safe (Heczey et al., 2017).

1.7.5.3 Immune-related Adverse Events (irAE)
Immune-related adverse events which are potential risks of using Immune Checkpoint Inhibitor Therapy have been published (**Brahmer et al., 2018; Haanen et al., 2017**) and have been summarized below. Please refer to the pembrolizumab package insert for expected toxicities of this therapy.

irAE Body Category *Toxicity*
Skin Toxicities
Rash/Inflammatory dermatitis
Bullous dermatoses

irAE Body Category *Toxicity*
SCARS (Severe cutaneous adverse reactions)- including Steve-Johnson Syndrome (SJS), toxic epidermal necrolysis (TEN), acute generalized exanthematous pustulosis, and drug reaction with eosinophilia and systemic symptoms (DRESS) or drug-induced hypersensitivity (DIHS)
GI Toxicities
Colitis
Hepatitis
Lung Toxicities
Pneumonitis
Endocrine Toxicities
Primary hypothyroidism
Hyperthyroidism
Adrenal- primary adrenal insufficiency
Pituitary- hypophysitis
Diabetes
Musculoskeletal Toxicities
Inflammatory arthritis
Myositis
Polymyalgia-like syndrome
Renal Toxicities
Nephritis
Nervous System Toxicities
Myasthenia gravis
Guillain-Barre syndrome
Peripheral neuropathy
Autonomic neuropathy
Aseptic meningitis
Encephalitis
Transverse myelitis
Hematologic Toxicities
Autoimmune hemolytic anemia
Acquired TTP
Lymphopenia

irAE Body Category *Toxicity*
Immune thrombocytopenia
Acquired hemophilia
Cardiovascular Toxicities
Myocarditis, pericarditis, arrhythmias, impaired ventricular function with heart failure and vasculitis
Venous thromboembolism
Ocular Toxicities
Uveitis/iritis
Episcleritis
Blepharitis

1.7.5.4 Radiation Therapy

Early Reactions **(Occurring during or shortly after treatment)**	**Late Reactions** **(Occurring months to years after treatment)**
• Skin changes including redness, irritation, scaliness, blistering, change in color of the skin, ulceration, thickening of the skin, or hair loss to the area being treated • Loss of appetite, nausea or vomiting • A change in the sense of taste or smell • Weight loss, weakness, tiredness (fatigue), or drowsiness • Swelling of the ear canal, feeling of a "stopped up" ear, hearing loss, or dizziness • Blurred Vision • Headache. • Problems with short term memory • Damage to the brain causing alteration of thinking ability or memory • Lowered blood counts, possibly leading to a greater risk of infection, bleeding, and/or the need for transfusion	• Changes in the texture or color of the skin, scars on the skin in the treated area, changes in the texture or color of the hair, a change in the re-growth of hair, or permanent hair loss in the area treated • Persistent drowsiness and tiredness, including excessive sleeping • Ear damage causing dryness of the ear canal, fluid collection in the middle ear, dizziness, or hearing loss • Damage to the eye(s) or optic nerve(s) causing cataracts, loss of vision or blindness • Permanent loss of smell • Damage to the pituitary gland that could require long term or permanent hormone replacement therapy • Bone damage that could lead to small cracks (fractures) in the bone • Damage to the spinal cord or nerves causing loss of strength, feeling, or coordination in the arms and legs, or loss of movement (paralysis) of the arms and legs and/or loss of bladder or bowel control

Early Reactions (Occurring during or shortly after treatment)	Late Reactions (Occurring months to years after treatment)
	• Permanent damage to short term memory • Brain damage causing a loss of intellectual ability, learning capacity, and reduced intelligence quotients • Radiation necrosis leading to swelling and inflammation in the brain tissue which may require medications and/or surgery. This may lead to permanent brain damage. • A second tumor caused by the radiation

1.7.5.5 Potential Benefits

It is possible that the CART-EGFRvIII cells will exert an anti-tumor effect. CART cells directed to CD19 have exerted dramatic and sustained responses in many patients with ALL and CLL (**Brentjens et al., 2013; Grupp et al., 2013; Kalos et al., 2011; Porter et al., 2011a**). Addition of the immune checkpoint inhibitor pembrolizumab has the potential to increase the anti-tumor effect of CAR T cells by reducing the immunosuppressive environment of the tumor.

Given that the patient population recruited for this protocol have limited therapeutic options, we believe that the above risks are acceptable.

2. STUDY OBJECTIVES

Primary Objective:

1) Determine the safety and tolerability of administering multiple infusions of CART-EGFRvIII cells in combination with a PD-1 inhibitor (pembrolizumab) in the treatment of newly diagnosed, *MGMT*-unmethylated GBM post-surgical resection.

Secondary Objectives:

1) Describe overall survival
2) Describe progression-free survival (PFS) based on standard MRI evaluation using modified RANO criteria.
3) Describe objective response rate (ORR)

Exploratory/Correlative Objectives:

1) Determine the persistence of modified CART-EGFRvIII cells in the peripheral blood and tumor.
2) Determine bioactivity of modified CART-EGFRvIII cells in the peripheral blood by modulation of systemic soluble factors (cytokines, chemokines, growth factors).
3) Evaluate development of secondary anti-tumor responses as a consequence of CART-EGFRvIII cells induced epitope spreading (if feasible) by:
    a. Using high throughput antibody screening.
    b. Performing exome and transcriptome gene analyses of the resected tumor by next generation sequencing (NGS) and evaluating whether cellular (i.e., T-cell) and/or

humoral (i.e., B-cell) responses are elicited against neo-antigens that are found by the NGS (i.e., detection of epitope spreading against neo-antigens).

4) Use plasma cell-free DNA (cfDNA) and RNA (cfRNA) as correlative measures of EGFRvIII-directed activity.
5) Where post-treatment tumor material or CSF is obtained as part of routine care, a portion of the sample will be collected for research purposes for the following testing:
    a. Measure trafficking of EGFRvIII-transduced cells to tumor by Q-PCR.
    b. Measure EGFRvIII expression to determine escape variants by next generation sequencing, immunohistochemistry, and/or fluorescent in situ hybridization (FISH).
    c. Determine evidence of anti-tumor immune activity using high throughput assays.
    d. Use advanced magnetic resonance imaging sequences and analysis to assess relative tumor blood volume (which has been shown to correlate with expression of EGFRvIII) by perfusion imaging; assess markers of pseudoprogression by MR spectroscopy; and assess axonal pathway integrity by diffusion tensor imaging.
    e. Use a newly developed plasma cell-free DNA assay as a correlative measure of disease response and EGFRvIII-directed activity.

3. STUDY DESIGN

3.1 General Design

This is a single-center, single-arm, open-label phase 1 study to determine the safety and tolerability of CART-EGFRvIII cells in combination with pembrolizumab in patients with newly diagnosed, EGFRvIII+, *MGMT*-unmethylated GBM. This population includes patients with diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV. This requires patients have one of the following: a). high-level amplification of EGFR; b). combined whole chromosome 7 gain and whole chromosome 10 loss (+7/-10); or c). *TERT* promoter mutation. This testing is performed prior to study participation as part of routine care practice, at either the Center for Personalized Diagnostics in the Department of Pathology at the University of Pennsylvania or by NeoGenomics Laboratories, which are both CLIA certified laboratories. While the *TERT* promoter mutation testing is being performed per routine care practice and the results will be known prior to study consent, this testing has not yet been FDA approved, thus its use in this study as an eligibility parameter is considered investigational.

All patients with GBM tumors resected at the Hospital of the University of Pennsylvania undergo EGFRvIII expression testing and *MGMT* promoter methylation testing as part of the standard neuropathology work flow for newly diagnosed gliomas. This testing is performed prior to study participation as part of routine care practice, at either the Center for Personalized Diagnostics in the Department of Pathology at the University of Pennsylvania or by NeoGenomics Laboratories, which are both CLIA certified laboratories. In order to participate in this study, the subject's tumor must be EGFRvIII positive and negative for *MGMT* promoter methylation according to tests performed by one of these laboratory facilities. While this testing is being performed per routine care practice and the results will be known prior to study consent, this testing has not yet been FDA approved, thus its use in this study as an eligibility parameter is considered investigational.

Subjects will receive a short course of adjuvant radiation to the brain with a total dose of 40 Gy, administered over 3 weeks (15 fractions). Study treatment (CART-EGFRvIII + pembrolizumab infusions)

will begin 2-3 weeks after completing a short course regimen of adjuvant radiation therapy (Cycle 1/Day 1). Thereafter, subjects will receive CART-EGFRvIII cells + pembrolizumab in 3 week cycles. Up to 3 infusions of CART-EGFRvIII cells may be received depending on product availability. Up to 4 infusions of Pembrolizumab may be received, as long as the subject is deriving clinical benefit.

For the first three subjects enrolled, infusion will be staggered such that a new subject is not infused until 21 days after the 1st infusion of CART-EGFRvIII cells + pembrolizumab for the previous subject to allow for DLTs to be evaluated. A safety pause will occur until all three subjects have completed 21 days of follow-up and a formal DLT assessment is performed by the Medical Director and Principal Investigator, protocol stopping rules have been assessed (per **Section 8.4.1**), and any recommended protocol changes have been implemented and approved by appropriate oversight bodies. If no DLTs were identified per Medical Director and PI assessment, subsequent subjects may be enrolled without timing restrictions.

Subjects will be followed for up to 15 years after initiation of study treatment to assess long-term adverse effects of the CAR T cells (See **Section 6.14**).

3.2 Primary Study Endpoints

This study's primary endpoint is the safety and tolerability of using multiple CART-EGFRvIII (autologous T cells transduced with a lentiviral vector to express a chimeric antigen receptor specific for EGFRvIII) in combination with pembrolizumab (anti-PD-1 monoclonal antibody) for the treatment of EGFRvIII+, *MGMT*-unmethylated GBM.

Safety will be evaluated based on the occurrence of study-related adverse events, using appropriate grading criteria as per Section 8, that occur during the adverse event reporting period and that are determined to be related to the CART-EGFRvIII T-cell infusion, pembrolizumab, or the combination thereof. This includes infusional toxicities, and any toxicity at least possibly related to these agents.

The primary safety endpoint of the study is the occurrence of adverse events related to study treatment.

3.3 Secondary Study Endpoints

Clinical

1. Describe the overall survival (OS).
2. Describe progression-free survival (PFS). Determination of disease progression will be based on standard MRI evaluation and modified RANO criteria.
3. Describe objective response rate (ORR). Response status will be determined by modified RANO criteria.

Correlative studies

1) Determine the persistence of modified CART-EGFRvIII cells in the peripheral blood and tumor.
2) Determine bioactivity of modified CART-EGFRvIII cells in the peripheral blood by modulation of systemic soluble factors (cytokines, chemokines, growth factors).
3) If feasible, evaluate development of secondary anti-tumor responses as a consequence of CART-EGFRvIII cells induced epitope spreading by:
    a. Using high throughput antibody screening.

b. Performing exome and transcriptome gene analyses of the resected tumor by next generation sequencing (NGS) and evaluating whether cellular (i.e., T-cell) and/or humoral (i.e., B-cell) responses are elicited against neo-antigens that are found by the NGS (i.e., detection of epitope spreading against neo-antigens).

4) Use plasma cell-free DNA (cfDNA) and RNA (cfRNA) as correlative measures of EGFRvIII-directed activity.

Where post-treatment tumor material or CSF is available:

1) Measure trafficking of EGFRvIII-transduced cells to tumor by Q-PCR.
2) Measure EGFRvIII expression to determine escape variants by next generation sequencing, immunohistochemistry, and/or fluorescent in situ hybridization (FISH).
3) Determine evidence of anti-tumor immune activity using high throughput assays.
4) Use advanced magnetic resonance imaging sequences and analysis to assess relative tumor blood volume (which has been shown to correlate with expression of EGFRvIII) by perfusion imaging; assess markers of pseudoprogression by MR spectroscopy; and assess axonal pathway integrity by diffusion tensor imaging.
5) Use a newly developed plasma cell-free DNA assay as a correlative measure of disease response and EGFRvIII-directed activity.

4. SUBJECT SELECTION AND WITHDRAWAL

4.1 Inclusion Criteria

1. One of the following diagnoses of GBM:
    a. Newly diagnosed glioblastoma multiforme that is histologically confirmed by pathology review of surgically resected tissue; OR
    b. An integrated molecular/pathologic diagnosis of diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV. This diagnosis requires patients have one of the following:
        i. High-level amplification of EGFR; OR
        ii. Combined whole chromosome 7 gain and whole chromosome 10 loss (+7/-10); OR
        iii. *TERT* promoter mutation.
2. Undergone tumor resection.
3. No prior systemic therapies, radiation, tumor-treating fields, or intratumoral therapeutic agents including Gliadel wafers are allowed. Tumor resection must be the only tumor-directed treatment that the patient has received for glioboblastoma.

    Note: Onstudy concomitant therapy restrictions are described in **Section 5.4**.
4. Tumor tissue is positive for EGFRvIII expression, as performed by either the University of Pennsylvania's in-house fusion transcript panel (RNA-based assay using Illumina HiSeq platform) or NeoGenomics Laboratories (quantitative RT-PCR assay).

5. Tumor tissue is negative for *MGMT* promoter methylation (i.e. the tumor is *MGMT*-unmethylated), as performed by either the University of Pennsylvania's in-house pyrosequencing protocol or NeoGenomics Laboratories.
6. Patients ≥ 18 years of age
7. ECOG performance status 0-1
8. Provides written informed consent
9. Must have adequate organ function as measured by:
    a. White blood count ≥ 2500/mm3; platelets ≥ 100,000/mm3, hemoglobin ≥ 9.0 g/dL; without transfusion or growth factor support
    b. AST, ALT, LDH, alkaline phosphatase within 2.5 x upper normal limit, and total bilirubin ≤ 2.0 mg/dL
    c. Serum creatinine ≤ 1.5 x upper limit of normal
    d. Adequate cardiac function (LVEF ≥ 45%)
10. Subjects of reproductive potential must agree to use acceptable birth control methods, as described in protocol **Section 4.3**.

4.2 Exclusion Criteria

1. Pregnant or lactating women
2. Inadequate venous access for or contraindications to leukapheresis.
3. Active Hepatitis B, hepatitis C, or HIV infection, or other active, uncontrolled infection
4. History of allergy or hypersensitivity to study product excipients (human serum albumin, DMSO, and Dextran 40)
5. History of severe hypersensitivity reactions to other monoclonal antibodies which in the opinion of the investigator may post an increased risk of serious infusion reactions.
6. Requirement for immunosuppressive agents including but not limited to cyclosporine, MMF, tacrolimus, rapamycin, or anti-TNF agents within 4 weeks of eligibility confirmation by the physician-investigator. Please refer to the Concomitant Therapy **Section 5.4** for information related to onstudy requirements.
7. Subjects with a history of known or suspected, severe or uncontrolled autoimmune or connective tissue disease. Patients with vitiligo, controlled type 1 diabetes mellitus (on stable insulin dose), residual autoimmune-related hypothyroidism (due to autoimmune condition only requiring hormone replacement), or psoriasis (not requiring systemic treatment), or conditions not expected to recur in the absence of an external trigger, are permitted to enroll.
8. Known history or current interstitial lung disease or non-infectious pneumonitis
9. Prior allogenic bone marrow or solid organ transplant
10. ~~Any concurrent malignancy other than non-melanoma skin cancer that has been curatively treated, or carcinoma in situ of the cervix or bladder that has been curatively treated. For any prior invasive malignancy, at least 5 years must have elapsed since curative therapy and patients must not have received any radiation to the brain. Monoclonal gammopathy of undetermined significance (MGUS) is permitted.~~ RETIRED WITH PROTOCOL VERSION 4
11. Any uncontrolled active medical or psychiatric disorder that would preclude participation as outlined.

12. Severe, active co-morbidity in the opinion of the physician-investigator would preclude participation in this study, including but not limited to the following:
    a. Unstable angina within 6 months prior to eligibility confirmation by the physician-investigator
    b. Transmural myocardial infarction within the last 6 months prior to eligibility confirmation by the physician-investigator
    c. New York Heart Association grade II or greater congestive heart failure requiring hospitalization within 12 months prior to eligibility confirmation by the physician-investigator.

       Note: Please refer to NYHA Functional Classification in Appendix 6.
    d. Serious and inadequately controlled cardiac arrhythmia
    e. Serious or non-healing wound, ulcer, or history of abdominal fistula, gastrointestinal perforation, intra-abdominal abscess major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to eligibility confirmation by the physician-investigator, with the exception of the craniotomy for tumor resection.
13. Patients with tumors primarily localized to the brain stem or spinal cord.

Please refer to **Section 5.4** for onstudy concomitant therapy restrictions.

4.3 Reproductive Status

Female subjects of reproductive potential (women who have reached menarche and who have not been post-menopausal for at least 24 consecutive months, i.e., who have had menses within the preceding 24 months, or have not undergone a sterilization procedure such as hysterectomy, bilateral oophorectomy, or bilateral tubal ligation) must have a negative urine pregnancy test at the time of enrollment and a negative serum pregnancy test at the Pre-Treatment Visit.

Due to the unknown risks of the CAR T cells with respect to pregnancy, as well as risks associated with pembrolizumab and radiation therapy, it is recommended that all subjects of reproductive potential use at least two medically acceptable forms of contraception for at least 1 year after their last infusion of CART-EGFRvIII cells. Investigators shall counsel subjects on the importance of pregnancy prevention and the implications of an unexpected pregnancy.

Medically acceptable forms of birth control include two of the following methods:

- Condoms (male or female) with or without a spermicidal agent
- Hormone-based contraception- including oral contraceptive pills, vaginal ring, injectables, implants or intrauterine devices (IUDs)
- Nonhormonal IUDs (such as ParaGard)
- Surgical methods- tubal ligation or vasectomy
- Diaphragm or cervical cap with spermicide
- Vaginal sponge

Subjects who are not of reproductive potential (women who have been postmenopausal for at least 24 consecutive months or have undergone hysterectomy, salpingotomy, and/or bilateral oophorectomy or men who have documented azoospermia) are eligible without requiring the use of contraception. Acceptable documentation of sterilization, azoospermia, or menopause consists of written or oral attestation by a physician or a physician's staff in one of the following formats:

- Physician report/letter
- Operative report or other source documentation in the subject's medical record (a laboratory report of azoospermia is required to document successful vasectomy)
- Discharge summary
- Laboratory report of azoospermia
- Follicle stimulating hormone measurement elevated into the menopausal range

4.4 Subject Recruitment and Screening

Subjects will be recruited from the Medical Oncology, Radiation Oncology, and Neurosurgery practices at the Hospital of the University of Pennsylvania. The study will be publicized on clinicaltrials.gov and University of Pennsylvania websites, and via University of Pennsylvania or Abramson Cancer Center press releases. No direct-to-patient advertising will be performed.

