ACTION

MCC Protocol #: 18752

ClinicalTrials.gov Identifier: NCT03334305

Title: ACTION Trial
Adoptive **C**ellular **T**herapy following Dose-**I**ntensified Tem**o**zolomide in **N**ewly-diagnosed Pediatric High-grade Gliomas (Phase I).

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Sponsor: National Pediatric Cancer Foundation (NPCF)

NCI-Supplied Agent(s): N/A Other

Agent(s): Temozolomide (TMZ)

Total tumor mRNA-pulsed autologous Dendritic Cells (DCs) (TTRNA-DCs) and tumor-specific ex vivo expanded autologous lymphocyte transfer (TTRNA-xALT) with or without autologous hematopoietic stem cells (HSCs)

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ACTION

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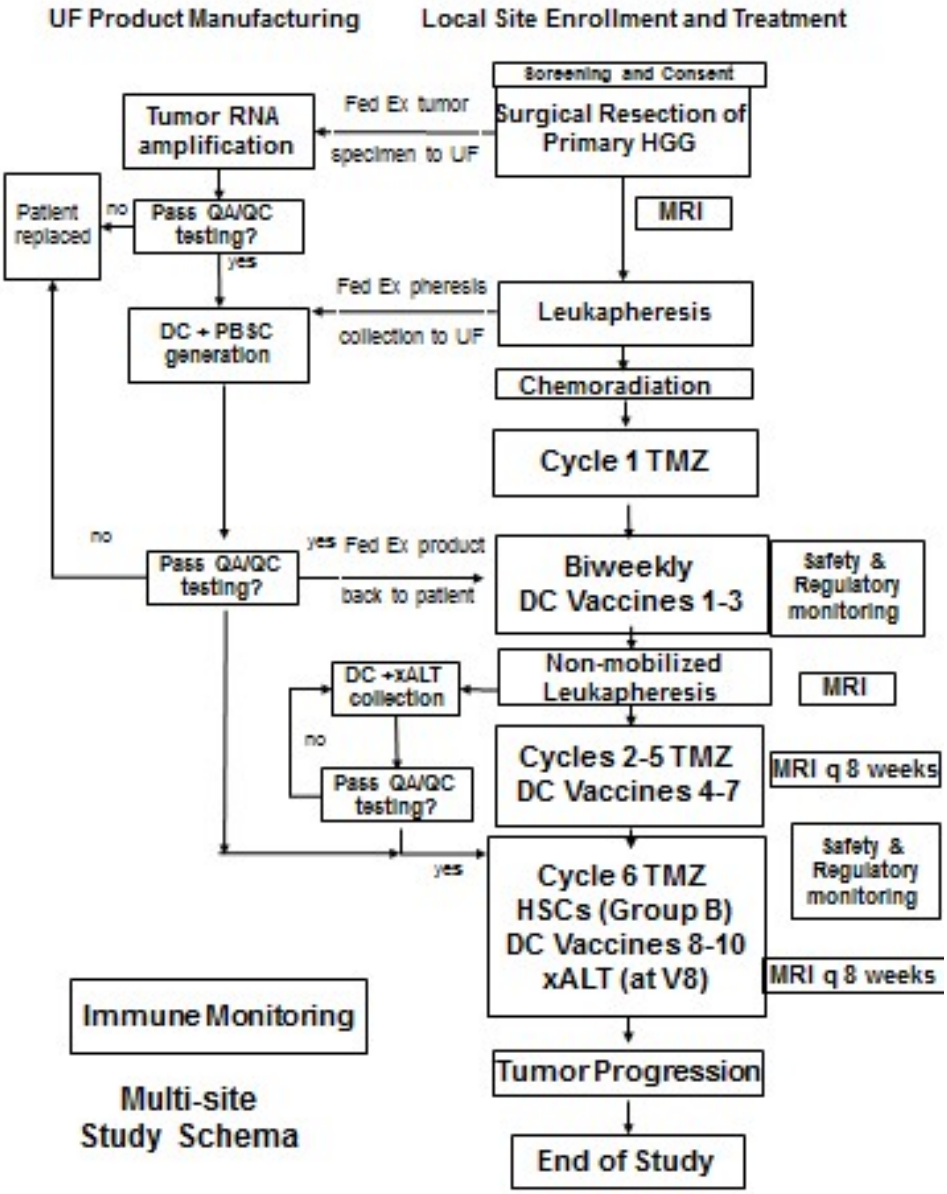


