Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Seattle Children's® Therapeutics

PHASE 1 STUDY OF B7-H3, EGFR806, HER2, AND IL13-zetakine (QUAD) CAR T CELL LOCOREGIONAL IMMUNOTHERAPY FOR PEDIATRIC DIFFUSE INTRINSIC PONTINE GLIOMA, DIFFUSE MIDLINE GLIOMA, AND RECURRENT OR REFRACTORY CENTRAL NERVOUS SYSTEM TUMORS

Protocol Number: BrainChild-04

Protocol Version: 3.0

Protocol Version Date: 06 Feb 2026

Investigational Product Name:	SC-CAR4BRAIN
Indication:	Recurrent or refractory pediatric central nervous system tumors, diffuse intrinsic pontine glioma, and diffuse midline glioma
IND/EudraCT Number:	29049
Sponsor:	Seattle Children's, Seattle Children's Therapeutics 1920 Terry Avenue M/S CURE-10 Seattle, WA 98101
Sponsor's Responsible Medical Officer:	Colleen Annesley, MD Medical Director, Seattle Children's Therapeutics 1920 Terry Avenue M/S CURE-10 Seattle, WA 98101

This study is to be performed in compliance with the protocol, Good Clinical Practices (GCP) and applicable regulatory requirements.

Confidentiality Statement: This confidential information about an investigational product is provided for the exclusive use of investigators of this product and is subject to recall at any time. Subject to the foregoing, this information may be disclosed only to those persons involved in the study who have a need to know, with the obligation not to further disseminate this information.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Seattle Children's Therapeutics

Protocol Version History

Document Version	Version Date (YYYY.MM.DD)	Key Changes
1.0	2023.01.23	N/A, Initial Version
1.1	2023.01.31	Minor correction to in-text cross reference.
1.2	2023.03.13	In line with discussions with the FDA, updates to inclusion/exclusion criteria, addition of CNS catheter experience on previous BrainChild studies, addition of post-infusion monitoring requirements, and update to definition of refractory or recurrent CNS disease. Addition of supportive care requirements and recommended interventions for non-CRS toxicities to align with clinical experience from other BrainChild studies. Additional minor administrative changes.
1.3	2023.12.18	Update to Sponsor Leadership, correlative laboratory name, and other minor administrative changes.
2.0	2024.09.25	Update to suggested bevacizumab use to apply to all treatment Arms per clinical discretion. Update to schedule of procedures to allow study assessments to be performed at Local Care Institutes (LCIs) for non-infusion visits. Removal of neurologic toxicity data reporting requirements and grading criteria. Removal of On-Target Off-Tumor toxicity definition and data collection requirements. Update to CSF sample collection requirements to not be mandatory during non-infusion visits. Addition of lost to follow-up definition. Lesion identification instruction and measurable disease criteria further defined. Study visit window terms updated for clarity.
2.1	2025.06.27	Post-infusion visits amended to be optional from course 7 onwards, conjugated bilirubin testing optional from course 3 onwards. Sponsor acknowledgement signature moved to align with SCTx standard.
3.0	2026.02.06	Update to visit schedule to reduce required assessments for subjects receiving more than 6 courses of treatment.

Sponsor Acknowledgement:

As the Sponsor representative, I confirm that Seattle Children's Therapeutics will comply with all Sponsor obligations as detailed in all applicable regulations and guidelines. I will ensure that the investigator is informed of all relevant information that becomes available during the conduct of this study.

Signed by:
Colleen E. Annesley, MD
Signer Name: Colleen E. Annesley, MD
Signing Reason: I approve this document
Signing Time: 2/6/2026 | 5:47:15 PM PST
FFD018C9EAA14B04B75C3F2B40C6508E

2/6/2026 | 5:47:16 PM PST

Colleen Annesley, MD
Medical Director, Seattle Children's Therapeutics

Date

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Seattle Children's Therapeutics

TABLE OF CONTENTS

TABLE OF CONTENTS 3
STUDY COMMITTEE 8
LIST OF ACRONYMS, ABBREVIATIONS, AND DEFINITION OF TERMS 9
ABSTRACT 11
1 BACKGROUND/RATIONALE 13
1.1 CNS Tumors of Children and Young Adults 13
1.2 Role of Immunotherapy in CNS Tumors 13
1.3 Redirected Specificity of T Cells Engineered to Express Chimeric Antigen Receptors 14
1.4 Clinical Experience Using CAR T Cells for Adult CNS Tumors 15
1.5 HER2, EGFR, B7-H3, and IL13Rα2 as Targets for CNS Tumors 16
1.5.1 HER2 16
1.5.2 EGFR 17
1.5.3 B7-H3 19
1.5.4 IL13Rα2 22
1.6 Rationale for Intraventricular CNS Dosing of CAR T cells 23
1.7 Rationale for Multi-antigen Targeting 23
1.8 Development of SC-CAR4BRAIN 24
1.9 Potential Toxicity Associated with SC-CAR4BRAIN 30
1.9.1 General symptoms associated with CNS administration of CAR T cells 30
1.9.2 Risk of Cytokine Release Syndrome (CRS) 30
1.9.3 Risk of neurologic toxicity 31
1.9.4 Potential long-term toxicity 31
1.9.5 Intra-Subject Dose Escalation 32
1.10 T Cell Dosing Rationale 33
1.11 CNS Catheters 33
1.12 Rationale for Use of This Product in Pediatric Subjects 34
2 STUDY OBJECTIVES 35
2.1 Primary Objectives 35
2.2 Secondary Objectives 35
2.3 Exploratory Objectives 35
3 ENROLLMENT AND ELIGIBILITY 36
3.1 Enrollment 36
3.2 Eligibility 36
3.2.1 Inclusion criteria 36
3.2.2 Exclusion criteria 38
4 DRUG INFORMATION 39
4.1 T Cell Product 39

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

4.2 Guidelines for Administering SC-CAR4BRAIN 39
4.3 Possible side effects of intracranially delivered CAR T cell therapy 40
4.4 Dose-Limiting Toxicity (DLT) 40
5 TREATMENT PROGRAM 41
5.1 Overview of Treatment Plan 41
5.2 Apheresis for T Cell Isolation 41
5.2.1 Requirements for Apheresis 41
5.3 Bridging Therapy 41
5.4 CAR T Cell Dosing 42
5.4.1 Treatment Arm Assignment 42
5.4.2 Dose Regimen Assignment 42
5.4.3 Dose modifications 45
5.5 CAR T Cell Product Infusion 46
5.5.1 Schedule of subsequent CAR T cell infusions 46
5.5.2 Requirements for initial CAR T cell infusion 46
5.5.3 Requirements for subsequent CAR T cell infusion 48
5.6 Concomitant Medication and Supportive Care 48
5.6.1 Anti-seizure medication 48
5.6.2 Blood product support 48
5.6.3 Antimicrobial prophylaxis 49
5.6.4 Prohibited medications 49
6 MANAGEMENT OF TOXICITIES AND COMPLICATIONS 50
6.1 Symptoms Associated with Apheresis 50
6.2 Symptoms Associated with CAR T Cell Infusion 50
6.3 Management of Cytokine Release Syndrome (CRS) 51
6.4 Management of Non-CRS Acute Toxicity Associated with Infused CAR T Cells into the CNS 52
6.5 Ablation of T Cells with Cetuximab 53
7 STUDY PROCEDURES AND ASSESSMENTS 56
7.1 Informed Consent/Assent 56
7.2 Demographics 56
7.3 Medical History and Active Medical Conditions 56
7.4 Performance Status 56
7.5 Physical Examination, Vital Signs, Weight, Height 56
7.6 Pulse Oximetry 56
7.7 Neurologic Exam 56
7.8 Concomitant Medications 56
7.9 Adverse Events 56
7.10 Hospitalization/ICU Admission 57

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

7.11 Pregnancy Test 57
7.12 Virology 57
7.13 Hematology 57
7.14 Chemistry 57
7.15 Cerebrospinal Fluid (CSF) Sampling 57
7.16 CRS Labs and Evaluation 57
7.17 Inflammatory markers 58
7.18 Correlative Sciences 58
7.19 CNS Imaging 58
7.20 Disease Response Evaluation 59
7.21 Post-Treatment Long-Term Follow-Up Testing 59
7.21.1 Autopsy 59
8 REMOVAL FROM PROTOCOL THERAPY & OFF STUDY CRITERIA 60
8.1 Removal from Protocol Therapy 60
8.2 Off-Study Criteria and Study Termination 60
9 STATISTICAL CONSIDERATIONS 62
9.1 Accrual and Study Duration 62
9.2 Primary Objectives 62
9.3 Secondary and Exploratory Objectives 63
9.4 Statistical Analysis 63
9.5 Safety and Toxicity 63
9.6 Survival Definitions 64
9.7 Evaluability for Disease Response 64
9.8 Statistical Study Design 65
9.8.1 BOIN design 65
9.8.2 Arms and DLT observation periods 67
9.8.3 Stopping rules 68
9.8.4 Definition and determination of MTDR and RP2DR 68
9.9 Expansion Cohort 69
9.10 Safety Monitoring 69
9.10.1 Weekly safety review 69
9.10.2 Data and Safety Monitoring Board 69
10 DISEASE RESPONSE 70
10.1 Disease Response Criteria 70
10.1.1 Measurable disease 70
10.1.2 Evaluable disease 70
10.1.3 Selection of target and non-target lesions 70
10.1.4 Response criteria for target lesions 70
10.1.5 Response criteria for non-target lesions 71

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

10.1.6 Evaluation of best overall response 71
11 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS 73
11.1 Definition of Adverse Event (AE) 73
11.2 Definition of Serious Adverse Event (SAE) 73
11.3 Classification of an Adverse Event 74
11.3.1 Grading of adverse events 74
11.3.2 Relationship of adverse event to treatment 74
11.4 Expectedness, Pre-Existing Conditions, and Persistent Adverse Events 75
11.5 Cytokine Release Syndrome Grading 76
11.6 Serious Adverse Event Reporting 76
11.6.1 Study-specific SAE reporting 77
11.7 Safety Reporting Contact Information 77
11.8 IND Safety Reporting 77
11.9 Reporting of Pregnancy 78
12 ADMINISTRATIVE, ETHICAL, AND REGULATORY CONSIDERATIONS 79
12.1 Good Clinical Practice 79
12.2 Institutional Review Boards (IRB) and Institutional Biosafety Committees (IBC) 79
12.3 Informed Consent/Assent and Other Informational Documents Provided to Study Participants 79
12.4 Data Handling and Record Keeping 80
12.4.1 Case report forms and source documents 80
12.4.2 Data quality assurance 80
12.4.3 Record retention 81
12.5 Investigational Product Accountability 81
12.6 Protocol Deviations 81
12.7 Subject-Specific Biologic Materials 82
12.8 Investigator's Responsibilities 82
12.9 Publication Policy 82
12.10 Financing and Insurance 82
13 REFERENCES 83
14 APPENDICES 92
APPENDIX 1 – PRINCIPAL INVESTIGATOR SIGNATURE PAGE 93
APPENDIX 2 – SCHEDULE OF PROCEDURES 94
APPENDIX 3 – PERFORMANCE STATUS SCALES 102
APPENDIX 4 –REFRACTORY AND RECURRENT DISEASE CATEGORIZATION 103
APPENDIX 5 – HIGHLY EFFECTIVE CONTRACEPTION 104

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

LIST OF FIGURES

Figure 1-1 BrainChild-03 Arm C by Dose Regimen 22
Figure 1-2 BrainChild-03 Arm C Dose Regimen 2 22
Figure 1-3 CAR distribution and construct diagrams in the SC-CAR4BRAIN product. 26
Figure 1-4 SC-CAR4BRAIN controls outgrowth of quad-antigen expressing U251T and U87 cell lines, as do individual single-CAR control lines 27
Figure 1-5 SC-CAR4BRAIN controls outgrowth of single-antigen K562 cell lines, as do individual single-CAR control lines. 27
Figure 1-6 SC-CAR4BRAIN and single CAR control lines respond to quad-antigen expressing target exposure by releasing cytokines. 28
Figure 1-7 SC-CAR4BRAIN and single CAR control lines release cytokine in response to single antigen-expressing K562 target cells only when target antigen is present. 29
Figure 1-8 Tumor progression and survival of engrafted NSG mice versus untreated mice. Bioluminescent signal (A) and survival (B) of mice treated with either mock transduced or SC-CAR4BRAIN T cells. Mice plotted individually for imaging data. 30
Figure 5-1 Dose Regimen 1 43
Figure 5-2 Dose Regimen 2 44
Figure 5-3 Dose Regimen 3 45

LIST OF TABLES

Table 3-1 Screening Hematologic Values 37
Table 3-2 Serum Creatinine ULN Based on Age and Sex 37
Table 5-1 CAR T Cell Dose Levels 42
Table 5-2 Pre-T Cell Therapy Wash Out Requirements 47
Table 6-1 Recommended Symptom Management for CRS 52
Table 6-2 Details adverse events observed in clinical trials using cetuximab 55
Table 9-1 Dose escalation/de-escalation rule for the BOIN design 67
Table 9-2 Stopping boundaries 67
Table 10-1 Best Overall Response 71

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

STUDY COMMITTEE

STUDY CHAIR
Rebecca Ronsley, MD
Pediatric Neuro-oncology
Seattle Children's Hospital
4800 Sand Point Way NE
Seattle, WA 98105
Phone: 206-987-2106
email: rebecca.ronsley@seattlechildrens.org

STUDY STATISTICIAN
Qian (Vicky) Wu, PhD
Associate Professor, Clinical Research Division
Fred Hutchinson Cancer Research Center
1100 Fairview Avenue N
Seattle, WA 98109
Phone: 206-667-3358
email: qwu@fredhutch.org

STUDY COMMITTEE MEMBERS
Nicholas Vitanza, MD
Pediatric Neuro-oncology
Seattle Children's Research Institute
1900 Ninth Avenue
Seattle, WA 98101
Phone: 206-987-2106
email: nicholas.vitanza@seattlechildrens.org

Juliane Gust, MD, PhD
Pediatric Neurology
Seattle Children's Hospital
4800 Sand Point Way NE
Seattle, WA 98105
Phone: 206-987-2087
email: juliane.gust@seattlechildrens.org

Hannah Goldstein, MD
Pediatric Neurosurgery
Seattle Children's Hospital
4800 Sand Point Way NE
Seattle, WA 98105
Phone: 206-987-6946
email: hannah.goldstein@seattlechildrens.org

Francisco Perez, MD, PhD
Neuroradiology
Seattle Children's Hospital
4800 Sand Point Way NE
Seattle, WA 98105
Phone: 206-987-2134
email: francisco.perez@seattlechildrens.org

Bonnie Cole, MD
Neuropathology
Seattle Children's Hospital
4800 Sand Point Way NE
Seattle, WA 98105
Phone: 206-987-2103
email: bonnie.cole2@seattlechildrens.org

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

LIST OF ACRONYMS, ABBREVIATIONS, AND DEFINITION OF TERMS

ACD	acid-citrate-dextrose
AE	adverse event
ALC	absolute lymphocyte count
ALL	acute lymphoblastic leukemia
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ATRT	atypical teratoid-rhabdoid tumor
BUN	blood urea nitrogen
CAR	chimeric antigen receptor
CED	convection-enhanced delivery
CFR	Code of Federal Regulations
CNS	central nervous system
CR	complete response
CRF	Case Report Form
CRS	cytokine release syndrome
CSF	cerebrospinal fluid
CSL	Correlative Sciences Laboratory
CTEP	Cancer Therapy Evaluation Program
DIPG	diffuse intrinsic pontine glioma
DL	dose level
DLT	dose-limiting toxicity
DMG	diffuse midline glioma, H3 K27M-altered
DR	Dose Regimen
DSMB	Data Safety Monitoring Board
EGFR	epidermal growth factor
FDA	Food and Drug Administration
GBM	glioblastoma
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HGG	high grade glioma
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
IBC	Institutional Biosafety Committee
ICF	informed consent form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Institutional Ethics Committee

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

IND	Investigational New Drug (application)
IRB	Institutional Review Board
irPD	immune-related progressive disease
IV	intravenous
IVC	intraventricular catheter
LCI	local care institution
LP	lumbar puncture
MRI	magnetic resonance imaging
MTDR	maximum tolerated dose regimen
NCI	National Cancer Institute
CTCAE	Common Terminology Criteria for Adverse Events
NRM	non-relapse mortality
OS	overall survival
PB	peripheral blood
PCR	polymerase chain reaction
PD	progressive disease
PFNS	preservative-free normal saline
PFS	progression-free survival
pHGG	pediatric high-grade glioma
PI	principal investigator
PR	partial response
RCL	replication-competent lentivirus
RP2DR	recommended Phase 2 dose regimen
SAE	serious adverse event
SCRI	Seattle Children's Research Institute
TCPC	therapeutic cell production core
ULN	upper limit of normal
US	United States

Definition of Terms

Investigational Product is defined as, "A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use" [from International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Harmonized Tripartite Guideline E6: Guideline for Good Clinical Practice]. The terms "Investigational Product" and "study drug" may be used interchangeably in the protocol.

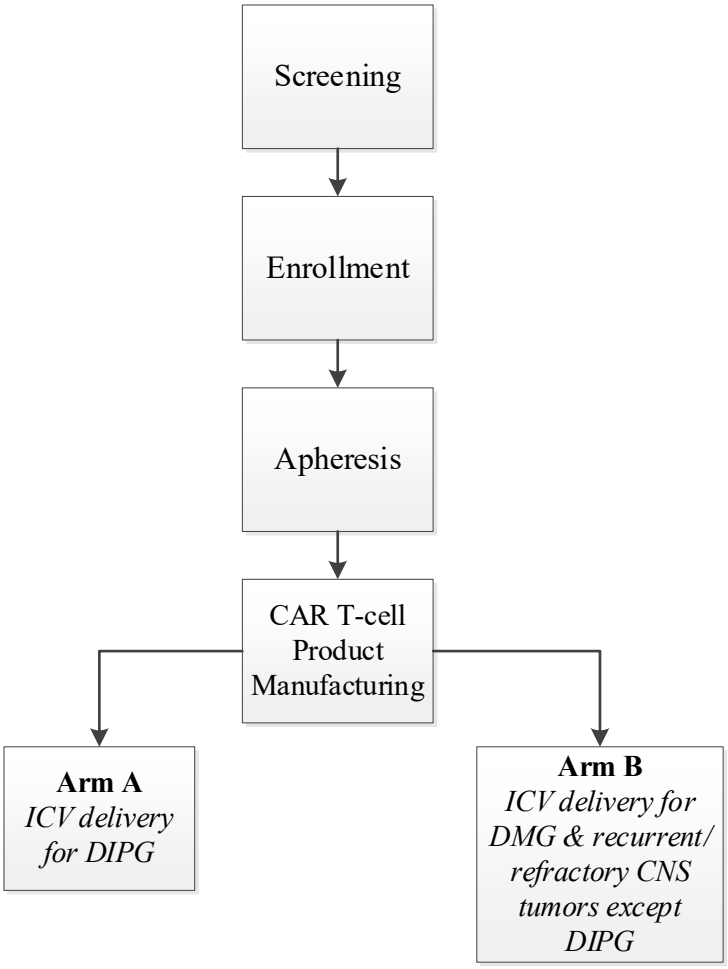
Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

ABSTRACT

This is a Phase 1 study of central nervous system (CNS) locoregional adoptive therapy with SC-CAR4BRAIN manufactured from autologous $CD4^+$ and $CD8^+$ T cells that are lentivirally transduced to express combinations of B7-H3, EGFR806, HER2 and IL13-zetakine chimeric antigen receptors (CAR) and EGFRt. CAR T cells are delivered via an indwelling catheter into the ventricular system in children and young adults with diffuse intrinsic pontine glioma (DIPG), diffuse midline glioma (DMG), and recurrent or refractory CNS tumors. The primary objectives of this protocol are to evaluate the feasibility of manufacturing and administering SC-CAR4BRAIN CAR T cells, to assess the safety and describe the associated toxicity, and to define the maximally tolerated dose regimen (MTDR) and recommended Phase 2 dose regimen (RP2DR) of repetitive CNS-delivered SC-CAR4BRAIN CAR T cell infusions. The statistical design employs intra-patient dose escalation, and a modified BOIN design for dose escalation and de-escalation. The secondary objectives are to assess SC-CAR4BRAIN CAR T cell distribution within the cerebrospinal fluid (CSF), the extent to which SC-CAR4BRAIN CAR T cells egress into the peripheral circulation, and disease response to SC-CAR4BRAIN CAR T cells. The exploratory objectives are to describe tumor surface antigen expression, to assess the presence of SC-CAR4BRAIN CAR T cells, and to explore biomarkers of SC-CAR4BRAIN CAR T cell expression, safety, and activity in tumor tissue and/or normal tissue or fluids.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

EXPERIMENTAL DESIGN SCHEMA



Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

1 BACKGROUND/RATIONALE

1.1 CNS Tumors of Children and Young Adults

Tumors of the central nervous system (CNS) are the most common cancer of childhood with an incidence of 5.57 per 100,000 annually and are the leading cause of pediatric cancer-related death in patients under 19 years of age [1, 2]. Outcomes for patients with newly diagnosed CNS tumors have steadily improved; however, this is not the case for all subtypes of CNS tumors. For example, children and young adults with diffuse intrinsic pontine glioma (DIPG)/diffuse midline glioma (DMG) or recurrent high-grade disease continue to have dismal outcomes and approximately 550 children die from brain and spinal cord tumors annually in the United States (US). Most pediatric CNS tumors are high grade glioma (HGG), DIPG/DMG, medulloblastoma, or atypical teratoid-rhabdoid tumor (ATRT). Five-year survival in children is only 15-35% for HGG, 15-25% for recurrent medulloblastoma, <10% for relapsed ATRT, and both DIPG/DMG remain universally fatal [3-6]. Other recurrent tumors, such as ependymoma, are often not curable and survivors are left with debilitating morbidity following multiple surgeries and multiple radiation treatments [7].

DIPG/DMG account for approximately 10% of all CNS tumors and 80% of all brainstem gliomas occurring in children, affecting ~400 children per year in the US [8]. The median age of onset is 6 years old. DIPG is a diffusely infiltrating tumor of the pons that cannot be resected and is universally fatal. DIPG is part of the broader molecular classification of DMG, which arise in the midline CNS and occur in the thalamus or spinal cord and are also universally fatal [9]. For DIPG/DMG, no standard therapy exists beyond initial radiotherapy and even at progression children are often left with re-irradiation as the only option to even marginally extend their life. The median progression free survival is 7 months and, when radiologic progression is included, the survival beyond progression is ~4 months [10]. Overall, the median survival at 1 year is 45.3% with a median overall survival of ~11 months [10]. Considering the median affected age, the incidence, and the fatal prognosis, DIPG is responsible for approximately 25,000 years of potential life lost in the US each year. In the setting of a uniformly fatal disease with no routine care procedures beyond radiation, DIPG/DMG will be eligible for this trial after completion of standard radiotherapy. Given the additional significance of primary location of DIPG within the brainstem, patients with DIPG tumors will be enrolled onto a dedicated study arm on this clinical trial.

Ultimately, while a recent molecular revolution within pediatric neuro-oncology led to fundamental discoveries, such as *BRAF* aberrations in low-grade glioma and *H3K27M* mutations in DMG, molecular targeting has improved survival for only a minority of patients[11]. The targeting of signal transduction pathways via small molecule drugs carries multiple challenges, including the complexity of molecular pathways, drug delivery across the blood-brain-barrier, tumor heterogeneity, and tumor escape via alternative signaling pathways. Targeting tumor-specific cell surface markers via locoregional delivery of T cells may circumvents these obstacles.

1.2 Role of Immunotherapy in CNS Tumors

Malignant CNS tumors represent a clinical entity that is highly attractive for immunotherapeutic intervention as (1) most patients with resection and radiation therapy achieve a state of minimal disease burden offering a safe time point for immunologic responses to occur in the CNS, and

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

(2) the anatomic location of these tumors within the confines of the CNS makes direct locoregional administration of effector cells possible. Furthermore, the paucity of therapies that can cure relapsed CNS disease demands novel approaches to therapy.

Multiple Phase 1 or 2 studies involving the administration of *ex vivo* activated lymphocytes to malignant glioma resection cavities or CNS ventricular system have been reported to date, using a variety of effector cell types [lymphokine-activated killer (LAK) cells, tumor infiltrating lymphocytes (TILs), and alloreactive cytotoxic T-lymphocytes (CTLs)] [12-24]. The practical obstacles to the success of these therapies include the time required to generate cell products, heterogeneity of tumor-specific antigen expression, local immune suppression, and difficulty interpreting imaging findings. However, these studies support the premise that a superior clinical effect of cellular immunotherapy for malignant CNS tumors might be expected with homogenous, highly potent effector cells.

1.3 Redirected Specificity of T Cells Engineered to Express Chimeric Antigen Receptors

To overcome some of the obstacles of cellular immunotherapy, multiple groups have focused on T cell genetic engineering strategies that serve to equip T cells with tumor specificity through the expression of chimeric antigen receptors (CARs). CARs are engineered to consist of an extracellular single chain antibody fused to the intracellular signaling domain of the T cell antigen receptor complex zeta chain. When expressed in T cells, CARs are able to redirect antigen recognition based on the monoclonal antibody's specificity [16]. These chimeric cell-surface molecules have the ability to bind antigen and to transduce activation signals via immunoreceptor tyrosine activation motifs (ITAMs) present in the CD3-ζ cytoplasmic tail [17], referred to as first generation CARs, or both activation and a co-stimulatory signal such as CD28 or 4-1BB domain are incorporated in series with CD3-ζ, referred to as second generation CARs [12, 25]. CAR molecules that include both CD28 and 4-1BB stimulatory signaling molecules are referred to as third generation CARs.

The design of CARs with target specificities for tumor cell-surface epitopes is a conceptually attractive strategy to generate antitumor immune effector cells for adoptive therapy, as it does not rely on pre-existing anti-tumor immunity. These receptors are "universal" in that they bind antigen in a major histocompatibility complex (MHC)-independent fashion; thus, one receptor construct can be used to treat a population of patients with antigen-positive tumors. This provides the additional advantage of being able to target tumors of divergent biology, but common surface marker expression. In addition to the highly successful CAR T cell therapies for hematologic malignancies, an increasing number of constructs for targeting human solid tumors have been described in the literature, including receptors with specificities for carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR)vIII, fibroblast activation protein (FAP), disialoganglioside GD2, human epidermal receptor 2 (HER2), interleukin13 receptor alpha 2 (IL13Rα2), mesothelin, receptor tyrosine kinase like orphan receptor 1 (ROR1), and L1 cell adhesion molecule (L1CAM) [13, 19, 26-29]. These epitopes all share the common characteristic of being cell-surface moieties accessible to scFv binding by the chimeric T cell receptor. The function of primary human T cells expressing tumor-specific scFvFc:ζ receptors have been evaluated *in vitro*; these cells specifically lyse tumor targets and secrete an array of pro-inflammatory cytokines, including IL-2, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and granulocyte macrophage-colony stimulating factor (GM-CSF) [30].

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Adoptively transferred CAR T cells can eradicate established tumors in a variety of animal models [13, 31-41]. Moreover, T cells expressing second or third generation CARs consisting of co-stimulatory signaling domains appear to be more resistant to activation-induced cell death, exhaustion, and T_{REG}-mediated functional anergy [12, 25, 32, 42-47]. Previous studies have shown that CD28 significantly increases cytokine production, whereas 4-1BB seems to improve survival of the T cells.

1.4 Clinical Experience Using CAR T Cells for Adult CNS Tumors

There are hundreds of open CAR T cell trials listed on clinicaltrials.gov for liquid, solid, and CNS malignancies. Studies employing CD19-directed CAR T cells against refractory pediatric and adult acute lymphoblastic leukemia (ALL), chronic lymphoblastic leukemia (CLL), and non-Hodgkin lymphoma (NHL) have demonstrated dramatic response rates, leading to commercialization of several CD19 CAR T cell therapies [48]. While the CNS experience is more limited, early reports suggest tolerability of intracavitary and intraventricular fractionated doses of IL13-zetakine CAR T cells specific for IL13Rα2, a cell surface receptor expressed in approximately 50% of adult glioblastoma multiforme (GBM) [49]. A first-in-human pilot and Phase 1 study evaluated IL13(E13Y)-zetakine CD8+ CAR T clones and CD4/CD8+ lines (NCT00730613). Patients received as many as 12 locally delivered infusions at a maximum dose of 1 x 10^8 cells via a catheter/reservoir system [49]. Intracranial CAR T cell instillations were found to be well tolerated with manageable, transient CNS inflammation and, notably, no systemic toxicities, including no cytokine release syndrome (CRS) or global neurotoxicity, both of which are frequently encountered in leukemia patients receiving systemically delivered CD19-specific CAR T cells [48]. Reported in the pilot study (NCT02208362), two of the three patients received planned full doses of 12 escalating intracavitary doses of 10^7 - 10^8 cells/dose. The other patient received all but one dose, which was suspended due to transient, worsening headache. No toxicities > Grade 3 were observed at doses up to 5 x 10^7 total cells/dose. At the maximum dose level of 1 x 10^8 cells, there were two cases of temporally limited headache (Grade 3) and one case of neurologic deficit (tongue deviation and shuffling gait) that resolved following treatment with dexamethasone. This neurologic event was seen in the patient with the highest degree of tumor IL13Rα2 antigen expression and the greatest intensity of CAR T cell-attributed fluid-attenuated inversion recovery (FLAIR) response on magnetic resonance imaging (MRI). Of the three patients, only one had a local recurrence at the previous resection cavity and the recurrent tumor displayed low IL13Rα2 expression.

Subsequently, a patient with recurrent GBM was treated with intracavitary and intraventricular dosing of the IL13Rα2-targeted CAR T cells (NCT02208362) [50]. Initially, intracavitary infusions controlled local disease but did not effectively control distant metastatic progression. Ten intraventricular doses were subsequently administered and by Dose 5 (Day 190), there was a 77-100% decrease in size of all measurable tumor sites, including elimination of all spinal tumors. This response was maintained for 7.5 months prior to new sites of metastatic disease, which preliminarily appear to demonstrate low IL13Rα2 expression. In total, this patient received six intracavitary and ten intraventricular cycles at a maximum dose of 1 x 10^7 cells/dose and no infusions were associated with toxicities of ≥ Grade 3. This dramatic response and the prolonged tolerability of treatment suggest feasibility of intraventricular dosing for leptomeningeal and spinal disease. In this single patient, total immune cells in the CSF increased by a factor of 7 +/- 3.6 while the immune response was limited to the CNS,

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

suggesting that local effects are robust, and that systemic CRS may not be a concern in CNS-direct T cell therapy.

1.5 HER2, EGFR, B7-H3, and IL13Rα2 as Targets for CNS Tumors

1.5.1 HER2

HER2 has been appreciated as a potential target in cancer cells since the 1980's and has been developed as a target for CAR T cells for over a decade [51, 52]. HER2 is an ideal target for treatment of CNS tumors as it is not expressed on normal CNS tissue but is expressed across a broad spectrum of biologic classes of brain tumors including ependymoma, GBM, and medulloblastoma [53, 54]. Interestingly, the level of HER2 expression is also associated with a worse survival [53, 54]. Importantly, HER2 expression is present on the cancer stem cell population of CD133 cells in these tumors [55]. While antibodies to HER2, such as trastuzumab, are clinically available, they most likely are ineffective in treating the HER2 low-expressing tumors most commonly found in children [56]. The most common malignant brain tumor of childhood is medulloblastoma, of which at least 40-80% express HER2. Glioblastoma, another common malignant brain tumor of childhood, expresses HER2 in 80% of cases and is present in CD133 stem cell populations which are known to be resistant to radiation, the most effective known treatment for GBM which extends beyond the margins of resection [57, 58].