4.5 Subject Withdrawal/Discontinuation

4.5.1 Reasons for Subject Discontinuation

Subjects who enroll but do not receive study treatment will be prematurely discontinued from the study, will not be followed, and will be replaced in the study. Reasons for premature discontinuation prior to receipt of study treatment may include, but are not limited to, the following:

1. The subject is lost to follow-up.
2. The judgment of the principal investigator that the subject is too ill to continue if this occurs prior to study treatment.
3. Pregnancy is documented prior to study treatment. If pregnancy occurs after the subject has received study treatment, they will remain active in the study for safety and pregnancy follow-up and pregnancy outcome. No subsequent study treatment will be administered.
4. Voluntary withdrawal: a subject may remove himself/herself from the study at any time without prejudice. A subject may withdraw from the study at any time.
5. Significant and rapid progression of disease requiring alternative intervention that would preclude study treatment.
6. A serious adverse event prior to study treatment that precludes study participation.
7. Technical difficulties are encountered during CART-EGFRvIII T cell manufacturing that precludes production of the cell product that meets all release criteria specified by the FDA.
8. Termination of the study

Reasons for discontinuation of subjects from the treatment phase after receipt of study drugs may include, but are not limited to, the below. Subjects may not be discontinued from the treatment phase of the study prior to the End of Study Treatment Visit for reasons other than subject withdrawal of all study consent or death.

1. The subject is lost to follow-up.
2. Voluntary withdrawal: a subject may remove himself/herself from the study at any time.
3. Completion of study treatment per protocol
4. Premature discontinuation of study treatment due to toxicity, disease progression of targeted malignancy, receipt of alternative therapy, or ineligibility to receive additional infusions (see **Section 5.2**).
5. Death

6. Termination of the study

Subjects who are discontinued from the treatment phase, will go into the long-term follow-up phase. Reasons for discontinuation of subjects from long-term follow-up after receipt of study treatment may include, but are not limited to, the following:

1. The subject is lost to follow-up.
2. Voluntary withdrawal: a subject may remove himself/herself from the study at any time.
3. Completion of protocol required long-term follow-up.
4. Death
5. Termination of the study

The reasons for discontinuation from both primary and long-term follow-up (for example, voluntary withdrawal, toxicity, death) must be recorded appropriately.

4.5.2 Data Collection and Follow-up

Follow-up data collection after gene-modified cell therapy clinical trials is specified by FDA. As long as subjects have detectable cells transduced with the lentiviral vector, they should be followed for toxicity, immune reactions, and any long-term adverse events.

In the event that a subject cannot return to the study site for follow-up visits because of subject preference or geographical concerns, the subject's primary care physician and/or local oncologist will be asked to provide information from the subject's medical record to the study team at protocol defined time points (including the results of any routine care examinations and/or laboratory assessments), and assist in the collection of protocol required blood samples (if applicable) which will be sent to the University of Pennsylvania for protocol required analysis. The subject and local provider will also be contacted via telephone by a member of the study team to assess any potential toxicity.

In numerous previous cell therapy trials at the University of Pennsylvania, loss of follow-up is estimated to occur in less than 5% of cases. Every effort will be made to contact subjects who appear to be lost to follow-up in order to at least obtain survival data. During the long-term follow-up period, there will be no attempt to withdraw subjects early from this protocol. In the event of poor compliance or temporary loss to long-term follow-up, best attempts will be made to continue contacting the subject to complete the long-term follow-up for up to 5 years. However, subjects can withdraw consent at any time.

4.5.3 Replacement of Subjects

All subjects who receive CART-EGFRvIII cells as part of this study will be considered evaluable. Subjects who do not receive CART-EGFRvIII cells are considered non-evaluable and will be replaced.

5. STUDY DRUGS

5.1 Description

5.1.1 CART-EGFRvIII cells

CART-EGFRvIII cells are autologous T cells that have been engineered to express an extracellular humanized single chain antibody (scFv) with specificity for EGFRvIII linked to an intracellular signaling

molecule comprised of a tandem signaling domain of the 4-1BB and TCRζ signaling modules. The CART-EGFRvIII cells are cryopreserved in infusible cryomedia, and administered in 1-2 bags.

Each dose will consist of 2 x 10^8 CART-EGFRvIII transduced T cells, which will be given by rapid IV infusion. The minimum infusible dose is $2x10^7$.

In the event of DLTs meeting the stopping criteria in **Section 8.4.1**, the CART-EGFRvIII cell dose may be dose de-escalated to $2x10^7$.

Please refer to the CART-EGFRvIII IB for additional information.

5.1.2 Pembrolizumab (Keytruda®)

Pembrolizumab (or Keytruda®) is a humanized monoclonal immunoglobulin (Ig) G4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or programmed cell death-1) with potential immune checkpoint inhibitory and antineoplastic activities. Full details on its mechanisms of action, standard clinical usages, and toxicity profiles can be found in the US prescribing information.

Pembrolizumab will be administered at a dose of 200mg via IV infusion in 3 week cycles. Commercial pembrolizumab will be obtained through the site-designated pharmacy for research purposes. It will be stored according to the manufacturing instructions in the approved package insert. While administered for research purposes as part of this study, it will be prepared and infused in accordance with its FDA approved label and standard institutional practice.

Please refer to the pembrolizumab package insert for additional information.

5.1.3 Radiation Therapy

A short course of adjuvant radiation to the brain will be administered. Radiation therapy should start no later than 6 weeks post-surgery and end approximately 2-3 weeks prior to Cycle 1/Day 1. A total dose of 40 Gy will be administered over 3 weeks (15 fractions). Please see **Appendix 3** for Radiation Parameters. There are no planned dose modifications. Radiation may be held for radiation induced toxicity per institutional guidelines, but the total dose of radiation must be 40 Gy, even if elongation of the treatment period with radiation therapy is required.

5.2 Subject Eligibility to Receive Study Treatment

The criteria below will be assessed by the investigator before administration of pembrolizumab and/or CART-EGFRvIII cells as indicated below. Subjects who do not satisfy criteria to receive study treatment may have their infusion(s) delayed until such time that all criteria are satisfied in the judgment of the treating and principal investigators. Note: Subjects who cannot receive one of the investigational products for any reason may still receive the other investigational product as scheduled. Missed doses will not be made up.

<u>Cycle 1/Day 1</u>

1) Subjects must not have developed new disease complications, deterioration in performance status or overall clinical condition, new laboratory abnormalities, or new toxicities of therapy that would, in the opinion of the treating investigator, render it unsafe to proceed with study treatment. The following are specific conditions that warrant delaying study treatment:

a. Pulmonary: New requirement for supplemental oxygen or presence of progressive radiographic abnormalities on chest x-ray (chest x-ray not required at this juncture, but should be evaluated if performed for clinical purposes).
b. Cardiac: New cardiac arrhythmia not controlled with medical management. EKG is not required to evaluate for arrhythmia in the absence of suggestive symptoms or exam findings.
c. Hypotension requiring vasopressor support.
d. Active Infection: Positive blood cultures for bacteria, fungus, or virus within 48 hours of study treatment.

2) Subjects must have adhered to restrictions outlined in **Section 5.4** on pre-infusion therapy.
3) Female subjects of child-bearing potential must not be pregnant as assessed by a negative serum beta HCG test drawn within 7 days prior to Cycle 1/Day 1.

Cycle 2 and Beyond

1) Pembrolizumab
   a. Subject should not experience a significant change in performance or clinical status compared to their previous study visit that would, in the opinion of the treating physician or PI, increase the risk of experimental cell infusion.
   b. Subject experiencing new laboratory abnormalities that, in the opinion of the treating investigator or PI may impact subject safety or the subjects' ability to receive study treatment, may have their infusion delayed until both the treating investigator and PI determine it is clinically appropriate to proceed.
   c. Subject must not have any immune-related adverse events (irAEs) determined to be related to pembrolizumab that require treatment hold/discontinuation as follows:
      i. Grade 2 irAE (other than pneumonitis): Hold pembrolizumab. May resume pembrolizumab if the irAE resolves to grade 1 or less within 6 weeks.
      ii. Grade 2 irAE (pneumonitis): Hold pembrolizumab. May resume pembrolizumab if the irAE resolves to grade 1 or less within 2 weeks. If grade 2 pneumonitis is recurrent, permanently discontinue pembrolizumab.
      iii. Grade 3 irAE: Hold pembrolizumab. May resume if the irAE resolves to grade 1 or less within 6 weeks AND subject has successfully tapered corticosteroid dose to <10mg/day of prednisone or equivalent within 6 weeks
      iv. Grade 4 irAE: Permanently discontinue pembrolizumab.
      v. Grade 3 irAEs pneumonitis, nephritis, myocarditis, encephalitis, or Guillain-Barre syndrome: Permanently discontinue pembrolizumab.

2) CART-EGFRvIII cells
   a. Subject should not experience a significant change in performance or clinical status compared to their previous study visit that would, in the opinion of the treating physician or PI, increase the risk of experimental cell infusion.
   b. Subject experiencing new laboratory abnormalities that, in the opinion of the treating investigator or PI may impact subject safety or the subjects' ability to receive study treatment, may have their infusion delayed until both the treating investigator and PI determine it is clinically appropriate to proceed.
   c. Any immune-related adverse events (irAEs), regardless of attribution, must be resolved to grade 2 or less.
   d. Subjects must meet concomitant therapy restrictions as described in **Section 5.4**.

e. If the treating physician and/or PI feels the patient may be experiencing signs/symptoms of cytokine release syndrome (CRS) or other severe CAR T cell related toxicities, subsequent infusions may be preemptively delayed/omitted to allow for longer observation and monitoring prior to each subsequent infusion. A single isolated temperature elevation does not in itself define CRS, but the investigator should consider delaying the infusion for 24 hours to observe the subject in such instances.
f. Availability of CART-EGFRvIII cells which meets the minimum acceptable dose for infusion.

5.3 Preparation and Administration of Study Drugs

5.3.1 CART-EGFRvIII cells

In addition to the language below, please see the Investigational Product Handling Manual for further details on product thawing, transport, and labeling.

5.3.1.1 Manufacturing

CAR T cell product manufacturing is performed in the Clinical Cell Vaccine Production Facility (CVPF) at the University of Pennsylvania. CART-EGFRvIII cells are not released from the CVPF until release criteria for the infused cells (e.g., cell dose, cell purity, sterility, average copy number of vectors/cell, etc.) are met. CART-EGFRvIII cells will be cryopreserved at the target dose: $2x10^8$ per bag. The minimum infusible dose is $2x10^7$ cells.

5.3.1.2 Release and Preparation

The CVPF will release the CART-EGFRvIII cells to the bedside for administration on the day of infusion. The cells will be released in cryopreserved infusion bag(s) requiring thaw at the bedside, and dispensed to the clinical team for administration per CVPF SOPs. If dose de-escalation is required after manufacturing/cryopreservation, the CART-EGFRvIII cells may be thawed in the CVPF, reformulated for dose, and released in a syringe to bedside for administration.

Packaging and Labeling

The investigational product will be affixed with a label containing information regarding the dose, the method of manipulation, the vector and the following statements: “FOR AUTOLOGOUS USE ONLY” and “Caution- New Drug- Limited by Federal Law to Investigational Use”. In addition the label will have at least two unique identifiers and other information required by law. Prior to each infusion, two individuals will independently verify all unique identifier information in the presence of the patient and to confirm that the information is correctly matched to the patient.

Cell thawing

The frozen cells will be transported in dry ice to the subject’s bedside. The cells will be thawed at the bedside using a water bath maintained between 36°C to 38°C. There should be no frozen clumps left in the container at the time of infusion. If the CAR T cell product appears to have a damaged or leaking bag, or otherwise appears to be compromised, it should not be infused and should be returned to the CVPF as specified below.

Return or Destruction of Study Drug

CAR T cells may need to be returned to the CVPF for a variety of reasons, including but not limited to: 1) Mislabeled product; 2) Condition of patient prohibits infusion/injection, and 3) Subject refuses infusion.

Any unused product will be returned to CVPF. Final disposition of the investigational product is to be documented.

5.3.1.3 Administration

Premedication

Side effects following T cell infusions include transient fever, chills, and/or nausea. It is recommended that the subject be pre-medicated with acetaminophen and diphenhydramine hydrochloride prior to each CART-EGFRvIII cell infusion, unless contraindicated in the judgment of the investigator (e.g. due to allergy or history of allergic reaction). These medications may be repeated every six hours as needed. A course of non-steroidal anti-inflammatory medication may be prescribed if the patient continues to have fever not relieved by acetaminophen. It is recommended that patients not receive systemic corticosteroids such as hydrocortisone, prednisone, methylprednisolone or dexamethasone at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on T cells. If corticosteroids are required for an acute infusional reaction, an initial dose of hydrocortisone 100 mg or equivalent is recommended.

Febrile reaction

In the event of febrile reaction, an evaluation for infection should be initiated, and patients managed appropriately with antibiotics, fluids and other supportive care as medically indicated and determined by the treating physician. In the unlikely event that the subject develops sepsis or systemic bacteremia following cell infusion, appropriate cultures and medical management should be initiated. If a contaminated CAR T cell product is suspected, the product can be retested for sterility using archived samples that are stored in the CVPF.

Administration

The CART-EGFRvIII cells will be administered via IV infusion at the Hospital of the University of Pennsylvania using precautions for immunosuppressed patients. T-cell infusion(s) will be performed by a licensed Registered Nurse at the Hospital of the University of Pennsylvania.

Prior to the infusion, two individuals will independently verify the information on the label of each bag in the presence of the subject, and confirm that the information correctly matches the participant. Subjects will also be evaluated by a physician-investigator for eligibility to receive the CART-EGFRvIII cells according to criteria in **Section 5.2**.

The CART-EGFRvIII cells must remain on dry ice until ready for thaw/administration. The investigational CART-EGFRvIII cells should be infused into the subject immediately after they are thawed (i.e. must be infused within 30 minutes of product thaw). There should be no frozen clumps left in the bag prior to the infusion. The CART-EGFRvIII cells will be infused at a rate of 10-20 ml/minute into an intravenous catheter, either through a peripheral vein (preferred) or central vein. A macrodrip intravenous tubing will be used to infuse the CART cells by gravity (i.e. no infusion pump). The macrodrip intravenous tubing will be connected to a "Y" adapter with one end of the adapter spiked to the CART cell product bag and the other to a normal saline solution bag. **A leukoreduction filter must not be used for the infusion of the T cell product**.

At cycles where CART-EGFRvIII cells will be administered concurrently with pembrolizumab, pembrolizumab will be infused first. Once the pembrolizumab infusion is completed and post-infusion

vital signs are completed/assessed and it is determined safe to proceed, the CART-EGFRvIII cells may be thawed and infused.

Please refer to Section 5.3.3 for complete details on pre/post-infusion monitoring requirements.

Emergency medical equipment (i.e., emergency trolley) must be available during the infusion in case the subject has an allergic response, or severe hypotensive crisis, or any other reaction to the infusion.

5.3.2 Pembrolizumab (Keytruda®)

Please refer to the pembrolizumab package insert for additional information.

Availability
Commercial pembrolizumab will be obtained through the site-designated pharmacy for research purposes.

Storage/Dispensing
Pembrolizumab will be stored and dispensed in accordance with the manufacturing instructions in the approved package insert.

Premedication
Premedication prior to receipt of pembrolizumab is not required.

Administration
Pembrolizumab will be administered at a dose of 200mg via IV infusion over 30 minutes. Infusion(s) will be performed by a licensed Registered Nurse at the Hospital of the University of Pennsylvania. While administered for research purposes as part of this study, it will be prepared and infused in accordance with its FDA approved label and standard institutional practice.

Subjects must also be evaluated by a physician-investigator for eligibility to receive pembrolizumab according to criteria in **Section 5.2**.
At cycles where pembrolizumab will be administered concurrently with CART-EGFRvIII cells, pembrolizumab will be infused first. Once the pembrolizumab infusion is completed and post-infusion vital signs are assessed and it is determined safe to proceed, the CART-EGFRvIII cells may be thawed and infused.

Please refer to Section 5.3.3 for complete details on pre/post-infusion monitoring requirements.

Emergency medical equipment (i.e., emergency trolley) must be available during the infusion in case the subject has an allergic response, or severe hypotensive crisis, or any other reaction to the infusion.

Return or Destruction of Study Drug
For this study, partially used study drug containers, vials and syringes may be destroyed on site. Unused study product will be stored in the site-designated pharmacy until expiration and/or study closure.

5.3.3 Pre/Post-Infusion Monitoring Requirements

Pembrolizumab Monotherapy Administration

Vital signs (temperature, respiration rate, pulse, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior to the infusion, during the pembrolizumab infusion as clinically indicated, and within 15 minutes after the end of infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion, until these signs are satisfactory and stable. If the subject's vital signs are not satisfactory and stable one hour after the end of the infusion, vital signs will continue to be monitored as clinically indicated until stable. The subject will be discharged when medically stable and in accordance with hospital policy.

Research blood samples will be collected 1-2 hours post-infusion. Please refer to the Schedule of Evaluations in **Appendix 1** for additional information.

CART-EGFRvIII Monotherapy Administration

A baseline neurologic exam performed by a qualified provider must be performed on the day of the infusion prior to administration of CART-EGFRvIII cells.

Neuro checks and vital signs (temperature, respiration rate, pulse, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior to the infusion and within 15 minutes after the end of the infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the infusion, and then every hour (+/- 10 minutes) for the next 2 hours until these signs are satisfactory and stable. If the subject's vital signs are not satisfactory and stable three hours post-CAR T cell infusion, vital signs will continue to be monitored as clinically indicated until stable. A second neurologic exam must be performed by a qualified provider after infusion of CART-EGFRvIII cells. Clinically significant changes in vital signs and any significant change in neurologic examination must be reported immediately to the treating investigator. The subject will be discharged when medically stable and in accordance with hospital policy.

Research blood samples will be collected 1-2 hours post-infusion. Please refer to the Schedule of Evaluations in Appendix 1 for additional information.

Pembrolizumab + CART-EGFRvIII Cells

A baseline neurologic exam performed by a qualified provider must be performed on the day of the infusion prior to administration of pembrolizumab and CART-EGFRvIII cells.

Neuro checks and vital signs (temperature, respiration rate, pulse, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior to the pembrolizumab infusion, during the pembrolizumab infusion as clinically indicated, and within 15 minutes after the end of the pembrolizumab infusion. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and 60 (+/- 5) minutes after the pembrolizumab infusion, until these signs are satisfactory and stable. If the subject's vital signs are not satisfactory and stable one hour after the end of the infusion, vital signs will continue to be monitored as clinically indicated until stable.

A neuro check and vital signs (temperature, respiration rate, pulse, blood pressure, and oxygen saturation by pulse oximetry) will be measured within 10 minutes prior to the CART-EGFRvIII infusion and within 15 minutes after the end of the CART-EGFRvIII infusion. Vital signs must be stable prior to administration of CART-EGFRvIII cells. Thereafter, vital signs will be measured at 30 (+/- 5) minutes, 45 (+/- 5) minutes, and

60 (+/- 5) minutes after the CART-EGFRvIII infusion, and then every hour (+/- 10 minutes) for the next 2 hours until these signs are satisfactory and stable. If the subject's vital signs are not satisfactory and stable three hours post-CAR T cell infusion, vital signs will continue to be monitored as clinically indicated until stable. A second neurologic exam must be performed by a qualified provider after infusion of CART-EGFRvIII cells. Clinically significant changes in vital signs and any significant change in neurologic examination must be reported immediately to the treating investigator. The subject will be discharged when medically stable and in accordance with hospital policy.