- Screening consent will be obtained prior to surgical resection for tumor preservation. If pathology is appropriate and tumor tissue available with screening consent, treatment consent will be obtained .

CONTENTS

1. BACKGROUND 20

1.1 Study Disease(s) 20

1.2 Investigational Agent(s) 20

1.3 Summary of Background and Supporting Data 21

1.4 Pre-clinical Experience 25

1.5 Clinical Experience 27
1.5.1 Lymphopenia and Homeostatic Proliferation 27
1.5.2 RNA-Loaded Dendritic Cells 29

1.6 DC + ALT Therapy 29
1.6.1 RNA loaded DC vaccines and amplified tumor RNA (preclinical and clinical studies) 29
1.6.2 Amplified Total Tumor RNA from human malignant brain tumors 30

1.7 Autoimmune Encephalomyelitis 30

1.8 Rationale 31

1.9 Re-MATCH phase 1 clinical trial of adoptive cellular therapy in recurrent medulloblastoma (reMB) and PNETs 33

1.10 Improved T cell Expansion 33

1.11 Study Design 33

1.12 On-Study Tests and Procedures 34

1.13 Correlative Studies Background 36

2. PATIENT SELECTION 36

2.1 Inclusion Criteria Screening 36

2.2 Exclusion Criteria 37

2.3 Inclusion of Women and Minorities 37

2.4 Consent/Assent Procedures 37

3. REGISTRATION PROCEDURES 38

3.1 Site and Investigator Registration with Moffitt Cancer Center 38

3.2 Patient Registration 38

ACTION

3.3 General Guidelines 39

4. TREATMENT PLAN 39

4.1 Agent Administration 39
4.1.1 Radiation Guidelines 40
4.1.2 Dose Escalation for Temozolomide Cycles 1-6 following radiation 41
4.1.3 Criteria for Starting Subsequent Cycles 42
4.1.4 Immune Monitoring 43
4.1.5 Follow-up 43

4.2 General Concomitant Medication and Supportive Care Guidelines 44
4.2.1 Treatment of pseudo-progression 44
4.2.2 PCP Prophylaxis 44
4.2.3 Neurosurgical Procedures 44

4.3 Duration of Therapy 44
4.3.1 Subject Withdrawal 45
4.3.2 Subject Death 45
4.3.3 Off Treatment Criteria 46
4.3.4 Off Study Criteria 46

4.4 Duration of Follow Up 46

4.5 Criteria for Removal from Study 46

4.6 Criteria for Evaluable Subjects 46

4.7 Replacement 47

5. DOSING DELAYS/DOSE MODIFICATIONS 47

5.1 Definition of Dose-Limiting Toxicity (DLT) 47

5.2 Dose Modification 48

5.3 Adverse Events and Management 48

6. SAFETY CONSIDERATIONS 50

6.1 Allergic Reactions to DC Immunization or TTRNA-xALT 50

6.2 Cerebral Edema 50

6.3 Infection 51

6.4 Delayed Autoimmune Diseases 51

6.5 Leukapheresis 51
6.5.1 Central Venous Catheter Placement 52

7. ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS 52

ACTION

7.1 Adverse Event Characteristics 52

7.2 Expedited Adverse Event Reporting (Serious Adverse Event) 53
7.2.1 Determination of Reporting Requirements: 53
7.2.2 Steps to Determine if an Adverse Event is to be reported in an Expedited Manner 53
7.2.3 Serious Adverse Event Reporting 54
7.2.4 Distribution of Adverse Event and Serious Adverse Event Reports 55
7.2.5 Expedited Reporting Guidelines - Death 55