The tolerability for HER2-specific CAR T cell therapy in the setting of a Phase 1 trial (NCT00902044) of sarcoma patients receiving intravenous infusions of a second generation (CD28:ζ) CAR was reported by Ahmed et al [59]. Intravenously (IV) administered cell doses up to 1 x 10^8 cells/m^2 were found to be well tolerated without evidence of on-target or off-tumor toxicities. Reported toxicities were predominantly Grade 1 and 2 nonspecific symptomatic findings, and no Grade 4 or 5 events occurred in the 19 dosed patients. This study was performed without lymphodepleting chemotherapy thus persistence of CAR T cells was modest and tracked by polymerase chain reaction (PCR). This group is conducting a Phase 1 study in patients with HER2-expressing GBM, and data presented in symposia to-date also indicate tolerability without evidence of on-target toxicity [60, 61]. This is in contrast to the report of a patient treated at NCI with high-dose CAR T cells after lymphodepletion and in combination with IL-2, who succumbed to pulmonary complications of CRS [62]. Data from Baylor College of Medicine trials indicate that lung HER2 expression is not a cause for pulmonary toxicity, at least in the context of patients who get lower CAR T cell doses without lymphodepletion and supplemental IL-2.

In July of 2018, Seattle Children's Therapeutics opened BrainChild-01 (BC-01) (NCT03500991), a Phase 1 study of central nervous system (CNS) locoregional adoptive therapy with autologous $CD4^+$ and $CD8^+$ T cells that are lentivirally transduced to express a HER2-specific chimeric antigen receptor (CAR) and EGFRt (IND 17857). Patients received weekly locoregional CAR T cells into the tumor cavity (Arm A) or intraventricularly (Arm B) for 3 weeks out of 4 weeks with intrapatient dose escalation. Subjects had to have received at least one of the highest intended CAR T cell dose level or experienced a DLT to be evaluable for dose escalation.

- As of September 1, 2022, 9 subjects have been enrolled, all had successful manufacture of cell product, and 7 subjects are evaluable for dose escalation on BrainChild-01. The 2

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

unevaluable subjects did not begin treatment because they no longer met infusion eligibility when their cell product was available.

- On Arm A (intracavitary delivery), 2 evaluable subjects completed the minimum 2 courses (6 doses) - with a maximum dose of 2.5 x 10^7 CAR T cells - without experiencing a dose limiting toxicity (DLT). On Arm B (intraventricular delivery), 5 evaluable subjects completed the minimum 2 courses (6 doses) - with a maximum dose of 5 x 10^7 CAR T cells - without experiencing a dose limiting toxicity (DLT). Arm A continues to enroll at DR1, while Arm B continues to enroll at DR2.
- A preliminary report of the initial 3 subjects enrolled onto BrainChild-01 represented the first pediatric experience of locoregional CAR T cell therapy for CNS tumors[23]. In summary, these subjects had evidence of local immune activation without any dose limiting toxicities. The primary adverse events (AEs) observed included self-resolving fever and headaches. Concurrent elevations in relevant chemokines/cytokines (e.g. CCL2, CXCL10) and serum c-reactive protein (CRP) were observed during treatment.

1.5.2 EGFR

The epidermal growth factor receptor (EGFR) is a cell surface tyrosine kinase receptor that is expressed in a diverse group of epithelial and non-epithelial tissues. EGFR signaling, which modulates cell growth, survival, and migration, is one of the crucial pathways implicated in cancer biology[63, 64]. Constitutive activation of EGFR signaling, as well as EGFR amplification and overexpression, are common to many cancers seen primarily in adults, such as non-small cell lung cancer and colorectal cancer, as well as GBM. The interstitial deletion mutation variant EGFRvIII is commonly expressed in glioblastoma and has become a therapeutic target of great interest[65]. In pediatric brain tumors, EGFR overexpression has been described in ATRT, DIPG/DMG, ependymoma, and HGG[66]. Overall, EGFR is likely a viable target in approximately one half of all malignant brain tumors[67]. Across cancers, EGFR has been associated with aggressive disease, chemotherapy resistance, and increased metastatic potential[68, 69].

While EGFR is expressed in many normal extraneural tissues, including the skin, skeletal muscle, liver, bladder, testis, and placenta, its expression is likely limited in the postnatal human brain[70]. Expression in multiple neuronal subtypes was reported in older studies by immunohistochemistry using polyclonal sera[70, 71]. In modern studies of EGFR expression in CNS tumors, EGFR immunolabeling was absent in normal control brain using multiple different antibodies[72, 73]. Therefore, the earlier results were likely due to nonspecific labeling. There are no reports of any of the EGFR-targeting antibody drugs binding to nontumor brain tissue, and no reports attributing neurotoxicity to these agents when given systemically[74, 75].

Therefore, EGFR has been the focus of numerous targeted cancer therapy initiatives, primarily targeted via tyrosine kinase inhibitors (TKIs) and anti-EGFR antibodies[76]. Employment of these agents has yielded some efficacy, but not against all cancer-associated EGFR mutations or tumors with overexpression of wild type EGFR and are frequently associated with notable off adverse events[77]. The unique EGFR monoclonal antibody (mAb) 806 selectively binds to an epitope on the extracellular portion of human EGFR expressed on the surface of tumor cells[78]. Though this antibody was raised against the deletion mutant EGFRvIII commonly

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

present in GBM and other adult solid tumors, it also binds to wild type EGFR that is untethered (which accounts for approximately 10% of EGFR-expressing cancer cells due to aberrant post-translational processing that is not present in non-malignant EGFR-expressing cells)[78-80]. A Phase 1 trial utilizing a radiolabeled chimeric version of this antibody in varied tumor types confirmed reliable tumor tissue uptake with no evidence of toxicity related to uptake in normal tissues known to express EGFR[81]. A more recent Phase 1 trial in adults with solid tumors characterized by EGFRvIII mutation or wild type EGFR overexpression studied the effects of a humanized mAb 806, ABT-806[82]. Treatment of was well tolerated with frequent AEs (≥10%) possibly/probably related to ABT-806 of fatigue (18%), nausea (16%), dermatitis acneiform (12%), and vomiting (10%) and occurrence of dose-limiting toxicity (Grade 3 morbilliform rash) in only one patient of 49 treated. Two subjects achieved prolonged stable disease. Given tumor specificity of this antibody, its ability to recognize an epitope on cells with a variety of EGFR aberrations and favorable toxicity profile in multiple adult trials, humanized mAb 806 is an attractive candidate for use in the development of an EGFR-specific CAR for employment in adoptive T cell therapy for EGFR-expressing relapsed or refractory pediatric solid tumors. ABT-414 (depatuxizumab mafodotin) is an antibody-drug conjugate that uses mAB806 as the binder, and displays similar binding characteristics as the unconjugated antibody, as well as efficacy in xenograft mouse models[83]. The drug was examined in clinical trials for GBM[84]. The most frequent side effects of the antibody-drug conjugate have been ocular and were seen in 91% of the 66 patients treated, with 33% experiencing Grade 3 and 2% experiencing grade 4 ocular toxicity, requiring treatment discontinuation in 6% of patients. The pathophysiology was determined to be due to microcystic keratinopathy and tended to improve with treatment discontinuation. Ocular side effects have not been commonly observed with other EGFR-targeted treatments and may be related to the antibody-conjugated drug. Similar side effects have been seen with other antibody-drug conjugates using MMAF toxin[85].

Given tumor specificity of this antibody, its ability to recognize an epitope on cells with a variety of EGFR aberrations and favorable toxicity profile in multiple adult trials, humanized mAb 806 is an attractive candidate for use in the development of an EGFR-specific CAR for employment in adoptive T cell therapy for EGFR-expressing relapsed or refractory pediatric solid and CNS tumors. To that end, children and young adults with EGFR-expressing recurrent/refractory solid tumors were enrolled on a SCTx Phase 1 trial to examine the safety and feasibility of administering autologous chimeric antigen receptor (CAR) T cells derived from autologous T cells genetically modified to express a second generation EGFR806-specific scFV-IgG4hinge-CD28tm/cyto-4-1-BB-zeta and EGFRt tracking/suicide construct (STRIvE-01, NCT03618381)[86]. All subjects received lymphodepleting chemotherapy with fludarabine and cyclophosphamide prior to intravenous administration of cryopreserved CAR T cells. The biologically effective dose (BED) or maximum tolerated dose was determined based upon observed toxicity through day 28 from initial CAR-T infusion and using a 3+3 statistical design. Eleven subjects (n=10 evaluable, age range 9-25, median 18) were enrolled and received either dose level (DL) 1 (0.5 x 10^6 CAR T cells/kg, n=4) or DL2 (1 x 10^6 CAR T cells/kg, n=7). CAR T were manufactured successfully in all subjects. Most common toxicities were fatigue, tumor-related pain and cytokine release syndrome (n=2, maximum CTCAE grade 1). Dose limiting toxicity of CTCAE grade 4 transaminase level and hyperbilirubinemia occurred at DL2 (n=1). Maximum circulating CAR-T expansion was 29.66 cells/uL (range 0.05-29.66 cells/uL)

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

with median persistence of 28 days (range 0-90). Two subjects on DL1 and one subject on DL2 demonstrated mixed response on day 28 and tolerated additional CAR T infusion without dose limiting toxicity.

In November of 2018, Seattle Children's Therapeutics opened BrainChild-02 (NCT03638167), a Phase 1 study of central nervous system (CNS) locoregional adoptive therapy with autologous $CD4^+$ and $CD8^+$ T cells that are lentivirally transduced to express an EGFR806-specific chimeric antigen receptor (CAR) and EGFRt (IND 18234). Patients received weekly locoregional CAR T cells into the tumor cavity (Arm A) or intraventricularly (Arm B) for 3 weeks out of 4 weeks with intrapatient dose escalation.

- As of September 1, 2022, 11 subjects have been enrolled, 10 had successful manufacture of cell product and 4 subjects are evaluable on BrainChild-02. The 6 unevaluable subjects did not begin treatment because they no longer met infusion eligibility when their cell product was available.
- On Arm A (intracavitary delivery), 1 evaluable subject completed the minimum 2 courses (received 5 of 6 planned doses) - with a maximum dose of 2.5×10^7 CAR T cells - without experiencing a dose limiting toxicity (DLT). On Arm B (intraventricular delivery), 3 evaluable subjects completed the minimum 2 courses (6 doses) – with a maximum dose of 2.5×10^7 CAR T cells - without experiencing a dose limiting toxicity (DLT). Arm A continues to enroll at DR1, while Arm B has advanced to DR2.

1.5.3 B7-H3

B7-H3 (CD276) is a member of the B7-CD28 immunoregulatory family of proteins whose expression drives IFN-gamma production and modulates T and NK cell killing [87]. In neuroblastoma, a common pediatric solid tumor, B7-H3 inhibits natural killer (NK) cell killing of tumor cells [88]. Its expression is known to be higher in malignant versus normal human tissue [89]. Amongst adult cancers, its expression also correlates with a poor prognosis in pancreatic, prostate, and urothelial cell carcinoma of the bladder [90-92]. In adult GBM, B7-H3 expression is correlated with a worse prognosis [93]. In mantle cell lymphoma, B7-H3 silencing induces sensitivity to conventional chemotherapy [94]. In melanoma, B7-H3 expression also correlates with metastatic potential [95]. B7-H3 is not identified in serum in healthy subjects supporting its lack of expression on normal tissue, while in patients with B7-H3-positive CNS tumors B7-H3 is detectable in the CSF and its expression level correlates directly to histologic tumor grade [87, 96].

Relevant to CNS tumors of childhood, B7-H3 is universally expressed in ATRT, choroid plexus carcinoma DIPG/DMG, ependymoma, HGG, and medulloblastoma [40, 97-99]. Regarding ATRT, investigators reported B7-H3 expression in all samples and cell lines and demonstrated that B7-H3CAR T cells delivered intracranially eradicated orthotopic ATRT xenografts in animal models[99]. Preclinical studies of B7-H3 CARs by independent research teams at the University of North Carolina and Stanford University demonstrated that the on-target effect of B7-H3 CAR is directly correlated with degree of surface B7-H3 expression that results in sparing of normal tissue targeting and providing an appropriate therapeutic window. Studies have confirmed the expression of B7-H3 in an overwhelming percentage of CNS tumor samples and *in vivo* studies against pediatric CNS tumor models, with confirmed on target effect and anti-tumor efficacy leading to eradication of disease, did not cause toxicity to normal tissue[39-41, 100]. While other immune modulators, such as CTLA4 (which acts synergistically with

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

B7-H3), are also expressed on tumor tissue, B7-H3 is comparatively expressed at a lower overall number and density on normal tissue [101]. Ultimately, it has been proven that B7-H3 density on normal tissue is insufficient to result in toxicity in animal models [99]. Primate toxicity studies have also been completed with the B7-H3 antibody from which our scFv binders are derived and found no significant test article-related safety findings [102]. Memorial Sloan Kettering has extensive experience with a radioimmunotherapy agent targeting B7-H3 which has been used in multiple tumor types. Even in the setting of an intensive multi-agent regimen, B7-H3 monoclonal antibody given intrathecally to patients with aggressive, metastatic neuroblastoma was well tolerated [103]. In a Phase 1 study of B7-H3 antibody administered directly into the pons, no Grade 4 or 5 toxicities were reported, and only 1 patient experienced Grade 3 CNS-related toxicity (transient hemiparesis) [104]. The event of hemiparesis may have been related to direct administration of B7-H3 antibody directly into the pons, a procedure that will not occur on BrainChild-04. A Phase 1 study of B7-H3 antibody (MGA271) performed by Macrogenics revealed no dose-limiting toxicity even in frequently heavily pre-treated patients [105]. The most common toxicities seen were fatigue, infusion-related reaction, nausea, and vomiting without any normal tissue directed toxicity observed.

Children and young adult patients with relapsed/refractory solid tumors were enrolled onto a SCTx Phase 1 trial to examine the safety of autologous T cells genetically modified to express scFV-IgG4hinge-CD28tm-4-1BB-zeta B7-H3-specific CAR with the methotrexate resistance/selection cassette DHFRdm and the tracking/suicide construct EGFRt (STRIvE-01, NCT04483778)[86]. All patients received lymphodepleting fludarabine and cyclophosphamide prior to intravenous infusion of cryopreserved CAR-T. The maximal tolerated dose or biologically effective dose (BED) was determined based upon observed toxicity through day 28 from initial CAR-T infusion and using a 3+3 statistical design. Sixteen subjects (age 11-24, median 17 years) enrolled and received dose level (DL) 1 (0.5×10^6 CAR T cells/kg, n=3) or DL2 (1×10^6 CAR T cells/kg, n=6). No dose limiting toxicity was observed following first infusion, most common toxicities were fatigue and cytokine release syndrome (CRS) (n=2, maximum CTCAE grade 2). Maximum circulating CAR T expansion on first infusion was 4.98 cells/uL (range 0.23-4.98 cells/uL) with median persistence of 28 days (range 14-90). Best overall response of Stable Disease was observed in 3 of the 9 subjects infused. Given observed expansion and persistence, DL2 was determined to be the BED. A second infusion at DL2 in one subject demonstrated CAR T expansion to 1590 cells/uL (86% of circulating CD3 cells) with CTCAE grade 2 CRS and transient dose limiting CTCAE grade 4 liver enzyme elevation. A partial metabolic response on FDG-PET by PERCIST criteria was observed in this subject at Day 28.

In November of 2019, Seattle Children's Therapeutics opened BrainChild-03 (NCT04185038), a Phase 1 study of central nervous system (CNS) locoregional adoptive therapy with autologous $CD4^+$ and $CD8^+$ T cells that are lentivirally transduced to express a B7-H3-specific chimeric antigen receptor (CAR) and EGFRt (IND 19043). The incorporation of a methotrexate-resistant human DHFR mutein ($huDHFR^{FS}$; L22F,F31S) in a single transcript in combination with the B7-H3-specific CAR and EGFRt, each separated by a T2A linker, allowed for the use of methotrexate (MTX) to select and enrich activated, proliferating CAR T cells *in vitro*. Variants of dihydrofolate reductase (DHFR), which confer resistance to antifolates, are used as dominant selectable markers *in vitro* and *in vivo* [106]. Expression of $huDHFR^{FS}$ can render lentivirally

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

transduced primary human $CD45RO^{+}CD62L^{+}$ memory T cells resistant to lymphotoxic concentrations of MTX up to 0.1μM [107]. Patients received weekly locoregional CAR T cells into the tumor cavity (Arm A) or intraventricularly (Arm B) for 3 weeks out of 4 weeks with intrapatient dose escalation. In addition, Arm C enrolled patients with DIPG and DMG, utilizing an every-other-week intraventricular infusion of CAR T cells with intrapatient dose escalation.

- As of September 1, 2022, 40 subjects have been enrolled, with successful manufacturing of cell product in all but 1 subject, and 25 subjects are evaluable on BrainChild-03.
- On Arm A (intracavitary delivery), 1 evaluable subject completed the minimum 2 courses (6 doses) – with a maximum dose of 2.5 x 107 CAR T cells – without experiencing a dose limiting toxicity (DLT). Arm A continues to enroll at DR1. On Arm B (intraventricular delivery), 12 evaluable subjects completed the minimum 2 courses (6 doses) – with a maximum dose of 10 x 107 CAR T cells – without experiencing a dose limiting toxicity (DLT), confirming DR3, the highest planned DR, as the MTDR. A 12-patient expansion cohort at DR3 is enrolling.
- On Arm C (intraventricular delivery to subjects with DIPG), there are 12 evaluable subjects, eleven of whom completed the minimum 2 courses (4 doses) – with a maximum dose of 5 x 107 CAR T cells without experiencing DLT. There has been 1 DLT of pontine hemorrhage that was experienced 8 days following CAR T cell infusion dose of 1x10 7. Of note, spontaneous intratumor hemorrhage has been documented in patients with DIPG[108]. Our initial Arm C experience has been published along with being presented nationally and internationally[23, 109, 110]. Overall, across BC-01, 02 and 03, 60 subjects have been enrolled, 57 of 59 subjects have had successful CAR T cell manufacturing (1 pending), 40 subjects have received CAR T cell doses, and 36 received at minimum 2 months of CAR T cell therapy. The 3 trials share a common delivery schema of weekly CAR T cell dosing given 3 weeks out of 4 weeks (or every other week dosing to subjects with DIPG on BrainChild-03 Arm C) with patients tolerating up to 29 doses (6-27 doses). The most common AE observed are metronomic fever and headache occurring approximately 6-24 hours post CAR T cell infusion. Arm C has advanced to the highest planned DR (DR4).

Subjects receiving CAR T cells have not exhibited differential AE based on a weekly schedule or other week schedule (BrainChild-03 Arm C) of infusions and the only DLT as of October 2022 across any of the three BrainChild studies was following a subject's first dose at DL1; therefore, BrainChild-04 will incorporate weekly CAR T cell infusions for all patients. There also have been no DLTs in the second Course of treatment, so the DLT window will be shortened from 8 weeks to 4 weeks.

Along with preliminary feasibility and safety data, potential clinical benefit has been observed in those subjects with DIPG treated on BC-03 Arm C (Figure 1-1), with radiographic local inflammatory changes seen even at Dose Regimen 1 and a suggestion of prolonged survival compared to that historically seen for this patient population of 11 months from diagnosis and <3 months from initial progression [10]. For example, in DR1, S005 who enrolled with progressive disease survived approximately 17 months from enrollment and 36 months from diagnosis, S008 with stable disease remains alive 16 months from enrollment and 20 months from diagnosis, S014 who enrolled with progressive disease survived approximately 17 months

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

from enrollment and 32 months from diagnosis. Overall, all five subjects with DIPG who enrolled on study prior to progression are still alive. In Dose Regimen 2, a 4-year-old girl enrolled after clinical and radiographic progression that required high dose steroids. Following 4 CAR T cell doses, she had a reduction both in overall size of the pons (29.7%) and its T2 hyperintense component (44.5%), which corresponded with clinical improvement (Figure 1-2).

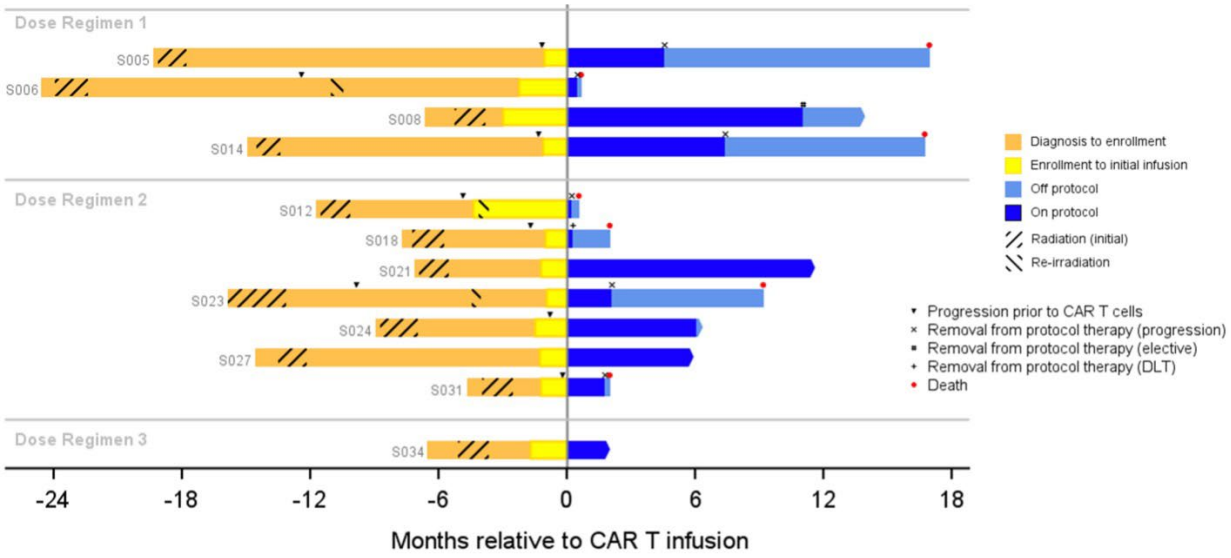
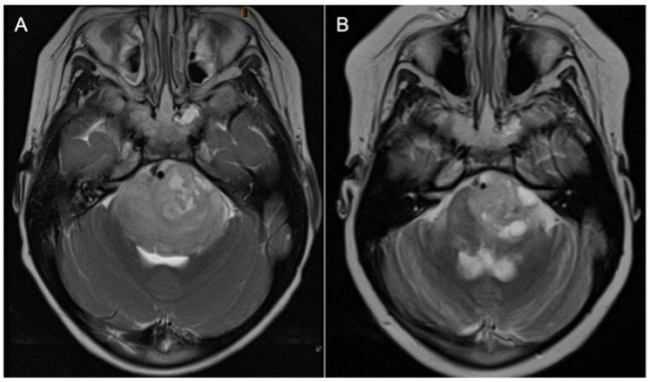


Figure 1-1 BrainChild-03 Arm C by Dose Regimen



4-year-old with DIPG treated with B7-H3 CAR T cells on BrainChild-03 Dose Regimen 2. (A) Axial T2 MRI post-progression at time of enrollment. (B) Axial T2 MRI after 4 doses of CAR T cells over 60 days on study.

Figure 1-2 BrainChild-03 Arm C Dose Regimen 2

1.5.4 IL13Rα2

IL13Rα2 is a monomeric high-affinity IL-13 receptor and is highly expressed on over 60% of glioblastoma, while not expressed on normal CNS tissue [50, 111]. Furthermore, IL13Rα2 expression has been correlated with poor patient survival, underscoring its presence in biologic subsets most in need of novel therapies. IL13Rα2 is also highly expressed in DMG, which otherwise have a fatal prognosis and multiple IL13Rα2 targeting therapeutics are being

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

investigated including antibody and cellular targeting approaches[112]. Of all intracranial cellular therapies, the largest experience has been with IL13Rα2 targeting CAR T cells described in Section 1.4 Clinical Experience Using CAR T Cells for Adult CNS Tumors. The IL13-zetakine CAR utilizes a membrane-tethered IL13 ligand mutated at a single site (E13Y) to recognizes IL13Rα2 but reduce potential binding to the more widely expressed IL13Rα2/IL4Rα complex[35, 113]. IL13-zetakine^{+} CTL have been shown to have IL13Rα2-specific cytolytic activity against CNS tumors in vitro and can mediate regression of GBM xenografts in vivo[114]. Clinical translation of the IL13-zetakine is reviewed in Section 1.4 [35, 113]

1.6 Rationale for Intraventricular CNS Dosing of CAR T cells

The intracavitary and intraventricular administration of T cells for the treatment of CNS tumors theoretically permits the expansion of CAR target epitopes to those expressed on tumor cells but not normal CNS cells, with less stringency on expression by other tissues outside the CNS. The concern regarding toxicity from cross-reactivity of tissues outside the CNS is mitigated by (1) the sequestration of cells in the CNS based on the intracavitary and intraventricular route of administration, and (2) the low cell numbers administered in comparison to cell doses typically administered systemically. CNS delivery of IL13-zetakine CAR T cells to patients with GBM or CNS delivery of HER2-specific CAR T cells to children with HER2+ CNS tumors did not demonstrate overt seeding of CAR T cells in the peripheral blood, or systemic toxicities which might be expected if this were to occur based on target antigen expression in tissues such as IL13Rα2 in lung epithelia [23, 49]. Our institutional experience with 36 evaluable patients on 3 distinct locoregional CNS CAR T cell trials, has confirmed locoregional CNS delivery does not result in serious systemic toxicities, on-target off-tumor toxicity, CRS, or ICANS[23, 110, 115, 116].

The BrainChild-04 regimen, in addition to direct intracranial administration of CAR T cells instead of IV, will not employ lymphodepletion or IL-2 supplementation as there have been clinical and radiographic responses in CNS patients receiving intracranial CAR T cells without lymphodepletion[50], there is no direct evidence systemic lymphodepletion improves local CNS CAR T activity, and with repeated locoregional delivery it is not feasible or safe to continuously lymphodeplete children.

1.7 Rationale for Multi-antigen Targeting

High grade CNS tumors of childhood, like other malignant tumors, have historically had limited response to single agent targeting of any kind[117]. Even with the advent of well-defined genetic, epigenetic, methylomic, and proteomic profiling of pediatric CNS tumors, the past decade of advancement has led to greater prognostication but limited therapeutic success outside of low-grade tumors.[118-120] For example, the greatest outcome of a molecularly targeted agent has been with MEK inhibition against low-grade gliomas, which already had a relatively large number of curative therapeutic options[11]. Unfortunately, high grade CNS tumors of childhood harbor significantly more complexity, so, as with other malignant childhood solid tumors, combinatorial targeting has been the most consistent path to improved outcomes[121].

A similar requirement for multi-targeting is emerging for cellular therapy across disease indications. From the most successful example of CAR T cell therapies, durable remissions in

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

pediatric B-cell ALL are limited in large part due to CD19 antigen negative relapse[48]. Moreover targeting of CD22 with CAR T cells had demonstrated the importance of down regulation of antigen density as a mechanism of tumor evasion, suggesting that there is a threshold of antigen binding that must occur to allow for CAR T cell cytotoxicity and providing an additional mechanism of resistance to cellular therapy[122]. Current efforts seeking to improve durable remissions are underway, with CAR T cell products targeting multiple antigens rather than single antigens.

CNS tumors such as high grade glioma display significant molecular and cell surface heterogeneity[123] and cells exhibit temporal phenotypic changes that complicate targeting[124], raising the distinct possibility that cellular therapy with single antigen targeting will result in insufficient tumor killing as demonstrated by Brown et al for IL13Ra CAR T cell therapy in patients with GBM[49]. The cell surface antigens that we have investigated for single-antigen targeting (i.e. HER2, EGFR, and B7-H3) are not ubiquitously expressed on all tumor cells and can exhibit temporal changes in antigen expression over time. In addition to heterogenous expression, the tumor cells may exhibit insufficient antigen density of the target protein to enable CAR T cell cytotoxicity[39].

SC-CAR4 BRAIN has the potential to address each of these barriers to efficacy. The presence of multiple distinct CAR T cells within the composition of the SC-CAR4BRAIN product ensures engagement of a multiplicity of tumor cell antigens, thereby overcoming de novo tumor heterogeneity, potentially decreasing the drive for tumor cells to down-regulate any one antigen, or at least minimizing the impact of any one event of antigen down-regulation by ensuring that alternative antigens will still be engaged to elicit CAR T cell cytotoxicity. SC-CAR4BRAIN will also allow for targeting of multiple distinct antigens on any individual tumor cell, thereby increases the likelihood that the relative threshold of antigen binding necessary for CAR T cell cytotoxicity will also be achieved even if the density of any one single antigen is insufficient for such activity.

The proposed trial will introduce multi-antigen targeting to pediatric CNS tumors with an objective to confirm safety and utilize a more biologically rationale foundation to improve outcomes.

1.8 Development of SC-CAR4BRAIN

SC-CAR4BRAIN was developed by transducing primary T cells with a mixture of four vectors simultaneously. Each vector encodes a CAR with specificity directed toward a distinct, high-value molecular target on brain tumors. The CAR constructs targeting HER2, EGFR806 and B7-H3 are the same constructs that have been utilized in Brainchild-01, 02, and 03 respectively. The specificity of these CARs is conferred by scFv binding domains derived from monoclonal antibodies Herceptin, mAb806, and MGA271 for HER2, EGFR806, and B7-H3 CARs respectively. The CAR using the IL-13 cytokine carrying a E13Y mutation in place of an scFv provides specificity for IL13Rα2 and is aligned with the construct investigated clinical in NCT00730613. The CAR's signaling domains all include CD3ζ chain as the primary stimulatory signal paired with a 4-1BB costimulatory domain. Through systematic testing of CAR constructs with different intracellular signaling domains and spacer lengths, the optimal spacers for B7-H3 CAR and HER2 CAR were found to be the medium spacer (IgG4hinge-CH3) while EGFR806 and IL13zetakine benefitted most from the short IgG4hinge only spacer.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

The surface expression of EGFRt in conjunction with each CAR provides a cell surface marker that allows easy examination of transduction efficiency [125]. Biotinylated or directly fluorochrome conjugated cetuximab binds to the EGFRt expressed on the cell surface and can be utilized for analysis with flow cytometry. Because the EGFRt lacks an intracellular domain, it does not have any signaling capacity, thus binding of cetuximab to the EGFRt does not affect the cell. Lastly, cetuximab has the potential to be used clinically as a suicide gene as it is a clinically available antibody with a low toxicity profile [26, 125]. After binding of cetuximab, cells undergo antibody-dependent cellular cytotoxicity *in vivo*. Therefore, cetuximab may be used to ablate CAR expressing T cells if required due to CAR T cell related toxicity.

Variants of dihydrofolate reductase (DHFR), which confer resistance to antifolates, are used as dominant selectable markers *in vitro* and *in vivo* [106]. A methotrexate-resistant human DHFR mutein (huDHFR^{FS}; L22F,F31S) is encoded in the B7-H3 CAR and IL13zetakine constructs along with EGFRt, each separated by a T2A linker, allowing the use of methotrexate (MTX) to select and enrich activated, proliferating CAR T cells during product manufacture. Expression of huDHFR^{FS} can render lentivirally transduced primary human CD45RO^{+}CD62L^{+} memory T cells resistant to lymphotoxic concentrations of MTX up to 0.1μM [107]. By combining the human-encoded DHFR mutein with pharmaceutical grade MTX, a purified population of lentivirally transduced CAR-expressing T cells harboring B7-H3 CAR or IL13zetakine, either singly expressed or in combination with HER2 CAR and EGFR806 CAR, can be produced for the clinic in the setting of current Good Manufacturing Practice (GMP)-compliant manufacturing.