Research blood samples will be collected 1-2 hours after the last infusion. Please refer to the Schedule of Evaluations in **Appendix 1** for additional information.

Emergency medical equipment (i.e., emergency trolley) must be available during the infusion(s) in case the subject has an allergic response, or severe hypotensive crisis, or any other reaction to the infusion.

5.4 Prior and Concomitant Therapy

All prescription and nonprescription medication, supplements/vitamins, devices, and herbal and nutritional supplements taken by the subject during the 30 days prior to consent will be recorded. At every visit following initiation of radiation therapy and until the subject has completed the end of treatment visit, concomitant medications will be recorded in the medical record and on the appropriate CRF. Any additions, deletions, or changes of these medications will be documented.

Concomitant medications and therapies deemed necessary for the supportive care and safety of the patient are allowed. However the following concomitant therapy guidelines must be adhered to during the study:

- Anticancer medications other than the protocol-described study treatment, including chemotherapy, biologic therapy (including bevacizumab), and non-protocol required radiation therapy, is prohibited after enrollment and during the Treatment Phase of this study. Subjects, who in the assessment of the investigator, require the use of these therapies for clinical management, should be discontinued from study treatment. Once a subject enters the Long-Term Follow-up Phase, they may receive anti-cancer treatment per routine care.
- Use of other concurrent investigational drug is not allowed.
- During the 7 days prior to apheresis, the use of steroids or other immunosuppressant drugs should be avoided. If however, steroids are necessary for safety, they should be limited to 2 mg or less dexamethasone/day (or equivalent dose of other corticosteroid).

  If steroids are required for symptom management after apheresis but prior to Cycle 1/Day 1, the treating clinician should aim to limit the total daily dose to 2 mg dexamethasone (or equivalent dose of other corticosteroid). However, if doses > 2 mg/day are required for patient safety, this will not limit the subject from continuing with Cycle 1/Day 1 study treatment as planned if eligibility criteria for infusion (**Section 5.2**) are met.

  Steroids or other immunosuppressant drugs should NOT be used as pre-medication for CAR T cell therapy or following CAR T cell infusion except to manage disease-related and/or immune-related toxicity. Additional checkpoint inhibitors are not permitted.
- GM-CSF or G-CSF must not be used 14 days prior to apheresis, as it may increase neutrophils in the collected product and make it difficult to process. GM-CSF and G-CSF should be avoided in the

21 days following CAR T cell infusion due to concern that these agents may provoke or exacerbate CRS.

- Use of any live vaccines against infectious disease is not allowable within 4 weeks of Cycle 1/Day 1 and while receiving study treatment.
- All subjects must either be taking at least one anti-epileptic medication at the time of enrollment or be started on one medication at least 24 hours prior to initiation of study treatment (Cycle 1/Day 1). The type of anti-epileptic medication will be at the discretion of the treating investigator, but a drug that is expected to be in the therapeutic range by the time of CART-EGFRvIII infusion would be most appropriate. Subject must remain on at least one anti-epileptic drug through 28 days post the last CART-EGFRvIII infusion.

6. STUDY PROCEDURES

The study consists of (1) a screening phase, (2) a manufacturing phase consisting of apheresis and preparation of the CART-EGFRvIII cell product(s), (3) a treatment phase, and (4) follow-up. Please refer to the Schedule of Evaluations (**Appendix 1**) for additional information.

6.1 Screening/Enrollment (~Week -12 to -8)

Informed consent must be obtained before the patient can undergo any research related procedures. Screening/enrollment assessments are described in this section and the Schedule of Evaluations (**Appendix 1**).

6.1.1 EGFRvIII Expression Testing/MGMT Promoter Methylation Testing

All GBM tumors resected at the Hospital of the University of Pennsylvania undergo EGFRvIII expression and *MGMT* promoter methylation testing as part of the standard neuropathology work flow for newly diagnosed gliomas. This testing is performed prior to study participation as per routine care practice, at either the Center for Personalized Diagnostics in the Department of Pathology at the University of Pennsylvania or by NeoGenomics Laboratories, which are both CLIA certified laboratories. Therefore the results of this testing will be known prior to subject consent and these results will be used by an appropriately licensed investigator to confirm study eligibility. This testing does not need to be repeated for research purposes. While this testing is performed as per routine care practice, this testing has not yet been FDA approved, thus its use in this study is considered investigational.

<u>EGFRvIII Expression</u>
Specifically, tumor tissue from formalin-fixed paraffin embedded blocks will be tested for the presence of EGFRvIII in a CLIA certified laboratory: either the Center for Personalized Diagnosis in the Department of Pathology at the University of Pennsylvania or NeoGenomics Laboratories. A Board-Certified neuropathologist will select a tissue block representative of the tumor with enough material for nucleic acid extraction. RNA will be obtained from this selected block according to manufacturer's guidelines. cDNA is synthesized complementary to the RNA, allowing for direct detection of EGFRvIII variant gene expression. Patients must have a positive EGFRvIII expression result to enroll on this study.

<u>MGMT Promoter Methylation</u>
Specifically, tumor tissue from formalin-fixed paraffin embedded blocks will be tested for the presence of *MGMT* promoter methylation in a CLIA certified laboratory. This will occur at either the Center for

Personalized Diagnosis in the Department of Pathology at the University of Pennsylvania or NeoGenomics Laboratories. A Board-Certified neuropathologist will select a tissue block representative of the tumor with enough material for nucleic acid extraction.

Genomic DNA will be extracted, undergo bisulfite conversion, and will then be amplified with primers which target DMR2 of the *MGMT* promoter including 4 CpG sites. Using pyrosequencing, the PCR product is evaluated to assess for percent methylation across the 4 CpG sites. The result is considered positive when the mean and median percent methylation across the 4 interrogated CpG sites are greater than or equal to 10%. The result is considered low positive when the mean and median level of DNA methylation seen are either relatively low (ie above the limit of detection but below 10%) or highly variable across the 4 CpG sites. A not detected result occurs when the mean and median percent methylation across the 4 CpG sites are below the limit of detection (4.5%). **Patients must have a NOT DETECTED result to enroll on this trial.** Low positive patients will still be considered positive and will NOT be eligible for this trial.

6.1.2 Additional Screening Evaluations

The following screening evaluations will be performed to determine eligibility to participate in this study:

1. Medical History and Physical Examination (including standard neurological examination) including an assessment of vital signs, ECOG performance status, and review of concomitant medications. Routine vital sign assessments include weight, temperature, pulse, respiratory rate, blood pressure, and oxygen saturation by pulse oximetry. Height will be collected at screening only.
2. Complete blood count and differential
3. Comprehensive chemistry panel- including Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, Calcium, Total Protein, Albumin, Total Bilirubin, Alk Phos, AST, ALT, Mg, Phos, LDH, Uric Acid.
4. Viral serologies (Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody, Hepatitis B core antibody, Hepatitis C antibody, HIV). If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 60 days prior to screening is provided.
5. Urine pregnancy test for females of child-bearing potential
6. Leukapheresis screening: a pre-donor evaluation will be performed to assess venous access for leukapheresis.
7. 12 lead electrocardiogram (EKG)
8. ECHO/MUGA- must be performed within 12 weeks of the initiation of study treatment (Cycle 1/Day 1).

In the event that the time between screening/enrollment and Cycle 1/Day 1 exceeds 12 weeks, the following will be repeated: Physical Examination, Performance Status Assessment, Complete Blood Count, Differential and Platelet Count, Chemistry Panel, Pregnancy test, ECHO or MUGA, and EKG. Other evaluations to be repeated at the discretion of the PI.

6.2 Enrollment

Assignment of subject numbers will occur at consent, will be in ascending order (i.e. 13318-XX), and no numbers will be omitted. Subject numbers will be used on all study documentation. Once assigned, the Subject Number must not be reused for any other subject and the Subject Number for that individual must not be changed, even if the subject is re-screened.

At the time a subject consents to participate in this study, a Consent Notification Form should be completed. Once required screening tests have been completed and the subject has been determined eligible by a physician-investigator, provide the documents listed below to:

Protocol Monitor and Sponsor Project Manager Center for Cellular Immunotherapies (CCI)

Documents required:

1. Completed Eligibility Notification
2. Redacted copy of signed patient consent and HIPAA authorization
3. Redacted source documentation to confirm enrollment/eligibility (including patient past medical history, laboratory, radiological reports, physical exam, concomitant medications and any other documentation to support patient meets eligibility criteria and has completed all required screening assessments).

Upon receipt of screening and eligibility documentation, the Sponsor Protocol Monitor will review and provide documentation that the monitoring visit for eligibility has been completed. This documentation must be received prior to apheresis and cell product manufacturing. If T cells have already been collected during a previous apheresis and are available for study manufacturing, it is not necessary to repeat the apheresis procedure.

6.3 Apheresis (~Week -8 to -4)

A large volume apheresis procedure is carried out at the Hospital of the University of Pennsylvania apheresis center in accordance with their policies/procedures. PBMC are obtained for CAR T cells during this procedure. From a single leukapheresis, the intention is to harvest at least 5 x 10^9 white blood cells to manufacture CAR T cells. If a single apheresis does not yield the adequate number of cells for manufacturing, then subjects can undergo an additional apheresis as needed. Baseline blood leukocytes for FDA look-back requirements and for research are also obtained and cryopreserved. **Section 5.4** enumerates medications/therapies prohibited prior to apheresis. The apheresis procedure will be performed approximately 4-8 weeks prior to the planned Cycle 1/Day 1 and may be performed any time after the Monitoring Visit for Eligibility is completed. Apheresis may be performed after initiation of radiation therapy at the physician-investigator's discretion.

It is recommended that the patient have an absolute lymphocyte count (ALC) ≥500/µl prior to undergoing apheresis. If the patient's ALC is <500/µl, it is recommended that a lymphocyte subset analysis (CD3, CD4, CD8 counts) be performed to confirm that the patient has an absolute CD3 count of ≥150/µl. If the absolute CD3 count is <150/µl, it is recommended that the leukapheresis procedure be delayed until their ALC is ≥500/µl or absolute CD3 count is ≥150/µl. Up to a 4 week delay may occur; following this, further discussion is needed with the study PI and the CVPF prior to proceeding.

Historical Apheresis Sample

Cryopreserved historical apheresis products collected from the patient prior to study entry are usable for CAR T cell manufacturing if collected at an appropriately certified apheresis center and the product meets adequate mononuclear cell yields. If a historical apheresis product is not available, an apheresis procedure (as described above) will be performed for cell procurement after study eligibility has been confirmed by an appropriately licensed investigator and the monitoring visit for eligibility has been completed.

6.4 Radiation Therapy (~Week -6 to Week -3)

A short course of adjuvant radiation to the brain will be administered. Radiation therapy should start no later than 6 weeks post-surgery and end approximately 2-3 weeks prior to Cycle 1/Day 1. A total dose of 40 Gy will be administered over 3 weeks (15 fractions). Please see Appendix 3 for Radiation Parameters.

There are no planned dose modifications. Radiation may be held for radiation induced toxicity per institutional guidelines, but the total dose of radiation must be 40 Gy, even if elongation of the treatment period with radiation therapy is required.

6.5 Pre-Treatment Visit (Day -7 to -1)

Subjects will undergo the following evaluations within 7 days prior to initiation study treatment (Cycle 1/Day 1), to obtain pre-infusion baseline clinical and disease status and assess eligibility to proceed with pembrolizumab and CART-EGFRvIII cell infusions (**Section 5.2**):

a) Review of current medical conditions, physical examination including standard neurological examination, and assessment of vital signs, ECOG performance status, and concomitant medications. Routine vital sign assessments include weight, temperature, pulse, respiratory rate, blood pressure, and oxygen saturation by pulse oximetry.

b) Complete blood count and differential

c) Chemistry panel- including Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, Calcium, Total Protein, Albumin, Total Bilirubin, Alk Phos, AST, ALT, Mg, Phos, LDH, Uric Acid

d) Serum pregnancy test (Females of childbearing potential only)

e) Baseline screens for HLH/MAS: ferritin, triglycerides, haptoglobin and CRP

f) Coagulation factors: PT, PTT, INR, fibrinogen, D-dimer

g) Endocrine evaluation: TSH, T4, free T4, cortisol, lipase

h) 12 lead electrocardiogram (EKG)

i) Brain MRI: to assess baseline disease response. Performed ~ 2-3 weeks following conclusion of radiation therapy. Brain MRI will include advanced sequences and analysis for correlative studies (Please refer to **Section 6.16**).

j) Research Blood Samples: blood samples will be collected for research analysis. Results of this analysis are not needed prior to initiating study treatment.

All subjects must either be taking at least one anti-epileptic medication at the time of enrollment or be started on one medication at least 24 hours prior to initiation of study treatment (Cycle 1/Day 1). The type of anti-epileptic medication will be at the discretion of the treating investigator, but a drug that is expected to be in the therapeutic range by the time of CART-EGFRvIII infusion (Cycle 1/Day 8) would be most

appropriate. Subject must remain on at least one anti-epileptic drug through 28 days post the last CART-EGFRvIII infusion.

6.6 Cycle 1/Day 1 (Pembrolizumab + CART-EGFRvIII cell Infusions)

Subjects will receive their first infusions of pembrolizumab and CART-EGFRvIII cells on Cycle 1/Day 1. Subjects will also be asked to undergo tests/procedures in accordance with the Schedule of Evaluations (**Appendix 1**). These assessments will be performed prior to administration of study treatment unless otherwise indicated.

Please refer to **Sections 5.3.1**, **5.3.2**, and **5.3.3** for information on pembrolizumab and CART-EGFRvIII administration, and pre/post-infusion monitoring requirements.

Subjects must also be evaluated by a physician-investigator for eligibility to receive study treatment according to criteria in **Section 5.2**. Subjects who do not satisfy criteria to receive study treatment(s) may have their infusion(s) delayed until such time that all criteria are satisfied in the judgment of the treating and principal investigators.

Subjects will continue to receive pembrolizumab + CART-EGFRvIII in 3 week cycles. Up to 4 infusions of pembrolizumab may be received, as long as the subject is deriving clinical benefit. Up to 3 infusions of CART-EGFRvIII cells may be received depending on product availability.

6.7 Cycle 1 Post-infusion Evaluations

Subjects will return for safety follow-up visits at the following timepoints after pembrolizumab + CART-EGFRvIII infusions: Cycle 1/Day 2, Cycle 1/Day 4 (+/- 1 day), Cycle 1/Day 8 (+/- 1 day), Cycle 1/Day 11 (+/- 1 day), and Cycle 1/Day 15 (+/- 3 days). Subjects will also be asked to undergo tests/procedures in accordance with the Schedule of Evaluations (**Appendix 1)**.

If at any point throughout their study participation, the subject undergoes surgical resection/biopsy as part of their routine care, portions of these samples will be used for research analysis.

6.8 Cycle 2/Day 1 (+/- 3 days)

Subjects will receive their second infusions of pembrolizumab and CART-EGFRvIII cells on Cycle 2/Day 1. If a second CART-EGFRvIII cell dose is not available (based on the availability of CAR T cell doses manufactured for the subject), the subject will receive pembrolizumab alone.

Subjects will be asked to undergo tests/procedures in accordance with the Schedule of Evaluations (**Appendix 1**). These assessments will be performed prior to the pembrolizumab and CART-EGFRvIII infusions unless otherwise indicated.

Subjects must also be evaluated by a physician-investigator for eligibility to receive study treatment according to criteria in **Section 5.2**. Subjects who do not satisfy criteria to receive study treatment may have their infusion(s) delayed until such time that all criteria are satisfied in the judgment of the treating and principal investigators. If delayed, the start of the next cycle will be shifted accordingly. Subjects who cannot receive one of the investigational products for any reason may still receive the other investigational product as scheduled. Missed doses will not be made up.

Administration and post-infusion monitoring will be performed in accordance with **Sections 5.3.1**, **5.3.2**, and **5.3.3**.

6.9 Cycle 2 Post-infusion Evaluations

If the subject received CART-EGFRvIII cells at Cycle 2/Day 1, they will be asked to return for post-infusion safety visits at the following timepoints post CART-EGFRvIII infusion: Cycle 2/Day 2, Cycle 2/Day 4 (+/- 1 day), Cycle 2/Day 8 (+/- 1 day), Cycle 2/Day 11 (+/- 1 day), and Cycle 2/Day 15 (+/- 3 days). At these visits subjects will be asked to undergo tests/procedures in accordance with the Schedule of Evaluations (Appendix 1). If subjects only received pembrolizumab at Cycle 2/Day 1, these safety follow-up visits are not required.

6.10 Cycle 3/Day 1 (+/- 3 days)

Subjects will continue to receive study treatment in 3 week (21 day) cycles. Up to 3 infusions of CART-EGFRvIII cells may be received depending on product availability. Up to 4 infusions of pembrolizumab may be received as long as the subject is deriving clinical benefit. Subjects must also be evaluated by a physician-investigator for eligibility to receive study treatment according to criteria in **Section 5.2**.

At these visits, subjects will also be asked to undergo tests/procedures in accordance with the Schedule of Evaluations (**Appendix 1**). These assessments will be performed prior to the pembrolizumab and CART-EGFRvIII infusions unless otherwise indicated. Subjects who do not satisfy criteria to receive study treatment (**Section 5.2**) may have their infusion(s) delayed until such time that all criteria are satisfied in the judgment of the treating and principal investigators. If delayed, the start of the next cycle will be shifted accordingly. Subjects who cannot receive one of the investigational products for any reason may still receive the other investigational product as scheduled. Missed doses will not be made up.

6.11 Cycle 3 Post-infusion Evaluations

If the subject received CART-EGFRvIII cells at Cycle 3/Day 1, they will be asked to return for post-infusion safety visits at the following timepoints post CART-EGFRvIII infusion: Cycle 3/Day 2, Cycle 3/Day 4 (+/- 1 day), Cycle 3/Day 8 (+/- 1 day), Cycle 3/Day 11 (+/- 1 day), and Cycle 3/Day 15 (+/- 3 days). At these visits subjects will be asked to undergo tests/procedures in accordance with the Schedule of Evaluations (Appendix 1). If subjects only received pembrolizumab at Cycle 3/Day 1, these safety follow-up visits are not required.

6.12 Cycle 4/Day 1 (+/- 3 days)

A 4th and final infusion of pembrolizumab may be administered if the subject is deriving clinical benefit and if they meet eligibility to receive study treatment according to criteria in **Section 5.2**.

At this visit, subjects will also be asked to undergo tests/procedures in accordance with the Schedule of Evaluations (**Appendix 1**). These assessments will be performed prior to the pembrolizumab infusion unless otherwise indicated. Subjects who do not satisfy criteria to receive study treatment may have their infusion delayed until such time that all criteria are satisfied in the judgment of the treating and principal investigators.

6.13 End of Study Treatment Visit

An End of Study Treatment Visit will be performed approximately 30 days (+/- 5 days) after the last study treatment and prior to initiation of a new anti-cancer therapy, whichever comes first. At the End of Study Treatment Visit, subjects will also be asked to undergo tests/procedures in accordance with the Schedule of Evaluations (**Appendix 1**).

All subjects discontinuing from the treatment phase will enter into long-term follow-up as part of this study.

6.14 Long-term Follow-up

Subjects who discontinue from primary study follow-up will enter into long-term follow-up for up to 15 years after their first CAR T cell infusion per FDA guidance.

The visit schedule during long-term follow-up is built from the initiation of study treatment (Cycle 1/Day 1) and the time of discontinuation from the treatment phase. **Table 6.14** indicates the **1st** LTFU study visit that would need to be completed based on Cycle 1/Day 1.

Table 6.1 – 1st LTFU study visit to be completed

Last study visit in treatment phase	1st LTFU study visit
<3 months	3 mo
≥3 months and <6 months	6 mo
≥6 months and <9 months	9 mo
≥9 months and <1 year	1 yr
>1 year and <1.5 years	1.5 yr
>1.5 years and <2 years	2 yr
= 2 year	2.5 yr

During long-term follow-up, subjects will undergo tests and assessments in accordance with the Schedule of Evaluations in **Appendix 1**.

In the event that a subject cannot return to the University of Pennsylvania for follow-up visits, the subject's local provider will also be asked to provide information from the patient's medical record to the study team at protocol-defined time points (i.e. the results of routine care physical examinations and/or laboratory assessments), and assist in the collection of protocol-required blood samples for RCL and persistence testing, which will be sent to the University of Pennsylvania Translational and Correlative Studies Laboratory (TCSL). The patient's local provider will also be asked to assist in the monitoring and reporting of protocol defined adverse events, and provide copies of documentation pertaining to the absence or presence of delayed adverse events, along with the relevant reports of tests and procedures. After the Month 60 (5 year) follow-up visit, subjects will only be asked to return for study visits if there is evidence of ongoing persistence of CAR T-cells in the previous year. If there is no evidence of CAR T-cell persistence in the previous year, follow-up will be conducted via phone/email/mail. The Follow-up Survey in **Appendix 4** may be used to facilitate contact with the subject as required. The template Physician Letter in **Appendix 5** may be used to contact the patient's local provider and facilitate their assistance in identifying any protocol-defined adverse events.

Subjects who enter long-term follow-up in the absence of disease progression after study treatment, will also be followed for disease response as per standard of care. These evaluations will continue to be performed until disease progression is confirmed.

Additional research blood may also be collected during the primary or long-term follow-up phases of the study. The total amount of extra blood that may be collected will not exceed 3 tablespoons of blood twice in one week. In addition, tumor tissue from tumor biopsies that are performed during long-term follow-up as part of routine care may be provided to TCSL for correlative studies.

6.15 Tumor Response Assessments

Tumor response assessments will be done according to standard of care practices at baseline and approximately every 6 weeks (every 2 treatment cycles). Hospital of the University of Pennsylvania policies will apply with regard to the use of contrast agents in subjects with known moderate to severe kidney disease.

Modified Response Assessment in Neuro-Oncology (RANO) criteria (**Ellingson et al., 2017**) will be used to assess for tumor response and progression, with definitions as outlined below. MRI scans will be interpreted by the attending neuro-radiologist at the University of Pennsylvania assigned as a sub-investigator on this study.

Measurable disease should be defined as contrast enhancing lesions with a minimum size of ***both*** perpendicular measurements greater than or equal to 10mm. For example, if the largest diameter is 15 mm but the perpendicular diameter is 8 mm, this would constitute ***non-measurable disease***. Up to a total of five target measurable lesions should be defined and ranked from largest to smallest. Non-measurable disease should be defined as lesions that are too small to be measured (less than 1 cm in both perpendicular dimensions), lesions that lack contrast enhancement (non-enhancing disease), or lesions that contain a poorly defined margin that cannot be measured or segmented with confidence.

The following definitions for disease response and progression will be used according to modified RANO criteria:

***Complete Response* (*CR*): Requires *all* of the following:**

1. Disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. The first scan exhibiting disappearance of all enhancing measurable and non-measurable disease is considered “preliminary CR”. If the second scan exhibits measurable enhancing disease with respect to the “preliminary CR” scan, then the response is not sustained, noted as pseudoresponse, PsR, and is now considered “preliminary PD” (note confirmed PD requires at least two sequential increases in tumor volume). If the second scan continues to exhibit disappearance of enhancing disease or emergence of non-measurable disease (less than 10mm bidimensional product), it is considered a ***durable CR*** and the patient should continue on therapy until confirmed PD is observed.
2. Patients must be off corticosteroids (or on physiologic replacement doses only).
3. Stable or improved clinical assessments (i.e. neurological examinations).

Note: Patients with non-measurable disease only at baseline cannot have CR; the best response possible is stable disease (SD).

***Partial Response* (*PR*): Requires *all* of the following:**

1. ≥50% decrease in sum of products of perpendicular diameters or ≥65% decrease in total volume of all measurable enhancing lesions compared with baseline, sustained for at least 4 weeks. The first scan exhibiting ≥50% decrease in sum of products of perpendicular diameters or ≥65% decrease in total volume of all measurable enhancing lesions compared with baseline is considered "preliminary PR". If the second scan exhibits PD with respect to the "preliminary PR" scan, then the response is not sustained, noted as pseudoresponse, PsR, and is now considered "preliminary PD" (note confirmed PD requires at least two sequential increases in tumor volume). If the second scan exhibits SD, PR, or CR, it is considered a ***durable PR*** and the patient should continue on therapy until confirmed PD is observed.
2. Steroid dose should be the same or lower compared with baseline scan.
3. Stable or improved clinical assessments.

Note: Patients with non-measurable disease only at baseline cannot have PR; the best response possible is stable disease (SD).

***Progressive Disease (PD)* is defined by any of the following:**

1. At least 2 sequential scans separated by at ≥4 weeks both exhibiting ≥25% increase in sum of products of perpendicular diameters of enhancing lesions. The first scan exhibiting ≥25% increase in sum of products of perpendicular diameters of enhancing lesions should be compared to the smallest tumor measurement obtained either at baseline (if no decrease) or best response (on stable or increasing steroid dose) and is noted as "preliminary PD." If the second scan at least 4 weeks later exhibits a subsequent ≥25% increase in sum of products of perpendicular diameters of enhancing lesions relative to the "preliminary PD" scan it is considered "confirmed PD" and the patient should discontinue therapy. If the second scan at least 4 weeks later exhibits SD or PR/CR, this scan showing "preliminary PD" is noted as "pseudo-progression", PsP, and the patient should continue on therapy until a second increase in tumor size relative to the PsP scan is observed. Note that any new *measurable* (>10mm x 10mm) enhancing lesions should *not* be immediately considered PD, but instead should be added to the sum of bidimensional products representing the entire enhancing tumor burden.
2. In the case where the best response demonstrates no measurable enhancing disease (visible or not visible), then any new *measurable* (>10mm x 10mm) enhancing lesions are considered PD *after* confirmed by a subsequent scan ≥4 weeks exhibiting ≥25% increase in sum of products of perpendicular diameters of enhancing lesions relative to the scan first illustrating new measurable disease. The first scan exhibiting new measurable disease is noted as "preliminary PD." If the second scan at least 4 weeks later exhibits a subsequent ≥25% increase in sum of products of perpendicular diameters of enhancing lesions relative to the "preliminary PD" scan it is considered "confirmed PD" and the patient should discontinue therapy. If the second scan at least 4 weeks later exhibits SD, CR, PR, or becomes non-measurable, this scan showing "preliminary PD" is noted as "pseudo-progression", PsP, and the patient should continue on therapy until a second increase in tumor size relative to the "preliminary PD", or PsP, scan is observed. Note that any new *measurable* (>10mm x 10mm) enhancing lesions on the subsequent scan following the preliminary PD scan should *not* be immediately considered confirmed PD, but instead should be added to the sum of bidimensional products representing the entire enhancing tumor burden.

3. Clear clinical deterioration not attributable to other causes apart from tumor (e.g. seizures, medication adverse effects, therapy complications, stroke, infection) or attributable to changes in steroid dose. Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration. Neurological exam data should be provided to the neuroradiologist as "stable, better, worse" in case report forms or from study sponsor. Clinical status should be recorded as "worse" if the neurological exam is worse, otherwise the clinical status should be set to "not worse." In the event that necessary clinical data is not available, clinical status should be recorded as "not available" and that particular time point can only be reviewed for PD (otherwise "non-evaluable"). Neurological data must be within ±7 days of the time-point response date, otherwise the data is considered "not available". Steroid use should be derived from the concomitant medications on the case report forms and recorded as "Yes", "No", or "not available". A value of "No" should be assigned if, at the time-point, the subject is not on steroids or on physiologic replacement doses only (<1.5 mg dexamethasone or equivalent per day). Steroid dose should be derived from the concomitant medications on the case report forms. Average steroid dose no greater than 2 mg change from baseline should be abstracted to "stable". If outside this range the steroid dose should be abstracted to "increased" or "decreased" accordingly. Steroid data should be within ±5 days of the time-point response date, otherwise the data is considered "not available".
4. Failure to return for evaluation as a result of death or deteriorating condition.

***Stable Disease* (*SD*): Requires *all* of the following:**

1. Does not qualify for CR, PR, or PD as defined above.
2. In the event that corticosteroid dose was increased (for new symptoms/signs) without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that the steroid increase was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

Overall Objective Status:

The overall objective status for an evaluation should be determined by combining the patient's radiographic response on target lesions, new disease, neurological status, and steroid dose/usage as defined in **the following table:**

Target lesions (current scan)	Target lesions (previous scan)	New sites of measurable disease[a]	Neurological status	Steroid usage	Steroid dose	Overall objective status
CR	Not Evaluated	No	Stable/Better	No	N/A	Preliminary CR
PR	Not Evaluated	No	Stable/Better	Any	Stable/Decreasing	Preliminary PR
PD	Not Evaluated	Yes or No	Stable/Better	Any	Stable/Increasing	Preliminary PD

Target lesions (current scan)	**Target lesions (previous scan)**	**New sites of measurable disease[a]**	**Neurological status**	**Steroid usage**	**Steroid dose**	**Overall objective status**
PD	Preliminary or Confirmed PR/CR	No	Stable/Better	Any	Stable/Increasing	Preliminary PD
SD	Preliminary or Confirmed CR/PR or SD/NE	No	Stable/Better	Any	N/A	SD
PR	Preliminary PR	Yes or No	Stable/Better	Any	Stable/Decreasing	Confirmed PR
SD	Preliminary PR	Yes or No	Stable/Better	Any	Stable/Decreasing	SD (Preliminary PR →Confirmed PR)
SD	Preliminary CR	Yes or No	Stable/Better	Any	Stable/Decreasing	SD (Preliminary CR →Confirmed CR)
CR	Preliminary CR	No	Stable/Better	No	N/A	Confirmed CR
SD	Preliminary PD	No	Stable/Better	Any	Stable/Decreasing	SD (Confirmed PsP)
CR/PR/SD PD/NE	CR/PR/SD/ PD/NE	Yes or No	Worse	Any	Stable/Increasing	Confirmed PD
PD	Preliminary PD	Yes or No	Any	Yes	Stable/Increasing	Confirmed PD

[a] Note that new sites of measurable disease are added to the sum of bidimensional products or total lesion volume, or constitutes preliminary PD in the case of no measurable disease at baseline or best response

All subjects infused with CART-EGFRvIII will be evaluable for PFS.

6.16 Imaging Studies

Standard post-contrast FLAIR images will be used to define RANO criteria and disease staging.

In addition, advanced magnetic resonance imaging sequences and analysis will be used to assess relative tumor blood volume (which has been shown to correlate with expression of EGFRvIII120) by perfusion imaging; assess markers of pseudoprogression by MR spectroscopy; and assess axonal pathway integrity by diffusion tensor imaging. These evaluations will be collected in tandem with standard of care MRI evaluations for assessment of tumor response and will be exploratory in nature.

The specific sequences to be used and the analyses and interpretation of the studies will be performed by the study neuro-radiologists. Advanced imaging studies will be performed at baseline and with each follow-up MRI while on study.

Details of the advanced imaging protocol is further described in **Appendix 2**.

6.17 Research Correlative Studies

Blood, body fluid, and tissue samples

Determination of the persistence and bioactivity of CART-EGFRvIII will be based on peripheral blood samples. For molecular studies (Q-PCR), immune phenotyping and functional assays, peripheral blood will be collected in Lavender top (K2EDTA) tubes. For cytokine analyses peripheral blood samples will be collected in red top (no additive) tubes. Samples will be collected according to the protocol Schedule of Evaluations (**Appendix 1**).

Additional samples may be collected at the discretion of the investigators, and is encouraged as part of the evaluation of any significant clinical events that may be related to the CART-EGFRvIII cells. Additional testing may be done depending on the clinical condition; for instance, patients with symptoms suggestive of cytokine release syndrome or with skin rash or other findings that may concern the investigators may have more frequent monitoring to enhance the safety of this trial.

Unscheduled Research Sample Collections

Beyond the research sample collections scheduled for specific time points, up to 45 mL (3 tablespoons) of additional peripheral blood may be drawn twice per week to better characterize correlates of clinical events such as cytokine release syndrome. In addition, if at any point throughout their study participation, the subject undergoes surgical resection/biopsy as part of their routine care, portions of these samples will be used for research analysis.

In the event that tumor tissue or CSF becomes available as part of routine clinical care, a sample will be collected for research analysis. Tissue or CSF samples will be analyzed for the presence of CART-EGFRvIII cells by PCR and/or immunohistochemistry. CSF samples may also be analyzed for cytokine levels by luminex technology. Tumor tissue will be analyzed for expression of EGFRvIII by PCR, next generation sequencing, immunohistochemistry, and/or FISH.

6.17.1 Sample handling

Samples will be delivered, processed, and frozen as per SOP to the Translational and Correlative Studies Laboratory (TCSL) (University of Pennsylvania). Samples will be stored in the TCSL at the University of Pennsylvania for storage and bulk analyses. Documentation for sample receipt, processing, and storage and primary data from the research analyses will be collected and stored in the TCSL.

Translational and Correlative Studies Laboratory (TCSL),
A Division of the Product Development and Correlative Sciences (PDCS) Laboratory
University of Pennsylvania
Smilow Center for Translational Research; South Tower
3400 Civic Center Blvd, Bldg. 421; Lab 9-303
Philadelphia, PA 19104-5157

The schedule of blood draws for various laboratory correlative studies is depicted in the Schedule of Evaluations (**Appendix 1**)

Circulating Tumor Material Assays:
Plasma cell-free DNA (cfDNA) and RNA (cfRNA) will be delivered, processed, and frozen as per SOP to the Circulating Tumor Material Center. Documentation for sample receipt, processing and storage, and primary data from the research analyses will be collected and stored in the University of Pennsylvania Circulating Tumor Material Center.

Please refer to the laboratory manual for additional instructions.

7. STATISTICAL PLAN

7.1 General Design

This is an open label, phase 1 study to evaluate the safety and tolerability, and persistence and engraftment of autologous T cells engineered to express a chimeric antigen receptor targeting EGFRvIII, that is linked to the CD3ζ-4-1BB signaling chains in patients with newly diagnosed EGFRvIII+ GBM. Failure to complete the study due to the stopping rules being invoked will be the main basis for determining safety of this study. This study is primarily intended to provide data that might allow the investigators to conduct a preliminary assessment of safety and feasibility. This study aims to evaluate 7 patients.

7.2 Sample Size

This is a phase I study with a primary goal of estimating safety and tolerability. Given the costs associated with CAR T cell production, we plan to enroll 7 patients that are evaluable for the study's primary endpoint. Subjects that have received at least one EGFRvIII CAR T cell infusion will be considered as evaluable. A larger follow-up trial will be designed that has the statistical power to assess the potential efficacy of the regimen if the safety profile of this study warrants a larger study design.

7.3 Endpoints for Primary Objectives

The primary objective of this study is to determine the safety and tolerability of multiple CART-EGFRvIII cells in combination with pembrolizumab for the treatment of GBM. Safety will be evaluated based on the occurrence of study related adverse events that are determined to be related to the study treatment. Endpoints of tolerability will include completion of scheduled infusion, laboratory values, and vital signs from time of the first infusion.

7.4 Endpoints for Secondary Objectives

The secondary endpoints are aimed to evaluate the anti-tumor responses to CART-EGFRvIII cells, assessed by overall survival (OS) and progression-free survival (PFS), objective response rate (ORR) based on standard MRI evaluation and the modified RANO criteria. We will also measure median progression free survival.

7.5 Subject Population(s) for Analysis

The subject population to be analyzed for primary, secondary, and correlative endpoints will include all patients infused with at least one dose of CART-EGFRvIII cells.

The **Screen Set** comprises all patients who are screened for the study. The **Enrolled Set** comprises all subjects who sign an informed consent form and are confirmed eligible for the study (i.e. excluding screen failure subjects). Screening failure is defined as failure of meeting the inclusion/exclusion criteria specified by the protocol. The **Safety Set** comprises all subjects who received minimum acceptable dose of CART-EGFRvIII cells as specified above and will be used for the primary safety endpoints. The **Efficacy Set** will include all subjects infused with minimum acceptable dose of CART-EGFRvIII cells and had at least one disease assessment available and will be used for the secondary endpoints, and other correlative/exploratory endpoints. Subjects with manufactured cells that do not meet the manufacturing release criteria and/or reach the minimum dose of $2x10^7$ will be considered a manufacturing failure.

7.6 Statistical Analysis of Endpoints

The statistical analysis will be primarily descriptive in keeping with the exploratory nature of the study. All adverse events will be described and exact 90% confidence intervals will be produced for adverse event rates, both overall and within major categories. DLT rate and exact 90% will be computed. For tolerability, the proportion of subjects that completed the scheduled infusions will be computed. Changes or abnormal laboratory values and vital signs from time of infusion will be summarized descriptively including mean, medians, standard deviation, and inter-quartile range (IQR).

Secondary endpoints include overall survival (OS), progression-free survival (PFS) and objective response rate (ORR). OS is defined as the number of days from the date of the first CART-EGFRvIII infusion to the date of death of any causes, or censored at last known date of alive. PFS is defined as the number of days from the date of the first CART-EGFRvIII infusion to the first documented disease progression (by the modified RANO criteria) or date of death, whichever occurs first. Patients who are lost to follow-up without a known date of progression will be censored in the analysis at the date of their last available tumor assessment. The survival function of OS and PFS will be estimated by Kaplan-Meier method. 90% confidence interval for the survival probability at a specific time point will be computed based on the log-log transformation. Median survival time along with the associated 90% confidence intervals will be presented if appropriate. ORR will be computed as the proportion of patients with complete response (CR) or partial response (PR) out of the total number of efficacy evaluable subjects. Exact 90% CI for ORR will be computed.

Descriptive statistics will be calculated for exploratory/correlative endpoints including mean, median, standard deviation, and inter-quartile range (IQR) for continuous variables (e.g., number or percent of EGFRvIII-transduced cells) and frequency and proportions for discrete variables. Most exploratory/correlative endpoints will be continuous. 90% confidence interval appropriate for each statistic will be used. The overall profile and individual-level change of CART-EGFRvIII cells or other measures of anti-tumor activities and biomarkers over time will be examined graphically.