Preclinical data characterizing the distribution of CAR expression suggests a well-balanced product composition between the two MTX-selectable CARs (B7-H3CAR and IL13zetakine) at a higher level within the product, with even distribution of HER2CAR and EGFR806CAR at a lower level given that neither construct alone nor in combination with each other would resist MTX selection. Overall ratio of distribution of each construct across the product is shown in Figure 1-3.

Due to challenges with staining the various CARs directly with completely differential signals, a portion of the B7-H3 proportion, while overwhelmingly likely to be B7-H3, cannot equivocally be guaranteed as such and instead depends on subtraction of HER2-specific staining from total staining of anti-human Fc, which would label both B7H3CAR and HER2CAR. To provide controls for preclinical studies, single-specificity products made by TCPC with each of the four aforementioned lentiviral vector lots singly (PD0316, PD0447, PD0352, and PD0375) were used as controls in the following studies as well as a cell line which was mock transduced (PP-055)

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

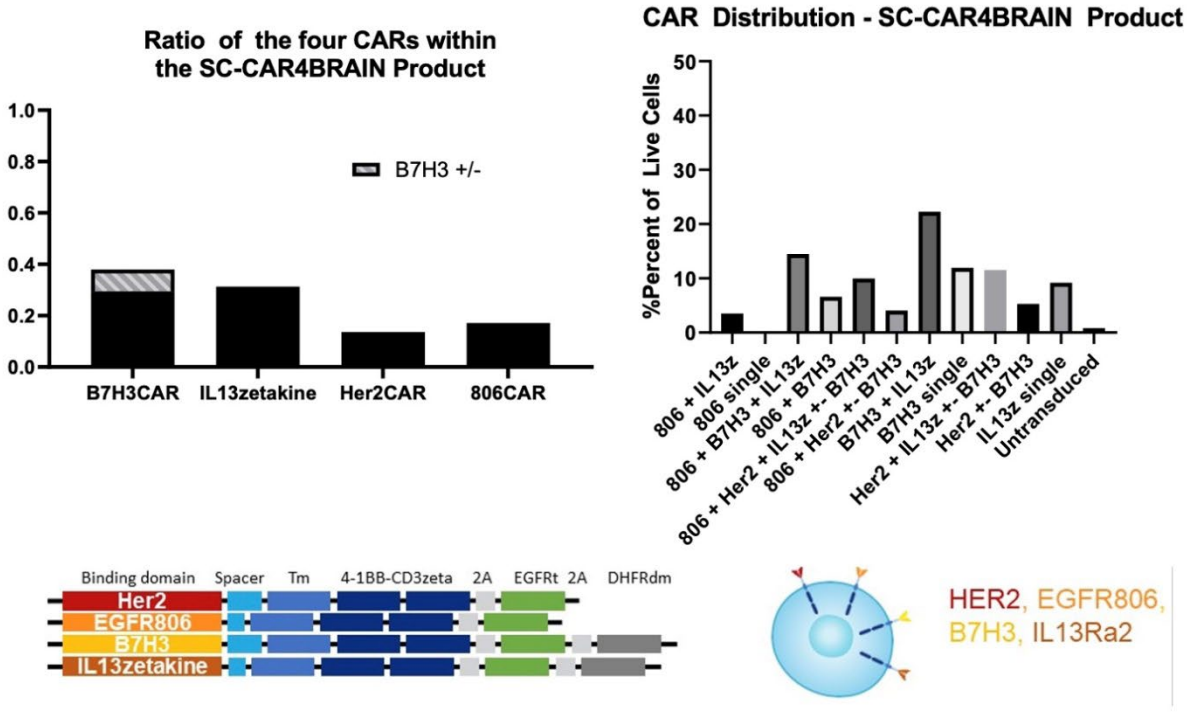


Figure 1-3 CAR distribution and construct diagrams in the SC-CAR4BRAIN product.

Assays were performed to demonstrate the activity of SC-CAR4BRAIN against its intended targets, both in combination and as single expressing cell lines. The cell lines were U251T, which natively expresses all four targets, U87 which was transduced with HER2 so that all targets were expressed, and K562 cell lines which were B7H3-deleted and transduced to each express mCherry and only one of HER2, EGFRvIII, and IL13R2α. K562 with no B7H3 deletion was used as the B7H3 single target line. The ability of SC-CAR4BRAIN and each individual CAR product to control growth of these cell lines was measured in a 72-hour Incucyte assay. SC-CAR4BRAIN controlled tumor cell growth in both U251T and U87 Her2+ (Figure 1-4) and each of the single antigen control lines (Figure 1-5).

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

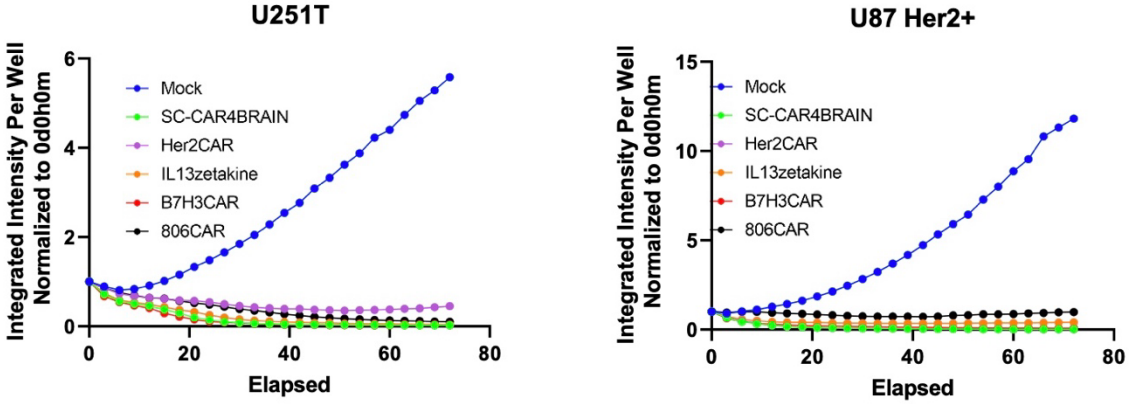


Figure 1-4 SC-CAR4BRAIN controls outgrowth of quad-antigen expressing U251T and U87 cell lines, as do individual single-CAR control lines

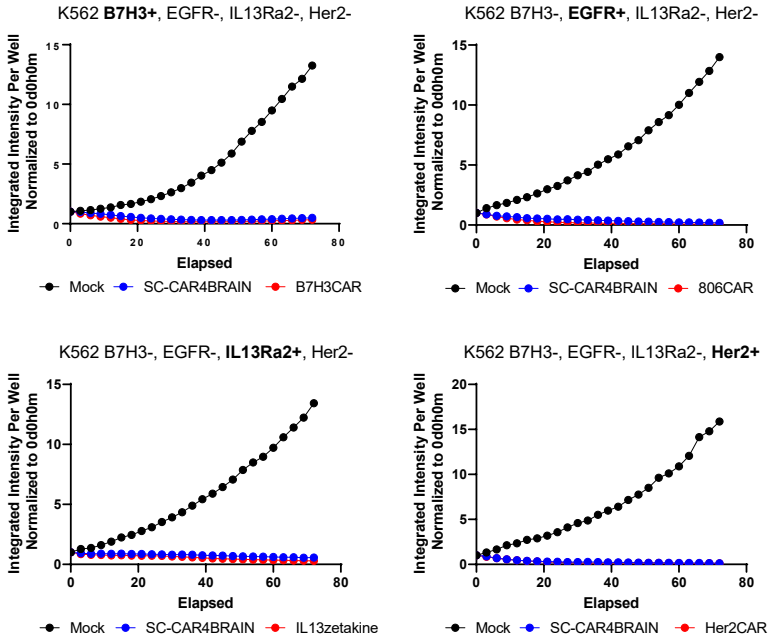


Figure 1-5 SC-CAR4BRAIN controls outgrowth of single-antigen K562 cell lines, as do individual single-CAR control lines.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

The release of T-cell effector cytokines upon target exposure was also measured for SC-CAR4BRAIN against the same combination of U251T Parental, U87 +HER2, and K562 single target expressing lines that was used for cytotoxicity analysis. SC-CAR4BRAIN or single-specificity control lines were exposed to targets at a 2:1 Effector:Target ratio for 24 hours, after which supernatants were collected and measured for TNFα, IFNγ, and IL-2. SC-CAR4BRAIN and single-specificity CARs released cytokines in response to quad-antigen expressing target lines (Figure 1-6). Results in the single-specificity K562 lines showed a high level of selectivity for targeted constructs, and strong cytokine secretion when the target of any CAR expressed on the effector line was present in the target line (Figure 1-7). This suggests a highly effective T-cell product and stringently target-specific CARs.

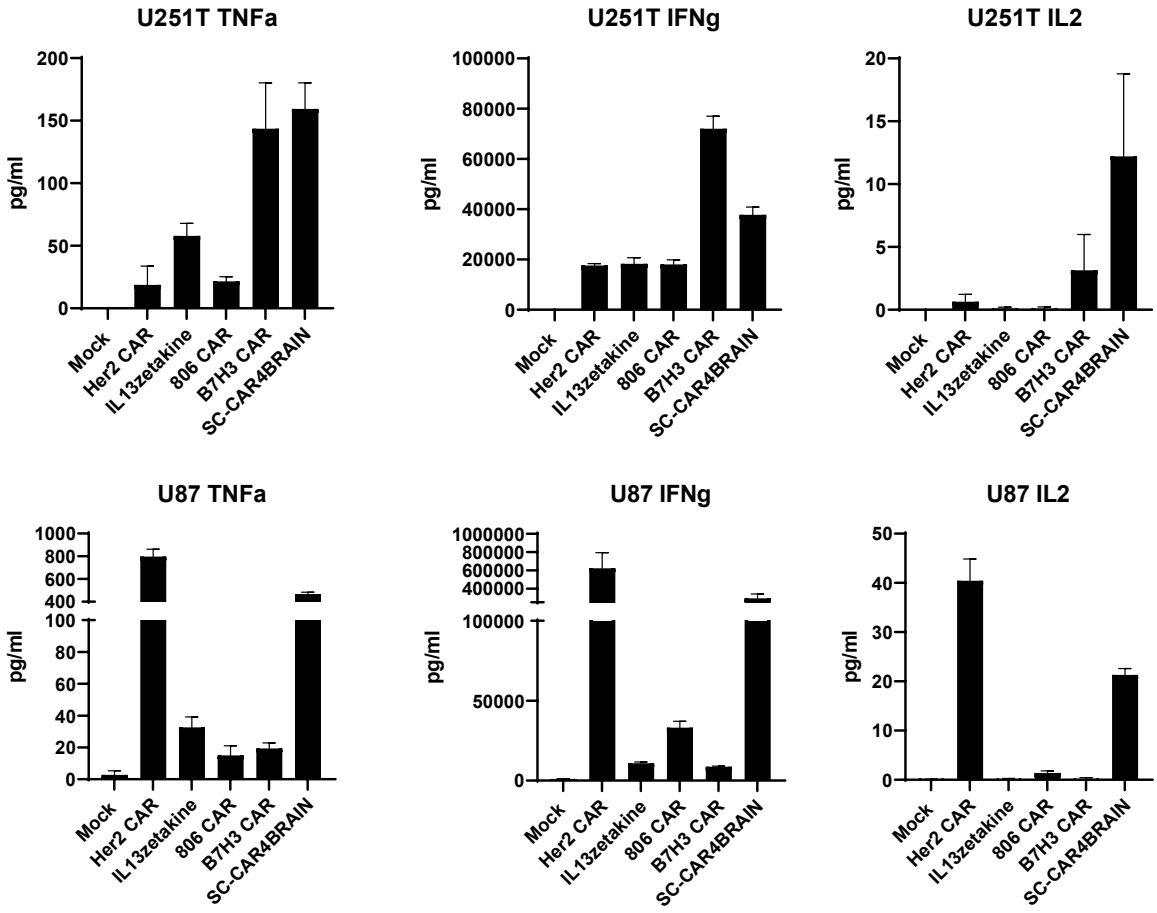


Figure 1-6 SC-CAR4BRAIN and single CAR control lines respond to quad-antigen expressing target exposure by releasing cytokines.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

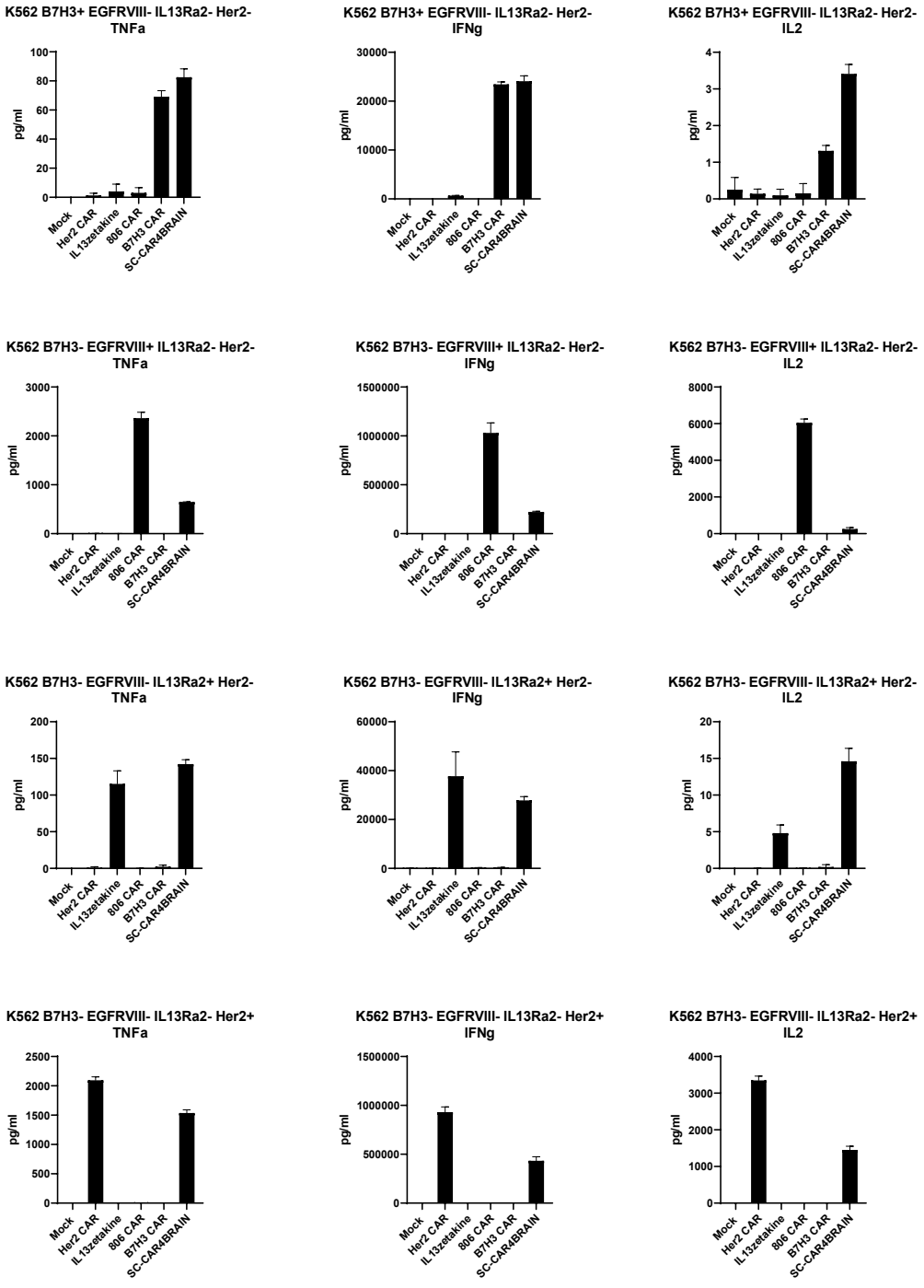


Figure 1-7 SC-CAR4BRAIN and single CAR control lines release cytokine in response to single antigen-expressing K562 target cells only when target antigen is present.

In order to demonstrate the ability of SC-CAR4BRAIN to eradicate established tumors in currently available *in vivo* models, an orthotopic intracranially-implanted glioblastoma mouse model was established using the U87 cell line expressing the GFP:ffluc reporter construct.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

$2x10^5$ U87 cells which had been transduced with a vector to induce HER2 expression were implanted intracranially in mice. Once mice had engrafted intracranial tumor confirmed by bioluminescent imaging (Day 6 post implantation), a single dose of $2x10^6$ SC-CAR4BRAIN T cells was administered intracranially, and mice were imaged serially for up to 84 days. A control group in which $2x10^6$ mock transduced T cells were administered needed to be euthanized completely by Day 36 of the study. The results of this study suggest a highly potent product for the control of aggressive, established intracranial tumors, with only a single mouse being euthanized prior to prescribed study endpoint with luminescence data suggesting a possible technical error in T cell administration (Figure 1-8). Accompanying survival data further demonstrates the efficacy of SC-CAR4BRAIN.

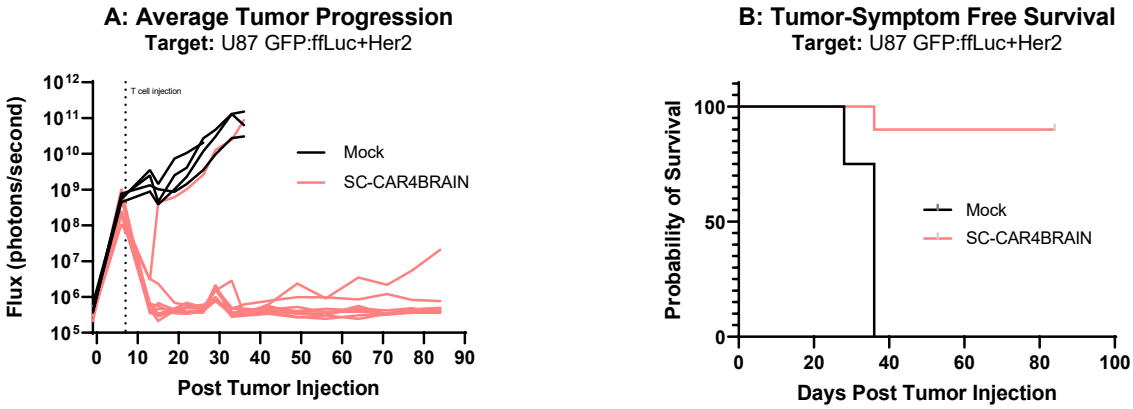


Figure 1-8 Tumor progression and survival of engrafted NSG mice versus untreated mice. Bioluminescent signal (A) and survival (B) of mice treated with either mock transduced or SC-CAR4BRAIN T cells. Mice plotted individually for imaging data.

1.9 Potential Toxicity Associated with SC-CAR4BRAIN

This is a first-in-human study of SC-CAR4BRAIN; therefore, the adverse effects of this product are not yet known. Similar studies of CAR T cells in pediatric and young adult patients with CNS tumors have seen the most common toxicity arising from a mild neurotoxicity syndrome that includes fever, headache, nausea, emesis, and mild to moderate worsening of baseline toxicities occurring 12-24 hours after CAR T cell administration.

1.9.1 General symptoms associated with CNS administration of CAR T cells

Risks of locoregional CNS CAR T cell infusion include nausea, emesis, chills, rigors, fever, headaches, and/or pain at metastatic CNS sites occurring within initial 72 hours of infusion. Concurrent symptoms of inflammation include elevation in CRP and worsening of baseline neurologic symptoms.

1.9.2 Risk of Cytokine Release Syndrome (CRS)

In patients with leukemia and solid tumors receiving intravenous therapeutic T cells, cytokine release syndrome (CRS) may occur shortly following infusion of the product, with symptoms of fever, chills, rigor, mild hypotension and mild respiratory distress. Although these toxicities can occur immediately following the T cell infusion, they typically have an onset of 3-10 days after the T cell infusion and can last for up to 1-2 weeks.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

CRS has not been commonly observed in subjects enrolled onto BC-01, BC-02, and BC-03 trials. In subjects for whom there is concern for CRS will be admitted to the hospital for observation. In subjects with adverse events (AEs) during *in vivo* expansion of CAR T cells, additional laboratory testing will be requested. Subjects may receive cytokine directed therapy for symptom control including, but not limited to, tocilizumab, an IL-6 antagonist. The use of corticosteroids may be given to prevent a more serious toxicity.

1.9.3 Risk of neurologic toxicity

A distinct syndrome of neurotoxicity can occur post infusion of CAR T cells. This syndrome consists predominantly of encephalopathy, tremor, aphasia, hallucinations, and seizures and is now termed Immune effector Cell Associated Neurotoxicity Syndrome (ICANS)[126-129]. ICANS has not been described among subjects receiving CNS intraventricular CAR T cell products. More commonly, mild, transient symptoms have been observed greater than 24 hours after receiving CAR T cell treatment that include fevers, headache, nausea, vomiting, increase in neurologic changes from baseline, and new onset or exacerbation of baseline seizure disorders. Neurologic changes may occur due to direct neurologic toxicity or due to tumor pseudoprogression. Pseudoprogression is a phenomenon where primary and/or metastatic tumor sites increase in size following antibody and/or cellular immunotherapy, including CAR T cells [130-132]. Pseudoprogression results from infiltration of CAR T cells and/or recruitment of immune cells into the tumor microenvironment. The increase in tumor size may result in pain, or increased mass effect of surrounding normal tissue causing worsening of baseline tumor associated symptoms, or new symptoms associated with the specific site of malignant cell involvement.

Baseline neurologic and neurocognitive evaluation is essential prior to treatment with SC-CAR4BRAIN in order to assess acute and long-term changes if they occur. Anti-seizure prophylaxis will be administered to all subjects prior to infusion of CAR T cell due to potential risk of CNS inflammation and/or inflammation related to underlying tumor that may predispose this patient population to seizure.

1.9.4 Potential long-term toxicity

Secondary Malignancy/Insertional Mutagenesis: Data regarding the risks of gammaretroviral vectors for T cell modification, have been published[133]. The University of Pennsylvania analyzed 221 subject samples obtained over a decade from 43 subjects who had been enrolled in T cell trials in which CD4ζ CARs were expressed on autologous T cells (CD4 and CD8) through genetic modification with gene integration directed by gammaretroviral vectors. There was durable engraftment of the modified T cells in 212 of the samples. Through whole genome analysis, a thorough analysis of the integration sites was evaluated in 11 individuals with high-level marking. A total of 7222 unique integration sites were detected and were enriched for sites near the 5’ end of a gene. There did not appear to be any clonality in the samples, and integration sites over time in the same subject varied. Additionally, there did not appear to be enrichment of integration sites near genes involved in clonal expansion or persistence, nor was there enrichment of integration sites near the 5’ ends of cancer-related genes. Overall, this report had 540 subject-years without integration-mediated toxicity, placing the true adverse event rate at less than 0.0068 per person-year. These data suggest that the use of gamma retroviruses for gene-integration in T cells carries a much safer toxicity profile than when used

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

in hematopoietic stem cells, and that T cells appear to have an inherent protection to insertional genotoxicity.

In addition to transducing T cells rather than hematopoietic stem cells, we are using a third generation SIN lentiviral vector rather than a gammaretroviral vector. Additional safety data have been published regarding the use of lentiviral vectors in clinical trials. One report detailed 65 subjects who received transduced CD4+ cells. The mean copy number was 2.3 with a range of 0.2-5.4. Additionally, all tests for replication competent lentivirus (RCL) were negative. Previously some of these subjects had integration site analysis performed, which showed that there were >7000 unique insertion sites without evidence of enrichment near proto-oncogene 5' ends or within tumor suppressor genes[134, 135]. Experience from the National Gene Vector Biorepository in testing for RCL from a variety of clinical trials was recently published and evaluated 460 lentiviral transduced cell products from 375 subjects, all of which were negative for RCL[136]. Two hundred and ninety-six subjects also underwent testing at least one month after receiving a cellular product, and all were also negative for evidence of RCL. These reports suggest that lentiviral vectors are safer than gammaretroviral vectors in regard to malignant transformation potential.

In summary, we believe that the potential toxicities associated with the use of SC-CAR4BRAIN T cell therapy are outweighed by the need to develop novel treatment for patients with life-threatening CNS tumors.

1.9.5 Intra-Subject Dose Escalation

We and others have observed induction of localized and transient neuro-inflammation following intraventricular CAR T cell administration that requires careful monitoring and management. In aggregate, our BrainChild trials and the City of Hope Phase 1 trials support the utilization of intra-subject dose escalation as a means to address dose level safety in the context of CNS intraventricular locoregional infusions wherein tolerance is dictated by both the fixed volume of this anatomic compartment, the heterogeneity in tumor mass within the compartment, the effect of tumor location (supratentorial, infratentorial, midline, lateral, etc.) and adjacent functional brain anatomy [49, 50, 137, 138]. Rather than proposing single bolus dosing in which a potentially large dose of cells is administered, our dosing strategy is to perform fractionated dosing so that a cumulative tumor exposure to large numbers of CAR T cells is achieved over a period of weeks. The added biological advantage to this strategy is that, while T cells exhaust within solid tumors after 1-2 weeks, this dosing strategy provides continued exposure to non-exhausted T cells by weekly infusion.

Unlike intravenous dosing, which is simplified by predictable and uniform distribution from subject to subject, dosing into the CNS, based on the variables described above, makes tolerability of dosing a "subject-specific" function that is not well accommodated by classic cohort escalation strategies. Our intra-subject dose escalation accommodates these subject-specific variables that impact on tolerability and safety using a rule set that calibrates dose escalation, dose cancellation, and dose de-escalation to the specific study participant's neurologic tolerance. This strategy safeguards subject safety in light of concern for significant transient inflammatory changes to the tumor bed and surrounding brain that may accompany CAR T cell activity. We believe this is the safest means to explore T cell dose escalation in the three dosing regimens that we have designed for our pediatric and young adult protocol.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

1.10 T Cell Dosing Rationale

The starting and subsequent dose escalations proposed for CAR T cell infusion are built from prior experience at the City of Hope and at Seattle Children's [23, 49, 50]. City of Hope has used a rapid intrapatient intracavitary dose escalation strategy compared to our trial's dose escalation: Day 1, 1 x 10^7 cells; Day 3, 5 x 10^7 cells; Day 5, 10 x 10^7 cells. These were given in a 2 mL volume with a 2 mL preservative-free normal saline flush. There were no Grade 3 or higher adverse events with the 1 x 10^7 cells or 5 x 10^7 cells doses. With the 10 x 10^7 cells dose there were 2 cases of Grade 3 headache and 1 case of acute focal neurologic changes, including tongue deviation and shuffling gait which preceded confirmed tumor progression.

The starting doses of the SC-CAR4BRAIN CAR T cell infusion align with our clinical trials for patients with recurrent or refractory CNS tumors, BrainChild-01 (NCT03500991), BrainChild-02 (NCT03638167), and BrainChild-03 (NCT04185038) where we have adopted a slower dose escalation, but have used the same cell dosage as was used at City of Hope and target suspension volumes for the pediatric population because the volumes of total CSF, ventricles, and the intracranial cavity undergo much smaller changes during childhood compared to overall body growth. Total CSF volume changes vary little between ages 1 and adulthood, ranging from 100 to 180 mL, with a mean volume around 150 mL [139]. In a study of 71 healthy pediatric subjects, intraventricular volumes ranged from 10 to 34 cm^3 in children ages 1 to 6, and from 12 to 34 cm^3 in children ages 7 to 15 [140]. Intracranial volumes ranged from 873 to 1687 cm^3 for children ages one through six, and 1037 to 1666 cm^3 in the 7 through 15-year age group. The ratio of ventricular to intracranial volumes was relatively stable over time, 0.019 at 12 months, 0.015 at eight years, and 0.018 at 15 years.

As outlined above and in preparation for this trial, we have confirmed preliminary tolerability of distinct targeting of HER2 (BrainChild-01 Arm B enrolling at 5x10^7 CAR^+ cells/dose without a DLT, BrainChild-02 Arm B enrolling at 2.5x10^7 CAR^+ cells/dose without a DLT, and BrainChild-03 Arm B reaching the MTDR at 10x10^7 without a DLT), along with the already published safety of IL13-zetakine[49].

In line with our previous BrainChild-01, BrainChild-02, and BrainChild-03 Dose Regimens, we will utilize a weekly dosing for 3 weeks, followed by a week of observation in each Course. While DIPG/DMG patients have been treated every other week on BrainChild-03 Arm C, no patients have experienced significant treatment delays due to pseudoprogressive symptoms. Overall, tolerability was similar across all disease groups including DIPG/DMG [110, 116], so in this study all patients will receive weekly dosing.

1.11 CNS Catheters

Despite the fact that an estimated 15% of global disease is due to CNS disorders, treatments for a broad spectrum of CNS diseases have lagged behind other advancements due to several barriers, notably creating therapies that can traffic across the blood-brain barrier and deliver a therapeutic payload to a particular area of the CNS [141]. However, drug dosing via intracerebroventricular or intrathecal routes in a rat model has a success approaching 100% in delivering drug to the CNS target [138]. Beyond target delivery, direct CNS dosing may potentially limit adverse effects with particular agents, including CAR T cells for which a potential adverse event is systemic CRS. CNS catheters, such as Ommaya catheters, have been used since the 1960s for the aspiration of CSF and for the delivery of agents directly into the

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

ventricle. Traditional intrathecal and intracavitary injection of chemotherapies has relied on the stereotactic placement of silastic catheters into the targeted region, which is connected to an access port placed under the scalp. The intracranial silastic catheters may or may not be coated in antibiotic polymers, such as rifampin. The access port ranges from smaller Rickham reservoirs to larger Ommaya reservoirs, depending on the size of the patient and intended use of the system. These access ports allow for repeated, sterile, percutaneous access to the intracranial space for repeated aspiration or administration of agents using a pharmacologically inert delivery system. On BrainChild-01, -02, and -03, no patients have reported site pain at or near the CNS catheter site following the infusion procedure; no patients have developed meningitis at any point on protocol therapy; no patients have developed hematomas, bleeding, or cellulitis at the site of the CNS catheter site; and no patients have required removal of their CNS catheter during the study period[142].

1.12 Rationale for Use of This Product in Pediatric Subjects

Enrolling pediatric subjects in this study is in accordance with 21 CFR 50, Subpart D Additional Safeguards for Children in Clinical Investigations. Specifically, pediatric subjects with CNS tumors have distinct biological diseases that occur in specific anatomical compartments, distinct from adult CNS tumors, making this study absolutely necessary to answer an important scientific question about the health and welfare of children. Furthermore, we have minimized risks by eliminating research procedures which do not contribute to the scientific objective and, regarding Equitable Selection, believe enrolling children is essential to answer the scientific objectives. Enrolled subjects will be children with recurrent or refractory CNS tumors who have failed a routine care option or are diagnosed with DIPG/DMG, a universally fatal CNS tumor for which there are no routine care procedures beyond radiation. We will enforce justice and fair subject selection according to the Declaration of Helsinki (paragraph 24). Extrapolation is not possible due to the differences in disease biology and anatomical tumor location as noted above.