8. SAFETY AND ADVERSE EVENTS

8.1 Definitions

Adverse Event
An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Intercurrent illnesses or injuries should be regarded as adverse events.

Immune-related Adverse Event (irAE)
An irAE is defined as a clinically significant AE that is associated with study drug exposure of unknown etiology, and is consistent with an immune-mediated mechanism.

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE or PDAE (as defined below) that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- leads to a persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly or birth defect
- an important medical event

Hospitalizations that meet the following criteria should not be reported as serious adverse events:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, such as preplanned study visits and preplanned hospitalizations for study procedures or treatment administration
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Social reasons and respite care in the absence of any deterioration in the patient's general condition

Note: Treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event.

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Unexpected adverse events
An adverse event is considered unexpected if the event, and/or severity of the event (grade), is not consistent with the risk information described in the investigator brochure(s) or protocol.

Related adverse events
An adverse event is considered related to participation in the research if there is a reasonable possibility that an event was caused by an investigational product, intervention, or research-required procedures. For the purposes of this study, "reasonable possibility" means there is evidence to suggest a causal relationship. The relationship of the event to the study will be classified as possibly related, probably related, and definitely related.

- **Possibly Related**: There is some evidence to suggest a causal relationship, however other factors may have contributed to the event.
- **Probably Related:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.
- **Definitely Related:** There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.

Unanticipated Adverse Device Effect (UADE) (Note: Device refers to the EGFRvIII expression and *MGMT* promoter methylation tests)
Is any serious adverse effect on health or safety, or any life threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Protocol-defined Adverse Events (PDAEs)
During long-term follow-up, only protocol-defined adverse events (PDAEs) will be collected and reported. Protocol-defined adverse events that are also determined to be serious, as defined above, will be considered protocol-defined serious adverse events (PDSAEs) and require expedited reporting to the sponsor per **Section 8.3**.

The PDAEs are as follows:

1. New incidence or exacerbation of a pre-existing neurologic disorder
2. New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
3. New incidence of hematologic disorder
4. New malignancy (T cell & non T cell)
5. Any serious adverse event or condition the investigator believes may have a reasonable relationship to CAR T cell therapy (including unexpected illnesses or hospitalizations in this patient population)

The following correlative laboratory results will also constitute a protocol-defined adverse event, however these events will be identified by the Sponsor and subsequently reported to the PI/site and FDA:

1. Positive RCL test result
2. Vector insertion site sequencing result with a mono- or oligoclonal vector integration pattern or in a location near a known human oncogene

Adverse Event Reporting Period

For this study, collection of adverse events will begin at the time of apheresis and continue until the subject is off-study. For subjects who do not undergo apheresis on this study (i.e. historical apheresis product available), the adverse event reporting period will begin with initiation of study treatment (Cycle 1/Day 1) and will continue until the subject is off study.

All adverse events will be collected through the End of Treatment Visit. Subjects may not be discontinued from the treatment phase of the study prior to the End of Study Treatment Visit for reasons other than subject withdrawal of all study consent or death. Once a subject discontinues from the treatment phase of the study, the subject will continue to be followed for PDAEs (defined above) in long-term follow-up until the subject is off-study.

Dose-Limiting Toxicity (DLT)

A dose limiting toxicity is defined as grade ≥ 3 toxicity [per NCI Common Terminology Criteria for Adverse Events (CTCAE v5.0)] occurring within 21 days after Cycle 1/Day 1, which develops or worsens following dosing (not existent before study treatment), at least possibly related to CART-EGFRvIII cells and/or pembrolizumab, and does not improve to Grade ≤ 1 within 7 days of optimal medical management.

Events excluded from the above DLT definition are:

- Grade 3 rash or fever
- Grade 3 immune-related adverse event (irAE) that resolves to a grade 1 within 14 days.
- Grade 3 neurologic event that resolves to grade 1 within 14 days
- Grade 3 nausea/vomiting that resolves to grade 1 within 14 days
- Grade ≥ 3 laboratory abnormalities as follows: 1) grade 3 electrolyte abnormalities; 2) hyperglycemia; 3) grade 4 neutropenia or thrombocytopenia lasting < 21 days from the time of CAR T cell infusion.

Preexisting Condition/General Physical Examination Findings

A preexisting condition is one that is present at the start of the Adverse Event Reporting Period. All clinically significant abnormalities should be recorded as a preexisting condition on the medical history eCRF. During the course of the study, a preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens. Preexisting conditions that improve should also be recorded appropriately.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to

separately record the lab/test result as an additional event. Laboratory abnormalities that do not meet the definition of an adverse event, should not be reported as adverse events. A Grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious defined above and/or as per investigator's discretion. Whenever possible, a diagnosis, rather than a symptom should be provided (i.e. anemia instead of low hemoglobin).

8.2 Recording of Adverse Events

Safety will be assessed by monitoring and recording potential adverse effects of the treatment using the Common Terminology Criteria version 5.0 at each study visit. Patients will be monitored by medical histories, physical examinations, and blood studies to detect potential toxicities from the treatment. If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, will be used whenever possible.

At each contact with the subject, the investigator must seek information on adverse events by non-directive questioning and, as appropriate, by examination. Adverse events may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory tests, or other assessments. Information on all adverse events should be recorded in the source documentation. All clearly related signs, symptoms, and abnormal diagnostic procedures results should be recorded as a diagnosis and symptoms used to make the diagnosis recorded within the diagnosis event. Do not list symptoms or abnormal diagnostic results separately if a diagnosis can be assigned. The safety team may require events be reported separately if they occur as SAEs (or in the context of a SAE) even if they can also be considered a constituent of another AE such as CRS.

All adverse events occurring during the adverse event reporting period (defined in **Section 8.1** above) must be recorded. Adverse events that begin in Primary Follow-up and are ongoing at the time the subject enters the LTFU phase of the study will continue to be followed in LTFU until: a) the adverse event resolves; b) the subject discontinues participation (i.e. End of Study); or c) there is a change in the adverse event that would normally require the event be captured as a new event (i.e. change in attribution). Please refer to the CRF Completion Guidelines (CCG) for specific instructions on data entry.

As much as possible, each adverse event should be evaluated to determine:

1. The severity grade (CTCAE Grade 1-5)
2. Its duration (Start and end dates)
3. Its relationship to the study treatment- [Reasonable possibility that AE is related: No (unrelated/ not suspected) or Yes (a suspected adverse reaction)]. If yes (suspected)- is the event possibly, probably or definitely related to the investigational treatment?
4. Expectedness to study treatment- [Unexpected- if the event severity and/or frequency is not described in the investigator brochure or protocol].
5. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in **Section 8.1**.

All adverse events should be treated appropriately. If a concomitant medication or non-drug therapy is given, this action should be recorded. Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

Progression of malignancy, documented appropriately in the medical records, should not be reported as a serious adverse event. Adverse events that occur concurrently with the progression of malignancy but that are not related to disease progression (i.e. deep vein thrombosis or hemoptysis) will be reported as an adverse event as described above. Progression of malignancy resulting in death that occurs during Primary Follow-up should be reported as a serious adverse event. During long-term follow-up, any death determined to be related to disease progression would not qualify as a protocol-defined adverse event.

Given the unique nature of the patient population under investigation, a modified approach for grading events of cerebral edema will be implemented as part of this protocol. These events will be graded as "Nervous system disorders- Other, Specify" according to the CTCAE V5 by severity of mild, moderate, severe and life-threatening, corresponding to Grades 1-4.

Serious adverse events that are still ongoing at the end of the adverse event reporting period must be followed to determine the final outcome. Any serious adverse event that occurs after the adverse event reporting period and is considered to be possibly related to the study treatment or study participation, should be recorded and reported.

Grading System of Cytokine Release Syndrome (CRS)
The ASTCT Consensus Grading Scale (**Table 8.2-1**) will be used to capture cytokine release syndrome (CRS) in CAR T-cell protocols. Please refer to Investigator's Brochure for additional detail on CRS.

The start date of CRS is a retrospective assessment of the date of onset of persistent fevers and/or myalgia consistent with CRS and not explained by other events (i.e. sepsis). The stop date of CRS is defined as the date when the patient has been afebrile for 24 hours and off vasopressors for 24 hours. For the purposes of defining the CRS start date, a fever is defined as a temperature of 100.4°F/38°C.

Table 8.2-1: ASTCT CRS Consensus Grading Criteria (Lee et al., 2019)

CRS parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever[1]	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C
With either				
Hypotension	None	Not requiring vasopressors	Requiring one vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/Or[2]				
Hypoxia	None	Requiring low-flow nasal cannula[3] or blow-by	Requiring high-flow nasal cannula, facemask, non-rebreather mask, or Venturi mask	Requiring positive pressure (eg: CPAP, BiPAP, intubation and mechanical ventilation)

CPAP = Continuous positive airway pressure; BiPAP= Bilevel positive airway pressure

#Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.

[1] Fever is defined as temperature ≥38°C not attributable to any other cause. In subjects who have CRS then receive antipyretics or anti-cytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

[2] CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a subject with temperature of 39.5°C, hypotension requiring one vasopressor and hypoxia requiring low-flow nasal cannula is classified as having Grade 3 CRS.

[3] Low-flow nasal cannula is defined as oxygen delivered at ≤6 liters/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at > 6 liters/minute.

Pregnancies

To ensure patient safety, each pregnancy occurring while the patient is on study must be reported to protocol sponsor within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. If a pregnancy occurs on study, this will be reported as an SAE using the SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males who took study treatment in this study. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

8.3 Reporting of Serious Adverse Events

Every SAE, UADE, and PDSAE (during LTFU), **regardless of suspected causality**, occurring during the adverse event reporting period defined in **Section 8.1** above must be reported to the sponsor within 24 hours of learning of its occurrence. The original SAE notification may take place by email to meet the 24 hour reporting window. However, within 3 business days of knowledge of the event, the investigator must submit a complete SAE form to the Sponsor along with any other diagnostic information that will assist the understanding of the event. The Investigator will keep a copy of this SAE Form on file at the study site.

Follow-up information on SAEs should be reported when updates are available, as a follow-up to the initial SAE form, and should include both the follow-up number and report date. New information on ongoing serious adverse events should be provided promptly to the sponsor. The follow-up information should describe whether the event has resolved or continues, if there are any changes in assessment, if and how it was treated, and whether the patient continued or withdrew from study participation.

Report serious adverse events by email to:

Attention: Clinical Safety Manager or designee
Center for Cellular Immunotherapies (CCI)
University of Pennsylvania

At the time of the initial report, the following information should be provided:

- Study identifier
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason the event is classified as serious
- Investigator assessment of the association between the event and study treatment
- Expectedness relative to investigational product(s)

8.3.1 Investigator Reporting: Local Regulatory Review Committees

Report events to local regulatory review committees per institutional requirements.

8.4 Toxicity Management, Stopping Rules, and Study Termination

It is expected that AEs will occur frequently in this population based on the underlying malignancy and that these can be SAEs. Therefore, there is no specific occurrence of SAEs that define a stopping rule, but the review of SAEs will form the basis for potential early stopping of the study. The review of these adverse events, and any decision to prematurely stop subject enrollment, will be determined by the Sponsor.

In addition to the above, premature termination of the clinical trial may also occur because of a regulatory authority decision, change in opinion of the IRB, determination that there are problems in the cell product generation, or as a result of safety concern. Additionally, recruitment may be stopped for reasons of particularly low recruitment, protocol violations, or inadequate data recording.

8.4.1 Criteria for Pausing/Stopping the Study

If 2 or more subjects have confirmed DLTs at a given dose level, the study will be paused for review by the DSMB and dose de-escalation (as applicable). If 2 or more subjects have confirmed DLTs at the de-escalated dose level, the study may be stopped prematurely.

This rule is based on upon the following: If the true DLT rate is <30%, the probability of observing 2 or more DLT (i.e., CART-EGFRvIII dose will be de-escalated) in 3 subjects is <22%. Based on this rule, the probability of pausing (i.e., 2 or more) early will be 0.03, 0.11, 0.34, 0.59, 0.76 if the true DLT rate is 5%, 10%, 20%, 30%, and 40%, respectively. The calculations were done based on 5000 simulations".

In the event a subject is on-study and receiving CART-EGFRvIII infusions when a decision is made to proceed to the dose de-escalated cohort, the subject may continue to be treated with the same dose as previously received if this was well tolerated, unless otherwise specified by the Investigators, Sponsor Medical Director and/or Regulatory Authorities.

Dose reductions are not permitted for pembrolizumab. Please refer to **Section 5.2** for events that may delay/interrupt pembrolizumab dosing.

The study will also be reviewed for potential termination if:

- Any subject develops uncontrolled T cell proliferation that does not respond to management.
- Premature study termination may occur if the Investigator, Sponsor, Study Funder, DSMC, Medical Director, DSMB, or any independent review board or regulatory body decides for any reason that subject safety may be compromised by continuing the study.
- Premature study termination may occur if the Sponsor decides to discontinue the development of the intervention to be used in this study.

Subject accrual will be paused if a death occurs within 30 days of study treatment that is felt to be possibly related to study treatment. Accrual will be held until an investigation is performed and the safety data is evaluated by the DSMB and applicable changes are implemented (as appropriate). The outcome of the DSMB review will be shared with the Sponsor. This information will then be provided to the FDA and site (for local regulatory submission) in the form of an outcome letter. If all parties are in agreement as to the event resolution, then the pause will be lifted.

The protocol will be paused and subject accrual suspended to review the manufacturing process should there be ≥33% failed manufacturing products (i.e. failure to release the product and/or failure to reach the target dose).

8.4.2 General Toxicity Management Considerations

8.4.2.1 CART-EGFRvIII

- Acute Neurological Toxicity
  In case of acute neurologic toxicity that is, in the investigator's opinion, probably related to CART-EGFRvIII effects, management may include dexamethasone and a cytokine blockade agent such as tocilizumab, anakinra and/or siltuximab. These cytokine-blocking agents are FDA approved for rheumatologic disease and Castleman's disease, respectively; the rationale for using these agents as medical practice is based on the rapid reversal of systemic toxicity (cytokine release syndrome) with tocilizumab when CART-19 cells are used in hematologic malignancies. In case of severe, acute neurologic toxicity possibly related to CART-EGFRvIII cells, the investigator may choose to use any of the above agents using standard dosing schedules; the agent that is most easily available at their institution may be used as first line, with either of the other agents used as second and third line. Each cytokine blocking agent functions via a different mechanism, and in case of refractory severe toxicity, they may be considered for use sequentially at the treating physician's discretion. There is a paucity of data regarding CNS penetration differences among the three cytokine blockade agents.

- Infusion Reactions
  Several reactions may develop during and immediately after T cell infusions such as flu-like symptoms, hemodynamic effects, and dermatologic reactions.

- Flu-like symptoms
  - Fever/arthralgia/myalgia: The onset of these symptoms usually occurs 2 to 4 hours after the cell product administration. Management: Administration of acetaminophen or non-steroidal anti-inflammatory drugs (NSAIDs) is effective in controlling and preventing symptoms. All subjects will be treated with diphenhydramine and/or an H2-blocker such as ranitidine to avoid transfusion or and dermatologic reactions.
  - Rigors/chills: These symptoms may occur during and/or after cell infusion administration. Management: The agent of choice to improve severe chills or rigors is an opioid such as meperidine hydrochloride or hydromorphone hydrochloride. If subjects develop rigors consistently after cell product administration, prophylactic administration of NSAIDS (e.g. ibuprofen) may prevent these reactions.

- Hemodynamic Effects
  - Edema: Adequate hydration is necessary to ensure renal perfusion, but over-hydration should be avoided. Subjects should be encouraged to drink electrolyte-containing fluids such as sport drinks and soups. Management: If diuretics are to be used to manage edema, they should be used with caution to avoid decreasing intravascular volume. If participants develop hypotension, IV fluids should be administered and appropriate evaluation with observation in an acute hospital bed is advisable.

- Hypotension: Organ dysfunction, oliguria, and increases in BUN and creatinine are usually reversible upon discontinuation of cell product and 24 to 48 hours of supportive treatment. Management: On rare occasions, low doses of dopamine and fluid support may be warranted. Educating the subject to slowly rise from a supine to a sitting position and then to a standing position can prevent orthostatic hypotension. Evaluation in the Emergency Department if symptoms occur is advisable.

- Dermatologic Reactions
  - Skin reaction: Dry skin, pruritis, erythema, and sloughing occur commonly following cell infusions. No interruption of therapy is usually needed. Management: Anti-histamines, water or oil-based lotions, and oatmeal baths may help control the symptoms. Diphenhydramine hydrochloride (Benadryl) and/or an H2-blocker, will be given before the administration of the T cell product to prevent skin reactions.

- Off-tumor toxicity manifested as rash or diarrhea
  Cross-reactivity with wild-type EGFR which is expressed on most epithelial tissues, particularly in lung epithelium and skin has been seen when wild type EGFR is targeted with other agents such as cetuximab. The expected toxicity is development of skin rash and diarrhea. If a biopsy of the affected organ is clinically indicated, a tissue sample should be sent to the TCSL for research evaluation. These may be managed with anti-diarrheal agents, oral budesonide, systemic steroids for diarrhea and topical, systemic steroids for skin rash according to the clinical decision of study PI.

- Tumor pseudo-progression
  In malignant glioma vaccine studies by us (**Okada et al., 2011**) and others (**Sampson et al., 2010**) some vaccine-recipients have shown enlargement or appearance of new contrast-enhancement on MRI that resolved with or without concomitant steroids. These findings may be attributed to immunologic anti-tumor response rather than tumor progression (i.e., tumor pseudoprogression). If pseudoprogression is suspected, the patient should be observed, if asymptomatic, or may be placed on dexamethasone (4 mg/d or lower) for one month, at which time an MRI scan should be repeated. If the steroid dose is ≤ 4 mg/d and repeat imaging indicates that the criteria for disease progression have not been met, patients will continue on study as prescribed by the protocol; otherwise, they will be considered progression and taken off study.

  Patients who meet the criteria for pseudo-progression will be classified as stable disease (SD) or partial response/complete response (PR/CR), depending on the imaging response after administration of CAR therapy, when compared to the pretreatment baseline. Patients on increased steroid doses, who show no change/worsening upon repeat imaging or no improvement in clinical status over a period of 1 month, will be considered for biopsy or resection (if clinically warranted) in order to differentiate between pseudo- and true tumor progression. Obtained histopathological specimens will be examined for evidence of inflammatory/lymphocytic infiltration that is indicative of pseudoprogression. If inflammatory infiltration and/or necrosis comprise the majority of the specimen, patients may remain on study, once they are clinically stable and on < 4 mg/day Decadron for at least 1 week. If the majority of the resected specimen consists of tumor, or if the patient does not have further surgery, the patient will be considered to have true progression and will be taken off follow-up study procedures but followed for OS. Patients who have progressively enlarging tumor size or

worsening symptoms, despite increasing corticosteroid doses, will be considered to have progressive disease and be taken off follow-up study procedures but followed for OS. So will patients who show enlargement that does not regress within 1 month, are judged not to be candidates for biopsy/resection.