In this study, we have appropriately balanced risk and benefit. While intraventricular dosing of CAR T cells in children carries more than minimal risk, the eligibility criteria are clear in ensuring that these subjects have failed a routine care option or in the case of DIPG/DMG have a universally fatal disease. Finally, the anticipated potential benefit and possible risk will be discussed in depth with each subject and adequate provisions will be made for soliciting the assent of the subject.

This clinical trial will be discontinued in the event of new findings that indicate a relevant deterioration of the risk-benefit assessment. Based on the data available to date, the conduct of this clinical trial is regarded as justified.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

2 STUDY OBJECTIVES

2.1 Primary Objectives

- To assess the feasibility of manufacturing and administering fractionated intraventricular CNS doses of adoptive therapy with autologous SC-CAR4BRAIN in children and young adults with DIPG, DMG, or recurrent/refractory CNS tumors.
- To assess the safety and describe the toxicity of fractionated intraventricular CNS administration of adoptive therapy with SC-CAR4BRAIN in children and young adults with DIPG, DMG, or recurrent/refractory CNS tumors.
- To define the maximally tolerated dose regimen (MTDR) and recommended Phase 2 dose regimen (RP2DR) of fractionated intraventricular CNS administered SC-CAR4BRAIN infusions.

2.2 Secondary Objectives

- To assess SC-CAR4BRAIN distribution within the cerebrospinal fluid (CSF) and the extent to which SC-CAR4BRAIN cells egress into the peripheral circulation.
- To assess disease response following fractionated intraventricular CNS administration of SC-CAR4BRAIN children and young adults with DIPG, DMG, or recurrent/refractory CNS tumors.

2.3 Exploratory Objectives

- To evaluate surface antigen expression in tumor tissue and/or normal tissue or fluids if a tissue biopsy, tumor biopsy, or resection is available.
- To evaluate for presence of SC-CAR4BRAIN in tumor tissue and/or normal tissue or fluids if a tissue biopsy, fluid collection, tumor biopsy or resection is clinically indicated post-treatment.
- To analyze blood, CSF, and tumor or normal tissue or fluids for biomarkers of SC-CAR4BRAIN tumor targeting, safety, and anti-tumor activity.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

3 ENROLLMENT AND ELIGIBILITY

3.1 Enrollment

Upon consent for participation in a Seattle Children's Therapeutics immunotherapy clinical trial, subject demographic information is entered into the Immunotherapy Registration Portal and a unique Registration ID is appointed. Subjects are then transferred to the study-specific database where they are assigned a unique Study ID. Screening and eligibility information will be entered in the study-specific database where it will be reviewed by the Study Chair or their designee who will subsequently approve/deny subject enrollment to the study (consult the sponsor and/or case report form (CRF) Completion Guidelines for instructions related to the study-specific databases).

3.2 Eligibility

3.2.1 Inclusion criteria

1. Subjects must be age ≥ 1 and ≤ 26 years (except for the first 3 subjects, who must be age ≥ 12 and ≤ 26 years).
2. Subject disease classified as one of the following:
    a. DIPG at any timepoint following completion of standard radiotherapy
    b. DMG at any timepoint following completion of standard radiotherapy
    c. Evidence of refractory or recurrent CNS disease for which there is no routine therapy, defined by either of the following (further categorization by disease type is listed in Appendix 4 –Refractory and Recurrent Disease Categorization):
        i. New site or sites of measurable or evaluable disease by radiographic imaging or histologic confirmation following completion of routine care first-line therapy for which curative salvage therapy is not available or amenable, OR
        ii. Measurable or evaluable disease that persists following completion of routine care first-line therapy for which curative salvage therapy is not available or amenable
3. Histopathological confirmation of primary malignant CNS tumor at either the time of diagnosis or recurrence (except for subjects with DIPG or with marker (+) CNS germ cell tumors)
4. Able to tolerate apheresis or already has an apheresis product available for use in manufacturing
5. CNS reservoir catheter, such as an Ommaya or Rickham catheter, present in the proper location for CNS-directed therapy delivered as specified for BrainChild-04
6. Life expectancy ≥ 8 weeks
7. Lansky or Karnofsky score ≥ 60. Subjects who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for purposes of assessing performance status
8. If subject does not have a previously obtained apheresis product that is acceptable and available for manufacturing of CAR T cells, subject must discontinue all anticancer agents and radiotherapy and, in the opinion of the investigator, have fully recovered from significant acute toxic effects of the following:
    a. *Cytotoxic chemotherapy/biologic therapy:* All cytotoxic chemotherapy/biologic therapy must be discontinued ≥ 7 days prior to enrollment.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

b. *Antibody therapy:* All anti-tumor antibody therapy must be discontinued ≥ 30 days or 3 half-lives (whichever is shorter) prior to enrollment. The last dose of bevacizumab may be given ≥ 7 days prior to enrollment.
c. *Cellular therapy:* All cellular therapy must be discontinued ≥ 30 days prior to enrollment.
d. *Corticosteroids:* All systemically administered (i.e. Subcutaneous, Intramuscular, PO or IV) corticosteroids (unless physiologic replacement dosing) must be stable or decreasing for ≥ 1 week prior to enrollment, with a maximum dexamethasone dose of 2.5 mg/m^2/day ongoing at enrollment. Corticosteroid physiologic replacement therapy for management of pituitary/adrenal axis insufficiency and/or topical administration (e.g. inhaled or dermatologic) is allowed.

9. Adequate hematologic values, all of the following in Table 3-1 must be true:

Table 3-1 Screening Hematologic Values

Analyte	Value
Absolute lymphocyte count (ALC) [1]	≥ 100 cells/uL
Absolute neutrophil count (ANC)	≥ 500 cells/uL
Hemoglobin [2]	≥ 9 g/dL
Platelets [2]	≥ 100,000/µL

[1]Value not required to be met if subject has previously obtained apheresis product acceptable and available for manufacturing of CAR T cells
[2]Subjects receiving blood product transfusion(s) are acceptable as long as they are not determined to be transfusion refractory

10. Adequate renal function, as indicated by serum creatinine ≤ the upper limit of normal (ULN), per Table 3-2:

Table 3-2 Serum Creatinine ULN Based on Age and Sex

Serum Creatinine (mg/dL) ULN		
Age	**Male**	**Female**
1 to < 2 yrs	0.6	0.6
2 to < 6 yrs	0.8	0.8
6 to <10 yrs	1	1
10 to < 13 yrs	1.2	1.2
13 to < 16 yrs	1.5	1.4
≥ 16 yrs	1.7	1.4

11. Adequate hepatic function as indicated by either of the following:
    a. Total bilirubin < 3 times ULN for age, OR
    b. Conjugated bilirubin < 2 mg/dL
12. Adequate respiratory function as indicated by BOTH of the following:
    a. Oxygen saturation ≥ 90% on room air without supplemental oxygen or mechanical ventilation, AND
    b. No dyspnea at rest
13. Adequate neurologic function as indicated by all of the following:

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

a. Signs and symptoms of neurologic deficit must be stable for ≥ 1 week prior to enrollment, AND
b. ≤ two anti-epileptic agents are required to control seizure activity, AND
c. No clinically evident encephalopathy present

14. Virology negative within 3 months prior to enrollment, to include all of the following:
    a. HIV antigen & antibody, AND
    b. Hepatitis B surface antigen, AND
    c. Hepatitis C antibody OR if antibody positive, Hepatitis C PCR is negative
15. Subjects of childbearing/fathering potential must agree to use highly effective contraception (Appendix 5 – Highly Effective Contraception) from the time of enrollment through 12 months following the last T cell infusion

3.2.2 Exclusion criteria

1. Presence of ≥ Grade 3 cardiac dysfunction or symptomatic arrhythmia requiring intervention
2. Presence of primary immunodeficiency/bone marrow failure syndrome
3. Presence of clinical and/or radiographic evidence of impending herniation in the CNS
4. For Arm A subjects only: Presence of > Grade 3 dysphagia
5. Presence of active malignancy other than the CNS tumor under study
6. Presence of active severe infection, defined as either of the following:
    a. Positive blood culture within 48 hours of enrollment, OR
    b. Fever > 38.2ºC AND clinical signs of infection within 48 hours of enrollment
7. Pregnant or breastfeeding
8. Subject and/or authorized legal representative unwilling to provide consent/assent for study participation, including participation in the 15-year follow-up period, which is required if CAR T cell therapy is administered
9. Presence of any condition that, in the opinion of the investigator, would prohibit the subject from undergoing treatment under this protocol

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

4 DRUG INFORMATION

4.1 T Cell Product

SC-CAR4BRAIN is an investigational CAR T cell product composed of autologous CD4+ and CD8+ T cells that express individual CARs directed against HER2, EGFR806, B7-H3, and IL13Rα2 or a combination thereof.

T cell product manufacturing will be conducted in the SCTx Therapeutic Cell Production Core (TCPC) GMP facility under approved Investigational New Drug (IND) processes. Cryopreserved cells will be stored in vapor phase in a controlled access LN2 (liquid nitrogen) freezer until released for clinical use. On the day of T cell product infusion, cryopreserved unit(s) will be thawed and formulated in a sterile, single use syringe as specified in thaw and dilute procedures. Formulated CAR T cell product must be administered prior to the expiration date/time specified in standard thaw procedures and recorded on the syringe label.

4.2 Guidelines for Administering SC-CAR4BRAIN

The administered dose of CAR T cell therapy may be within ±10% of the protocol prescribed dose. Administering a dose of CAR T cell therapy varying by greater than ±10% of the protocol prescribed dose is not permitted without prior Sponsor approval. All dosing deviances should be communicated to the Sponsor and the occurrence clearly documented in the subject files. Please refer to Section 5.1 Overview of Treatment Plan for full details of the study dosing requirement.

CAR T cells will be administered through a CNS reservoir catheter, such as an Ommaya or Rickham catheter, present in the proper location for CNS-directed therapy. Subjects may undergo CAR T cell infusion in the outpatient or inpatient setting. When infusion occurs in the outpatient setting, subjects are monitored for 3 hours post infusion prior to discharge from the clinic. When clinically prudent, subjects may be admitted to the hospital following the CAR T cell infusion for observation and monitoring if they exhibit minor symptoms which, if to worsen, could place the subject at risk of an adverse medical outcome.

In addition to institutional guidelines for infusion of cellular products, subjects are to be administered CAR T cell product according to the following guidelines:

- Prior to CAR T cell product administration (in instances of documented allergy or sensitivity to any of the below pre-medications clinical discretion may be used for appropriate substitution):
- Required: acetaminophen
    - Recommended dosing: 12.5 mg/kg (maximum dose 650 mg), PO
- Optional: diphenhydramine
    - Recommended dosing: 0.5 mg/kg (maximum dose 25 mg), IV or PO
- Optional: ondansetron
    - Recommended dosing: 0.15 mg/kg (maximum dose 8 mg), IV or PO
- Thawed, formulated CAR T cell product will be administered slowly through a catheter placed into the CNS ventricular system.
- Microaggregate filters and leukodepletion filters must not be used to infuse CAR T cell products.
- CAR T cell infusion must be followed by a preservative free normal saline (PFNS) flush delivered slowly via a manual push technique to clear the administration line and catheter.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

The volume of PFNS is dependent on catheter size and should be kept consistent for each CAR T cell infusion the subject receives.

4.3 Possible side effects of intracranially delivered CAR T cell therapy

There is a risk of permanent disability or death from treatment administered under this protocol. Experimental immunotherapy research has been conducted in animals and humans with a variety of diseases, however all the risks and side effects are not known. Because this CAR T cell product has not previously been administered to patients with CNS tumors, the frequency, time of onset and duration of side effects is unknown. The risks and side effects may be related to the dose given and are unpredictable. Please see the SC-CAR4BRAIN Investigator's Brochure for safety information.

4.4 Dose-Limiting Toxicity (DLT)

A DLT is an event, defined below, which, in the opinion of the investigator, is possibly, probably, or definitely attributable to the CAR T cell product and which occurs from the time of initial CAR T cell infusion through 28 days following the final CAR T cell infusion. The maximum tolerated dose regimen (MTDR) will be established by the occurrence of DLTs which occur from the time of initial CAR T cell infusion through 7 days following the final CAR T cell infusion received during Course 1. See Section 9.8.4 Definition and determination of MTDR and RP2DR, for information on establishing MTDR.

The definition of a DLT includes all ≥ Grade 3 Common Terminology for AEs (CTCAE) v5 toxicities **except** ≥ Grade 3 toxicities that are known to be related to CAR T cells [49], listed below.

Exceptions to ≥ Grade 3 DLTs:

- Grade 3 CRS that decreases to ≤ Grade 2 within 72 hours of onset
- Up to Grade 4 hypotension, fever, chills not controlled with medical intervention that decrease to ≤ Grade 2 within 72 hours
- Up to Grade 4 activated PTT, fibrinogen, and/or INR that are asymptomatic and resolve within 72 hours
- Up to Grade 4 hypoglycemia and/or electrolyte imbalance that are asymptomatic and resolve within 72 hours
- Up to Grade 4 nausea and/or vomiting that decrease to ≤ Grade 2 within 7 days
- Grade 3 neurological adverse event that decreases to ≤ Grade 2 within 21 days (treatment with dexamethasone and/or bevacizumab is allowed)
- Grade 3 headache that decreases to ≤ Grade 2 within 21 days (treatment with dexamethasone and/or bevacizumab is allowed)

The definition of a DLT also includes any possibly, probably, or definitely related toxicity lasting > 21 days which prohibits the subject from meeting criteria for subsequent CAR T cell infusion.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

5 TREATMENT PROGRAM

5.1 Overview of Treatment Plan

Following enrollment, subjects who do not already have an apheresis product available for manufacturing will undergo apheresis. During the period between apheresis (or completion of screening procedures for subjects not undergoing apheresis) until the commencement of CAR T cell infusions, subjects may receive bridging therapy at the direction of their physician.

Following confirmation of the availability of SC-CAR4BRAIN cell product for infusion, subjects will be assigned a dose regimen on their specific treatment arm based on their primary disease.

Depending on initial tolerance to SC-CAR4BRAIN, subjects may proceed with subsequent courses of CAR T cell infusions for as long as it is providing benefit to the subject and doses of CAR T cells remain available.

All subjects who receive at least one infusion of SC-CAR4BRAIN will be followed for long term follow up requirements for 15 years following the final CAR T cell infusion.

5.2 Apheresis for T Cell Isolation

Subjects who do not already have an apheresis product available for use in manufacturing will undergo apheresis to obtain T cells from which individualized CAR T cell therapy will be manufactured. Apheresis may take place in either an outpatient or inpatient setting and will be performed per institutional standard operating procedure. After meeting requirements for apheresis, subjects will undergo apheresis with a target total lymphocyte collection of 1×10^9.

In instances of manufacturing failure or insufficient availability of starting material, subjects may undergo a repeat apheresis procedure if the Principal Investigator feels it may yield a successful product. Subjects who have already initiated CAR T cell infusion and are eligible for ongoing CAR T cell therapy but no longer have available CAR T cell product and who do not have sufficient starting material for additional manufacture may undergo repeat apheresis. All subjects undergoing apheresis or repeat apheresis must meet requirements for apheresis, noted in Section 5.2.1 Requirements for Apheresis.

5.2.1 Requirements for Apheresis

Subjects with active, severe, infection may not undergo apheresis. For the purpose of this trial, active, severe infection is defined as:

- Positive blood culture within 48 hours of apheresis, OR
- Fever > 38.2°C AND clinical signs of infection within 48 hours of apheresis

5.3 Bridging Therapy

During the time from apheresis to infusion of CAR T cells, subjects may return to the care of their primary physician and may receive additional cancer directed therapy (see Section 5.1 Overview of Treatment Plan). The site team will be notified when the subject's CAR T cell product is released and available so that the CAR T cell infusion may be scheduled.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Additional therapy as directed by the primary physician aimed at controlling disease burden to allow the subject to meet criteria for CAR T cell infusion may be given. Refer to Section 5.5.2 Requirements for initial CAR T cell infusion and Table 5-2 for Pre-T Cell Therapy Wash-Out Requirements.

5.4 CAR T Cell Dosing

5.4.1 Treatment Arm Assignment

Subjects will be enrolled into one of two Treatment Arms:

- **Arm A** will include subjects with DIPG.
- **Arm B** will include subjects with non-pontine DMG or recurrent/refractory CNS tumors.

Within each Arm, subjects will be enrolled into a defined Dose Regimen (DR). Subjects will receive a different combination of Dose Levels (DL) depending on the DR into which they are enrolled.

The specific Dose Levels within each Dose Regimen are:

Table 5-1 CAR T Cell Dose Levels

Dose Level (DL)	Dose
DL 1	1 x 10^7 EGFRt+ T cells
DL 2	2.5 x 10^7 EGFRt+ T cells
DL 3	5 x 10^7 EGFRt+ T cells
DL 4	10 x 10^7 EGFRt+ T cells

5.4.2 Dose Regimen Assignment

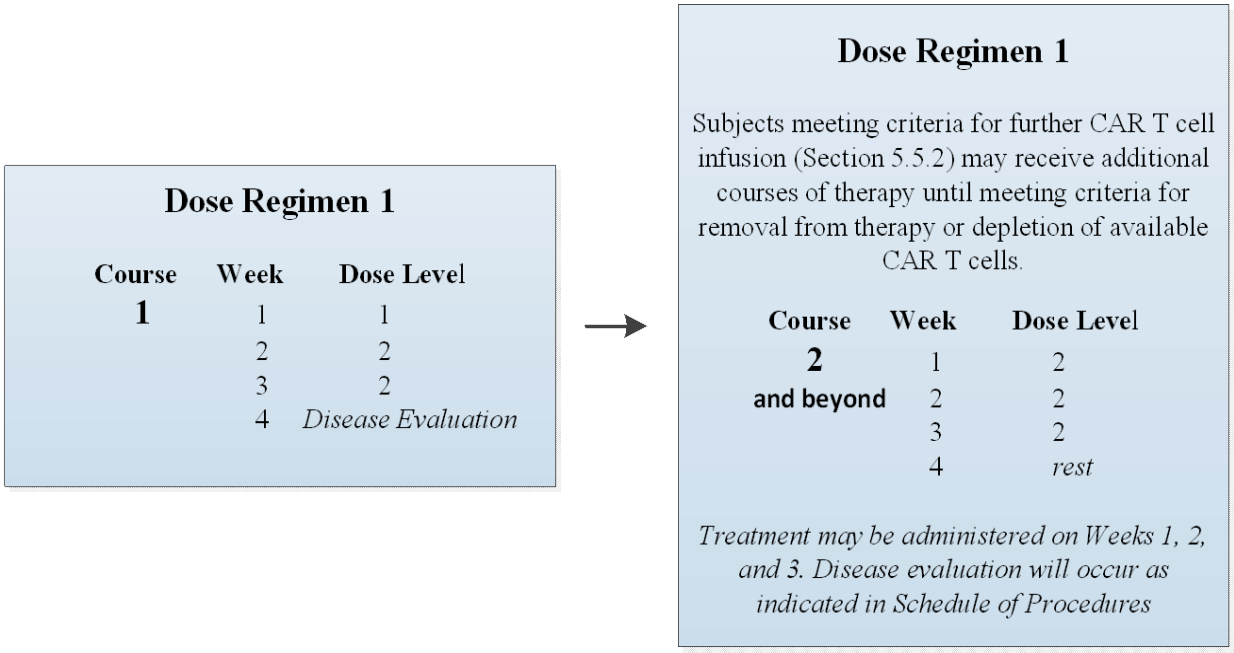
Detailed rules for dose regimen (DR) assignment are described in Section 9.8.2 Arms and DLT observation periods.

If the quantity of CAR T cell product is less than the specified for a particular DR, but is otherwise releasable, it may be administered as long as the subject meets criteria to undergo initial T cell infusion (Section 5.5.2 Requirements for initial CAR T cell infusion). However, in the absence of a DLT during Course 1, subjects who do not receive all 3 intended doses per DR will not be evaluable for DR escalation per Section 9.5 Safety and Toxicity.

If a subject has a releasable CAR T cell product but treatment is suspended (during the mandated period between participants, or while the data are under review by the Data Safety Monitoring Board [DSMB] or Food and Drug Administration [FDA]) and the subject is not medically stable enough to wait until treatment is reinitiated, the subject may receive their CAR T cell product at the maximum DR that was deemed tolerable.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Figure 5-1 Dose Regimen 1

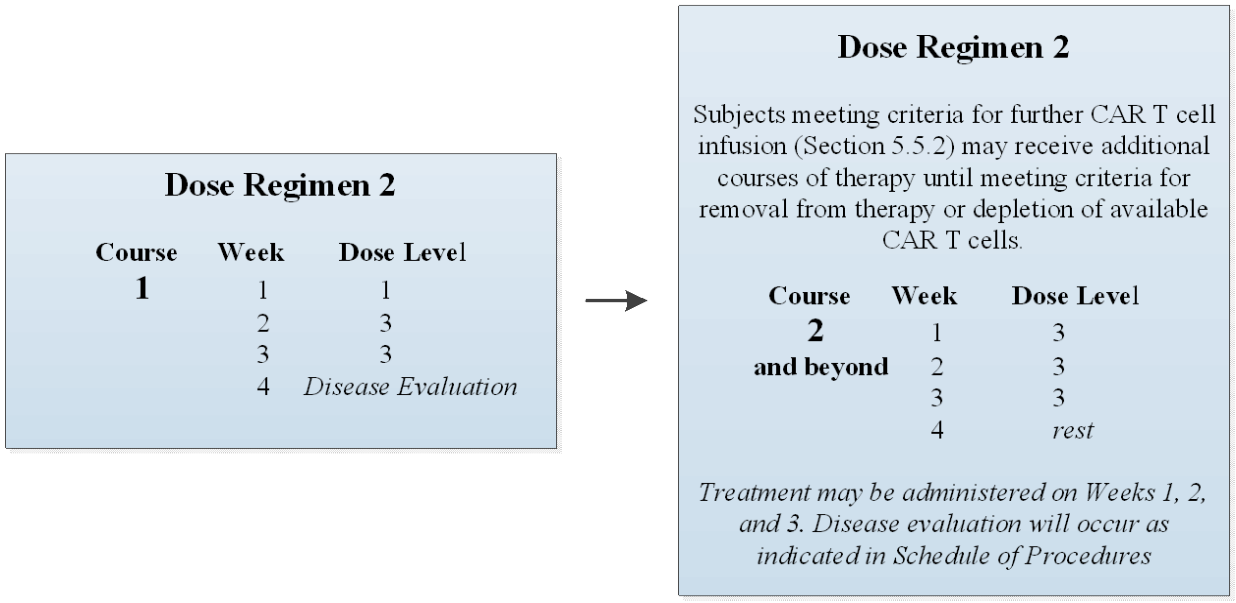


In DR 1 Course 1, subjects will receive DL1 on Week 1, followed by DL2 on Weeks 2, and 3, followed by a rest during Week 4 with disease restaging evaluation, including radiographic restaging.

Following Course 1, subjects meeting criteria for further CAR T cell infusion (Section 5.5.3 Requirements for subsequent CAR T cell infusion) may receive additional Courses of therapy until subject meets criteria for removal from protocol therapy. The DLs for Courses 2 and beyond will be no higher than DL2, unless a subsequent DR has been cleared for escalation or for selection as the MTDR. In that case, the subject may increase to the highest DL on the subsequent DR. A lower DL may be administered based on availability of CAR T cells, see above. Treatment may be administered as frequently as on Weeks 1, 2, and 3 followed by a rest week every fourth week. Disease restaging evaluations will occur as indicated in the schedule of procedures.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

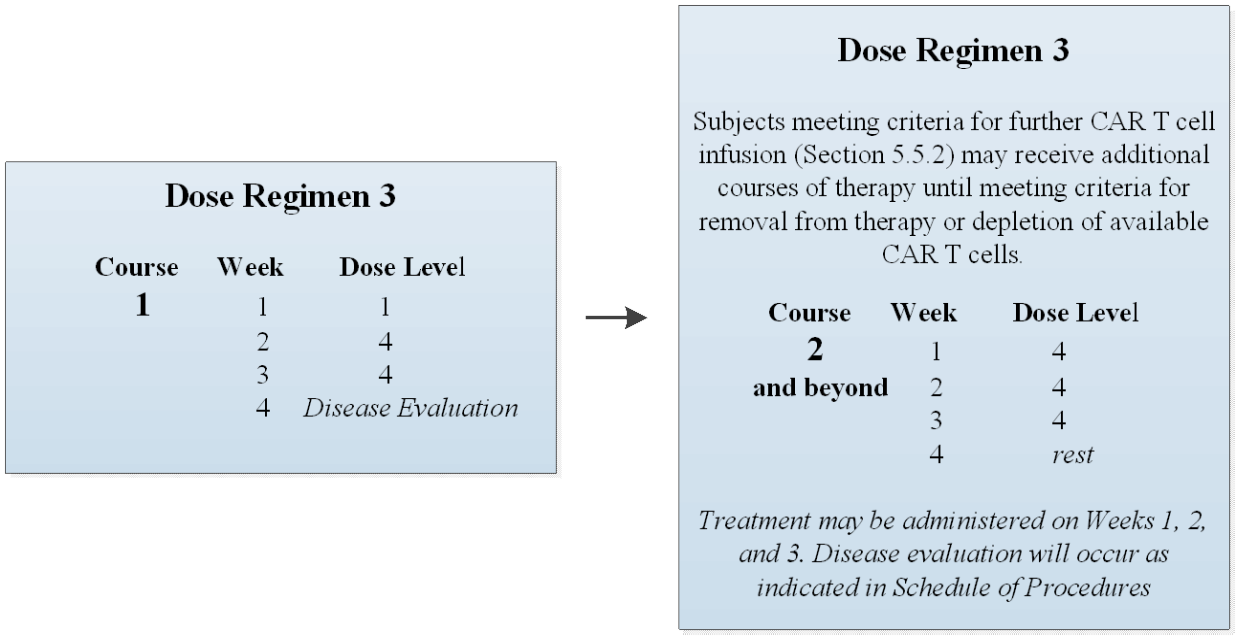
Figure 5-2 Dose Regimen 2



In DR 2 Course 1, subjects will receive DL1 on Week 1, followed by DL3 on Weeks 2 and 3, followed by a rest during Week 4 with disease evaluation, including radiographic restaging. Following Course 1, subjects meeting criteria for further CAR T cell infusion (Section 5.5.3 Requirements for subsequent CAR T cell infusion) may receive additional Courses of therapy until subject meets criteria for removal from protocol therapy. The DLs for Courses 2 and beyond will be no higher than DL 3, unless a subsequent DR has been cleared for escalation or for selection as the MTDR. In that case, the subject may increase to the highest DL on the subsequent DR. A lower DL may be administered based on availability of CAR T cells, see above. Treatment may be administered as frequently as on Weeks 1, 2, and 3 followed by a rest week every fourth week. Disease restaging evaluations will occur as indicated in the schedule of procedures.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Figure 5-3 Dose Regimen 3



In DR 3 Course 1, subjects will receive DL1 on Week 1, followed by DL4 on Weeks 2 and 3, followed by a rest during Week 4 with disease evaluation, including radiographic restaging. Following Course 1, subjects meeting criteria for further CAR T cell infusion (Section 5.5.3 Requirements for subsequent CAR T cell infusion) may receive additional Courses of therapy until subject meets criteria for removal from protocol therapy. The DLs for Courses 2 and beyond will be no higher than DL4. A lower DL may be administered based on availability of CAR T cells, see above. Treatment may be administered as frequently as on Weeks 1, 2, and 3 followed by a rest week every fourth week. Disease restaging evaluations will occur as indicated in the schedule of procedures.

5.4.3 Dose modifications

If a subject experiences an AE that meets DLT criteria following administration of CAR T cells at DL1, then that subject will be removed from protocol therapy.
If a subject experiences an AE that meets DLT criteria following administration of CAR T cells at a dose > DL1, then that subject will be removed from protocol therapy, unless:

- the subject's AE recovers ≤ Grade 2 toxicity or to baseline in ≤ 3 weeks from planned next infusion AND
- the subject meets all other requirements for subsequent CAR T cell infusion except the DLT requirement as listed in Section 5.5.3.

If above criteria are met, the subject may receive subsequent CAR T cell administrations at one DL lower than that which resulted in a DLT. If the DLT recurs at the lower DL, then the subject will be removed from protocol therapy.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

5.5 CAR T Cell Product Infusion

5.5.1 Schedule of subsequent CAR T cell infusions

During each Course, the time interval between each CAR T cell infusion will be according to the Schedule of Procedures and based upon the date of the most recent CAR T cell infusion. The end date of each Course will be the date of completion of Week 4 evaluations per the Schedule of Procedures. The start date of each Course will be the date that the Week 1 CAR T cell infusion required evaluations are performed.

Within Course 1, if a subject does not meet criteria to proceed with a CAR T cell infusion on the planned infusion day of a Course regardless of attribution to the CAR T cells, the infusion may be delayed no more than 3 weeks from the planned infusion date. If delayed greater than 3 weeks from planned next infusion date, the subject will be removed from protocol therapy (Section 8.1 Removal from Protocol Therapy) and will not receive further CAR T cell treatment.

Between Course 1 and Course 2, delays due to family/subject preference, travel disruptions or due to subject recovery from a surgical procedure (e.g., tumor biopsy) are allowed. During the delay subjects must continue to be followed for concomitant medications (Section 7.8) and adverse events (Section 7.9).

Beyond Course 1, subjects must receive at least the Week 1 infusion of any Course but may skip the other scheduled infusions. If a subject does not receive an infusion within 3 weeks of the Week 1 infusion, the next infusion will be considered a new Course.

If an infusion is not given, the pre- and post-visit procedures for that infusion are not required. If the subject does not receive the next CAR T cell infusion within 5 weeks of the prior CAR T cell infusion, the subject will be removed from protocol therapy (see Section 8.1 Removal from Protocol Therapy) unless the delay was due to travel disruptions or subject recovery from a surgical procedure (e.g., tumor biopsy). During the delay subjects must continue to be followed for concomitant medications (Section 7.8) and adverse events (Section 7.9).

5.5.2 Requirements for initial CAR T cell infusion

The following criteria must be met prior to administration of initial CAR T cell product:

- First three treated subjects are required to be 12 years or older
- Subject is ≥ 5 days from CNS surgery, including catheter placement
- Subject has confirmed evidence of persistent, evaluable disease
- Regarding bridging therapy:
  - If the subject has achieved defined partial or complete remission with bridging therapy, the Study Chair or designee will recommend to the subject's treating oncologist that the bridging therapy be continued. The subject may be re-evaluated and would be eligible to receive CAR T cell treatment upon meeting requirements for CAR T cell infusion.
  - The following treatments must be discontinued for the specified duration (wash-out period) prior to infusion of the CAR T cell product, and subject must have recovered from acute therapy-associated toxicities.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Table 5-2 Pre-T Cell Therapy Wash Out Requirements

Treatment	Wash-out Period (prior to CAR T cell product infusion)
Radiation therapy	≥ 6 weeks
Bevacizumab	≥ 28 days
Cytotoxic chemotherapy	≥ 21 days
Biologic agents	≥ 7 days
Antibody therapy Interferons T cell growth factors Vaccines	≥ 3 half-life or 30 days (whichever is shorter)
Cellular therapy	≥ 30 days since last infusion
Any investigational agent	≥ 3 half-life or 30 days (whichever is shorter)

- If the subject is receiving corticosteroids for symptomatic relief from their CNS disease, steroid dosing must be stable or decreasing for ≥ 1 week with a maximum dexamethasone dose of 2.5 mg/m^2/day on the day of the infusion. Corticosteroid physiologic replacement therapy for management of pituitary/adrenal axis insufficiency and/or topical administration (e.g. inhaled or dermatologic) is allowed.
- Cryopreserved CAR T cells are available and released for infusion
- Subject must have adequate organ function as indicated by:
    - Renal: Serum creatinine ≤ ULN per Table 3-2 Serum Creatinine ULN Based on Age and Sex
    - Hepatic: Total bilirubin < 3 times ULN for age OR conjugated bilirubin < 2 mg/dL
    - Respiratory: Oxygen saturation ≥ 90% on room air without supplemental oxygen or mechanical ventilation, and no dyspnea at rest
    - Hematologic: Platelets ≥ 100,000/µL (transfusion(s) allowed to meet criteria)
- No evidence of clinically significant progressive encephalopathy or uncontrolled seizure activity.
- Subject is taking an anti-seizure medication.
- No evidence of active severe infection, defined as: 1) positive blood culture within 48 hours of CAR T cell infusion; or 2) fever > 38.2°C AND clinical evidence of infection within 48 hours of CAR T cell infusion
- Subject is not breastfeeding
- Female subjects of childbearing potential only: subject has a negative pregnancy test within 2 days prior to initial CAR T cell infusion

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

5.5.3 Requirements for subsequent CAR T cell infusion

The following criteria must be met prior to administration of subsequent CAR T cell product:

- No DLT (Section 4.4) unless the subject meets criteria as defined in Section 5.4.3 Dose modifications (see also Section 8 Removal from Protocol Therapy & Off Study Criteria).
- Cryopreserved CAR T cells are available and released for infusion
- Subject must have adequate organ function as indicated by:
    - Renal: Serum creatinine ≤ ULN per Table 3-2 Serum Creatinine ULN Based on Age and Sex
    - Hepatic: Total bilirubin < 3 times ULN for age OR conjugated bilirubin < 2 mg/dL
    - Respiratory: Oxygen saturation ≥ 90% on room air without supplemental oxygen or mechanical ventilation, and no dyspnea at rest
    - Hematologic: Platelets ≥ 100,000/µL (transfusion(s) allowed to meet criteria)
- No evidence of clinically significant progressive encephalopathy or uncontrolled seizure activity
- Subject is taking an anti-seizure medication
- No evidence of active severe infection, defined as: 1) positive blood culture within 48 hours of CAR T cell infusion; or 2) fever > 38.2°C AND clinical evidence of infection within 48 hours of CAR T cell infusion
- Subject is not breastfeeding
- Female subjects of childbearing potential only: subject has a negative pregnancy test within 2 days prior to Week 1 CAR T cell infusion of each Course

5.6 Concomitant Medication and Supportive Care

5.6.1 Anti-seizure medication

Seizures may cause significant short and long-term impairment, however treatment-related seizures may be a preventable complication in subjects receiving localized therapy for intra-cranial tumors. It is required that seizure prophylaxis begin prior to the subject's initial CAR T cell infusion on study and continue until 1 month following the final CAR T cell infusion on study. Levetiracetam is recommended; however, an alternative anti-seizure medication may be given per investigator discretion.

5.6.2 Blood product support

All blood products will be transfused per institutional standards.

It is strongly recommended that platelet transfusions be given to maintain a platelet count ≥ 100,000/µL prior to initial, and between, CAR T cell infusions.

It is recommended that packed red blood cells (PRBCs) be given to maintain a hematocrit of ≥ 25% during the immediate post-CAR T cell infusion periods (to include the first 48 hours) and during periods of Grade 2-4 CRS, unless clinically the subject has a higher established transfusion threshold.

Cryoprecipitate should be given to maintain fibrinogen > 150 mg/dL.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

5.6.3 Antimicrobial prophylaxis

Pneumocystis jiroveci pneumonia (PJP) prophylaxis should be given per institutional standards. Meningeal dosing of antibiotics should be considered based on history, clinical presentation, and physical examination for subjects who develop fever > 38.2°C within 72 hours of CAR T cell infusion.

Infectious disease management should follow institutional standards but may be individualized as clinically indicated. Institutional standards for infectious diseases will guide addition of antimicrobial agents in cases of persistent or recurrent fever.
Additionally, if the CAR T cell product has a positive culture which is discovered after being infused into the subject, blood cultures will be drawn and appropriate antibiotic coverage will be initiated, as follows:

- For gram negative organisms: ceftazidime and gentamicin should be started until identification and susceptibility have been determined and antibiotic coverage can then be tailored to the specific bacteria. If the subject has a cephalosporin allergy, in infectious disease consult should be obtained to determine appropriate antibiotic coverage. Additionally, ciprofloxacin may be substituted for gentamicin if the subject has impaired renal function
- For fungal organisms: an infectious disease consult should be obtained to determine appropriate antibiotic coverage

If the subject's blood cultures remain negative for 5 days and the subject remains clinically well, antimicrobials may be discontinued.

5.6.4 Prohibited medications

The following agents are not allowed from the time of initial CAR T cell infusion through 28 days following the final CAR T cell infusion or time of removal from protocol therapy (Section 8.1 Removal from Protocol Therapy), whichever comes first:
Any tumor directed therapy

- Systemic immunosuppressive agents (other than corticosteroids) unless given to treat symptoms attributed to CAR T cells as noted in Section 6.3 Management of Cytokine Release Syndrome (CRS) and Section 6.4 Management of Non-CRS Acute Toxicity Associated with Infused CAR T Cells into the CNS.
- Immunotherapy (unless used for the treatment of toxicity)
- Other investigational agents, unless used to treat or prevent symptoms related to protocol CAR T cell infusions

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

6 MANAGEMENT OF TOXICITIES AND COMPLICATIONS

6.1 Symptoms Associated with Apheresis

Side effects that may occur during cell collection include nausea, vomiting, fainting or dizziness, seizures, skin rash, hives, flushing (redness and warmness of the skin, usually the face), blood loss, and infection. Tingling of the lips, muscle cramping, and, very rarely, changes in heart rhythm, may occur. These symptoms may be prevented or made milder by giving calcium supplements, either IV or PO during the apheresis procedure. Very rarely (< 1 in 1,000 procedures) clotting may occur in the apheresis machine or in the subject and is potentially life-threatening. To reduce the risk of clotting, acid-citrate-dextrose (ACD) and heparin may be given during the apheresis procedure. ACD may increase the risk of bleeding and may cause temporary tingling of the lips and limbs, muscle cramping, seizures, or changes in heart rhythm. Heparin may also increase the risk of bleeding. Transfusions of both PRBC and platelets may be required surrounding the procedure.

6.2 Symptoms Associated with CAR T Cell Infusion

Mild, transient symptoms have been observed while receiving or within 24 hours of receiving CAR T cell therapy including fevers, chills, rigors, headache, and, rarely, nausea, vomiting, hypotension, and pulmonary toxicity. The management of these symptoms is outlined below.

Fever, chills and temperature elevations > 38.2°C may be managed with additional acetaminophen as clinically indicated, and/or 1 mg/kg meperidine (Demerol) IV for chills (maximum 50 mg). Additional methods such as cooling blankets may be employed for fevers resistant to these measures. All subjects who develop fever and/or chills should have a blood culture drawn and be admitted for IV antibiotics and supportive care.

Headache may be managed with additional acetaminophen as clinically indicated, and magnesium, diphenhydramine/metoclopramide, NS fluid and/or narcotics may be considered.

Nausea and vomiting may be treated with additional ondansetron, and diphenhydramine/metoclopramide, scopolamine, lorazepam, and/or olanzapine may be considered.

Hypotension:

- Transient hypotension may initially be managed by intravenous fluid administration; however, subjects with persistent hypotension may require transfer to the intensive care unit (ICU) for definitive medical treatment.
- If significant hypotension occurs during the CAR T cell infusion, the infusion should be immediately suspended. Significant hypotension is defined as symptomatic and/or systolic blood pressure < 80 mm/Hg for age > 12 years, < 70 mm/Hg for age < 12 years, or a 15% drop from baseline, whichever value is lower.
- Treatment for significant hypotension will follow institution standard practice.

Hypoxemia may be managed by standard clinical practice.
If the CAR T cell infusion is stopped before completion and the symptoms causing cessation return to baseline within 30 minutes, the CAR T cell infusion may be restarted at a slower rate. All product must be infused prior to the expiration time/date listed on the syringe label.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

If the CAR T cell infusion is terminated due to acute toxicity occurring during the infusion, the residual CAR T cell product should be returned to the TCPC for analysis. Investigation of possible causes of observed symptoms should proceed and, if necessary, additional medical treatment should be instituted.

6.3 Management of Cytokine Release Syndrome (CRS)

All subjects for whom there is concern for CRS will be admitted to the hospital for observation. In subjects with adverse events (AEs) during in vivo expansion of CAR T cells, additional laboratory testing will be requested. If a subject is suspected of having CRS, additional samples may be sent for clinical cytokine analysis per treating physician discretion and additional correlative studies specimens may be requested. Subjects may receive cytokine-directed therapy for symptom control including, but not limited to, tocilizumab, an IL-6 antagonist. The use of corticosteroids may be given to prevent a more serious toxicity. Table 6-1 provides guidelines for CRS management.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Table 6-1 Recommended Symptom Management for CRS

Symptom related to CRS	Suggested Intervention
Fever > 38.2°C	Acetaminophen (12.5 mg/kg) PO/IV up to every 4 hrs
Persistent fever ≥ 39 °C for 6 hrs that is unresponsive to acetaminophen	Tocilizumab (8-12 mg/kg) IV
Persistent fevers ≥ 39 °C after Tocilizumab	Dexamethasone 5-10 mg IV/PO up to every 6-12 hrs with continued fevers
Recurrence of symptoms 48 hrs after initial dose of Tocilizumab	Tocilizumab (8-12 mg/kg) IV
Hypotension	Fluid bolus, target hematocrit > 24%
Persistent/recurrent hypotension after initial fluid bolus (within 6 hrs)	Tocilizumab (8-12 mg/kg) IV
Use of low dose vasopressors for hypotension for longer than 12 hrs	Dexamethasone 5-10 mg IV/PO up to every 6 hrs with continued use of vasopressors
Initiation of higher dose vasopressors or addition of a second pressor for hypotension	Dexamethasone 5-10 mg IV/PO up to every 6 hrs with continued use of vasopressors
Initiation of oxygen supplementation	Tocilizumab (8-12 mg/kg) IV
Increasing respiratory support with concern for impending intubation	Dexamethasone 5-10 mg IV/PO up to every 6-12 hrs with continued use of vasopressors
Recurrence/Persistence of symptoms for which Tocilizumab was given ≥ 48 hrs after initial dose was administered	Tocilizumab (8-12 mg/kg) IV

6.4 Management of Non-CRS Acute Toxicity Associated with Infused CAR T Cells into the CNS

When clinically prudent, subjects may be admitted to the hospital following the CAR T cell infusion for observation and monitoring if they exhibit minor symptoms which, if to worsen, could place the subject at risk of an adverse medical outcome.

Mild, transient symptoms have been observed greater than 24 hours after receiving CAR T cell treatment including fevers, headache, nausea, vomiting, and increase in neurologic changes from baseline. Suggested management of these symptoms is outlined below.

Fever may be managed with acetaminophen as clinically indicated. Avoid the use of NSAIDs. Consider obtaining blood culture and/or initiation of antibiotic therapy based upon clinical symptoms and institutional standards.

Headache may be managed with acetaminophen. If unresponsive to acetaminophen, consider magnesium, diphenhydramine/metoclopramide, NS fluid, and/or narcotics.

Nausea and vomiting may be treated with ondansetron. If unresponsive to ondansetron, consider diphenhydramine/metoclopramide, scopolamine, lorazepam, and/or olanzapine.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Seizures may require a medical evaluation with recommended neurology consult, hospital admission, and appropriate anti-seizure medication(s). Dexamethasone (10 mg x 1 dose) and neuroimaging should be considered.

Neurologic changes require symptomatic care and a review of the anti-epileptic therapy are recommended. Discussion with the PI, PI designee, and/or neuro-oncologist is recommended. For subjects with ≥ Grade 2 neurologic changes, the addition of corticosteroids should be considered, with initial dosing recommendations of dexamethasone 5-10 mg IV every 6 to 12 hours, often until resolution of symptoms. Bevacizumab may be used based on clinical judgment. Tocilizumab, and/or other cytokine-directed therapies may be used based on clinical judgment. Cerebrospinal fluid assessments and CNS imaging should be considered as clinically indicated. See Section 7.18 Correlative Sciences for correlative study requirements during periods of systemic toxicity possibly, probably, or definitely related to CAR T cells.

Subjects who develop other serious medically significant toxicity attributable to the infused CAR T cell product will also be hospitalized for observation and treatment. In addition, subjects who develop a new toxicity ≥ Grade 3 that is possibly, probably, or definitely attributable to the CAR T cell infusion other than those noted above, meets the definition of a DLT in Section 4.4, and, in the opinion of the PI or PI designee puts the subject at significant risk of an untoward outcome if measures are not taken to ameliorate the toxicity, should be given corticosteroids and/or tocilizumab.

Symptom related to Non-CRS Toxicity	Suggested Intervention
Fever > 38.2°C	Acetaminophen
Headache	• Acetaminophen • Magnesium • Diphenhydramine/metoclopramide • NS fluids
Nausea/vomiting	• Ondansetron • Diphenhydramine/metoclopramide • Lorazepam • Olanzapine
Seizures	• Anti-seizure medication • Dexamethasone (10 mg) up to every 6 hours
Neurologic changes (≥ Grade 2)	• Anti-seizure medication • Bevacizumab (10 mg/kg) every 2 weeks

6.5 Ablation of T Cells with Cetuximab

Cetuximab therapy may be initiated to ablate CAR expressing T cells in subjects under the following circumstances:

- Subject is experiencing a toxicity that meets the definition of DLT in Section 4.4 Dose-Limiting Toxicity (DLT), lasts ≥ 48 hours, AND, in the opinion of the PI or designee, cannot be controlled and puts the subject at significant risk of an untoward outcome if measures are not taken to ameliorate the toxicity, OR

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

- Subject is experiencing any duration or grade of toxicity which, in the opinion of the PI or designee, cannot be controlled and puts the subject at significant risk of an untoward outcome if measures are not taken to ameliorate the toxicity, OR
- Subject molecular studies indicate a lymphoproliferative disorder arising from the CAR T cells

The Study Chair or designee must be consulted prior to administering cetuximab.
Cetuximab is to be administered according to the current package insert. See Table 6-2 Details adverse events observed in clinical trials using cetuximab [143-147].
Pretreatment with diphenhydramine is recommended 30-60 minutes before each dose of cetuximab (recommended diphenhydramine dosing: 1 mg/kg, maximum dose of 50 mg).
Cetuximab dosing recommendations are as follows:

- Subjects age ≥ 18 years, based on FDA approved dosing: loading dose of 400 mg/m^2 IV, followed by additional weekly doses of 250 mg/m^2 IV, if required
- Subjects age < 18 years: 250 mg/m^2 IV administered over 1 hour weekly [143]

If after 2 cetuximab doses are administered, there is no appreciable decrease in the CAR T cell burden, no further doses will be given. For subjects who have an appreciable response to cetuximab, up to a total of 4 weekly doses may be given. Any further cetuximab therapy following the 4 doses, if deemed necessary by the Sponsor and PI, will be administered only after Sponsor consultation with the FDA.

For all subjects who receive cetuximab, Correlative Sciences samples (peripheral blood and CSF) will be collected to determine CAR T cell persistence.

All Correlative Sciences samples drawn in response to cetuximab administration have a ± 3-day window, however a distinct sample must be drawn for each time point (samples may not be shared across time points: for instance, 1 sample drawn on day 4 following cetuximab dosing could not be used for both Day 1 and Day 3 despite being within the draw window). Refer to the study specific lab manual for specimen requirements of all correlative studies samples:

- *Peripheral Blood*: Peripheral blood for Correlative Sciences should be collected prior to the initial dose of cetuximab and again on days 1, 3, 7, 10, 14, and 28 following the initial dose of cetuximab.
- *Cerebrospinal Fluid:* CSF for Correlative Sciences should be collected prior to the initial dose of cetuximab and again on days 1, 3, 7, 10, 14, and 28 days following the initial dose of cetuximab.

Additional measures may also be taken to resolve toxicity should the protocol-specified cetuximab treatment plan fail to abate the side effects associated with the CAR T cell product such as, but not limited to, immunosuppressive medications or agents (such as ATG/Campath and calcineurin inhibitors) or chemotherapy agents with immunosuppressive properties.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Table 6-2 Details adverse events observed in clinical trials using cetuximab

Common	Less Common	Rare
• Abdominal pain • Anemia • Constipation • Cough • Diarrhea • Difficulty breathing • Fatigue • Headache • Hypomagnesemia • Infection • Leukopenia • Liver enzyme elevation • Nail changes • Nausea/vomiting • Neutropenia • Peripheral neuropathy • Skin reactions • Stomatitis • Weakness • Weight loss	• Anxiety • Bone pain • Chills • Confusion • Dehydration • Depression • Dry mouth • Eye irritation • Fever • Infusion reaction • Insomnia • Hypocalcemia • Hypokalemia • Joint pain • Thrombocytopenia • Upset stomach	• Cardiac abnormality • Severe infusion reaction leading to death

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

7 STUDY PROCEDURES AND ASSESSMENTS

Refer to Appendix 2 – Schedule of Procedures, for a summary of required study activities. Evaluations may be performed at Local Care Institutions (LCIs) as described in the Schedule of Procedures beyond Course 1. All apheresis visits, Course 1 visits inclusive of Post-Infusion and Week 4 visits, and subsequent course Infusion visits must be performed at the study site.

7.1 Informed Consent/Assent

Prior to conducting any tests or procedures performed solely for the purposes of the study, written informed consent, and assent if applicable, must be obtained from the subject and/or subject's legal representative.

7.2 Demographics

Demographic information (including date of birth, sex, race and ethnicity) will be transferred from the Immunotherapy Registration Portal at Screening.

7.3 Medical History and Active Medical Conditions

Relevant medical history and active medical conditions, including history and treatment of CNS disease, will be obtained per the Schedule of Procedures including at Screening and prior to CAR T cell infusion.

7.4 Performance Status

Lansky (for subjects < 16 years of age) or Karnofsky (for subjects ≥ 16 years of age) performance status will be assessed per the Schedule of Procedures. Subjects who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for purposes of assessing performance status. Reference Appendix 3 – Performance Status Scales.

7.5 Physical Examination, Vital Signs, Weight, Height

Physical examinations, vital signs (must include pulse, respiratory rate, blood pressure and temperature), weight and height will be done per the Schedule of Procedures.

7.6 Pulse Oximetry

Pulse oximetry will be performed as noted in the Schedule of Procedures.

7.7 Neurologic Exam

A neurological exam will be performed as indicated in the Schedule of Procedures.

7.8 Concomitant Medications

All concomitant medication and therapies will be documented at time of apheresis and then from the time of initial CAR T cell infusion through the end of course 6 or removal from protocol therapy, whichever occurs earlier. IV contrast is not considered a concomitant medication.

7.9 Adverse Events

Information regarding AEs will be captured following the start of the CAR T cell therapy per Section 11 Adverse Events and Serious Adverse Events.

All adverse events will be captured following the start of CAR T cell therapy through 28 days after their final course of CAR T cell treatment, up to a maximum of 6 courses of CAR T cell treatment. For subjects that receive CAR T cell treatment beyond course 6, grade 3 and higher adverse events and all serious adverse events will be captured from course 7 onwards through

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

28 days post-final infusion. Related AEs will continue to be followed through resolution, if possible.

This study will utilize the NCI CTCAE v5 for toxicity reporting and grading, except for CRS which will be assessed using the CRS Grading Scale in Section 11.5 Cytokine Release Syndrome Grading. The study team should have access to the CTCAE v5 which can be downloaded from the Cancer Therapy Evaluation Program (CTEP) website (http://ctep.cancer.gov). Refer to Section 11 for information on reporting AEs.

7.10 Hospitalization/ICU Admission

The study team will record any hospitalization or admission to the ICU at any time between initiation of SC-CAR4BRAIN removal from protocol therapy, whichever is earlier. The relationship to treatment with SC-CAR4BRAIN will be assessed by the PI or sub-investigator for all admissions.

7.11 Pregnancy Test

Female subjects of childbearing potential will have urine or serum pregnancy tests as indicated in the Schedule of Procedures. Additional pregnancy tests will be performed at any visit in which pregnancy status is in question. A serum pregnancy test will be performed in the event of a positive or equivocal urine pregnancy test result.

7.12 Virology

Virology must include human immunodeficiency virus (HIV) antigen and antibody, Hepatitis B surface antigen, and Hepatitis C antibody testing and will be performed per the Schedule of Procedures. If Hepatitis C antibody testing is positive, quantitative PCR will be performed. Results of virology testing obtained up to 3 months prior to enrollment will be accepted.

7.13 Hematology

Hematology must include complete blood count (CBC) including hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count and will be obtained according to the Schedule of Procedures.

7.14 Chemistry

Chemistry must include serum sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), total bilirubin, and conjugated bilirubin, will be obtained according to the Schedule of Procedures.

7.15 Cerebrospinal Fluid (CSF) Sampling

Cerebrospinal fluid sampling will be performed as clinically indicated or according to the time points indicated in the Schedule of Procedures. CSF samples may be obtained via intraventricular or CNS catheters. CSF sample collection is not required if not feasible during non-infusion visits.

7.16 CRS Labs and Evaluation

During periods of Grade 2-4 CRS, inflammatory response labs and evaluations should be obtained. Labs include LDH, ferritin, d-dimer, PT, PTT, fibrinogen, and absolute lymphocyte count. Evaluations must include temperature, heart rate and blood pressure.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

7.17 Inflammatory markers

Inflammatory marker labs will be obtained according to the Schedule of Procedures. During periods of clinical concern of CNS inflammation, serum C-reactive protein (CRP) and CSF studies should be considered.

7.18 Correlative Sciences

Refer to Schedule of Procedures and study specific laboratory manual for requirements of collection, processing, and shipment of correlative study samples. Correlative specimens, including Peripheral Blood and CSF, will be processed and may be used for prospective testing that may include:

- molecular analysis of CAR T cell persistence
- flow cytometric analysis based on detection of EGFRt in conjunction with T cell surface markers
- assessment of immune cell subsets for determinants of safety, toxicity and efficacy
- ex vivo activity of persistent CAR T cells
- cytokine analysis
- molecular and/or proteomic testing
- assessment of antigen expression on tumor cells
- RNA or DNA sequencing or phenotypic analysis of products and participant samples via multiparameter flow cytometry, CyTOF and/or gene expression profiling

Specimens to be obtained and used in the above-mentioned studies include:

- Peripheral blood: Peripheral blood samples will be drawn at the timepoints specified in the Schedule of Procedures and sent to CSL.
- Cerebrospinal fluid: CSF will be sent to CSL at the timepoints indicated in the Schedule of Procedures. CSF samples for CSL may be requested for non-protocol mandated lumbar punctures done for clinical indications.
- If a subject develops systemic toxicity or neurologic toxicity possibly, probably, or definitely related to CAR T cells (see section 6 Management of Toxicities and Complications), peripheral blood and/or CSF obtained for clinical purposes may be requested for correlative analysis and may be analyzed as above and, if indicated by clinical data, detection of an anti-CAR or EGFRt immune response.

Any material in excess of that needed for protocol-required correlative studies will be retained for potential additional analysis related to this trial or banked for future research upon appropriate authorization.

Archival tumor tissue, CSF, tumor biopsy/resection material or normal tissue biopsy material obtained for clinical purposes may be processed and shipped according to the laboratory manual and may be analyzed for tumor surface antigen expression, detection of CAR T cells, evaluation of markers of immune modulation and/or additional analysis related to this trial or banked for future research upon appropriate authorization.

7.19 CNS Imaging

Disease-specific routine care radiographic imaging (e.g., MRI brain and spine) will occur according to the Schedule of Procedures. Per Section 6.4 Management of Non-CRS Acute

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Toxicity Associated with Infused CAR T Cells into the CNS, additional CNS imaging may be considered in subjects who develop neurologic toxicity, if clinically indicated.

After the End of Therapy/Early Discontinuation Visit, MRI of the spine is required only for subjects with previous spinal disease, positive CSF cytology, or cause for clinical concern. After month 12 following the final CAR T cell infusion, MRIs will be performed only as clinically indicated.

7.20 Disease Response Evaluation

Disease Response will be assessed via MRI brain and spine and CSF according to the Schedule of Procedures and as further described in Section 10: Disease Response.

7.21 Post-Treatment Long-Term Follow-Up Testing

At time points specified in the Schedule of Procedures, up to 15 mL of peripheral blood will be collected and sent to CSL and, if applicable, will be tested for persistence of CAR T cells, replication-competent lentivirus (RCL), and/or clonality through year 1. During years 2 through 15, samples will be collected and sent to CSL annually for those subjects with evidence of persistent CAR T cells or who test positive for RCL. If a patient receives a different lentiviral gene therapy product following treatment on BrainChild-04, they will be expected to have persistence and RCL testing for that product and will no longer have specimens collected on BrainChild-04.

Persistence: PCR for the transgene vector sequence and/or flow cytometric analysis may be performed on mononuclear cells to determine the persistence of engineered T cells. Persistence testing will be discontinued if a patient has had a negative test post their final infusion.
RCL: RCL testing will be performed using a VSVg qPCR assay pre-infusion and at the following time points during the first year: 3, 6 and 12 months (Appendix 2 – Schedule of Procedures). If all post-treatment RCL tests are negative during the first year, then sample collection for RCL testing will be discontinued per FDA guidance. If a post-treatment RCL test is positive at any time during the first year, further time points will be determined by the sponsor after review with FDA.

Clonality: Subjects who at any time point in long-term follow-up have a >5% increase in cells positive for transgene expression will have a repeat test within 1 month of result availability. If the percentage of cells positive for transgene expression continues to increase, additional testing for clonality will be performed. Clonality may be measured by either examining integration sites or TCR diversity among vector positive cells. If there is evidence of clonality, repeat testing will be performed no later than 3 months.

7.21.1 Autopsy

If a research participant dies an autopsy will be requested. If excess autopsy material is available, tissue may be utilized for correlative analysis or banked for future research upon appropriate authorization.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

8 REMOVAL FROM PROTOCOL THERAPY & OFF STUDY CRITERIA

8.1 Removal from Protocol Therapy

A subject may be discontinued from protocol therapy at any time. Subjects will be removed from protocol therapy for the following reasons:

- Physician Decision
- Withdrawal by parent/guardian from protocol therapy but agrees to continue with LTFU
- Withdrawal by subject from protocol therapy but agrees to continue with LTFU
- Completed evaluations through final dose of CAR T cells and no further doses are planned
- Non-compliance with study schedule
- No longer clinically benefiting (an example would be subject receives medical intervention prohibited under this protocol or which will interfere with the ability to assess toxicity or response following CAR T cell infusion)
- Failure to meet criteria as defined in Section 5.5.2 Requirements for initial CAR T cell infusion
- Failure to meet continuation criteria as defined in Section 5.5.3 Requirements for subsequent CAR T cell infusion , or subject experiences a DLT following CAR T cell infusion (as defined in Section 5.4.3 Dose modifications)
- Pregnancy
- Protocol-Specified withdrawal criterion met (Section 8.2 Off-Study Criteria and Study Termination)

All subjects who discontinue protocol therapy should be encouraged to complete all remaining scheduled visits and procedures per Appendix 2 – Schedule of Procedures.

All subjects who have received SC-CAR4BRAIN on this protocol will be required to adhere to long-term follow-up (LTFU) adverse event and sample collection requirements for 15 years from the time of final SC-CAR4BRAIN administration, as mandated by the FDA. This could include entering an independent LTFU protocol separate from this trial.

8.2 Off-Study Criteria and Study Termination

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be recorded in the subject's source documents.

Subjects will be withdrawn/removed from the study for the following reasons:

- Withdrawal by subject
- Withdrawal by subject's parents/legal guardian
- Subject never received SC-CAR4BRAIN T cell product
- Enrolled onto a long-term follow-up protocol for gene therapy
- Completed the study inclusive of 15-year follow up following final SC-CAR4BRAIN infusion
- Death

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

- Lost to follow-up – a reasonable attempt to contact the subject must be made in order for subjects to be considered lost to follow-up. The Sponsor recommends a minimum of 3 documented attempts to contact the subject using at least 2 different contact methods (e.g., phone, email, or certified letter). Institutional standard operating procedures may be followed if approved by the Sponsor.

The study may be terminated at any time by the Sponsor or the FDA.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

9 STATISTICAL CONSIDERATIONS

A preliminary report may be generated once at least 3 subjects enrolled, regardless of arm, to allow for early reporting of novel toxicity, anti-tumor response and/or correlative analyses. Prior to the analysis of the final study data, a detailed statistical analysis plan (SAP) will be written describing all analysis that will be performed. The SAP will contain any modifications to the analysis plan described below.

9.1 Accrual and Study Duration

This is a Phase I open-label, dose-finding study, and will employ the Bayesian optimal interval (BOIN) design [148, 149] to assess safety and tolerability and to determine a dose regimen to be further explored in a future Phase II study. The BOIN design is implemented in a simple way similar to the traditional 3+3 design but is more flexible and possesses superior operating characteristics that are comparable to those of the more complex model-based designs, such as the continual reassessment method (CRM) [150]. Similar to a 3+3 design, we will enroll and treat patients in cohorts of 3. A total of 18 treated subjects are expected in this dose-escalation/de-escalation study for each arm (36 total). An optional expansion cohort with up to 12 subjects for each arm is allowed. Anticipated duration for BOIN design and expansion cohort is 2-4 years.

9.2 Primary Objectives

The primary objectives are:

- To assess the feasibility of manufacturing and administering fractionated intraventricular CNS doses of adoptive therapy with autologous SC-CAR4BRAIN in children and young adults with DIPG, DMG, or recurrent/refractory CNS tumors.
- To assess the safety and describe the toxicity of fractionated intraventricular CNS administration of adoptive therapy with SC-CAR4BRAIN in children and young adults with DIPG, DMG, or recurrent/refractory CNS tumors.
- To define the maximally tolerated dose regimen (MTDR) and recommended Phase 2 dose regimen (RP2DR) of fractionated intraventricular CNS administered SC-CAR4BRAIN infusions.

Feasibility will be described across all subjects and within each therapy Arm and assessed as ability to achieve criteria noted above versus inability to meet each criterion.
Feasibility data will include:

- Number and percent of subjects with sufficient therapeutic product generated to receive two courses at the intended DL per assigned DR after two attempts using a single apheresis product for starting material
- Number of subjects who meet criteria for initial CAR T cell infusion per Section 5.5.2 Requirements for initial CAR T cell infusion
- Number of subjects who meet criteria for at least two courses of CAR T cell infusions per Section 5.5.3 Requirements for subsequent CAR T cell infusion

Safety and toxicity data will include:

- History and physical exam (PE) occurring on study and occurring after the CAR T cell infusion

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

- Laboratory/radiographic evaluations taking place on study and occurring after CAR T cell infusions
- Adverse events using CTCAEv5 terminology

Within each Arm, the toxicities observed with each DR will be summarized in terms of type (organ affected or laboratory determination, such as ANC), severity (by NCI CTCAE v5), CRS grading, neurologic grading, and nadir or maximum values for the laboratory measures, date of onset, and attribution. Tables will be created to summarize these toxicities by Arm and DR with both number of subjects and number of incidences. The evaluation taking place within 2 days prior to initial CAR T cell infusion will serve as the baseline measurement.