- Replication-competent lentivirus (RCL) may be generated during the CAR T cell manufacturing phase or subsequently after introduction of vector transduced cells into the patient. However, an RCL resulting from the production phase is highly unlikely since elements are incorporated in the design of the vector system that minimize vector recombination and generation of RCL. Furthermore, the vector used to transduce the product undergoes sensitive assays for detection of RCL before it can be released to a subject. Nevertheless, generation of an RCL following infusion of the vector product remains a theoretical possibility. The consequences of such recombination events in subjects without a known lentiviral infection are unknown, and therefore subjects with coexistent HIV infection are excluded from participation in this study in order to minimize this possibility. The development of RCL could pose a risk to both the subject and their close contact(s), and therefore, monitoring for RCL will be conducted during the course of the trial.

  Regulatory agencies and the gene therapy community have previously discussed measures to be taken should an RCL be confirmed in a subject. However, because the probability and characteristics of an RCL are unknown, no guidelines have been put in place. Nevertheless, all agree that the subject must be isolated until an understanding of how to manage the subject becomes clear. Some considerations are

  - Intensive follow-up of subject in consultation with gene therapy experts, study investigators, FDA and NIH.
  - Inform local public health officials and CDC.
  - Identify sexual partners and provide appropriate counseling and intervention.

  RCL will be monitored by a suitable Q-PCR DNA assay for detection of the lentivirus (for example: HIV gag DNA or VSV-G DNA). If a positive RCL DNA assay result is obtained, the PI will be informed and the subject rescheduled for a retest for the DNA test. If the second DNA test is positive, then infusions of subsequent patients will be temporarily halted. The patient will undergo a blood draw for isolation of HIV from his/her cells. The virus will be sequenced and compared to sequences of the transfer vector and packaging constructs, as well as to available HIV sequences to determine the origin of the virus. Determination of the origin of the virus can be easily performed by evaluation for HIV accessory genes such as vif, vpr and vpu which are not present in the packaging constructs. If the sequence is derived from wt-HIV then infusions for all subjects can resume, and the patient will be referred to treatment for HIV. If an RCL is confirmed, or the virus cannot be isolated from the blood draw, the patient will be scheduled for apheresis and will undergo a full biological RCL testing for detection and/or characterization of the RCL.

- Clonality and insertional oncogenesis
  The occurrence of adverse events caused by insertional mutagenesis in five patients in a gene therapy trial for X-linked SCID following stem cell therapy emphasizes the potential for problems in translating this approach to the clinic(**Hacein-Bey-Abina et al., 2008; Hacein-Bey-Abina et al., 2010**). To date, clinically evident insertional mutagenesis has not been reported following adoptive transfer of engineered T cells. Lentiviral vectors may have a lower risk than oncoretroviral vectors based on several considerations (**Montini et al., 2006; Newrzela et al., 2008**). In case of continual increase of CART cells or if the CBC analysis reveals abnormal T cell counts, monitoring for T cell clonal outgrowth will be performed via TCR deep sequencing at multiple time points to assess evolution of the repertoire; we will follow if the repertoire is dominated by a single or few clones, and the persistence of these clones. If a subject's repertoire is found to be monoclonal or oligoclonal, the subject's T cells will be evaluated for the pattern of vector insertion (insertion site analysis) in order to locate association of the insertion site(s) with known human oncogenes. If the clone is persistent within 3 months and the pattern of insertion is found to favor a single dominant insertion site, the subject will be monitored for hematologic malignancies.

- Uncontrolled T cell proliferation
  CAR T cells could proliferate without control of normal homeostatic mechanisms. In pre-clinical studies, CAR T cells have only proliferated in response to physiologic signals or upon exposure to antigen. In the context of this protocol it is expected that the T cells will proliferate in response to signals from the malignant tumor or normal B cells. This could be beneficial or harmful depending on the extent of proliferation. Clonal dominance of adoptively transferred T cells has been associated with tumor reduction in adoptive transfer trials(**Dudley et al., 2002**). If uncontrolled T cell proliferation occurs, subjects may be treated with corticosteroids. Subjects will be treated with pulse methylprednisolone (2 mg/kg i.v. divided q8 hr x 2 days), followed by a rapid taper.

- Macrophage activation syndrome (MAS)
  Based on the observations of subjects treated on UPENN protocol UPCC 04409, there is some concern for macrophage activation syndrome (MAS). Treatment and timing of treatment of this toxicity will be at the discretion of the patient's physician and the study investigator. Suggested management might include: if the subject has a fever greater than 101°F that lasts more than 2 consecutive days and there is no evidence of infection (negative blood cultures, CXR or other source), tocilizumab 8 mg/kg can be considered. The addition of corticosteroids and anti-TNF therapy can be considered at the physician's discretion.

- Cytokine Release Syndrome (CRS) / Macrophage Activation Syndrome (MAS)
  Selective tocilizumab therapy has been utilized (described below) to manage CRS/MAS toxicity without precluding CAR T cell expansion in patients. Steroids or other immunosuppressant drugs should **NOT** be used as pre-medication for CAR T cell therapy but may be considered in the management of CRS.

  The moderate to severe cases of CRS observed required intervention with single dose tocilizumab with or without high dose corticosteroids, between 2 and 9 days after T cell infusion to date. This resulted in rapid reversal of the high persistent fevers and hemodynamic instability associated with CRS in most but not all patients.

Given the clinical improvement of patients after treatment with anti-cytokine therapy, patients with moderate to severe cytokine toxicities should be managed with administration of tocilizumab.

Tocilizumab should be used as a single, weight-based dose of 8 mg/kg at the time of hemodynamic instability. This management approach is designed to avoid life-threatening toxicities, while attempting to allow the CAR T cells to establish a proliferative phase that appears to correlate with clinical efficacy. Thus, the timing of the tocilizumab should be individualized, in close consultation with the Principal Investigator and/or expert consultants for the trial. Steroids have not always been effective in this setting and may not be necessary given the rapid response to tocilizumab. Because steroids are thought to interfere with CART cell function and efficacy, if used, they should be rapidly tapered.

Upon developing the prodrome of high-persistent fevers following CART–cell infusion, patients should be followed closely. Infection and tumor lysis syndrome work up should be immediately undertaken. The pharmacy should be notified of the potential need for tocilizumab. Patient management in an intensive care unit may be required and the timing is dependent upon local institutional practice. In addition to supportive care, tocilizumab may be administered in cases of moderate to severe CRS, especially if the patient exhibits any of the following:

- Hemodynamic instability despite intravenous fluid challenges and moderate stable vasopressor support
- Worsening respiratory distress, including pulmonary infiltrates, increasing oxygen requirement including high-flow O2, and/or need for mechanical ventilation.
- Any other signs or symptoms of rapid deterioration despite medical management

The recommended dosing for tocilizumab is 8 mg/kg (maximum individual dose of 800 mg) i.v. every 8 hours for a maximum of four doses; this is the dosing specified in the tocilizumab product label for management of CRS. Failure of CRS to improve after two tocilizumab doses, however, should also prompt consideration of adjunctive therapies, as described below, in addition to further tocilizumab doses. Not all Grade 4 CRS reactions following CAR T cell infusions have been immediately treated with tocilizumab and decisions are, in part, based upon the rapidity of the syndrome onset and the individual patient's physiologic reserve.

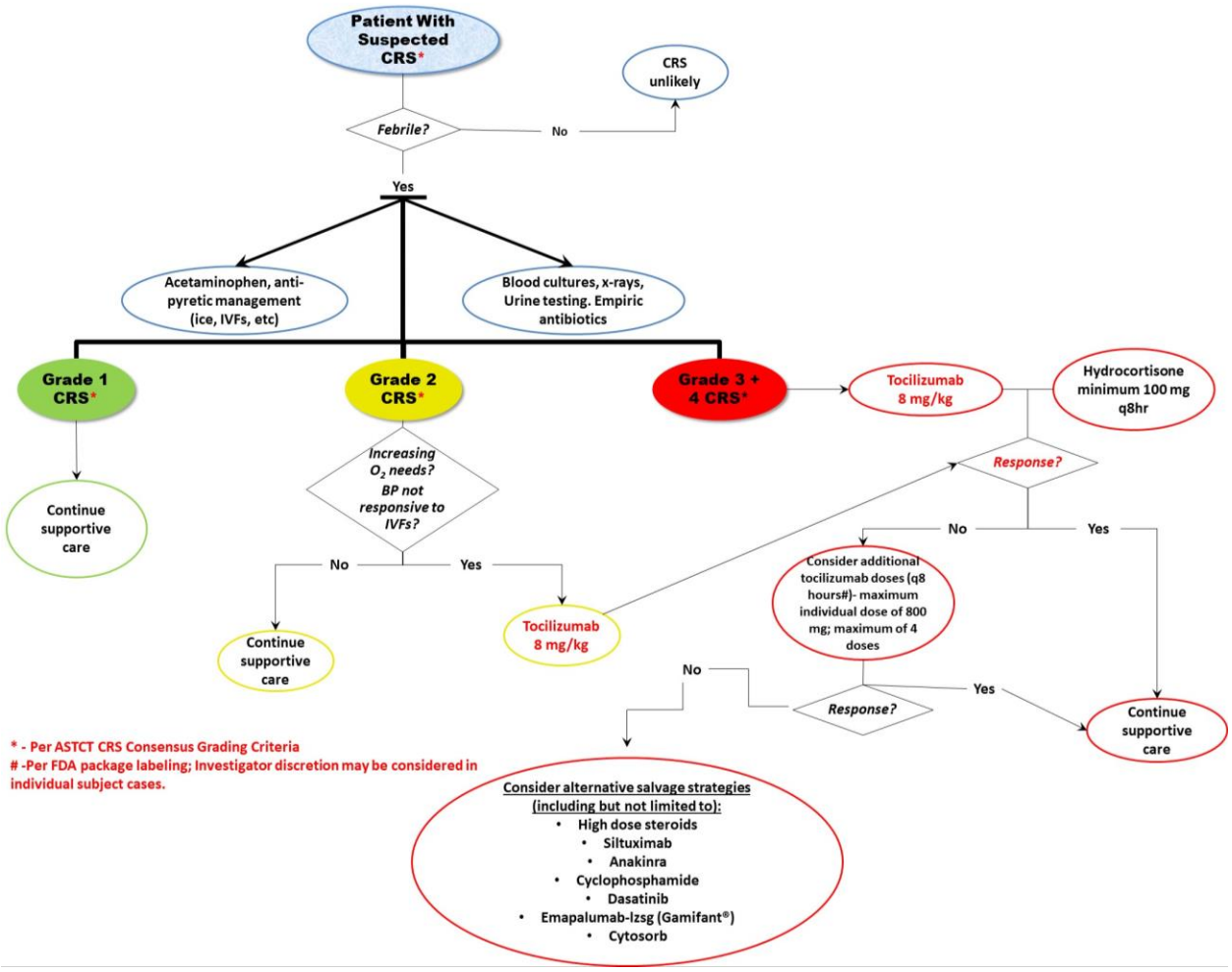
Other anti-cytokine therapies, such as repeat administration of tocilizumab or use of siltuximab or etanercept, may also be considered if the patient does not respond to the initial dose of tocilizumab. If the patient experiences ongoing CRS despite administration of anti-cytokine directed therapies, anti T-cell therapies such as cyclophosphamide, ATG, or alemtuzumab may be considered.

CRS has been associated with biochemical and physiologic abnormalities consistent with MAS. Moderate to extreme elevations in serum C-reactive protein (CRP) and ferritin have been seen with CART19 associated CRS, however the magnitude and kinetics vary greatly between individual patients. CRS management decisions should be based upon clinical signs and symptoms and response to interventions, not these laboratory values *per se*. **Refer to Figure 8.4.2-1 below for a CRS Management Algorithm.** Aggressive monitoring and management of cytopenias and coagulopathy is recommended for patients with CRS/MAS, especially those with neurologic

sequelae, in order to minimize bleeding risk. This may involve transfusing to maintain hemoglobin, platelets, and fibrinogen at higher-than-usual thresholds, as clinically indicated.

In all cases of CRS, we will perform serum cytokine analysis at baseline using a 30-plex Luminex assay. This analysis will be repeated at all time-points collected during the event until its clinical resolution. This analysis will be batched and not available in real time; thus, it will not be used for clinical decisions. We will report these results to FDA.

Figure 8.4.2-1 CRS Management Algorithm



8.4.2.2 Pembrolizumab

Please refer to the pembrolizumab package insert and the following publication (Brahmer, et al in the Journal of Clinical Oncology 2018; **http://ascopubs.org/doi/full/10.1200/JCO.2017.77.6385**) for toxicity management considerations.

8.5 Protocol Exceptions and Deviations

Exception
A one time, **intentional** action or process that departs from the IRB-approved study protocol, intended for **one** occurrence. If the action disrupts the study progress, such that the study design or outcome (endpoints) may be compromised, or the action compromises the safety and welfare of study subjects, **advance** documented approval from the Regulatory Sponsor and local regulatory review committees per institutional guidelines is required. Approval from the Regulatory Sponsor must be received prior to submission to local regulatory review committees for approval.

Deviation
A one time, **unintentional** action or process that departs from the IRB-approved study protocol, involving one incident and **identified retrospectively**, after the event occurred. If the impact on the protocol disrupts the study design, may affect the outcome (endpoints) or compromises the safety and welfare of the subjects, the deviation must be reported to the Regulatory Sponsor IRB within 10 business days of PI knowledge, and to local regulatory review committees per institutional guidelines. Acknowledgement from the Regulatory Sponsor must be received prior to submission to local regulatory review committees.

Other deviations should be appropriately documented per site policies/procedures (such as a subject missing a visit is not an issue unless a critical/important treatment or procedure was missed and must have been done at that specific time).

Include the following information on the Sponsor supplied exception/deviation form: protocol number, subject study number, comprehensive description of the exception/deviation from the protocol, rationale and corrective and preventative action plan (deviations only). Ensure all completed exception/deviation forms are signed by the Principal Investigator (or Physician Sub-Investigator) and submitted to the Sponsor Project Manager for review.

Attention: Sponsor Project Manager
Center for Cellular Immunotherapies (CCI)
University of Pennsylvania

Once approval of the exception request or acknowledgement of the deviation has been granted by the Regulatory Sponsor, the exception or deviation will be submitted to all applicable committees for review and approval.

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.7 Independent Data and Safety Monitoring Board

A Data and Safety Monitoring Board (DSMB), comprised of a minimum of 3 individuals including physicians with experience in oncology and/or gene transfer therapy, will be assembled and will work under a charter specifically developed for safety oversight of this study. The DSMB will provide guidance/advice to the Sponsor. The DSMB will evaluate patient-subject safety as specified in the DSMB Charter.

A DSMB meeting will review safety data approximately every 6 months throughout the study. If necessary, additional meeting of the DSMB may be held if safety issues arise in between scheduled meetings.

It is envisioned that the DSMB may make four types of recommendations, namely:

- No safety or efficacy issues, ethical to continue the study as planned
- Serious safety concerns precluding further study treatment, regardless of efficacy
- Overwhelming evidence for futility, recommend stopping the study.
- Recommendation to continue the study but proposing an amendment to the protocol (e.g., incorporate an additional safety assessments)

A sponsor representative will share the outcome of the DSMB meeting with the PI via email, for submission to local regulatory review committees as required per institutional policy.

9. DATA HANDLING AND RECORDKEEPING

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: 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 and complete, 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.

The investigator must maintain source documents for each subject in the study, consisting of case and visit notes (hospital or clinical medical records) containing demographic and medical information,

laboratory data, electrocardiograms and the results of any other tests or assessments. All information recorded on the eCRFs must be traceable to source documents in the patient's file. The investigator must also keep the original signed informed consent form, and a signed copy must be given to the patient.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All entries will be entered into an electronic data capture system (EDC) via PennCTMS. The Principal Investigator is responsible for assuring that the data entered into eCRF is complete, accurate, and that entry and updates are performed in a timely manner.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10. STUDY MONITORING, AUDITING, AND INSPECTING

10.1 Study Monitoring Plan

This study will be monitored according to the Sponsor Data and Safety Monitoring Plan.

Interim Monitoring Visits will be conducted during the course of the study. The Monitors will assure that submitted data are accurate and in agreement with source documentation; verify that investigational products are properly stored and accounted for, verify that subjects' consent for study participation has been properly obtained and documented, confirm that research subjects entered into the study meet inclusion and exclusion criteria, and assure that all essential documentation required by Good Clinical Practices (GCP) guidelines are appropriately filed.

At the end of the study, Monitors will conduct a close-out visit and will advise on storage of study records and disposition of unused investigational products.

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance offices.

The Principal Investigator must notify the Sponsor in real-time if an audit/inspection notification is received.

11. ETHICAL CONSIDERATIONS

This study is to be conducted according to Good Clinical Practice as implemented by the FDA (FDA Title 21 part 312, International Conference on Harmonization guidelines, and other applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject and the appropriately-licensed investigator obtaining the consent.

The protocol is listed under clinicaltrials.gov.

12. STUDY FINANCES

12.1 Funding Sources

This study will be funded by Novartis.

12.2 Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

12.3 Subject Stipends or Payments

There is there is no subject stipend/payment for participation in this protocol.

13. PUBLICATION PLAN

Publication of the results of this trial will be governed by University of Pennsylvania policies. Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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15. Appendix 1: Schedule of Evaluations

	~ Week -12 to -8 [1]	~ Week -8 to -4	~ Week -6 to -3	~ Day -7 to -1	Cycle 1 / Day 1	Cycle 1/Day 2, Cycle 1/ Day 4 (+/- 1d), Cycle 1/Day 8 (+/- 1d), Cycle 1/Day 11 (+/- 1d), and Cycle 1/Day 15 (+/- 3d)	Cycle 2 / Day 1 (+/- 3 days)	Cycle 2/Day 2, Cycle 2/ Day 4 (+/- 1d), Cycle 2/Day 8 (+/- 1d), Cycle 2/Day 11 (+/- 1d), and Cycle 2/Day 15 (+/- 3d)	Cycle 3 / Day 1 (+/- 3 days)	Cycle 3/Day 2, Cycle 3/ Day 4 (+/- 1d), Cycle 3/Day 8 (+/- 1d), Cycle 3/Day 11 (+/- 1d), and Cycle 3/Day 15 (+/- 3d)	Cycle 4/ day 1 (+/- 3 days)	30 Days (+/- 5 days) After Last Study Treatment	Months 3, 6, 9 (+/-1 month)	Months 12, 18, & every 6 months up to Month 60 (+/- 2 months)	Annual Visits Years 6 to 15 (if evidence of vector-modified cells) [2]	Annual Visits Years 6 to 15 (if no evidence of vector-modified cells) [2]
	Screening/ Enrollment	**Apheresis**	**Radiation Therapy**	**Pre-Treatment Visit**	**Cycle 1** Pembrolizumab + CART-EGFRvIII Infusions	**Cycle 1** Safety Follow-up	**Cycle 2** Pembrolizumab + CART-EGFRvIII Infusions	**Cycle 2** [3] Safety Follow-up	**Cycle 3** Pembrolizumab + CART-EGFRvIII Infusions	**Cycle 3** [3] Safety Follow-up	**Cycle 4** [29] Pembrolizumab Monotherapy	**End-of-Study Treatment Visit**	**Long-term Follow-up** [4]			
CLINICAL ASSESSMENTS																
Informed Consent	X															
Demographics	X															
Diagnosis and Extent of Cancer	X															
Relevant Medical History	X															
Antineoplastic Therapy [5]	X												X[5]---------- X[5]			
Recent Medical History Physical Examination (including Neurologic Exam) [6], ECOG Performance Status	X			X	X[7]	X	X[7]	X	X[7]	X	X	X	X	X	X	
Vital Sign Assessments [8]	X			X	X[8]	X	X[8]	X	X[8]	X	X[8]	X				
Prior/Concomitant Medications [9]	X		X---------- X										X[10]---------- X[10]			
Adverse Events		X[37]---------- X											X[11]---------- X[11]			
Leukapheresis Screening	X															
MRI Brain [12]				X[13]					X[14]			X[14]				
EGFRvIII Expression Testing [15]	X[16]															
MGMT Promoter Methylation Testing [15]	X[16]															

	~ Week -12 to -8 [1]	~ Week -8 to -4	~ Week -6 to -3	~ Day -7 to -1	Cycle 1 / Day 1	Cycle 1/Day 2, Cycle 1/ Day 4 (+/- 1d), Cycle 1/Day 8 (+/- 1d), Cycle 1/Day 11 (+/- 1d), and Cycle 1/Day 15 (+/- 3d)	Cycle 2 / Day 1 (+/- 3 days)	Cycle 2/Day 2, Cycle 2/ Day 4 (+/- 1d), Cycle 2/Day 8 (+/- 1d), Cycle 2/Day 11 (+/- 1d), and Cycle 2/Day 15 (+/- 3d)	Cycle 3 / Day 1 (+/- 3 days)	Cycle 3/Day 2, Cycle 3/ Day 4 (+/- 1d), Cycle 3/Day 8 (+/- 1d), Cycle 3/Day 11 (+/- 1d), and Cycle 3/Day 15 (+/- 3d)	Cycle 4/ day 1 (+/- 3 days)	30 Days (+/- 5 days) After Last Study Treatment	Months 3, 6, 9 (+/-1 month)	Months 12, 18, & every 6 months up to Month 60 (+/- 2 months)	Annual Visits Years 6 to 15 (if evidence of vector-modified cells) [2]	Annual Visits Years 6 to 15 (if no evidence of vector-modified cells) [2]
	Screening/ Enrollment	**Apheresis**	**Radiation Therapy**	**Pre-Treatment Visit**	**Cycle 1** Pembrolizumab + CART-EGFRvIII Infusions	**Cycle 1** Safety Follow-up	**Cycle 2** Pembrolizumab + CART-EGFRvIII Infusions	**Cycle 2** [3] Safety Follow-up	**Cycle 3** Pembrolizumab + CART-EGFRvIII Infusions	**Cycle 3** [3] Safety Follow-up	**Cycle 4** [29] Pembrolizumab Monotherapy	**End-of-Study Treatment Visit**	**Long-term Follow-up** [4]			
EKG	X			X												
ECHO or MUGA	X[18]						X[39]				X[39]					
Survival Follow-up													X----------X[20]			
CLINICAL LAB TESTS																
CBC, Differential (5 mL EDTA)	X	X[21]		X	X	X	X[22]	X	X[22]	X	X[22]	X	X	X	X	
Chemistry (3 mL SST) [23]	X			X	X	X	X[22]	X	X[22]	X	X[22]	X	X	X	X	
Serum Pregnancy Test [24] (1 mL SST)				X												
Urine Pregnancy Test [24]	X															
Viral Serology [25] (5 mL red top, serum)	X															
HIV Test (1 mL SST)	X															
Coagulation Factors (PT, PTT, INR, fibrinogen, D-dimer) (1 blue top – 5mL)				X[36]												
HLH/MAS Labs (serum ferritin, triglycerides, haptoglobin, CRP) (4mL SST; 3mL EDTA)				X[36]												
Endocrine Evaluation: TSH, T4, Free T4, cortisol, lipase				X	X		X		X		X	X				

	~ Week -12 to -8 [1]	~ Week -8 to -4	~ Week -6 to -3	~ Day -7 to -1	Cycle 1 / Day 1	Cycle 1/Day 2, Cycle 1/ Day 4 (+/- 1d), Cycle 1/Day 8 (+/- 1d), Cycle 1/Day 11 (+/- 1d), and Cycle 1/Day 15 (+/- 3d)	Cycle 2 / Day 1 (+/- 3 days)	Cycle 2/Day 2, Cycle 2/ Day 4 (+/- 1d), Cycle 2/Day 8 (+/- 1d), Cycle 2/Day 11 (+/- 1d), and Cycle 2/Day 15 (+/- 3d)	Cycle 3 / Day 1 (+/- 3 days)	Cycle 3/Day 2, Cycle 3/ Day 4 (+/- 1d), Cycle 3/Day 8 (+/- 1d), Cycle 3/Day 11 (+/- 1d), and Cycle 3/Day 15 (+/- 3d)	Cycle 4/ day 1 (+/- 3 days)	30 Days (+/- 5 days) After Last Study Treatment	Months 3, 6, 9 (+/-1 month)	Months 12, 18, & every 6 months up to Month 60 (+/- 2 months)	Annual Visits Years 6 to 15 (if evidence of vector-modified cells) [2]	Annual Visits Years 6 to 15 (if no evidence of vector-modified cells) [2]
	Screening/ Enrollment	**Apheresis**	**Radiation Therapy**	**Pre-Treatment Visit**	**Cycle 1** Pembrolizumab + CART-EGFRvIII Infusions	**Cycle 1** Safety Follow-up	**Cycle 2** Pembrolizumab + CART-EGFRvIII Infusions	**Cycle 2** [3] Safety Follow-up	**Cycle 3** Pembrolizumab + CART-EGFRvIII Infusions	**Cycle 3** [3] Safety Follow-up	**Cycle 4** [29] Pembrolizumab Monotherapy	**End-of-Study Treatment Visit**	**Long-term Follow-up** [4]			
INTERVENTIONS																
Anti-epileptic Medication					X[27] ----------					----------X[27]						
Radiation Therapy			X[28]													
Pembrolizumab Infusion[29, 30]					X		X		X		X[29]					
CART-EGFRvIII Cell Infusion[30]					X[26]		X[26]		X[26]							
Apheresis		X[31]														
RESEARCH SPECIMENS [32, 33]																
cfDNA and cfRNA (up to 30 mL)		X	X[38]	X			X[34]		X[34]		X[34]					
Peripheral Blood Mononuclear Cells ~25 mL (purple top tubes)				X	X[35]	X	X[35]	X	X[35]	X	X[35]	X	X	X	X	
DNA (qPCR persistence) [17]				X	X	X	X	X	X	X	X	X	X	X	X	
DNA RCL (VSV-G qPCR) [19]				X					X		X		X	X	X	
Peripheral Blood SERUM ~5 mL (Red top tube)				X	X[35]	X	X[35]	X	X[35]	X	X[35]	X				
TOTAL BLOOD DRAW (~mL)	~14	~35	60	~87	~73	~38	~103	~38	~103	~38	~103	~38	~33	~33	~33	0

[1] In the event that the time between screening/enrollment and Cycle 1/Day 1 exceeds 12 weeks, the following will be repeated: Physical Examination, ECOG Performance Status Assessment, Complete Blood Count, Differential and Platelet Count, Chemistry Panel, Pregnancy test, ECHO or MUGA, and EKG. Other evaluations to be repeated at the discretion of the PI.

[2] Visit window ±60 days

[3] Cycle 2 and Cycle 3 safety follow-up visits will only be required if CART-EGFRvIII cells are infused.

[4] Subjects who complete or prematurely discontinue from the Treatment Phase will enter into long-term follow-up for up to 15 years after their CAR T cell infusion. Please see **Section 6.14** for long-term follow-up requirements.

[5] Prior antineoplastic therapies up through study treatment and post-disease recurrence will be collected.

[6] To be performed by a qualified provider.

[7] Neurologic exam must be performed on the day of infusion prior to administration of CART-EGFRvIII cells, and again post infusion. Any clinically significant change in neurologic examination must be reported immediately to the treating investigator.

[8] Routine vital sign assessments include weight, temperature, pulse, respiratory rate, blood pressure, and oxygen saturation by pulse oximetry. Height will be collected at screening only. On infusion days, please refer to **Section 5.3.3** for pre- and post-infusion vital sign monitoring requirements for pembrolizumab and CART-EGFRvIII. Clinically significant changes in vital signs must be reported immediately to the treating investigator.

[9] Please refer to **Section 5.4** for complete information on concomitant therapy requirements.

[10] During long-term follow-up, only medications used to treat the subject's cancer will be collected.

[11] Please see **Section 8.1** for protocol-defined adverse event (PDAE) reporting requirements in long-term follow-up.

[12] Brain MRIs performed will include advanced sequences and analysis for correlative studies. Please refer to **Section 6.15** and **6.16** for complete information on Tumor Response Assessments and Imaging Studies.

[13] MRI brain performed to assess baseline disease response. This baseline scan should be performed ~2-3 weeks following conclusion of radiation therapy.

[14] Brain MRI to be performed ~q6 weeks (i.e. after every two treatment cycles).

[15] Please refer to **Section 6.1.1** for additional details.

[16] All GBM tumors resected at Penn undergo EGFRvIII expression and *MGMT* promoter methylation testing as part of the standard neuropathology work flow for newly diagnosed gliomas. This testing is performed at either the Center for Personalized Diagnostics in the Department of Pathology at the University of Pennsylvania or at NeoGenomics Laboratories as part of routine care practice. Therefore the results of this testing will be known prior to subject consent and these results will be used by the physician-investigator to confirm study eligibility. This testing does not need to be repeated for research purposes.

[17] Testing for CART-EGFRvIII persistence by Q-PCR will continue until any 2 sequential tests are negative documenting loss of CAR T cells.

[18] Must be performed within 12 weeks of initiation of study treatment.

[19] RCL VSV-G assay performed at Months 3, 6, 12, and 18 following Cycle 1 treatment with CART-EGFRvIII cells. If these results are negative, all subsequent samples under this protocol will be archived. Pre-infusion samples for subjects who do not reach the Month 3 time point will be archived and not routinely analyzed.

[20] Survival follow-up may be conducted by phone or email/mail. The Follow-up Survey in Appendix 4 may be used to facilitate contact with the subject as required.

[21] Performed per Apheresis Unit policy. It is recommended that the patient have an absolute lymphocyte count (ALC) ≥500/µl prior to undergoing apheresis. If the patient's ALC is <500/µl, it is recommended that a lymphocyte subset analysis (CD3, CD4, CD8 counts) be performed to confirm that the patient has an absolute CD3 count of ≥150/µl. If the absolute CD3 count is <150/µl, it is recommended that the leukapheresis procedure be delayed until their ALC is ≥500/µl or absolute CD3 count is ≥150/µl. Up to a 4 week delay may occur; following this, further discussion is needed with the study PI and the CVPF prior to proceeding.

[22] Results of CBC, differential, and chemistry panel must be reviewed by an investigator prior to CAR T cell infusion; otherwise, results of laboratory testing are not required prior to CAR T cell administration, except as necessary for the investigator to evaluate criteria to proceed with CAR T cell infusion as described in **Section 5.2**

[23] Chemistry – Glucose, BUN, Creatinine, Sodium, Potassium, Chloride, Calcium, Total Protein, Albumin, Total Bilirubin, Alk Phos, AST, ALT, Mg, Phos, LDH, Uric Acid

[24] Pregnancy test for females of childbearing potential only. The serum pregnancy test performed at the Pre-treatment Visit must occur prior to the first day of study treatment (Cycle 1/Day 1).

[25] To include Hepatitis B surface antibody, Hepatitis B core antibody, Hepatitis B surface antigen, and Hepatitis C antibody. If the HCV antibody is positive, a screening HCV RNA by any RT-PCR or bDNA assay must be performed. Eligibility will be determined based on the screening value. The test is not required if documentation of a negative result of a HCV RNA test performed within 60 days prior to screening is provided.

[26] CART-EGFRvIII cells are administered as an IV infusion. Up to 3 infusions of CART-EGFRvIII cells may be received depending on product availability. Please refer to **Sections 5.3.1** and **5.3.3** for information on CART-EGFRvIII administration and post-infusion monitoring.

[27] All subjects must either be taking at least one antiepileptic medication at the time of enrollment or be started on one at least 24 hours prior to initiation of study treatment (Cycle 1/Day 1). Please refer to **Section 6.5** for additional details.

[28] A total dose of 40 Gy will be administered over 3 weeks (15 fractions). Please refer to **Section 6.4** for additional details.

[29] Up to 4 infusions of pembrolizumab may be received, in 3 week (21 day) cycles of treatment, for as long as the subject is deriving clinical benefit. If a subject is required to discontinue pembrolizumab due to toxicity (See **Section 5.2**), the subject may continue to receive CART-EGFRvIII cells alone. Please refer to **Sections 5.3.2** and **5.3.3** for additional information on pembrolizumab administration and post-infusion monitoring.

[30] The criteria outlined in **Section 5.2** will be assessed by the investigator before administration of pembrolizumab and/or CART-EGFRvIII cells. Subjects who do not satisfy criteria to receive study treatment may have their infusion delayed until such time that all criteria are satisfied in the judgment of the treating and principal investigators. If delayed, the start of the next cycle will be shifted accordingly. Subjects who cannot receive one of the investigational products for any reason may still receive the other investigational product as scheduled. Missed doses will not be made up.

[31] Please refer to **Section 6.3** for additional information on apheresis collection.

[32] Please refer to **Section 6.17.1** for sampling handling requirements.

[33] Beyond the research sample collections scheduled for specific time points, up to 45 mL (3 tablespoons) of additional peripheral blood may be drawn twice per week to better characterize correlates of clinical events such as cytokine release syndrome. In addition, if at any point throughout their study participation, the subject undergoes surgical resection/biopsy as part of their routine care, portions of these samples will be used for research analysis.

[34] Collected prior to treatment on Day 1 of each cycle.

[35] On infusion days, samples will be collected pre-infusion and then approximately 1-2 hours after the end of the last infusion. The post-infusion sample will include ~5mL PBMC (purple top) and ~2mL serum (red top).

[36] Labs may be repeated post-study treatment as clinically indicated.

[37] Collection of adverse events will begin at the time of apheresis and continue until the subject is off-study. For subjects who do not undergo apheresis as part of this study, AE collection begins with initiation of study treatment (Cycle 1/Day 1) and continues until the subject is off study.

[38] Research specimens for cfDNA and cfRNA analysis will be collected at the start of radiation therapy (on the 1st day of RT, prior to the first dose) and at the end of radiation therapy [on the last day of RT, after the last dose is received (+ 3 days)]. If apheresis was performed within 1 week of the start of radiation therapy and samples for cfDNA/cfRNA analysis were collected at that timepoint, an additional research sample does not need to be collected at the start of radiation therapy.

[39] Performed within 7 days prior to each infusion.

16. Appendix 2: Correlative Imaging Studies

Imaging Protocol

MR Imaging: MR imaging will be performed on a 3T Tim Trio MR (MRD1) scanner (Siemens Medical System, Erlangen, Germany) using a twelve-channel phased array head coil. The Standard of care (SOC) imaging protocol includes:

1. Three-plane scout localizer,
2. 3D- magnetization prepared rapid gradient echo (MPRAGE) [Repetition time (TR)/echo time (TE)/inversion time (TI) = 1620/3.9/950ms)], 192x256 matrix, slice thickness= 1mm, 192 slices per slab, flip angle=15°, number of excitations (NEX)=1, bandwidth (BW)=150Hz/pixel);
3. Axial proton density weighted images (TR/TE=4100/13 ms, NEX=1, slices=40, slice thickness=3.0mm, BW=130Hz, flip angle=120°);
4. Axial T2 weighted FLAIR image (TR/TE/TI= 9420/141/2500ms), slice thickness=3mm, slices=60, flip angle=170°, NEX=1, BW=287Hz/pixel;
5. Axial contrast enhanced T1 weighted MPRAGE images after administration of standard dose (0.1 mmol/kg) of contrast agent.

In addition for correlational analysis with therapeutic response or survival, the following sequences will also be used.

1. ***3D Echo-planar Spectroscopic Imaging (EPSI):*** TR/TE=1710/17ms, spatial points =50x50x18, FOV= 280x280x180mm^3, voxel size = 5.6x5.6x10mm^3 (0.31cm^3), excitation angle=73°, 512 complex points, spectral BW=2500Hz with radiofrequency excitation pulse centered at water resonance, NEX=1. Water suppression using frequency-selective saturation pulses and inversion-recovery nulling of lipid signal will be performed with TI of 198ms. This includes an interleaved water reference acquisition scan obtained using a gradient-echo acquisition with 20° excitation angle and a 6.3ms TE. This water-unsuppressed image is used to perform signal normalization, eddy current correction and image co-registration.
2. ***Perfusion imaging:*** Multihance (gadobenate dimeglumine) or other standard Gadolinium based contrast agents (GBCA) as per the standard clinical protocol will be used as a contrast agent for all contrast enhanced imaging sequences including dynamic contrast enhanced imaging (DCE) for measurement of tumor perfusion/permeability and dynamic susceptibility contrast (DSC) enhanced imaging for measuring cerebral blood volume. 50% of the total dose will be used for the DCE-MRI part, while the remaining 50% of the contrast agent will be used for DSC-MRI study.
3. ***Diffusion Tensor Imaging:*** DTI data will be acquired using 30 non-collinear/non-coplanar directions with a single-shot spin-echo, echo-planar read-out sequence with parallel imaging by using generalized auto-calibrating partially parallel acquisition (GRAPPA) and acceleration factor of 2. Sequence parameters will be as follows: TR/TE = 5000/86ms, number of acquisitions = 3, field of view=22x 22 cm^2, matrix size=128x128, slice thickness= 3mm, b=0, 1000 s/mm^2, slices= 40 covering the whole brain.
4. ***Quantitative Susceptibility Mapping:*** High-resolution, flow-compensated 3D-susceptibility weighted images (SWI) will be acquired with the following parameters: TR/TE=30/18ms, flip angle=15°, slice thickness=2mm, FOV=220×220mm^2, base

resolution=1024, voxel size=0.9×0.9×2mm^3, BW=120Hz/px, acquisition time=3:54min, and iPAT factor=2.

5. ***Resting Stage functional MRI:*** The resting stage-functional MRI (RS-fMRI) sequence will be acquired using an echo-planar imaging (EPI) sequence. A total of 35 contiguous slices with a slice thickness of 3.5 mm will be acquired. The other parameters are as follows: TR = 2000 ms, TE = 30 ms, flip angle = 90°, distance factor = 20%, FOV = 220 × 220 mm2, matrix size = 64 × 64, measurements = 100. During the RS-fMRI scanning, all participants will be instructed to close their eyes, and stay awake, and not to systematically think about anything during the whole session. The acquisition time ~ 5:00minutes.