9.3 Secondary and Exploratory Objectives

The secondary objectives are:

- To assess SC-CAR4BRAIN distribution within the cerebrospinal fluid (CSF) and the extent to which SC-CAR4BRAIN cells egress into the peripheral circulation.
- To assess disease response following fractionated intraventricular CNS administration of SC-CAR4BRAIN children and young adults with DIPG, DMG, or recurrent/refractory CNS tumors.

Data for evaluation of secondary objectives will include:

- CAR T cell egress will be assessed by analysis of peripheral blood and CSF.
- Tumor response will be assessed using disease response criteria (Section 10.1).
- The duration of overall response will be measured from the initial complete response (CR) or partial response (PR) (whichever is first recorded) until the first date treatment failure is clinically or objectively documented.
- Survival endpoints: progression-free survival (PFS), overall survival (OS), and non-relapse mortality (NRM)

The exploratory objectives are:

- To evaluate surface antigen expression in tumor tissue and/or normal tissue or fluids if a tissue biopsy, tumor biopsy, or resection is available.
- To evaluate for presence of SC-CAR4BRAIN in tumor tissue and/or normal tissue or fluid if a tissue biopsy, fluid collection, tumor biopsy or resection is clinically indicated post-treatment.
- To analyze blood, CSF, and tumor tissue or fluids for biomarkers of SC-CAR4BRAIN tumor targeting, safety, and anti-tumor activity.

9.4 Statistical Analysis

Descriptive statistics, such as mean, standard deviation, and range for continuous variables, and percent and number for categorical variables, will be summarized for baseline information (eg, the extent of prior therapy), and demographic information for each Arm singly and combined. This analysis includes the following variables: race, sex, age, height and weight, stage of disease, tumor characteristics at diagnosis, and others if applicable.

9.5 Safety and Toxicity

Subjects receiving any CAR T cell product will be eligible for safety and tolerability analyses. If a subject is removed from study prior to receiving the initial CAR T cell infusion, they will be replaced. For the purpose of assessing overall safety and tolerability, subjects will be evaluated

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

for toxicities for up to 28 days following final CAR T cell infusion. For the purposes of escalation of DR and defining MTDR, subjects in the existing cohort will be evaluated for toxicities from the time of initial CAR T cell infusion through 7 days following the final CAR T cell infusion received during Course 1, or until they commence with alternate therapies for their CNS tumor, whichever comes first. Subjects who are taken off study prior to Day 28 of Course 1 and have experienced a DLT will count towards that DR's evaluable accrual and will not be replaced. If a subject has not received all intended doses at the DL per the assigned DR in Course 1 due to toxicity or removal from protocol therapy or study for reasons other than toxicity (e.g., progressive disease requiring immediate alternative therapy), they will not count toward that DR's evaluable accrual and will be replaced before cohort advancement is permitted. Subjects who die before Day 28 of Course 1, and before receiving alternative therapy, due to CAR T cell related toxicity will be considered to have experienced a DLT.

9.6 Survival Definitions

OS: Time from initial CAR T cell infusion to death from any cause. If a subject is alive at the last evaluation time period, survival time is censored at the time of last follow-up.

PFS: Time from initial CAR T cell infusion to the first observation of disease progression or death from any cause, whichever occurs first. If the subject has not progressed, relapsed or died, progression-free survival is censored at the time of last follow-up.

NRM: Time to death event where cause of death is not attributable to underlying disease. Relapse and progression, including death attributable to underlying disease, are treated as competing risks for NRM and censored at time of last follow-up for those who do not relapse, progress or die.

Analysis of survival endpoints will include all subjects who receive at least one SC-CAR4BRAIN infusion and will be summarized for all subjects and by cohort. PFS and OS will be assessed using Kaplan Meier (K-M) estimates, and NRM will be assessed using cumulative incidence estimates. The following will be presented for both PFS and OS:

- K-M curves
- The median K-M expected survival time and 95% confidence interval
- Six-month and 12-month K-M survival estimates with 95% confidence intervals

The following will be presented for NRM:

- Cumulative Incidence curves
- Median expected survival and 95% confidence interval
- Six-month and 12-month and 95% confidence intervals

9.7 Evaluability for Disease Response

A subject will be considered evaluable for disease response if: (1) the subject meets the eligibility criteria to receive the CAR T cell infusion; (2) the subject receives any CAR T cell infusion; and (3) the subject is under follow-up for a sufficient period to evaluate the disease and meets criteria for having evaluable disease (Section 10.1 Disease Response Criteria). For the purposes of the study, response will be assessed in comparison to the Treatment Response Evaluation performed within 28 days prior to the initial T cell infusion. Response will be characterized following Course 1 and, if available, at the completion of additional Courses. A

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

subject who dies as a result of toxicity after receiving T cell product but prior to initial response assessment following Course 1 will be considered a non-responder.

9.8 Statistical Study Design

9.8.1 BOIN design

The target toxicity rate for the MTD is 0.3 and the maximum sample size is 18. We will enroll and treat patients in cohorts of size 3. DLTs are defined in Section 4.4 Dose-Limiting Toxicity (DLT). As shown in Table 9-1, the BOIN design uses the following rules, optimized to minimize the probability of incorrect dose regimen assignment, to guide dose regimen escalation/de-escalation:

- if the observed DLT rate at the current dose regimen is ≤ 0.236, escalate the dose to the next higher dose regimen level;
- if the observed DLT rate at the current dose regimen is > 0.359, de-escalate the dose to the next lower dose regimen level;
- otherwise, stay at the current dose regimen.

For the purpose of overdose control, dose regimens j and higher levels will be eliminated from further examination if Pr (pj > 0.3 | data) > 0.95 and at least 3 evaluable patients have been treated at dose regimen level, where pj is the true DLT rate of dose regimen level j, j=1, 2, 3. This posterior probability is evaluated based on the beta-binomial model yj | pj ~binomial (pj) with pj ~uniform(0,1) , where yj is the number of patients experienced DLT at dose regimen level j. When the lowest dose regimen is eliminated, stop the trial for safety. The probability cutoff 0.95 is chosen to be consistent with the common practice that when the target DLT rate <=1/6, a dose with 2/3 patients experienced DLT is eliminated. The above dose regimen escalation/de-escalation and elimination rule can be equivalently presented in Table 9-1, which will be used to conduct the trial.

The steps to implement the BOIN design are described as follows:

1. Patients in the first cohort are treated at Dose Regimen 1.
2. To assign a dose regimen to the next cohort of patients, conduct dose regimen escalation/de-escalation according to the rule displayed in Table 9-1. When using Table 9-1, please note the following:
    a. “Eliminate” means eliminate the current and higher dose regimens from the trial to prevent treating any future patients at these doses because they are overly toxic.
    b. When we eliminate a dose regimen, automatically de-escalate the dose regimen to the next lower level. When the lowest dose regimen is eliminated, stop the trial for safety. In this case, no dose should be selected as the MTDR.
    c. If none of the actions (i.e., escalation, de-escalation or elimination) is triggered, treat the new patients at the current dose regimen.
    d. If the current dose regimen is the lowest dose regimen and the rule indicates dose regimen de-escalation, treat the new patients at the lowest dose regimen unless the number of DLTs reaches the elimination boundary, at which point terminate the trial for safety.
    e. If the current dose regimen is the highest dose regimen and the rule indicates dose regimen escalation, treat the new patients at the highest dose regimen.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

f. If all 3 treated patients in the first cohort of a dose regimen level are non-evaluable, the next cohort of 3 will be treated at the same dose regimen level as the new first cohort. If there are 1 or 2 non-evaluable patients in the first cohort, we will continue enrollment and treat patients one by one until at least 3 evaluable patients have been treated at the same dose regimen level, and follow Table 9-1 for a decision.

3. Repeat step 2 until the maximum sample size of 18 is reached, or stop the trial early when one of the following two conditions is satisfied:
   a. The number of patients who experienced DLTs at the lowest dose regimen level reaches the stopping boundaries listed in Table 9-2 below. In this case, no dose regimen should be selected as the MTD.
   b. The number of patients treated at the current dose regimen >=12 and the decision according to Table 9-1 is to stay at the current dose regimen.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Table 9-1 Dose escalation/de-escalation rule for the BOIN design

Number of evaluable patients treated at current dose regimen	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***
Escalate if # of DLT <=	NA	NA	0	0	1	1	1	1	2	2	2	2
Deescalate if # of DLT >=	NA	2	2	2	2	3	3	3	4	4	4	5
Eliminate if # of DLT >=	NA	NA	3	3	4	4	5	5	5	6	6	7

Note: # of DLT is the number of patients with at least 1 DLT. When none of the actions (i.e., escalate, de-escalate or eliminate) is triggered, stay at the current dose regimen for treating the next cohort of patients. "NA" means that a dose regimen cannot be eliminated before treating 3 evaluable patients.

Table 9-2 Stopping boundaries

The number of evaluable patients treated at the lowest dose regimen	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***
Stop the trial if # of DLT >=	**NA**	**NA**	**2**	**3**	**3**	**4**	**4**	**4**	**5**	**5**	**6**	**6**

Note: # of DLT is the number of patients with at least 1 DLT and "NA" means that a trial cannot be stopped before treating 3 evaluable patients at the lowest dose regimen.

Table 9-2 is used to implement a more stringent safety stopping rule, on top of the dose regimen elimination rule, to safeguard the case that the lowest dose regimen is overly toxic. The stopping boundaries displayed in Table 9-2 correspond to the following Bayesian stopping rule: stop the trial if Pr(p1 > 0.3 | data) >0.9 where p1 is the true DLT rate of the lowest dose regimen. Note that this rule is applied only if three or more evaluable patients have been treated at the lowest dose regimen level.

9.8.2 Arms and DLT observation periods

Subjects will be enrolled onto one of two Arms, as described in Section 5.4.1 Treatment Arm Assignment. The first 3 subjects treated on the trial, regardless of arm, will be ≥ 12 years and ≤ 26 years at time of enrollment, following which treatment of subjects ≥ 1 and ≤ 26 years will occur. Within each Arm, subjects will be assigned to successive DRs in Cohorts of up to 3 subjects. Dose regimen escalation rules will be applied separately for each Arm. There will be a ≥ 7-day interval before enrollment of the initial subject onto the other Arm. [For example, if the initial subject on Arm A is enrolled on 7 April 2023, the initial subject for Arm B cannot be enrolled until ≥ 14 April 2023.]

A modified BOIN design will be used for determining DR assignment of a maximally tolerated dose regimen (MTDR). Within each Arm, treatment will begin with DR 1, Course 1. After the first subject completes Course 1 (i.e., completes the seven-day observation period following the last CAR T cell infusion of Course 1) the subsequent 2 subjects may be treated. Subjects within the first cohort at DR1 of each arm may only receive their initial CAR T cell infusion after the previously treated subject completes Course 1. Subjects enrolled in subsequent cohorts may

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

only receive their initial CAR T cell infusion no sooner than 14 days following the previously treated subject received their initial CAR T Cell infusion. Before the cohorts for the next (higher) DR may open, all 3 subjects of the prior (lower) DR must have received all Course 1 doses of the intended highest dose level and completed a 7-day observation period following the final infusion occurring in Course 1 and not have experienced DR closure as noted below.

If the current Cohort is fully accrued, receiving therapy, but is not yet completely evaluated for DR DLTs, or if the cumulative number of DLTs at the current DR (including subjects still undergoing evaluation) lead to a dose regimen de-escalation or eliminate decision based on Table 9-1, a new subject cannot be assigned to the current DR. Instead, the subject may be offered (a) treatment at the DR below the DR currently being evaluated or (b) to wait until that current Cohort has been completely evaluated to receive treatment in the next DR as per study design.

9.8.3 Stopping rules

T cell infusions will be suspended pending review by the DSMB and consultation with the FDA if death unrelated to disease progression or accidental trauma that occurs within 30 days of CAR T cell administration.

To further protect the safety of subjects, infusions will also be suspended pending review by the DSMB and consultation with the FDA if the lower bound of a 1-sided 80% exact binomial confidence interval of the discontinuation rate due to medically significant Grade 4 or greater events that are possibly, probably, or definitely attributable to CAR T cells is > 10%. Operationally, any of the following would trigger such a rule: 2 out of the first ≤ 5 subjects or 3 out of the first ≤ 11 subjects or 4 out of the first ≤ 18 subjects or 5 out of the first ≤ 24 subjects have unacceptable toxicity or ≥ Grade 4 AEs that are possible, probably, or definitely attributable to CAR T cells. If the true probability of ≥ Grade 4 AE is 2.5%, the probability of study suspension under the above rule is approximately 0.007; if the true probability is 30%, the probability of suspension is approximately 0.93 (probabilities estimated from 5,000 simulations).

- This rule will apply to medically significant (requiring medical intervention) ≥ Grade 4 toxicities that are possibly, probably, or definitely attributable to CAR T cells, with the exception of Grade 4 CRS for < 72 hours, and Grade 4 fever.
- If this rule is met, further T cell infusions for all subjects enrolled will be immediately halted. A safety evaluation will be performed and reviewed with the FDA and DSMB prior to resuming any CAR T cell infusions.

Additionally, the Study Chair may shut down a dose regimen at any time due to toxicities observed or efficacy observed at lower DRs.

9.8.4 Definition and determination of MTDR and RP2DR

The MTDR is defined as the highest DR with at least six DLT-evaluable subjects, which DLT rate is closest to 30% and the cumulative DLT rate during Courses 1 and 2 is below 34%. The MTDR will be estimated separately for each Arm.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Per investigator discretion and with DSMB approval, if a DR lower than the selected MTDR displays satisfactory disease response and has at least six DLT-evaluable subjects, then this DR can be chosen as the RP2DR instead of the MTDR.

9.9 Expansion Cohort

Following the determination of the safety and toxicity of each Arm based on our modified BOIN design, Per PI discretion an expansion cohort of 3 to 12 subjects in each arm starting with treatment at the MTDR/RP2DR will be undertaken. We will still enroll and treat patients in cohorts of size 3 and follow the BOIN design described in Section 9.8.1 to decide whether to stay at the MTDR/RP2DR or DR de-escalation. The expansion cohort will not consider dose-escalation unless recommended by PI and DSMB. The expansion cohort might stop early if at least 9 treated subjects are evaluable at the MTDR/RP2DR per PI's recommendation. The purpose of the expansion cohort is to provide a more precise assessment of the tolerability and safety/toxicity of this treatment when dosed at the MTDR/RP2DR as well as gain additional knowledge regarding secondary and exploratory objectives with a particular focus on the duration and magnitude of in vivo persistence of CAR T cells in the cerebral spinal fluid including descriptions of cellular and protein components. Accrual to the expansion cohort will be continuous and will not depend upon the completion of treatment of the previous patients in the expansion cohort. Ongoing safety monitoring will occur during the expansion cohort with analysis in combination with all subjects treated at the MTDR/RP2DR following BOIN design.

9.10 Safety Monitoring

9.10.1 Weekly safety review

The Study Chair, Investigators, study statistician (if needed), CRCs, research nurses, and other site and Sponsor staff will meet weekly (or as needed when there are active subjects) to review subject enrollment and conduct subject safety review. This group is responsible for monitoring the data and safety of this study, including implementation of the stopping rules for dose escalation. During this meeting, this group will review as applicable enrollment, AEs, DLTs, and protocol compliance, CAR T cell persistence analysis, feasibility data and follow-up information for each subject.

9.10.2 Data and Safety Monitoring Board

The study will be monitored by a DSMB. This is an independent committee with no affiliation to the protocol. The DSMB will meet prior to the study opening to review and approve the study protocol and DSMB Charter. The DSMB will review toxicity data approximately every 6 months. In addition, the DSMB will review study conduct including accrual, drop-outs, data completeness, any inability to generate CAR T cell product that meets all Quality Control criteria, positive bacterial cultures from the CAR T cell product, subjects not meeting eligibility criteria for CAR T cell infusion after enrollment, protocol compliance, and treatment efficacy measures.

DSMB meetings may be called at any time by the DSMB chair or sponsor for additional safety review if indicated. Following any temporary suspension of accrual for a safety event, the DSMB will be convened and will further review the safety data to determine if continuation of accrual is appropriate. Applicable regulatory agencies will receive copies of the DSMB's recommendations as they become available.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

10 DISEASE RESPONSE

10.1 Disease Response Criteria

Radiographic responses will be assessed based on a modified iRANO criteria[151].

10.1.1 Measurable disease

Measurable disease is defined as a lesion that can be accurately measured at minimum 8 mm in two dimensions on standard MRI. Serial measurements should be performed with the same modality (standard MRI) at each assessment. For those tumors other than DIPG or non-pontine DMG, previously irradiated lesions may be considered measurable and followed for response but must demonstrate clear progression following completion of most recent radiotherapy or have biopsy confirmed disease following radiotherapy. For those patients with DIPG or non-pontine DMG, lesions that meet criteria for measurable disease may be followed for response at any timepoint through disease course, with or without progressions after radiotherapy.

10.1.2 Evaluable disease

Evaluable disease is defined as a lesion or multiple lesions, with no lesion that can be accurately measured in at least 2 dimensions and does not meet criteria to be measurable. Such lesions may be evaluable by pathology, MRI, immunocytochemistry techniques, tumor markers, CSF cytology, or other reliable measures.

10.1.3 Selection of target and non-target lesions

Target lesions: For most CNS tumors, only 1 lesion/mass is present and therefore is considered a “target” for measurement/follow up to assess for tumor progression/response. If multiple measurable lesions are present, up to 5 may be selected as target lesions. Target lesions should be selected on the basis of size and suitability for accurate repeated measurements. The lower size limit of the target lesion(s) should be at least twice the thickness of the slices showing the tumor to decrease the partial volume effect (e.g., 8 mm lesion for a 4-mm slice).

Non-target lesions: All evaluable disease (or sites of disease) plus any measurable lesions not selected as target lesion (below lower size limit or if over 5 target lesions present) will be followed as non-target lesions. Measurements are not required but these lesions should be noted at baseline and should be followed as “present”, “absent” or in rare cases “unequivocal progression”.

10.1.4 Response criteria for target lesions

Complete Response (CR): Disappearance of all target lesions

Partial Response (PR): ≥ 50% decrease in the sum of the products of the two perpendicular diameters of all target lesions (taking as reference the initial baseline measurements)

Stable Disease (SD): Neither sufficient decrease in the sum of all target lesions to qualify for PR, nor sufficient increase to qualify for progressive disease

Progressive Disease (PD): ≥ 25% increase in the sum of the products of the perpendicular diameters of the target lesions (taking as reference the smallest sum of the products observed since the start of treatment)

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Immune-Related Progressive Disease (irPD) may occur in the setting of CAR T cell therapy and can manifest as increased disease and/or new lesions and is distinct from PD [151]. irPD will be documented as SD. irPD must occur within 6 months of the initiation of immunotherapy, cannot last longer than 6 months, and cannot correspond to neurologic decline that is definitely attributable to worsening disease. If irPD is suspected:

- Re-imaging is recommended in 3 months and if progressive disease is confirmed, subject will be determined to have PD
- Biopsy should be considered and if pathology is consistent with progressive disease, subject will be determined to have PD

10.1.5 Response criteria for non-target lesions

Complete Response (CR): Disappearance of all non-target lesions

Stable Disease (SD): The persistence of one or more non-target lesions

Progressive Disease (PD): The appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Immune-Related Progressive Disease (irPD) may occur in the setting of CAR T cell therapy and can manifest as increased disease and/or new lesions and is distinct from PD [151]. irPD will be documented as SD. irPD must occur within 6 months of the initiation of immunotherapy, cannot last longer than 6 months, and cannot correspond to neurologic decline that is definitely attributable to worsening disease. If irPD is suspected:

- Re-imaging is recommended in 3 months and if progressive disease is confirmed, subject will be determined to have PD
- Biopsy should be considered and if pathology is consistent with progressive disease, subject will be determined to have PD

10.1.6 Evaluation of best overall response

The overall response assessment takes into account response in both target and non-target lesions and the appearance of one or more new lesions, according to the criteria described in Table 10-1. The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the subject's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 10-1 Best Overall Response

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	SD	No	PR
PR	CR or SD	No	PR
SD	CR or SD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Subjects with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

"symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (biopsy) to confirm the complete response status.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

11 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

11.1 Definition of Adverse Event (AE)

According to 21 CFR 312.32(a): "An adverse event is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related." An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational products. AEs may also include pre- or post-treatment complications that occur as a result of protocol specified procedures, lack of efficacy, overdose, drug abuse/misuse reports, or occupational exposure. Pre-existing events that increase in severity or change in nature during or as a consequence of participation in the clinical study will also be considered AEs. The subject's baseline for purposes of assessing toxicity is established by the active medical conditions present directly prior to initiation of Course 1, Week 1 investigational product administration.

An AE does not include the following:

- Medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. The condition that led to the procedure may be an adverse event and must be reported.
- Pre-existing diseases, conditions, or laboratory abnormalities present or detected before the infusion of investigational product that do not worsen.
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions).
- Overdose without clinical sequelae.
- Any medical condition or clinically significant laboratory abnormality with an onset date before infusion of investigational product and not related to a protocol-associated procedure is not an AE. It is considered to be pre-existing and should be documented on the medical history CRF.
- Abnormal laboratory values or tests that are not clinically significant or do not require therapy.

A suspected adverse reaction is any AE for which there is a reasonable possibility that the investigational product caused the AE. For the purposes of IND safety reporting, "*reasonable possibility*" means there is evidence to suggest a causal relationship between the investigational product and the AE. *Suspected* adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by an investigational product. Life-threatening AE or life-threatening suspected adverse reaction is an AE or suspected adverse reaction that, in the view of either the investigator or study Sponsor, places the study participant at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

11.2 Definition of Serious Adverse Event (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the study Sponsor, it results in any of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- A secondary malignancy

Important medical events that may not result in death, be immediately life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the study participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

11.3 Classification of an Adverse Event

11.3.1 Grading of adverse events

Wherever possible, all AEs will be graded using the NCI CTCAE v5, except grading of CRS which will be according to the CRS Grading Scale (Section 11.5 Cytokine Release Syndrome Grading). Individual neurologic symptoms should be graded using CTCAE v5. The majority of AEs can be graded using these scales.

If an AE cannot be graded using the NCI CTCAE criteria, it should be graded as mild, moderate, severe, life-threatening, or death using the following definitions.

- *Mild (Grade 1):* Awareness of signs or symptoms, but easily tolerated and of a minor irritant type, causing no loss of time from normal activities. Symptoms do not require therapy or a medical evaluation. Signs and symptoms are transient.
- *Moderate (Grade 2):* Events that introduce a low level of inconvenience or concern to the participant and may interfere with daily activities but are usually improved by simple therapeutic measures. Moderate experiences may cause some interference with functioning.
- *Severe (Grade 3):* Events that interrupt the participant's normal daily activities and generally require systemic drug therapy or other treatment. They are usually incapacitating.
- *Life-threatening (Grade 4):* Events that place the participant at immediate risk of death or are disabling.
- *Death (Grade 5):* Events that result in death.

To make sure there is no confusion or misunderstanding of the difference between the terms "serious" and "severe," which are not synonymous, the following note of clarification is provided. The term "*severe*" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "*serious*", which is based on participant/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

11.3.2 Relationship of adverse event to treatment

The investigator will assess the potential relationship of the AE to investigational product using the following descriptions.

- *Not Related:* This category applies to an AE that is clearly not related to the investigational agent/procedure, beyond a reasonable doubt. That is, another cause of the

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

event is most plausible and/or a clinically plausible temporal sequence is inconsistent with the onset of the event and the exposure to study drug and/or a causal relationship is considered biologically implausible.

- *Unlikely Related:* This category applies to an AE that is doubtfully related to the investigational agent/procedure. That is, another cause of the event is highly likely and/or there is not a reasonable temporal sequence from administration of the study drug or one that follows a known or expected response pattern to the suspected study drug. The event could readily have been produced by a number of other factors.
- *Possibly Related:* This category applies to an AE that follows a reasonable temporal sequence from administration of the study drug and that follows a known or expected response pattern to the suspected study drug, but that could readily have been produced by a number of other factors.
- *Probably Related:* This category applies to an AE that is likely related to the investigational agent/procedure. That is, the AE has a temporal relationship to the administration of the investigational agent(s) or research intervention and follows a known or suspected pattern of response.
- *Definitely Related:* This category applies to an AE that is clearly related to the investigational agent/procedure. That is, the AE has a temporal relationship to the administration of the investigational agent(s) or research intervention and follows a known or suspected pattern of response.

11.4 Expectedness, Pre-Existing Conditions, and Persistent Adverse Events

Expectedness: The study Sponsor will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the investigational product.

Pre-Existing Conditions: If a pre-existing condition worsens in severity, the worsening may require reporting as an AE or SAE. In addition, if a condition present at baseline resolves and then recurs, the reoccurrence may require reporting as an AE or SAE.

Persistent Adverse Events: A persistent AE is one that extends continuously, without resolution (is ongoing). A persistent AE is reported only once unless the grade and/or frequency become more severe. If the grade becomes more severe the original AE will be considered to have stopped on the date the severity increased and the AE must be reported again with the higher grade and/or frequency.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

11.5 Cytokine Release Syndrome Grading

Grade [1]	Description of Symptoms
1: Mild	Not life-threatening, require only symptomatic treatment such as antipyretics and anti-emetics (e.g., fever [2] ≥ 38.0°C, nausea, emesis, fatigue, headache, myalgia, malaise)
2: Moderate	Require and respond to moderate intervention: • Oxygen requirement for low flow nasal cannula[3] or blow by oxygen, or • Hypotension responsive to fluids
3: Severe	Require and respond to aggressive intervention: • Oxygen requirement for high flow nasal canula, face mask, non-rebreather or Venturi mask, and/or • Hypotension requiring a one vasopressor with or without vasopressin
4: Life threatening	Life-threatening: • Requirement for positive pressure support including ventilator support, CPAP or BiPAP • Hypotension requiring multiple vasopressors (excluding vasopressin)
5: Fatal	Death

[1]CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring one vasopressor and hypoxia requiring low-flow nasal cannula is classified as having Grade 3 CRS. Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.[2]Fever is defined as temperature ≥38.0°C not attributable to any other cause. In patients who have CRS then receive antipyretics or anti-cytokine therapy such as tocilizumab or corticosteroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.
[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

11.6 Serious Adverse Event Reporting

SAEs occurring from the beginning of the first infusion of the investigational product up to and including 30 days after the last infusion of the investigational product will be reported to the study Sponsor or its designee within 24 hours of the investigator becoming aware of the event. SAEs occurring after 30 days following the last treatment with the investigational product, which, in the judgement of the investigator or treating physician, are ***possibly***, ***probably, or definitely*** related to treatment with the investigational product, will be reported to the study Sponsor or its designee within 24 hours of the investigator becoming aware of the event.

Initial SAE reports must be followed by detailed descriptions. These should include copies of hospital case records and other documents when requested. Telephone reports must be confirmed promptly by written report.

Additionally, the Investigator is responsible for submitting follow-up reports for all SAEs until the SAE has resolved or until the subject's condition stabilizes (in case of persistent impairment), or the study participant dies.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

A death (including death due to progressive disease) occurring within 30 days after the last T cell infusion must be reported to the Sponsor or its designee within 24 hours of site awareness of the death whether or not it is considered treatment-related. Any death occurring greater than 30 days after the last T cell infusion must be reported to the Sponsor or its designee within 24 hours of site awareness of the death only if it is possibly, probably, or definitely related to treatment with the investigational product.

The investigator also must notify the IRB/Institutional Ethics Committee (IEC) and/or Institutional Biosafety Committee (IBC) of the occurrence of the SAE, in writing, as soon as is practicable and in accordance with local law. A copy of this notification must be provided to the study Sponsor or its designee.

11.6.1 Study-specific SAE reporting

Planned hospitalization will not be reported to the Sponsor as an SAE. Planned hospitalizations may include admission for:

- entry into the study
- elective treatment of a pre-existing condition
- routine monitoring of the subject not associated with any deterioration in condition
- monitoring following CAR T cell infusion if minor (non-serious) symptoms indicate the potential for more significant medical problems

11.7 Safety Reporting Contact Information

An SAE Report Form will be provided by Seattle Children's Therapeutics for sites to use in reporting SAEs to the Sponsor.

The site will provide the completed SAE Report Form to SCTx per the instructions on the SAE report form.

11.8 IND Safety Reporting

This study will comply with 21 CFR 312.32, which requires that the sponsor notify the FDA and participating investigators in an IND Safety Report of potentially serious risks from clinical trials or any other source. Reports must be submitted no later than 15 calendar days after the Sponsor becomes aware of the information and determines it is reportable.
The Sponsor will submit an IND Safety Report for individual events meeting the following criteria:

- There is a reasonable probability the drug under study caused the event (ie, there is evidence to suggest a causal relationship between the drug and the AE)
- The event meets the criteria in Section 11.2 Definition of Serious Adverse Event (SAE) for a serious adverse event
- The event is unexpected (i.e., it is not consistent with the risk information in the protocol or other information submitted to the FDA in the IND)

Analysis of one or more occurrences of the same event, and/or aggregate analysis of specific events, may lead the Sponsor to determine that information requires reporting via an IND Safety Report.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

11.9 Reporting of Pregnancy

Pregnancies in study participants or partners that occur from time of consent through 28 days following administration of investigational product or in subjects with continued persistence of CAR T cells (whichever is later) must be reported within 24 hours of site awareness of the event to the study Sponsor or its designee. Subjects who become pregnant prior to the initial administration of investigational product will be taken off study. If pregnancy in subject's partner occurs after administration of investigational product, the investigator must make every effort to follow the pregnancy of either the participant or partner through resolution of the pregnancy (delivery or termination) and report the resolution to the study sponsor or its designee. In the event of a pregnancy in the partner of a participant, the investigator should make every effort to obtain the female partner's consent for release of protected health information.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

12 ADMINISTRATIVE, ETHICAL, AND REGULATORY CONSIDERATIONS

12.1 Good Clinical Practice

This protocol is written in accordance with the principles established by the 18th World Medical Assembly General Assembly (Helsinki, 1964) and subsequent amendments and clarifications adopted by the General Assemblies. The investigator will make every effort that the study described in this protocol is conducted in full conformance with those principles, current FDA regulations, ICH Good Clinical Practices (GCP) guidelines, and local ethical and regulatory requirements. Should a conflict arise, the investigator will follow whichever law or guideline affords the greater protection to the individual subject. The investigator will also make sure he or she is thoroughly familiar with the appropriate administration and potential risks of administration of the study drug, as described in this protocol, prior to the initiation of the study.

12.2 Institutional Review Boards (IRB) and Institutional Biosafety Committees (IBC)

The protocol and consent form, and any accompanying material to be provided to participants, will be reviewed and approved by the IRB/IEC and/or IBC of the participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC and/or IBC in accordance with the standard operating procedures and policies of the IRB/IEC and/or IBC, and the Investigator will keep the IRB/IEC and/or IBC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC and/or IBC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure (IB), consent forms, information concerning participant recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC and/or IBC. The IRB/IEC's and/or IBC's written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated.

Before implementation, the investigator will submit to and receive documented approval from the IRB/IEC for any modifications made to the protocol or any accompanying material to be provided to participants after initial IRB/IECapproval, with the exception of those necessary to reduce immediate risk to study subjects.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB/IEC; new information that may affect adversely the safety of the participants of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

Research modifications will be submitted to the IBC according to National Institutes of Health (NIH) guidelines, standard operating procedures and policies of the IBC.