These imaging studies, which include both the standard of care clinical imaging as well as the sequences described above, will take about 1 hour to complete.

Data processing:

1. ***EPSI:*** 3D-EPSI data will be processed offline using the metabolic imaging and data analysis system (MIDAS) package. The data will be corrected for inhomogeneity in the B_0 field, followed by eddy current correction and interpolation to a spatial resolution of 64x64x32 voxels. The processing also includes steps to reduce ringing artifacts from subcutaneous lipids using a mask of the scalp region derived from the T1-weighted images. Signal intensity normalization of metabolite maps will be performed using tissue water as an internal reference. The automated MIDAS tool will be used to compute parametric maps of NAA, Cr and Cho.
2. ***Perfusion imaging:*** CBV will be calculated based on the intravascular indicator dilution theory (IIDT). The assumption of negligible leakage of contrast agent is often violated in tumor such that some form of corrections, such as gamma variate function fitting or baseline subtraction, is required. We will use NordicICE (NordicImagingLab AS, Bergen, Norway) to compute leakage corrected and uncorrected CBV maps with the method proposed by Boxerman *et al*. DCE-MRI data will be processed using a pharmacokinetic modeling approach proposed by Johnson et al to measure vascular transfer constant, K^{trans}, fractional plasma volume, v_p, and fractional extravascular extracellular space volume, v_e. The advantage of such pharmacokinetic modeling approaches is that it is not based on the assumptions for negligible recirculation and negligible leakage. As the first step, contrast agent concentration, *C*, in arbitrary units will be calculated using the standard formula $C = -\ln\left(S/S_0\right)$ where *S* is the MRI signal intensity and S_0 is the pre-contrast signal (Rosen et al., 1991). Assuming that the leakage in the normal white matter is negligible, the signal enhancement in white matter region of interest (ROI) will be used to define the arterial input function, C_p, based on an extended gamma variate function:

$$C_p = Ag\left(t\right) + \lambda\int_0^t g\left(t - t'\right)dt' \quad \text{where } g = \begin{cases} \left(t - t_0\right)^{\alpha}\exp\left(-\beta\left(t - t_0\right)\right) & t > t_0 \\ 0 & t \le t_0 \end{cases}, \qquad (1)$$

t_0 is the start time of the bolus, α and β are parameters that determine the shape of the bolus, and *A* is a scale factor. Then, the estimated arterial input function will be used to estimate the total tissue concentration in the lesion using the generalized kinetic model (GKM) with vascular term:

$$C_t = v_p C_p + v_e C_e = v_p C_p + K^{trans} \int_0^t C_p\left(t'\right) \exp\left(-K^{trans}\left(t - t'\right) / v_e\right) dt' . \quad (2)$$

3. **DTI:** The diffusion-weighted images will be co-registered to the non-diffusion weighted (b = 0 s/mm^2) images to minimize eddy-current and/or subject motion induced artifacts using a 3D affine transformation estimated by maximizing the mutual information between the images as described previously.(Wang et al., 2009b) The corrected raw images will be combined to compute MD, FA, CL, CP and CS maps.
4. **QSM:** Susceptibility weighted imaging and mapping (SWIM) algorithm will be used to reconstruct QSM maps from high resolution 3D-SWI data. The post-processing involves skull stripping to remove the artifacts caused by skull and brain tissue interface using the brain extraction tool, followed by phase unwrapping using a Laplacian operator. To remove background field in homogeneity, a variable high-pass filter of 32 pixels size will be applied and, finally, inverse filtering will be performed to generate QSM maps.
5. To process the resting stage-fMRI data, an in-house developed matlab script will be used.

Unsuppressed water images, corresponding 3D-EPSI maps (NAA, Cr, Cho), DTI maps (MD, FA, CL, CP and CS), CBV maps and FLAIR images will be co-registered to contrast enhanced T1-weighted images using a 3D non-rigid transformation and mutual information by combining affine transformation and discrete sine bases. All image processing procedures will be performed using in-house developed interactive data language (IDL) routines (ITT Visual Information Solutions, Boulder, CO).

17. Appendix 3: Radiation Therapy Parameters

Dose: 40 Gy in 15 daily fractions

1. A total dose of 40 Gy in 15 fractions of 2.67 Gy per day shall be delivered. Greater than or equal to 95% of the PTV should receive greater than or equal to 40 Gy. Undercoverage of the PTV at greater than or equal to 90% at 40 Gy is an acceptable variation in order to meet OAR constraints as above.
2. IMRT plan heterogeneity should ideally be 110% (44 Gy Dmax) or less and preferably under 105% (42 Gy Dmax). A Dmax of 114% (45.6 Gy) is an acceptable variation.

Parameters

1. Positioning, Immobilization and Simulation:
    a. The patient will be simulated in the supine position in most situations, immobilized typically with a thermoplastic mask. The treatment planning simulation CT scan will be acquired in the treatment position. The treatment volumes will be defined by fusion with the pre- and post-resection MRI scans.
    b. CT contrast may be omitted if medically indicated. The MRI sequences should include a T1-weighted post-contrast sequence (preferably stereotactic, thin slice, contiguous). Additionally, a T2/FLAIR sequence is helpful to identify non-enhancing tumor.
2. Equipment and Technique:
    a. Radiation will be delivered with megavoltage equipment, typically of 4MV or greater energy.
    b. Intensity modulated radiation (IMRT) via any method (eg, VMAT, static field IMRT, tomotherapy) is required. 3D planning is not allowed.
    c. Daily image-guidance is recommended to allow smaller planning target volume (PTV) margins, but is not required. The PTV margin should reflect if imageguided radiation (IGRT) is used.
3. Target Volume Delineation:
    a. A gross tumor volume (GTV) will be defined using the CT and MRI images. The GTV includes any enhancing tumor, if present following resection, as well as the post-operative resection cavity. The GTV also includes any non-enhancing tumor as identified on T2/FLAIR. T2/FLAIR signal consistent with edema is not specifically included in the GTV. Therefore a distinction is made between T2 edema (typically without mass effect, sparing the cortical ribbon, obeying the grey/white junction, etc) and T2 tumor (mass effect with sulcal effacement, involvement of the grey/white junction, obliteration of the cortical ribbon). Fusion of the pre-operative MRI to determine initial extent of the tumor is helpful.
    b. The clinical target volume (CTV) is created by anatomically expanding the GTV by 15 to 20 mm, per physician preference. The CTV should be trimmed at anatomic boundaries to rational tumor spread, such as the tentorium, falx if not near the corpus callosum, and skull. At these boundaries, the CTV may be 0 mm.
    c. A geometric PTV expansion of 3 to 5 mm will be applied to the CTV that is justified based on image guidance and immobilization. For treatment utilizing IGRT techniques, the PTV may be as small as 3mm. For non-IGRT approaches, a 5 mm expansion is recommended.
4. Organs at Risk (OAR) and OAR Constraints

a. OARs shall be contoured, including: optic nerves, optic chiasm, eye globe, brainstem, and cochlea.
b. Radiation plan heterogeneity shall be constrained so that the above OARs all receive less than or equal to 41 Gy Dmax (102.5% of prescribed dose).

18. Appendix 4: Long-Term Follow-up Survey

This follow-up survey is for:

- Subjects who cannot return for an onsite visit at any time point(s).
- Subjects to be contacted annually because they had no sign of gene therapy product at the 5-year follow-up visit or later and are, therefore, not required to return for any remaining future onsite visits.

Follow-Up Survey
Name of Participant: _____ **Method of Contact.** Document method of contact ☐ Mail: _____ ☐ Email: _____ ☐ Phone: _____ Above information to be filled out by Coordinator
1) Today's Date:_____/_____/_____ MM DD YYYY
2) Update contact information. Document any changes below since your last study visit or last contact with the study team. ☐ **Phone:** _____ ☐ **Email:** _____ ☐ **Address:** _____ **Preferred method of contact: _____**
3) Have you been diagnosed with any type of cancer since your last study visit or last contact with the study team? ☐ No, I have not been diagnosed with Cancer. ☐ Yes, I have been diagnosed with Cancer. [*Please complete the fields below*.] • Cancer Diagnosis: _____ • Type of Cancer: _____ • Date of Diagnosis: _____

Follow-Up Survey
4) Have you developed any of the following symptoms since your last study visit or last contact with the study team? ☐ Experienced a worsening or new loss of feeling in any part of your body, especially hands and feet ☐ Experienced a worsening or new loss of control of any body part (arms, legs…) ☐ Experienced a worsening or had a seizure ☐ Experienced a worsening or new experience memory loss **Please explain if necessary:**
5) Have you developed a worsening or arthritis or been recently diagnosed with arthritis by a doctor since your last study visit or last contact with the study team? ☐ **Yes** ☐ **No** **Arthritis:** Most kinds of arthritis cause pain and swelling in your joints.
6) Have you developed a worsening of your autoimmune disease or been recently diagnosed with an autoimmune disease by a doctor since your last study visit or last contact with the study team? ☐ **Yes** ☐ **No** **Autoimmune Disease:** The body's immune system protects you from disease and infection. Autoimmune diseases are when your immune system attacks healthy cells in your body by mistake. Autoimmune diseases can affect many parts of the body.
7) Have you had any new or unexpected illnesses or been hospitalized unexpectedly? ☐ **Yes** ☐ **No** **If yes, please explain:**

Completed By:

_____

Name **Signature** **Date**

19. Appendix 5: Example Physician Letter

EXAMPLE LETTER TO PHYSICIANS

[*date*]

[*name and address*]

Dear [*physician name*],

Your patient [*patient name*] is participating in a clinical research study that requires 15 year monitoring for adverse events. To aid in reporting of adverse events that are possibly related to the clinical research study, we are asking the patients on our research study to designate a primary care physician or local oncologist that may help in the monitoring and reporting of adverse events. Your patient has designated you. **If upon any of your visits with your patient, any of the following events are reported or discovered, please contact the study nurse or physician as soon as possible**:

1. New malignancies
2. New incidence of exacerbation of a pre-existing neurologic disorder
3. New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
4. New incidence of a hematologic disorder.
5. Events that may be unexpected in this patient population and thus potentially related to the investigational product they received.

If your patient experiences any of these events, please contact the study coordinator below as soon as you can so that they can record the event and then monitor your patient's health if necessary. When you call, remember to mention the protocol number of the study which is UPCC XXXXX, Subject ID (*XXX*) and the brief study title which is "**XXXX**".

Study Coordinator
Name
Address
Phone
Email

In addition, if your patient cannot return to the University of Pennsylvania for some their follow-up visits, we will contact you to provide information from their medical record, including the results of any routine care physical examinations and/or laboratory assessments performed. We may also ask for your assistance in the collection of protocol required blood samples, which will need to be sent to the University of Pennsylvania for required analysis.

If you have any questions about this letter or the study itself, please do not hesitate to contact the above study nurse or physician.

Thank you for your support in helping us to monitor for delayed adverse events.

Best regards,
[*study coordinator*]

20. Appendix 6: New York Heart Association (NYHA) Functional Classification

Class	Functional Capacity: How a patient with cardiac disease feels during physical activity
I	Patients with cardiac disease but resulting in no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort increases.

Statistical Analysis Plan

General Design

This is an open label, phase 1 study to evaluate the safety and tolerability, and persistence and engraftment of autologous T cells engineered to express a chimeric antigen receptor targeting EGFRvIII, that is linked to the CD3ζ-4-1BB signaling chains in patients with newly diagnosed EGFRvIII+ GBM. Failure to complete the study due to the stopping rules being invoked will be the main basis for determining safety of this study. This study is primarily intended to provide data that might allow the investigators to conduct a preliminary assessment of safety and feasibility. This study aims to evaluate 7 patients.

Sample Size

This is a phase I study with a primary goal of estimating safety and tolerability. Given the costs associated with CAR T cell production, we plan to enroll 7 patients that are evaluable for the study's primary endpoint. Subjects that have received at least one EGFRvIII CAR T cell infusion will be considered as evaluable. A larger follow-up trial will be designed that has the statistical power to assess the potential efficacy of the regimen if the safety profile of this study warrants a larger study design.

Endpoints for Primary Objectives

The primary objective of this study is to determine the safety and tolerability of multiple CART-EGFRvIII cells in combination with pembrolizumab for the treatment of GBM. Safety will be evaluated based on the occurrence of study related adverse events that are determined to be

related to the study treatment. Endpoints of tolerability will include completion of scheduled infusion, laboratory values, and vital signs from time of the first infusion.

Endpoints for Secondary Objectives

The secondary endpoints are aimed to evaluate the anti-tumor responses to CART-EGFRvIII cells, assessed by overall survival (OS) and progression-free survival (PFS), objective response rate (ORR) based on standard MRI evaluation and the modified RANO criteria. We will also measure median progression free survival. EGFRvIII + PD1 Inhibitor in GBM.

Subject Population(s) for Analysis

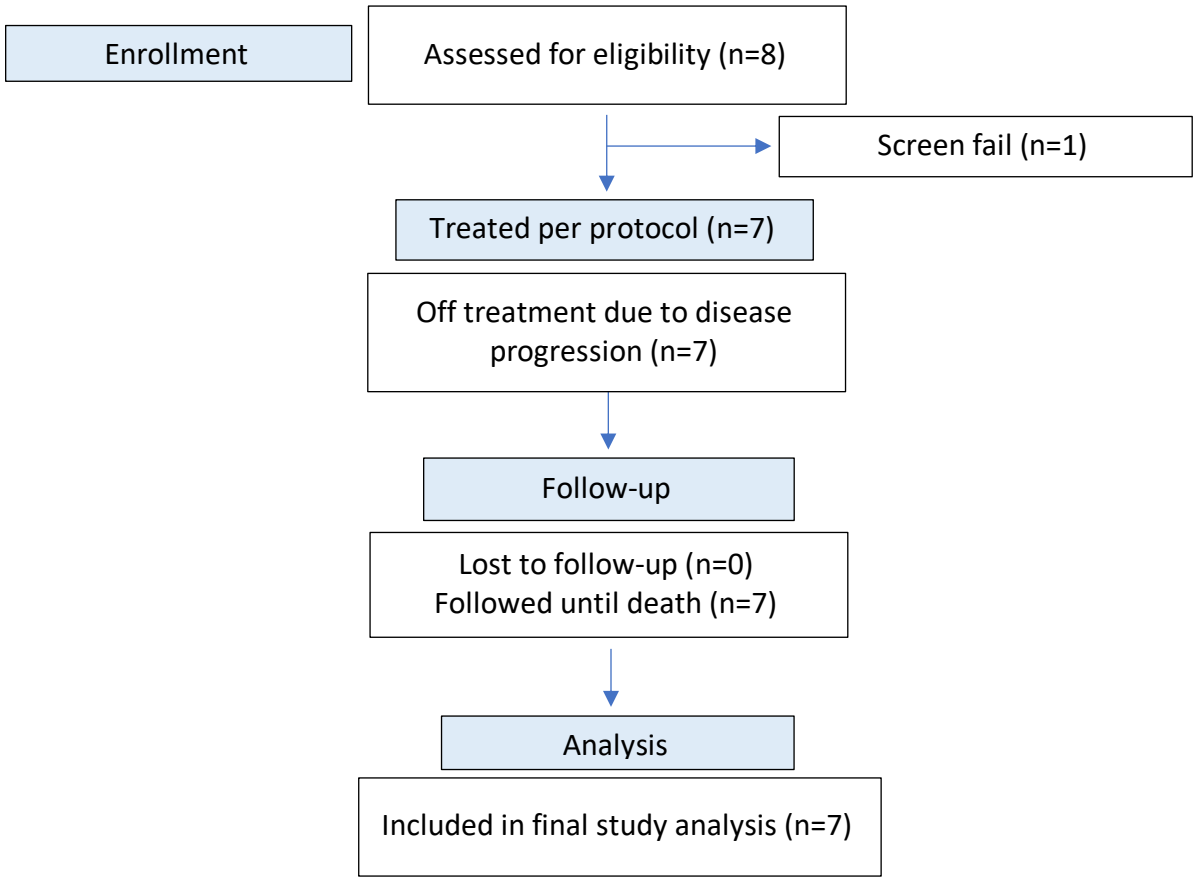
The subject population to be analyzed for primary, secondary, and correlative endpoints will include all patients infused with at least one dose of CART-EGFRvIII cells. The Screen Set comprises all patients who are screened for the study. The Enrolled Set comprises all subjects who sign an informed consent form and are confirmed eligible for the study (i.e. excluding screen failure subjects). Screening failure is defined as failure of meeting the inclusion/exclusion criteria specified by the protocol. The Safety Set comprises all subjects who received minimum acceptable dose of CART-EGFRvIII cells as specified above and will be used for the primary safety endpoints. The Efficacy Set will include all subjects infused with minimum acceptable dose of CART-EGFRvIII cells and had at least one disease assessment available and will be used for the secondary endpoints, and other correlative/exploratory endpoints. Subjects with manufactured cells that do not meet the manufacturing release criteria and/or reach the minimum dose of 2x107 will be considered a manufacturing failure.

Statistical Analysis of Endpoints

The statistical analysis will be primarily descriptive in keeping with the exploratory nature of the study. All adverse events will be described and exact 90% confidence intervals will be produced for adverse event rates, both overall and within major categories. DLT rate and exact 90% will be computed. For tolerability, the proportion of subjects that completed the scheduled infusions will be computed. Changes or abnormal laboratory values and vital signs from time of infusion will be summarized descriptively including mean, medians, standard deviation, and inter-quartile range (IQR).

Secondary endpoints include overall survival (OS), progression-free survival (PFS) and objective response rate (ORR). OS is defined as the number of days from the date of the first CART-EGFRvIII infusion to the date of death of any causes, or censored at last known date of alive. PFS is defined as the number of days from the date of the first CART-EGFRvIII infusion to the first documented disease progression (by the modified RANO criteria) or date of death, whichever occurs first. Patients who are lost to follow-up without a known date of progression will be censored in the analysis at the date of their last available tumor assessment. The survival function of OS and PFS will be estimated by Kaplan-Meier method. 90% confidence interval for the survival probability at a specific time point will be computed based on the log-log transformation. Median survival time along with the associated 90% confidence intervals will be presented if appropriate. ORR will be computed as the proportion of patients with complete response (CR) or partial response (PR) out of the total number of efficacy evaluable subjects. Exact 90% CI for ORR will be computed.

Descriptive statistics will be calculated for exploratory/correlative endpoints including mean, median, standard deviation, and inter-quartile range (IQR) for continuous variables (e.g., number or percent of EGFRvIII-transduced cells) and frequency and proportions for discrete variables. Most exploratory/correlative endpoints will be continuous. 90% confidence interval appropriate for each statistic will be used. The overall profile and individual-level change of CART-EGFRvIII cells or other measures of anti-tumor activities and biomarkers over time will be examined graphically.


Enrollment
Assessed for eligibility (n=8)
Screen fail (n=1)
Treated per protocol (n=7)
Off treatment due to disease progression (n=7)
Follow-up
Lost to follow-up (n=0)
Followed until death (n=7)
Analysis
Included in final study analysis (n=7)