12.3 Informed Consent/Assent and Other Informational Documents Provided to Study Participants

The Investigator will prepare the informed consent form, assent and the Health Insurance Portability and Accountability Act (HIPAA) authorization and provide the documents to the Sponsor or its designee for approval prior to submission to the IRB/IEC. The consent/assent

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB. The written consent documents will comply with the elements of informed consent as described in 21 CFR Part 50 and ICH E6, and will also comply with local regulations. The Investigator will send a copy of the IRB-approved Informed Consent/Assent Form to the Sponsor or its designee for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the participant into the trial. Information should be given in both oral and written form and participants or their legal representatives must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB, assent from the subject will also be obtained. If a participant is unable to sign the informed consent form (ICF) and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the participant or their legal representative and will be provided any new information during the course of the study that might affect their continued participation in the study.

If the protocol is amended and the ICF (and assent, if applicable) is revised, each participant or their legal representative will be required to provide written informed consent/assent again using the revised ICF (and assent, if applicable).

Receipt of written informed consent/assent will be documented in each potential participant's CRF. The signed ICF will remain in each participant's study file and must be available to the study monitor(s) at all times.

12.4 Data Handling and Record Keeping

12.4.1 Case report forms and source documents

The investigator is required to initiate and maintain, for each participant, an adequate and accurate case history that records all observations and other data related to the study for that participant. A validated electronic data capture system will be used for entry of the data into electronic Case Report Forms (CRFs). Data must be recorded on CRFs approved by the Sponsor or its designee. All information recorded on CRFs for this study must be consistent with the participant's source documentation.

Initial data entry and any changes to the data will be made only by SCTx-authorized users, and data entries and changes will be captured in an electronic audit trail. An explanation of any data change should be recorded in the CRF. All data entered in to the CRF must be verifiable; therefore, CRFs will be routinely checked for accuracy, completeness, and clarity and will be cross-checked for consistency with source documents, including laboratory test reports and other subject records by the Sponsor or its designee. The investigator must allow direct access to all source documents.

12.4.2 Data quality assurance

Quality assurance will be monitored by the Sponsor/Sponsor's designee at appropriate intervals determined by multiple factors including, but not limited to, the assessed risk level of the study, subject enrollment, reporting deadlines, and the findings of previous monitoring visits.
The trial will comply with the standard guidelines set forth by applicable regulatory committees and other institutional, state, and federal guidelines.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

12.4.3 Record retention

All study records must be retained for at least two years after the last approval of a marketing application in the US or an ICH region and until: 1) there are no pending or contemplated marketing applications in the US or an ICH region or 2) at least two years have elapsed since the formal discontinuation of clinical development of the investigational product under study. The investigator/institution should retain subject identifiers for at least 15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a Seattle Children's Therapeutics agreement. Seattle Children's Therapeutics must be notified and will assist with retention should the investigator/institution be unable to continue maintenance of subject files for the full 15 years. All study records must be stored in a secure and safe facility.

12.5 Investigational Product Accountability

While at the clinical site, investigational product must be stored in a secure limited access location at controlled temperature as required and according to product packaging. The storage facility must be available for inspection by the study monitor at any time during the study. A drug accountability record must be maintained for all investigational product received, dispensed, returned, and/or lost during the study. This record must be kept current and made available to the study monitor for inspection.

12.6 Protocol Deviations

A protocol *deviation* is any change, divergence, or departure from the study design or procedures defined in the protocol. In general, protocol deviations are classified as either *major* (or "*important*" per ICH E3 Structure and Content of Clinical Study Reports — Questions and Answers R1) or *minor*.

Major or important protocol deviations are a subset of protocol deviations that might significantly affect the completeness, accuracy, and/or reliability of the study data or that might significantly affect a participant's rights, safety, or well-being. For example, *major* or *important protocol deviations* might include enrolling participants in violation of key eligibility criteria or failing to collect data necessary to interpret primary objectives, as this may compromise the scientific value of the trial.

Minor protocol deviations are protocol deviations that do not have a substantive effect on the participant's rights, safety, or well-being or the integrity of the data. For example, *minor protocol deviations* might include a missed study visit "window."

Major or *important protocol deviations* of this study include, but are not limited to, the following reasons:

- Failure to meet inclusion/exclusion criteria
- Use of a prohibited concomitant medication
- Failure to comply with GCP guidelines

The Investigator will determine if a *major* or *important protocol deviation* will result in withdrawal of a subject. All deviations should be reported to the IRB/IBC in accordance to the IRB/IBC reporting requirements.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

12.7 Subject-Specific Biologic Materials

Leftover CAR T cell product, serum samples, cryopreserved peripheral blood mononuclear cells (PBMC), CSF, and other tissue and material will become the property of the Sponsor and may be used in non-therapeutic experiments.

12.8 Investigator's Responsibilities

By signing the Agreement of Investigator page (Appendix 1B – Principal Investigator Signature Page), the Principal Investigator agrees to:

- Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
- Personally conduct or supervise the study (or investigation).
- Ensure that the requirements relating to obtaining informed consent and IRB/IEC review and approval meet federal guidelines, as stated in 21CFR parts 50 and 56.
- Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with 21 CFR 312.64.
- Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
- Maintain adequate and accurate records in accordance with 21 CFR 312.62 and to make those records available for inspection with the Sponsor or its designee.
- Ensure that an IRB/IEC that complies with the requirements of 21 CFR 56 will be responsible for initial and continuing review and approval of the clinical study.
- Promptly report to the IRB/IEC and the Sponsor or its designee all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
- Ensure IRB/IEC approval before any changes are made in the research study, except when necessary to eliminate immediate hazards to the participants/subjects.
- Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in 21 CFR 312.

12.9 Publication Policy

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, HIPAA. Material must be reviewed and approved by the Sponsor prior to submission for publication.

12.10 Financing and Insurance

For participation at sites other than Seattle Children's Hospital, financing and insurance for this clinical trial will be addressed in clinical trial agreements with the study site.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

13 REFERENCES

1. Ostrom, Q.T., et al., *CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007-2011.* Neuro Oncol, 2014. **16 Suppl 4**: p. iv1-63.
2. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2015.* CA Cancer J Clin, 2015. **65**(1): p. 5-29.
3. Becker, A.P., et al., *KIAA1549: BRAF Gene Fusion and FGFR1 Hotspot Mutations Are Prognostic Factors in Pilocytic Astrocytomas.* J Neuropathol Exp Neurol, 2015. **74**(7): p. 743-54.
4. Kool, M., et al., *Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas.* Acta Neuropathol, 2012. **123**(4): p. 473-84.
5. Northcott, P.A., et al., *Medulloblastoma comprises four distinct molecular variants.* J Clin Oncol, 2011. **29**(11): p. 1408-14.
6. Wu, G., et al., *Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas.* Nat Genet, 2012. **44**(3): p. 251-3.
7. Pajtler, K.W., et al., *Molecular Classification of Ependymal Tumors across All CNS Compartments, Histopathological Grades, and Age Groups.* Cancer Cell, 2015. **27**(5): p. 728-43.
8. Freeman, C.R. and J.P. Farmer, *Pediatric brain stem gliomas: a review.* Int J Radiat Oncol Biol Phys, 1998. **40**(2): p. 265-71.
9. Louis, D.N., et al., *The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary.* Acta Neuropathol, 2016. **131**(6): p. 803-20.
10. Cooney, T., et al., *Contemporary survival endpoints: an International Diffuse Intrinsic Pontine Glioma Registry study.* Neuro Oncol, 2017. **19**(9): p. 1279-1280.
11. Banerjee, A., et al., *A phase I trial of the MEK inhibitor selumetinib (AZD6244) in pediatric patients with recurrent or refractory low-grade glioma: a Pediatric Brain Tumor Consortium (PBTC) study.* Neuro Oncol, 2017. **19**(8): p. 1135-1144.
12. Chang, L., et al., *Transgene-enforced co-stimulation of CD4+ T cells leads to enhanced and sustained anti-tumor effector functioning.* Cytotherapy, 2007. **9**(8): p. 771-84.
13. Cooper, L.J., et al., *Development and application of CD19-specific T cells for adoptive immunotherapy of B cell malignancies.* Blood Cells Mol Dis, 2004. **33**(1): p. 83-9.
14. Cooper, L.J., et al., *T-cell genetic modification for re-directed tumor recognition.* Cancer Chemother Biol Response Modif, 2005. **22**: p. 293-324.
15. Dudley, M.E., et al., *Adoptive transfer of cloned melanoma-reactive T lymphocytes for the treatment of patients with metastatic melanoma.* J Immunother, 2001. **24**(4): p. 363-73.
16. Eshhar, Z., et al., *Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors.* Proc Natl Acad Sci U S A, 1993. **90**(2): p. 720-4.
17. Irving, B.A. and A. Weiss, *The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways.* Cell, 1991. **64**(5): p. 891-901.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

18. Kusmartsev, S. and D.I. Gabrilovich, *Role of immature myeloid cells in mechanisms of immune evasion in cancer.* Cancer Immunol Immunother, 2006. **55**(3): p. 237-45.
19. Park, J.R., et al., *Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma.* Mol Ther, 2007. **15**(4): p. 825-33.
20. Thomas, D.A. and J. Massague, *TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance.* Cancer Cell, 2005. **8**(5): p. 369-80.
21. Vence, L., et al., *Circulating tumor antigen-specific regulatory T cells in patients with metastatic melanoma.* Proc Natl Acad Sci U S A, 2007. **104**(52): p. 20884-9.
22. Woo, E.Y., et al., *Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer.* Cancer Res, 2001. **61**(12): p. 4766-72.
23. Vitanza, N.A., et al., *Locoregional infusion of HER2-specific CAR T cells in children and young adults with recurrent or refractory CNS tumors: an interim analysis.* Nat Med, 2021. **27**(9): p. 1544-1552.
24. Majzner, R.G., et al., *GD2-CAR T cell therapy for H3K27M-mutated diffuse midline gliomas.* Nature, 2022. **603**(7903): p. 934-941.
25. Kowolik, C.M., et al., *CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells.* Cancer Res, 2006. **66**(22): p. 10995-1004.
26. Baselga, J., et al., *Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin.* J Clin Oncol, 2000. **18**(4): p. 904-14.
27. Berger, C., et al., *Safety of targeting ROR1 in primates with chimeric antigen receptor-modified T cells.* Cancer Immunol Res, 2015. **3**(2): p. 206-16.
28. Morello, A., M. Sadelain, and P.S. Adusumilli, *Mesothelin-Targeted CARs: Driving T Cells to Solid Tumors.* Cancer Discov, 2016. **6**(2): p. 133-46.
29. Xiao, X., et al., *Identification and characterization of fully human anti-CD22 monoclonal antibodies.* MAbs, 2009. **1**(3): p. 297-303.
30. Weijtens, M.E., et al., *Single chain Ig/gamma gene-redirected human T lymphocytes produce cytokines, specifically lyse tumor cells, and recycle lytic capacity.* J Immunol, 1996. **157**(2): p. 836-43.
31. Almasbak, H., T. Aarvak, and M.C. Vemuri, *CAR T Cell Therapy: A Game Changer in Cancer Treatment.* J Immunol Res, 2016. **2016**: p. 5474602.
32. Brentjens, R.J., et al., *Genetically targeted T cells eradicate systemic acute lymphoblastic leukemia xenografts.* Clin Cancer Res, 2007. **13**(18 Pt 1): p. 5426-35.
33. Gade, T.P., et al., *Targeted elimination of prostate cancer by genetically directed human T lymphocytes.* Cancer Res, 2005. **65**(19): p. 9080-8.
34. Gill, S., M.V. Maus, and D.L. Porter, *Chimeric antigen receptor T cell therapy: 25years in the making.* Blood Rev, 2016. **30**(3): p. 157-67.
35. Kahlon, K.S., et al., *Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells.* Cancer Res, 2004. **64**(24): p. 9160-6.
36. Stephan, M.T., et al., *T cell-encoded CD80 and 4-1BBL induce auto- and transcostimulation, resulting in potent tumor rejection.* Nat Med, 2007. **13**(12): p. 1440-9.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

37. Teng, M.W., et al., *Immunotherapy of cancer using systemically delivered gene-modified human T lymphocytes.* Hum Gene Ther, 2004. **15**(7): p. 699-708.
38. Westwood, J.A., et al., *Adoptive transfer of T cells modified with a humanized chimeric receptor gene inhibits growth of Lewis-Y-expressing tumors in mice.* Proc Natl Acad Sci U S A, 2005. **102**(52): p. 19051-6.
39. Majzner, R.G., et al., *CAR T Cells Targeting B7-H3, a Pan-Cancer Antigen, Demonstrate Potent Preclinical Activity Against Pediatric Solid Tumors and Brain Tumors.* Clin Cancer Res, 2019. **25**(8): p. 2560-2574.
40. Haydar, D., et al., *Cell-surface antigen profiling of pediatric brain tumors: B7-H3 is consistently expressed and can be targeted via local or systemic CAR T-cell delivery.* Neuro Oncol, 2021. **23**(6): p. 999-1011.
41. Vitanza, N.A., et al., *Intraventricular B7-H3 CAR T cells for diffuse intrinsic pontine glioma: preliminary first-in-human bioactivity and safety.* Cancer Discov, 2022.
42. Finney, H.M., et al., *Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product.* J Immunol, 1998. **161**(6): p. 2791-7.
43. Friedmann-Morvinski, D., et al., *Redirected primary T cells harboring a chimeric receptor require costimulation for their antigen-specific activation.* Blood, 2005. **105**(8): p. 3087-93.
44. Haynes, N.M., et al., *Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors.* Blood, 2002. **100**(9): p. 3155-63.
45. Loskog, A., et al., *Addition of the CD28 signaling domain to chimeric T-cell receptors enhances chimeric T-cell resistance to T regulatory cells.* Leukemia, 2006. **20**(10): p. 1819-28.
46. Moeller, M., et al., *A functional role for CD28 costimulation in tumor recognition by single-chain receptor-modified T cells.* Cancer Gene Ther, 2004. **11**(5): p. 371-9.
47. Pule, M.A., et al., *A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells.* Mol Ther, 2005. **12**(5): p. 933-41.
48. Gardner, R.A., et al., *Intent-to-treat leukemia remission by CD19 CAR T cells of defined formulation and dose in children and young adults.* Blood, 2017. **129**(25): p. 3322-3331.
49. Brown, C.E., et al., *Bioactivity and Safety of IL13Ralpha2-Redirected Chimeric Antigen Receptor CD8+ T Cells in Patients with Recurrent Glioblastoma.* Clin Cancer Res, 2015. **21**(18): p. 4062-72.
50. Brown, C.E., et al., *Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy.* N Engl J Med, 2016. **375**(26): p. 2561-9.
51. Ahmed, N.M., et al., *Immunotherapy for HER2-positive medulloblastoma: Regression of experimental tumors by transfer of HER2-redirected T cells in vivo.* J Clin Oncol, 2006. **24**(18_suppl): p. 9008.
52. Hudziak, R.M., J. Schlessinger, and A. Ullrich, *Increased expression of the putative growth factor receptor p185HER2 causes transformation and tumorigenesis of NIH 3T3 cells.* Proc Natl Acad Sci U S A, 1987. **84**(20): p. 7159-63.
53. Gilbertson, R.J., et al., *Prognostic significance of the c-erbB-2 oncogene product in childhood medulloblastoma.* Br J Cancer, 1995. **71**(3): p. 473-7.
54. Mineo, J.F., et al., *Low HER2-expressing glioblastomas are more often secondary to anaplastic transformation of low-grade glioma.* J Neurooncol, 2007. **85**(3): p. 281-7.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

55. Ahmed, N., et al., *Regression of experimental medulloblastoma following transfer of HER2-specific T cells.* Cancer Res, 2007. **67**(12): p. 5957-64.
56. Pegram, M., et al., *Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers.* Oncogene, 1999. **18**(13): p. 2241-51.
57. Ahmed, N., et al., *HER2-specific T cells target primary glioblastoma stem cells and induce regression of autologous experimental tumors.* Clin Cancer Res, 2010. **16**(2): p. 474-85.
58. Bao, S., et al., *Glioma stem cells promote radioresistance by preferential activation of the DNA damage response.* Nature, 2006. **444**(7120): p. 756-60.
59. Ahmed, N., et al., *Human Epidermal Growth Factor Receptor 2 (HER2) -Specific Chimeric Antigen Receptor-Modified T Cells for the Immunotherapy of HER2-Positive Sarcoma.* J Clin Oncol, 2015. **33**(15): p. 1688-96.
60. Ahmed, N., et al., *Autologous HER2 CMV bispecific CAR T cells are safe and demonstrate clinical benefit for glioblastoma in a Phase I trial.* Journal for ImmunoTherapy of Cancer, 2015. **3**(S2).
61. O'Rourke, D.N., M; Morrissette, JJ; Melenhorst, JJ; Lacey, SF; Mansfield, K; Martinez-Lage, M; Desai, AS; Brem, S; Maloney, E; Mohan, S; Wang, S; Verma, G; Navenot, JM; Shen, A; Zheng, Z; Levine, B; Okado, H; June, CH; Maus, MV., *Pilot study of T cells redirected to EGFRvIII with a chimeric antigen receptor in patients with EGFRvIII+ glioblastoma*, in *ASCO*. 2016: Chicago.
62. Ertl, H.C., et al., *Considerations for the clinical application of chimeric antigen receptor T cells: observations from a recombinant DNA Advisory Committee Symposium held June 15, 2010.* Cancer Res, 2011. **71**(9): p. 3175-81.
63. Barker, F.G., 2nd, et al., *EGFR overexpression and radiation response in glioblastoma multiforme.* Int J Radiat Oncol Biol Phys, 2001. **51**(2): p. 410-8.
64. Gilbertson, R.J., et al., *ERBB receptor signaling promotes ependymoma cell proliferation and represents a potential novel therapeutic target for this disease.* Clin Cancer Res, 2002. **8**(10): p. 3054-64.
65. An, Z., et al., *Epidermal growth factor receptor and EGFRvIII in glioblastoma: signaling pathways and targeted therapies.* Oncogene, 2018. **37**(12): p. 1561-1575.
66. Zhang, Y., et al., *A pediatric brain tumor atlas of genes deregulated by somatic genomic rearrangement.* Nat Commun, 2021. **12**(1): p. 937.
67. Wu, G., et al., *The genomic landscape of diffuse intrinsic pontine glioma and pediatric non-brainstem high-grade glioma.* Nat Genet, 2014. **46**(5): p. 444-450.
68. Ho, R., et al., *Proliferation of human neuroblastomas mediated by the epidermal growth factor receptor.* Cancer Res, 2005. **65**(21): p. 9868-75.
69. Hynes, N.E. and H.A. Lane, *ERBB receptors and cancer: the complexity of targeted inhibitors.* Nat Rev Cancer, 2005. **5**(5): p. 341-54.
70. Birecree, E., L.E. King, Jr., and L.B. Nanney, *Epidermal growth factor and its receptor in the developing human nervous system.* Brain Res Dev Brain Res, 1991. **60**(2): p. 145-54.
71. Werner, M.H., et al., *Localization of immunoreactive epidermal growth factor receptors in human nervous system.* J Histochem Cytochem, 1988. **36**(1): p. 81-6.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

72. Heimberger, A.B., et al., *The natural history of EGFR and EGFRvIII in glioblastoma patients.* J Transl Med, 2005. **3**: p. 38.
73. Liu, T.F., et al., *Growth factor receptor expression varies among high-grade gliomas and normal brain: epidermal growth factor receptor has excellent properties for interstitial fusion protein therapy.* Mol Cancer Ther, 2003. **2**(8): p. 783-7.
74. Gan, H.K., et al., *Antibody-drug conjugates in glioblastoma therapy: the right drugs to the right cells.* Nat Rev Clin Oncol, 2017. **14**(11): p. 695-707.
75. Harandi, A., et al., *Clinical Efficacy and Toxicity of Anti-EGFR Therapy in Common Cancers.* J Oncol, 2009. **2009**: p. 567486.
76. Mendelsohn, J. and J. Baselga, *Epidermal growth factor receptor targeting in cancer.* Semin Oncol, 2006. **33**(4): p. 369-85.
77. Li, T. and R. Perez-Soler, *Skin toxicities associated with epidermal growth factor receptor inhibitors.* Target Oncol, 2009. **4**(2): p. 107-19.
78. Luwor, R.B., et al., *Monoclonal antibody 806 inhibits the growth of tumor xenografts expressing either the de2-7 or amplified epidermal growth factor receptor (EGFR) but not wild-type EGFR.* Cancer Res, 2001. **61**(14): p. 5355-61.
79. Li, D., et al., *Therapeutic anti-EGFR antibody 806 generates responses in murine de novo EGFR mutant-dependent lung carcinomas.* J Clin Invest, 2007. **117**(2): p. 346-52.
80. Reilly, E.B., et al., *Characterization of ABT-806, a Humanized Tumor-Specific Anti-EGFR Monoclonal Antibody.* Mol Cancer Ther, 2015. **14**(5): p. 1141-51.
81. Scott, A.M., et al., *A phase I clinical trial with monoclonal antibody ch806 targeting transitional state and mutant epidermal growth factor receptors.* Proc Natl Acad Sci U S A, 2007. **104**(10): p. 4071-6.
82. Cleary, J.M., et al., *A phase 1 study of ABT-806 in subjects with advanced solid tumors.* Invest New Drugs, 2015. **33**(3): p. 671-8.
83. Phillips, A.C., et al., *ABT-414, an Antibody-Drug Conjugate Targeting a Tumor-Selective EGFR Epitope.* Mol Cancer Ther, 2016. **15**(4): p. 661-9.
84. van den Bent, M., et al., *Efficacy of depatuxizumab mafodotin (ABT-414) monotherapy in patients with EGFR-amplified, recurrent glioblastoma: results from a multi-center, international study.* Cancer Chemother Pharmacol, 2017. **80**(6): p. 1209-1217.
85. Donaghy, H., *Effects of antibody, drug and linker on the preclinical and clinical toxicities of antibody-drug conjugates.* MAbs, 2016. **8**(4): p. 659-71.
86. Albert, C.M., et al., *STRIvE-01: Phase I study of EGFR806 CAR T-cell immunotherapy for recurrent/refractory solid tumors in children and young adults*. 2022, American Society of Clinical Oncology.
87. Chapoval, A.I., et al., *B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production.* Nat Immunol, 2001. **2**(3): p. 269-74.
88. Castriconi, R., et al., *Identification of 4Ig-B7-H3 as a neuroblastoma-associated molecule that exerts a protective role from an NK cell-mediated lysis.* Proc Natl Acad Sci U S A, 2004. **101**(34): p. 12640-5.
89. Xu, H., et al., *MicroRNA miR-29 modulates expression of immunoinhibitory molecule B7-H3: potential implications for immune based therapy of human solid tumors.* Cancer Res, 2009. **69**(15): p. 6275-81.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

90. Benzon, B., et al., *Correlation of B7-H3 with androgen receptor, immune pathways and poor outcome in prostate cancer: an expression-based analysis.* Prostate Cancer Prostatic Dis, 2017. **20**(1): p. 28-35.
91. Inamura, K., et al., *Tumor B7-H3 (CD276) Expression and Survival in Pancreatic Cancer.* J Clin Med, 2018. **7**(7).
92. Xu, Z., et al., *High expression of B7-H3 and CD163 in cancer tissues indicates malignant clinicopathological status and poor prognosis of patients with urothelial cell carcinoma of the bladder.* Oncol Lett, 2018. **15**(5): p. 6519-6526.
93. Zhang, C., et al., *Large-scale analysis reveals the specific clinical and immune features of B7-H3 in glioma.* Oncoimmunology, 2018. **7**(11): p. e1461304.
94. Zhang, W., et al., *B7-H3 silencing inhibits tumor progression of mantle cell lymphoma and enhances chemosensitivity.* Int J Oncol, 2015. **46**(6): p. 2562-72.
95. Flem-Karlsen, K., et al., *Immunoregulatory protein B7-H3 promotes growth and decreases sensitivity to therapy in metastatic melanoma cells.* Pigment Cell Melanoma Res, 2017. **30**(5): p. 467-476.
96. Baral, A., et al., *B7-H3 and B7-H1 expression in cerebral spinal fluid and tumor tissue correlates with the malignancy grade of glioma patients.* Oncol Lett, 2014. **8**(3): p. 1195-1201.
97. Gregorio, A., et al., *Small round blue cell tumours: diagnostic and prognostic usefulness of the expression of B7-H3 surface molecule.* Histopathology, 2008. **53**(1): p. 73-80.
98. Zhou, Z., et al., *B7-H3, a potential therapeutic target, is expressed in diffuse intrinsic pontine glioma.* J Neurooncol, 2013. **111**(3): p. 257-64.
99. Majzner, R.G., et al., *CAR T cells targeting B7-H3, a Pan-Cancer Antigen, Demonstrate Potent Preclinical Activity Against Pediatric Solid Tumors and Brain Tumors.* Clin Cancer Res, 2019.
100. Theruvath, J., et al., *Locoregionally administered B7-H3-targeted CAR T cells for treatment of atypical teratoid/rhabdoid tumors.* Nat Med, 2020. **26**(5): p. 712-719.
101. Walker, A.J., et al., *Tumor Antigen and Receptor Densities Regulate Efficacy of a Chimeric Antigen Receptor Targeting Anaplastic Lymphoma Kinase.* Mol Ther, 2017. **25**(9): p. 2189-2201.
102. Loo, D., et al., *Development of an Fc-enhanced anti-B7-H3 monoclonal antibody with potent antitumor activity.* Clin Cancer Res, 2012. **18**(14): p. 3834-45.
103. Kramer, K., et al., *Compartmental intrathecal radioimmunotherapy: results for treatment for metastatic CNS neuroblastoma.* J Neurooncol, 2010. **97**(3): p. 409-18.
104. Souweidane, M.M., et al., *Convection-enhanced delivery for diffuse intrinsic pontine glioma: a single-centre, dose-escalation, phase 1 trial.* Lancet Oncol, 2018. **19**(8): p. 1040-1050.
105. Powderly, J., et al., *Interim results of an ongoing Phase I, dose escalation study of MGA271 (Fc-optimized humanized anti-B7-H3 monoclonal antibody) in patients with refractory B7-H3-expressing neoplasms or neoplasms whose vasculature expresses B7-H3.* Journal of ImmunoTherapy of Cancer, 2015. **3**(8).
106. Ercikan-Abali, E.A., et al., *Active site-directed double mutants of dihydrofolate reductase.* Cancer Res, 1996. **56**(18): p. 4142-5.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

107. Jonnalagadda, M., et al., *Efficient selection of genetically modified human T cells using methotrexate-resistant human dihydrofolate reductase.* Gene Ther, 2013. **20**(8): p. 853-60.
108. Su, J.M., et al., *A phase 2 study of valproic acid and radiation, followed by maintenance valproic acid and bevacizumab in children with newly diagnosed diffuse intrinsic pontine glioma or high-grade glioma.* Pediatr Blood Cancer, 2020. **67**(6): p. e28283.
109. Vitanza, N.A., et al., *Locoregional HER2CAR T cells for pediatric central nervous system tumors: preclinical efficacy to tolerability in first patient. IMMU-02, Oral Presentation*, in *Society of Neuro-Oncology Pediatric Basic and Translational Research Conference*. 2019: San Francisco, CA.
110. Vitanza, N.A., et al., *Interim analysis from BrainChild-03: Seattle Children's Locoregional B7-H3 CAR T Cell Trial for Children with Recurrent Central Nervous System Tumors and DIPG*, in *International Society of Pediatric Neuro-Oncology*. 2022: Hamburg, GER.
111. Berlow, N.E., et al., *IL-13 receptors as possible therapeutic targets in diffuse intrinsic pontine glioma.* PLoS One, 2018. **13**(4): p. e0193565.
112. Lian, X., et al., *Design considerations of an IL13Ralpha2 antibody-drug conjugate for diffuse intrinsic pontine glioma.* Acta Neuropathol Commun, 2021. **9**(1): p. 88.
113. Brown, C.E., et al., *Optimization of IL13Ralpha2-Targeted Chimeric Antigen Receptor T Cells for Improved Anti-tumor Efficacy against Glioblastoma.* Mol Ther, 2018. **26**(1): p. 31-44.
114. Brown, C.E., et al., *Stem-like tumor-initiating cells isolated from IL13Ralpha2 expressing gliomas are targeted and killed by IL13-zetakine-redirected T Cells.* Clin Cancer Res, 2012. **18**(8): p. 2199-209.
115. Vitanza, N.A., et al., *CAR T Cell Targeting of Pediatric Central Nervous System Tumors. Oral Presentation*, in *Society of Neuro-Oncology Pediatric Basic and Translational Research Conference*. 2019: San Francisco, CA.
116. Vitanza, N.A., et al., *Clinical updates and correlative findings from the first patient with DIPG treated with intracranial CAR T cells*, in *6th Biennial Pediatric Neuro-Oncology Research Conference*. 2021: Virtual.
117. Vitanza, N.A. and Y.J. Cho, *Advances in the biology and treatment of pediatric central nervous system tumors.* Curr Opin Pediatr, 2016. **28**(1): p. 34-9.
118. Mackay, A., et al., *Integrated Molecular Meta-Analysis of 1,000 Pediatric High-Grade and Diffuse Intrinsic Pontine Glioma.* Cancer Cell, 2017. **32**(4): p. 520-537 e5.
119. Grobner, S.N., et al., *The landscape of genomic alterations across childhood cancers.* Nature, 2018. **555**(7696): p. 321-327.
120. Mackay, A., et al., *Molecular, Pathological, Radiological, and Immune Profiling of Non-brainstem Pediatric High-Grade Glioma from the HERBY Phase II Randomized Trial.* Cancer Cell, 2018. **33**(5): p. 829-842 e5.
121. Park, J.R., et al., *Effect of Tandem Autologous Stem Cell Transplant vs Single Transplant on Event-Free Survival in Patients With High-Risk Neuroblastoma: A Randomized Clinical Trial.* JAMA, 2019. **322**(8): p. 746-755.
122. Fry, T.J., et al., *CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy.* Nat Med, 2018. **24**(1): p. 20-28.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

123. Vinci, M., et al., *Functional diversity and cooperativity between subclonal populations of pediatric glioblastoma and diffuse intrinsic pontine glioma cells.* Nat Med, 2018. **24**(8): p. 1204-1215.
124. Mende, A.L., et al., *Current Advances in Immunotherapy for Glioblastoma.* Curr Oncol Rep, 2021. **23**(2): p. 21.
125. Wang, X., et al., *A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells.* Blood, 2011. **118**(5): p. 1255-63.
126. Davila, M.L., et al., *Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia.* Sci Transl Med, 2014. **6**(224): p. 224ra25.
127. Kochenderfer, J.N., et al., *Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor.* J Clin Oncol, 2015. **33**(6): p. 540-9.
128. Lee, D.W., et al., *ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells.* Biol Blood Marrow Transplant, 2019. **25**(4): p. 625-638.
129. Maude, S.L., et al., *Chimeric antigen receptor T cells for sustained remissions in leukemia.* N Engl J Med, 2014. **371**(16): p. 1507-17.
130. Hodi, F.S., et al., *Evaluation of Immune-Related Response Criteria and RECIST v1.1 in Patients With Advanced Melanoma Treated With Pembrolizumab.* J Clin Oncol, 2016. **34**(13): p. 1510-7.
131. Wolchok, J.D., et al., *Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria.* Clin Cancer Res, 2009. **15**(23): p. 7412-20.
132. Danylesko, I., et al., *Immune imitation of tumor progression after anti-CD19 chimeric antigen receptor T cells treatment in aggressive B-cell lymphoma.* Bone Marrow Transplant, 2021. **56**(5): p. 1134-1143.
133. Scholler, J., et al., *Decade-long safety and function of retroviral-modified chimeric antigen receptor T cells.* Sci Transl Med, 2012. **4**(132): p. 132ra53.
134. Wang, G.P., et al., *Analysis of lentiviral vector integration in HIV+ study subjects receiving autologous infusions of gene modified CD4+ T cells.* Mol Ther, 2009. **17**(5): p. 844-50.
135. McGarrity, G.J., et al., *Patient monitoring and follow-up in lentiviral clinical trials.* J Gene Med, 2013. **15**(2): p. 78-82.
136. Cornetta, K., et al., *Absence of Replication-Competent Lentivirus in the Clinic: Analysis of Infused T Cell Products.* Mol Ther, 2018. **26**(1): p. 280-288.
137. Alizadeh, D., et al., *Induction of anti-glioma natural killer cell response following multiple low-dose intracerebral CpG therapy.* Clin Cancer Res, 2010. **16**(13): p. 3399-408.
138. Luger, T.J., et al., *Intracerebroventricular and intrathecal injectate spread in rats.* Eur J Anaesthesiol, 2005. **22**(3): p. 236-9.
139. Matsuzawa, J., et al., *Age-related volumetric changes of brain gray and white matter in healthy infants and children.* Cereb Cortex, 2001. **11**(4): p. 335-42.
140. Xenos, C., S. Sgouros, and K. Natarajan, *Ventricular volume change in childhood.* J Neurosurg, 2002. **97**(3): p. 584-90.
141. Collins, P.Y., et al., *Grand challenges in global mental health.* Nature, 2011. **475**(7354): p. 27-30.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

142. Vitanza, N.A., et al., *Locoregional CAR T cells for children with CNS tumors: Clinical procedure and catheter safety.* Neoplasia, 2023. **36**: p. 100870.
143. Trippett, T.M., et al., *Phase I and pharmacokinetic study of cetuximab and irinotecan in children with refractory solid tumors: a study of the pediatric oncology experimental therapeutic investigators' consortium.* J Clin Oncol, 2009. **27**(30): p. 5102-8.
144. Robert, F., et al., *Phase I study of anti--epidermal growth factor receptor antibody cetuximab in combination with radiation therapy in patients with advanced head and neck cancer.* J Clin Oncol, 2001. **19**(13): p. 3234-43.
145. Secord, A.A., et al., *Phase II trial of cetuximab and carboplatin in relapsed platinum-sensitive ovarian cancer and evaluation of epidermal growth factor receptor expression: a Gynecologic Oncology Group study.* Gynecol Oncol, 2008. **108**(3): p. 493-9.
146. Vermorken, J.B., et al., *Open-label, uncontrolled, multicenter phase II study to evaluate the efficacy and toxicity of cetuximab as a single agent in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck who failed to respond to platinum-based therapy.* J Clin Oncol, 2007. **25**(16): p. 2171-7.
147. Xiong, H.Q., et al., *Cetuximab, a monoclonal antibody targeting the epidermal growth factor receptor, in combination with gemcitabine for advanced pancreatic cancer: a multicenter phase II Trial.* J Clin Oncol, 2004. **22**(13): p. 2610-6.
148. Yuan, Y., et al., *Bayesian Optimal Interval Design: A Simple and Well-Performing Design for Phase I Oncology Trials.* Clin Cancer Res, 2016. **22**(17): p. 4291-301.
149. Liu, R., J. Lin, and P. Li, *Design considerations for phase I/II dose finding clinical trials in Immuno-oncology and cell therapy.* Contemp Clin Trials, 2020. **96**: p. 106083.
150. Zhou, H., Y. Yuan, and L. Nie, *Accuracy, Safety, and Reliability of Novel Phase I Trial Designs.* Clin Cancer Res, 2018. **24**(18): p. 4357-4364.
151. Okada, H., et al., *Immunotherapy response assessment in neuro-oncology: a report of the RANO working group.* Lancet Oncol, 2015. **16**(15): p. e534-e542.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

14 APPENDICES

Appendix 1A – Sponsor Signature Page
Appendix 1B – Principal Investigator Signature Page
Appendix 2 – Schedule of Procedures
Appendix 3 – Performance Status Scales
Appendix 4 –Refractory and Recurrent Disease Categorization
Appendix 5 – Highly Effective Contraception

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

APPENDIX 1 – Principal Investigator Signature Page

Protocol Title: PHASE 1 STUDY OF B7-H3, EGFR806, HER2, AND IL13-zetakine (QUAD) CAR T CELL LOCOREGIONAL IMMUNOTHERAPY FOR PEDICATRIC DIFFUSE INTRINSIC PONTINE GLIOMA, DIFFUSE MIDLINE GLIOMA, AND RECURRENT OR REFRACTORY CENTRAL NERVOUS SYSTEM TUMORS

Principal Investigator:
I have read the Protocol, including all appendices, and I agree to conduct the study as detailed in this protocol and in compliance with the Declaration of Helsinki, Good Clinical Practices (GCP) and all applicable regulatory requirements and guidelines.

Signed by:
Rebecca Ronsley, MD
Signer Name: Rebecca Ronsley, MD
Signing Reason: I approve this document
Signing Time: 10/20/2026 | 5:46:35 PM PDT
7F5B0AC30B9D4D5FAAC7727797D6BAED

Principal Investigator	10/20/2026 | 5:46:38 PM PDT Date

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Seattle Children's Therapeutics

APPENDIX 2 – SCHEDULE OF PROCEDURES

Appendix 2, Table 1 Schedule of Procedures Screening through Course 6/End of Therapy/Early Discontinuation

			Course 1							Course 2-6								
	Screening	Apheresis	Week 1 Infusion	Post-infusion	Week 2 Infusion	Post-infusion	Week 3 Infusion	Post-infusion	Week 4	Week 1 Infusion	Post-infusion	Week 2 Infusion	Post-infusion	Week 3 Infusion	Post-infusion	Week 4	End of Therapy / Early Discontinuation	28 Day Follow-up
Procedure Window → (unless otherwise noted)		e	f	h	f, g	h	f, g	h	i	f	h	f, g	h	f, g	h	i	l	m
Informed consent/assent	X [a]																	
Eligibility determination	X [a]	X	X		X		X			X		X		X				
Demography	X [a]																	
Medical history	X [a]		X [n]															
Disease history	X [a]		X [n]															
Treatment history	X [a]		X [n]															
Performance status [1]	X [b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Physical exam, vital signs [2], weight	X [b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Neurologic exam	X [b]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Height	X [b]																	
Pulse oximetry	X [b]		X		X		X			X		X		X				
Chemistry [3]	X [b]	X	X		X		X		X	X		X		X		X	X	
Hematology [4]	X [b]	X	X		X		X		X	X		X		X		X	X	
Pregnancy test [5]	X [b]		X							X								
Virology [6]	X [c]																	
CSF [7]	X [d]		X		X	X	X		X	X		X		X		X	X [k]	
MRI brain & spine [8]	X [d]		X [j]						X								X [k]	

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

	Screening	Apheresis	Course 1							Course 2-6							End of Therapy / Early Discontinuation	28 Day Follow-up
			Week 1		Week 2		Week 3			Week 1		Week 2		Week 3				
			Infusion	Post-infusion	Infusion	Post-infusion	Infusion	Post-infusion	Week 4	Infusion	Post-infusion	Infusion	Post-infusion	Infusion	Post-infusion	Week 4		
Procedure Window → (unless otherwise noted)		e	f	h	f, g	h	f, g	h	i	f	h	f, g	h	f, g	h	i	l	m
Inflammatory markers [9]			X		X		X		X	X		X		X		X	X	
CRS labs and evaluations [10]																		
Correlative Sciences: CSF [11]			X		X	X	X		X	X							X	
Correlative Sciences: PB [12]		X	X		X	X	X		X	X							X	
Correlative Sciences: other [13]																		
Apheresis		X																
CAR T cell infusion			X		X		X			X		X		X				
Adverse events [14]		X	*Continuous through day 28 post-final infusion*[o]							*Continuous through day 28 post-final infusion*[o]							X	X
Concomitant Medications		X	*Continuous through day 28 post-final infusion*							*Continuous through day 28 post-final infusion*							X	X

a Evaluations must be performed prior to study enrollment, but have no specific procedure window

b Evaluations must be performed within 7 days prior to study enrollment. Results of routine care tests or examinations performed for clinical purposes prior to obtaining informed consent may be used.

c Evaluations must be performed within 3 months prior to study enrollment. Results of routine care tests or examinations performed for clinical purposes prior to obtaining informed consent may be used.

d Evaluations must be performed within 28 days prior to study enrollment. Results of routine care tests or examinations performed for clinical purposes prior to obtaining informed consent may be used.

e Evaluations must be performed within 2 days prior to any apheresis. Correlative Sciences samples collected during repeat apheresis visits after initiating study therapy (see Section 5.2) may be used for other visit time points if collected within the required window. Refer to the study specific lab manual for requirements of all CSL samples.

f Evaluations must be performed within 2 days prior to CAR T cell infusion unless the infusion is omitted in which case evaluations are optional (see Section 5.5). Week 4 evaluations of a prior course, except Correlative Sciences evaluations, may be used for Week 1 pre-infusion if performed within the required window for both visits.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

g Infusion must be performed ≥ 7 days after the previous infusion

h Evaluations must be performed 2 to 5 days after CAR T cell infusion, unless the infusion is omitted in which case evaluations are optional (see Section 5.5). Post-infusion evaluations, except Correlative Sciences evaluations, may be used for infusion and/or Week 4 evaluations if performed within the required window. Distinct Correlative Sciences evaluations must be completed at every timepoint. Assessments may be obtained from local provider or treating oncologist in Course 2 and beyond. Post-infusion visits will be optional for course 7 and beyond.

i Evaluations must be performed 28 (+/- 7) days after Week 1 infusion of that course. Assessments may be obtained from a local provider or treating oncologist in Course 2 and beyond.

j MRI brain and spine must be performed within 28 days prior to Course 1, Week 1 CAR T cell infusion. The screening MRI may be used if performed within the required window.

k Disease evaluation CSF and MRI brain and spine must occur within 14 days following the last CAR T cell infusion but may be delayed up to 28 days due to clinical or scheduling issues. Disease Evaluations are no longer required if progressive disease was confirmed in a prior evaluation.

l End of Therapy/Early Discontinuation Evaluations must be performed within 14 days following the final CAR T cell infusion, EXCEPT MRI brain and spine which must be performed within 28 days following the final CAR T cell infusion. Any evaluations completed for a time point following the final T cell infusion may be used as End of Therapy/Early Discontinuation evaluations if performed within the indicated window. If greater than 14 days have elapsed since the subject's last dose and it is determined that subject will no longer receive protocol therapy, then assessments must be performed within 28 days of the subject's last dose. Assessments may be obtained from local provider or treating oncologist.

m Evaluations must be performed 28 (+/- 7) days post final CAR T cell infusion. The purpose of the 28-day follow-up visit is to ensure adverse event and concomitant medication information is collected per the time period specified by the protocol. Visit may be done in person, by the subject's LCI, or via phone.

n Include medical, disease and treatment history information from time of apheresis to Course 1, Week 1 CAR T cell infusion

o SAE collection must be ongoing until Day +30 post-final CAR T cell infusion (see Section 11.8).

1 Performance status will be assessed utilizing Lansky (for subjects < 16 years of age) or Karnofsky (for subjects ≥ 16 years of age). Subjects who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for purposes of assessing performance status

2 Vital signs must include pulse, respiratory rate, blood pressure and temperature

3 Chemistry must include serum sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), total bilirubin, & conjugated bilirubin. Conjugated bilirubin is optional for courses 3 and beyond.

4 Hematology must include complete blood count (CBC) including hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count

5 Female subjects of child-bearing potential only

6 Virology must include human immunodeficiency virus (HIV) antigen and antibody, Hepatitis B surface antigen, and Hepatitis C antibody testing; if Hepatitis C antibody testing is positive, quantitative PCR will be performed

7 CSF testing must include cell count, glucose, protein, and cytology, except at screening where only cell count and cytology are required. CSF may be obtained via intraventricular or CNS catheters. CSF sample collection is optional for all non-infusion visits.

8 Following Course 1, MRI is required every 12 weeks (+/- 2 weeks) from Course 1 Week 1 Infusion and at End of Therapy/Early Discontinuation visit. If clinically indicated Disease Evaluation CSF and MRI brain and spine should occur prior to receiving the next course. Disease Evaluations are no longer required if progressive disease was confirmed in a prior evaluation.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

9 Inflammatory markers must include CRP, and ferritin if clinically indicated

10 CRS labs and evaluation must be obtained daily during periods of grade 2-4 inflammation including CRS. Labs must include CRP, LDH, ferritin, d-dimer, fibrinogen and absolute lymphocyte count. Evaluation must include temperature, heart rate and blood pressure.

11 Refer to study specific lab manual for collection and shipping requirements of all correlative studies samples. In addition to samples required per the Schedule of Procedures, in the event of neurologic toxicity, CSF should be sent to CSL for cytokine analysis and CAR T cell detection (Section 7.18 for guidance). Also, subjects who receive cetuximab for ablation of T cells (Section 6.5) will require additional CSF to be sent to CSL for determination of CAR T cell persistence. CSF for correlative studies should be collected prior to the initial dose of cetuximab and again on days 1, 3, 7, 10, 14, and 28 days following the initial dose of cetuximab. CSF may be obtained via intraventricular or CNS catheters. CSF sample collection is optional for all non-infusion visits.

12 Refer to study specific lab manual for collection and shipping requirements of all Correlative Sciences studies samples. Pre-IP administration RCL testing will be performed on the pre-infusion sample. If testing is not successful, the pre-apheresis sample will be tested. In addition to samples required per the Schedule of Procedures, in the event of systemic toxicity related to CAR T cells or neurologic toxicity (Section 7.18), peripheral blood may be requested to be sent to the CSL up to once per day. All subjects who receive cetuximab for ablation of T cells will require PB Correlative Sciences studies samples be collected prior to initial dose of cetuximab and at days 1, 3, 7, 10, 14 and 28 after initial dose of cetuximab for determination of T cell persistence.

13 “Other” Correlative Sciences studies samples are not a regularly scheduled protocol procedure, however if archival tumor tissue, tumor biopsy/resection or other tissue/material is collected for clinical purposes, samples may be sent to the CSL for evaluation of marker expression, immune modulation and/or presence of CAR T cells (see Section 7.18 for further guidance); refer to lab manual for requirements of all CSL samples

14 Baseline for assessing adverse events is established by the evaluations performed within 2 days prior to the Course 1, Week 1 CAR T cell infusion.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Appendix 2, Table 2 Schedule of Procedures – Course 7 onwards

	Week 1 infusion	Week 2 infusion	Week 3 infusion	End of Therapy / Early Discontinuation	28 Day Follow-up
Procedure Window →	a	a, b	a, b	c	d
Eligibility determination	X	X	X		
Performance status [1]	X	X	X	X	
Physical exam, vital signs [2], weight	X	X	X	X	
Neurologic exam	X	X	X	X	
Pulse oximetry	X	X	X		
Chemistry [3]	X	X	X	X	
Hematology [4]	X	X	X	X	
Pregnancy test [5]	X				
CSF [6]	X	X	X	X[k]	
MRI brain & spine [7]				X[k]	
Inflammatory markers [8]	X	X	X	X	
CRS labs and evaluations [9]					
Correlative Sciences: CSF [10]	X			X	
Correlative Sciences: PB [11]	X			X	
Correlative Sciences: other [12]					
CAR T cell infusion	X	X	X		
Adverse events [13]	*Continuous through day 28 post-final infusion[o]*			X	X

a Evaluations must be performed within 2 days prior to CAR T cell infusion unless the infusion is omitted in which case evaluations are optional (see Section 5.5). Week 4 evaluations of a prior course, except Correlative Sciences evaluations, may be used for Week 1 pre-infusion if performed within the required window for both visits.

b Infusion must be performed ≥ 7 days after the previous infusion

c End of Therapy/Early Discontinuation Evaluations must be performed within 14 days following the final CAR T cell infusion, EXCEPT MRI brain and spine which must be performed within 28 days following the final CAR T cell infusion. Any evaluations completed for a time point following the final T cell infusion may be used as End of Therapy/Early Discontinuation evaluations if performed within the indicated window. If greater than 14 days have elapsed since the subject's last dose and it is determined that subject

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

will no longer receive protocol therapy, then assessments must be performed within 28 days of the subject's last dose. Assessments may be obtained from local provider or treating oncologist.

d Evaluations must be performed 28 (+/- 7) days post final CAR T cell infusion. The purpose of the 28-day follow-up visit is to ensure adverse event and concomitant medication information is collected per the time period specified by the protocol. Visit may be done in person, by the subject's LCI, or via phone.

1 Performance status will be assessed utilizing Lansky (for subjects < 16 years of age) or Karnofsky (for subjects ≥ 16 years of age). Subjects who are unable to walk because of paralysis, but who are up in a wheelchair, will be considered ambulatory for purposes of assessing performance status

2 Vital signs must include pulse, respiratory rate, blood pressure and temperature

3 Chemistry must include serum sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), total bilirubin, & conjugated bilirubin. Conjugated bilirubin is optional for courses 3 and beyond.

4 Hematology must include complete blood count (CBC) including hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count

5 Female subjects of child-bearing potential only

6 CSF testing must include cell count, glucose, protein, and cytology, except at screening where only cell count and cytology are required. CSF may be obtained via intraventricular or CNS catheters. CSF sample collection is optional for all non-infusion visits.

7 Following Course 1, MRI is required every 12 weeks (+/- 2 weeks) from Course 1 Week 1 Infusion and at End of Therapy/Early Discontinuation visit. If clinically indicated Disease Evaluation CSF and MRI brain and spine should occur prior to receiving the next course. Disease Evaluations are no longer required if progressive disease was confirmed in a prior evaluation.

8 Inflammatory markers must include CRP, and ferritin if clinically indicated

9 CRS labs and evaluation must be obtained daily during periods of grade 2-4 inflammation including CRS. Labs must include CRP, LDH, ferritin, d-dimer, fibrinogen and absolute lymphocyte count. Evaluation must include temperature, heart rate and blood pressure.

10 Refer to study specific lab manual for collection and shipping requirements of all correlative studies samples. In addition to samples required per the Schedule of Procedures, in the event of neurologic toxicity, CSF should be sent to CSL for cytokine analysis and CAR T cell detection (Section 7.18 for guidance). Also, subjects who receive cetuximab for ablation of T cells (Section 6.5) will require additional CSF to be sent to CSL for determination of CAR T cell persistence. CSF for correlative studies should be collected prior to the initial dose of cetuximab and again on days 1, 3, 7, 10, 14, and 28 days following the initial dose of cetuximab. CSF may be obtained via intraventricular or CNS catheters. CSF sample collection is optional for all non-infusion visits.

11 Refer to study specific lab manual for collection and shipping requirements of all Correlative Sciences studies samples. Pre-IP administration RCL testing will be performed on the pre-infusion sample. If testing is not successful, the pre-apheresis sample will be tested. In addition to samples required per the Schedule of Procedures, in the event of systemic toxicity related to CAR T cells or neurologic toxicity (Section 7.18), peripheral blood may be requested to be sent to the CSL up to once per day. All subjects who receive cetuximab for ablation of T cells will require PB Correlative Sciences studies samples be collected prior to initial dose of cetuximab and at days 1, 3, 7, 10, 14 and 28 after initial dose of cetuximab for determination of T cell persistence.

12 "Other" Correlative Sciences studies samples are not a regularly scheduled protocol procedure, however if archival tumor tissue, tumor biopsy/resection or other tissue/material is collected for clinical purposes, samples may be sent to the CSL for evaluation of marker expression, immune modulation and/or presence of CAR T cells (see Section 7.18 for further guidance); refer to lab manual for requirements of all CSL samples

13 Baseline for assessing adverse events is established by the evaluations performed within 2 days prior to the Course 1, Week 1 CAR T cell infusion.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Appendix 2, Table 2 Schedule of Procedures, All Arms Long-Term Follow-Up, Month 3 through Year 5

		Timepoint: months following final CAR T cell infusion											
		Month 3	**Month 6**	**Month 9**	**Month 12 (Year 1)**	**Month 18**	**Month 24 (Year 2)**	**Month 30**	**Month 36 (Year 3)**	**Month 42**	**Month 48 (Year 4)**	**Month 54**	**Month 60 (Year 5)**
Procedure Window →		± 3 months											
Medical history [1, 2]			X		X		X		X		X		X
Physical exam [1]			X		X		X		X		X		X
CSF [1, 3]		X	X	X	X								
Correlative Studies: PB	**CAR T cell persistence** [4]	X	X		X	X	X	X	X	X	X	X	X
	Replication-competent lentivirus (RCL) [7]	X	X		X								
MRI brain & spine [1, 5]		X	X	X	X								
Adverse events [6]		*Continuous*											

1 If performed for clinical indication. May be obtained from local provider or treating oncologist

2 Medical events relevant to subject's disease and treatment, inclusive of infectious disease. If relevant, records pertaining to disease response and/or relapse will be collected.

3 Testing at all time points must include cell count and cytology. Any additional material obtained for clinical purposes may be sent to CSL for research testing. Disease Evaluations are no longer required if progressive disease was confirmed in a prior evaluation.

4 Timepoints only applicable if ongoing CAR T cell persistence in PB at the end of protocol therapy/discontinuation visit. CAR T cell persistence testing will be discontinued after a negative result

5 MRI of the spine for subjects with previous spinal disease, positive CSF cytology, or cause for clinical concern. Disease Evaluations are no longer required if progressive disease was confirmed in a prior evaluation.

6 The following are Delayed Related AEs of Interest which must be reported to the Sponsor: development of a new malignancy, or neurologic, rheumatologic, autoimmune, or hematologic disorder.

7 RCL testing will be discontinued if no positive results during the first year. Further time points may be determined by sponsor after review with FDA if RCL testing is positive at any time during the first year.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

Appendix 2, Table 3 Schedule of Procedures, All Arms Long-Term Follow-up, Years 6 through 15

	Timepoint: years following final CAR T cell infusion									
	Year 6	**Year 7**	**Year 8**	**Year 9**	**Year 10**	**Year 11**	**Year 12**	**Year 13**	**Year 14**	**Year 15**
Procedure Window →	± 3 months									
Medical history [1, 2]	X	X	X	X	X	X	X	X	X	X
Physical exam [1, 5]	X	X	X	X	X	X	X	X	X	X
Correlative Studies: PB CAR T cell persistence [1,3]	X	X	X	X	X	X	X	X	X	X
Adverse events [4]	*Continuous*									

1 May be obtained from local provider or treating oncologist

2 Interim medical history relevant to subject's disease, treatment and status; this information may be obtained by phone, unless subject has evidence of ongoing CAR T cell persistence, in which case subject must be seen in person

3 Timepoints only applicable if ongoing CAR T cell persistence in PB at the 60-month visit. CAR T cell persistence testing will be discontinued after one negative result.

4 The following are Delayed Related AEs of interest which must be reported to the Sponsor: development of a new malignancy, or neurologic, rheumatologic, autoimmune, or hematologic disorder.

5 Only required for subjects with evidence of ongoing CAR T cell persistence or RCL.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

APPENDIX 3 – PERFORMANCE STATUS SCALES

Performance Status Criteria Karnofsky and Lansky performance scores are intended to be multiples of 10			
Karnofsky subjects ≥ 16 years of age		**Lansky** subjects < 16 years of age	
Score	***Description***	***Score***	***Description***
100	Normal, no complaints, no evidence of disease	100	Fully active, normal
90	Able to carry on normal activity, minor signs or symptoms of disease	90	Minor restrictions in physically strenuous activity
80	Normal activity with effort; some signs or symptoms of disease	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work	70	Both greater restriction of and less time spent in play activity
60	Required occasional assistance, but is able to care for most of his/her needs	60	Up and around, but minimal active play; keeps busy with quieter activities
50	Requires considerable assistance and frequent medical care	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities
40	Disabled, requires special care and assistance	40	Mostly in bed, participates in quiet activities
30	Severely disabled, hospitalization indicated. Death not imminent	30	In bed, needs assistance even for quiet play
20	Very sick, hospitalization indicated. Death not imminent	20	Often sleeping, play entirely limited to very passive activities
10	Moribund, fatal processes progressing rapidly	10	No play, does not get out of bed

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

APPENDIX 4 –REFRACTORY AND RECURRENT DISEASE CATEGORIZATION

- DIPG and DMG (WHO grade IV)
- Embryonal Tumor [including, but not limited to, medulloblastoma, atypical teratoid rhabdoid tumor (ATRT), pineoblastoma, embryonal tumor with multilayered rosettes (ETMR), and embryonal tumor NOS] that was refractory to standard first-line therapy or has recurred after standard first-line therapy.
- High-grade glioma [including, but not limited to, anaplastic astrocytoma (WHO grade III), glioblastoma (WHO grade IV)] that was refractory to standard first-line therapy or has recurred after standard first-line therapy.
- Ependymoma that was refractory to standard first-line therapy or has recurred after standard first-line therapy.
- Germ cell tumors (including but not limited to germinoma and nongerminomatous germ cell tumor) that was refractory to standard first-line therapy or has recurred after standard first-line therapy.
- Other WHO grade III or IV CNS tumor (including, but not limited to, choroid plexus carcinomas) that was refractory to standard first-line therapy or has recurred after standard first-line therapy.

Docusign Envelope ID: FEB36823-0ED7-89E4-81CE-413A871C1574

APPENDIX 5 – HIGHLY EFFECTIVE CONTRACEPTION

The following methods of birth control are considered highly effective in preventing pregnancy:

- Total abstinence when in line with the subject's preferred and usual lifestyle. Periodic abstinence like calendar, ovulation, symptothermal, post-ovulation methods, and withdrawal are not acceptable methods of contraception.
- Female sterilization, when the female subject has been surgically sterilized at least 6 weeks prior to enrollment (bilateral oophorectomy or bilateral salpingectomy).
- Male sterilization: the male subject, or female subject's sole sexual partner has been surgically sterilized at least 6 weeks be for enrollment (vasectomy). If the partner of a female subject, appropriate documentation of sterilization should be provided.
- Male subjects: use of a condom during intercourse. In addition, it is advised that the subject's female partner use an additional highly effective method of contraception (hormonal contraception, IUD, etc).
- Female subjects of childbearing potential: use of a combination of any two of the following:
    - Use of oral, injected or implanted hormonal methods of contraception or other forms of hormonal contraception that have comparable efficacy (failure rate < 1%), for example hormone vaginal ring or transdermal hormone contraception. If oral contraception, subject should be on a stable dose of the same medication for ≥ 3 months prior to enrollment;
    - Placement of an intrauterine device (IUD) or intrauterine system (IUS);
    - Use of an occlusive cap (diaphragm or cervical/vault cap) by a female subject, or a condom by a female subject's male partner, combined with a spermicidal foam/gel/film/cream/vaginal suppository

docusign

Certificate Of Completion

Envelope Id: 55315A9D-50AC-4E83-A6E0-4DDD91D0FAB0
Status: Completed
Subject: Complete with Docusign: BrainChild-04_Protocol_v3.0 2026.02.06 clean.pdf
Source Envelope:
Document Pages: 104
Signatures: 1
Certificate Pages: 1
Initials: 0
AutoNav: Enabled
EnvelopeId Stamping: Enabled
Time Zone: (UTC-08:00) Pacific Time (US & Canada)

Envelope Originator:
Thomas Roberts
4800 Sand Point Way NE
Seattle, WA 98105
Thomas.Roberts@seattlechildrens.org
IP Address: 136.226.54.189

Record Tracking

Status: Original
2/6/2026 4:56:44 PM
Holder: Thomas Roberts
Thomas.Roberts@seattlechildrens.org
Location: DocuSign

Signer Events	Signature	Timestamp
Colleen E. Annesley, MD Colleen.Annesley@seattlechildrens.org MD Seattle Children's Hospital - Part 11 Security Level: Email, Account Authentication (Required), Login with SSO	Colleen E. Annesley, MD Signature Adoption: Pre-selected Style Signature ID: FFD018C9-EAA1-4B04-B75C-3F2B40C6508E Using IP Address: 146.79.254.10 With Signing Authentication via Docusign password With Signing Reasons (on each tab): I approve this document	Sent: 2/6/2026 4:57:51 PM Viewed: 2/6/2026 5:09:36 PM Signed: 2/6/2026 5:47:16 PM
Electronic Record and Signature Disclosure: Not Offered via Docusign		
In Person Signer Events	**Signature**	**Timestamp**
Editor Delivery Events	**Status**	**Timestamp**
Agent Delivery Events	**Status**	**Timestamp**
Intermediary Delivery Events	**Status**	**Timestamp**
Certified Delivery Events	**Status**	**Timestamp**
Carbon Copy Events	**Status**	**Timestamp**
Witness Events	**Signature**	**Timestamp**
Notary Events	**Signature**	**Timestamp**
Envelope Summary Events	**Status**	**Timestamps**
Envelope Sent	Hashed/Encrypted	2/6/2026 4:57:51 PM
Certified Delivered	Security Checked	2/6/2026 5:09:36 PM
Signing Complete	Security Checked	2/6/2026 5:47:16 PM
Completed	Security Checked	2/6/2026 5:47:16 PM
Payment Events	**Status**	**Timestamps**

Certificate Of Completion

Envelope Id: FEB36823-0ED7-89E4-81CE-413A871C1574
Status: Completed
Subject: Complete with Docusign: BrainChild-04_Protocol_v3.0_2026.02.06.pdf
Source Envelope:
Document Pages: 105
Signatures: 1
Certificate Pages: 2
Initials: 0
AutoNav: Enabled
EnvelopeId Stamping: Enabled
Time Zone: (UTC-08:00) Pacific Time (US & Canada)

Envelope Originator:
Sam Bakotich
4800 Sand Point Way NE
Seattle, WA 98105
Sam.Bakotich@seattlechildrens.org
IP Address: 71.212.192.154

Record Tracking

Status: Original
10/20/2026 5:23:45 PM
Holder: Sam Bakotich
Sam.Bakotich@seattlechildrens.org
Location: DocuSign

Signer Events	Signature	Timestamp
Rebecca Ronsley, MD rebecca.ronsley@seattlechildrens.org Principal Investigator and Study Chair, Seattle Children's Hospital Security Level: Email, Account Authentication (Required), Login with SSO	Signed by: Rebecca Ronsley, MD Signer Name: Rebecca Ronsley, MD Signing Reason: I approve this document Signing Time: 10/20/2026 | 5:46:35 PM PDT 7F5B0AC30B9D4D5FAAC7727797D6BAED Signature Adoption: Pre-selected Style Signature ID: 7F5B0AC3-0B9D-4D5F-AAC7-727797D6BAED Using IP Address: 66.183.6.241 With Signing Authentication via Docusign password With Signing Reasons (on each tab): I approve this document	Sent: 10/20/2026 5:27:45 PM Resent: 10/20/2026 5:46:34 PM Viewed: 10/20/2026 5:46:38 PM Signed: 10/20/2026 5:46:38 PM

Electronic Record and Signature Disclosure:
Not Offered via Docusign

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	10/20/2026 5:27:45 PM
Certified Delivered	Security Checked	10/20/2026 5:46:38 PM
Signing Complete	Security Checked	10/20/2026 5:46:38 PM
Completed	Security Checked	10/20/2026 5:46:38 PM

Payment Events	Status	Timestamps