7.3 Routine Adverse Event Reporting 55

7.4 Secondary Malignancy 56

8. PHARMACEUTICAL INFORMATION 57

8.1 Names, Classification, and Mechanism of Action 57

8.2 Packaging and Labeling 57

8.3 Dispensing and Preparation 57

8.4 Commercial Agent(s) Non-investigational 58

8.5 GCSF, GMCSF, and Td Vaccine Risks 59

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES 61

10. STUDY CALENDAR 63

10.1 Study Calendar Descriptions 66

10.2 Laboratory Evaluations 67

10.3 Radiologic Evaluations 67

11. MEASUREMENT OF EFFECT 67

11.1 Immunologic confounders of radiographic response 67

11.2 Antitumor Effect – Solid Tumors 68
11.2.1 Definitions 68

11.3 General Methodology for Determining Tumor Measurements 68

11.4 Tumor Response Criteria 69
11.4.1 Evaluation of Best Overall Response 71
11.4.2 Progression-Free Survival 71

12. DATA REPORTING / REGULATORY REQUIREMENTS 72

12.1 Data Reporting 72
12.1.1 Method for Data Reporting and Monitoring 72
12.1.2 Responsibility for Data Submission 74

12.2 Moffitt Cancer Center Guidelines 74
12.2.1 Suspension/Termination 74
12.2.2 Trial Discontinuation 74
12.2.3 Protocol Modifications 74

13. STATISTICAL CONSIDERATIONS 75

13.1 Study Design/Endpoints 75
13.1.1 Analysis Sets 75
13.1.2 Patient Demographics and other Baseline Characteristics 75
13.1.3 Treatments 75
13.1.4 Primary Objective 75
13.1.5 Secondary Objectives 75
13.1.6 Interim Analysis 76
13.1.7 Safety endpoint statistical analysis and stopping criteria 76
13.1.8 Handling of missing values, censoring, and discontinuations 76

13.2 Sample Size/Accrual Rate 76

13.3 Stratification Factors 77

14. REFERENCES 78

15. APPENDIX A 86

16. APPENDIX B 87

LIST OF POSSIBLE ABBREVIATIONS

Ab	Antibody
AB	Alpha Beta/ Antibody
ABC	Automated Blood Count
ABMT	Autologous Bone Marrow Transplantation
ACTB	Beta Actin
ACD	Acid Citrate Dextrose
ACLS	Advanced Cardiac Life Support
ACTH	Adrenocorticotropic Hormone
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALL	Acute Lymphoblastic Leukemia
ALK PHOS	Alkaline Phosphatase
ANOVA	Analysis Of Variance
Anti-HB	Anti- Hepatitis B
ANC	Absolute Neutrophil Count
ANLL	Acute Non-Lymphatic Leukemia
AML	Acute Myelogenous Leukemia
APAAP	Alkaline Phosphatase Antialkaline Phosphatase Complex
APC	Allophycocyanin
ALPS	Autoimmune LymphoProliferative Syndrome
ALT	Autologous Lymphocyte Transfer
nALT	Naïve Autologous Lymphocyte Transfer
xALT	*Ex vivo* Activated Autologous Lymphocyte Transfer
αCD3	anti-cell differentiation antigen 3
αIFN-γ	anti-interferon alpha
αTNF-α	anti-tumor necrosis factor-alpha
αIL2	anti-interleukin 2
AST	Aspartate Aminotransferase
ACTIVATE	**A C**omplementary **T**rial of an **I**mmunotherapy **V**accine **A**gainst **T**umor-Specific **E**GFRvIII
AT	Ambient Temperature
AUC	Area under the concentration time curve
BD-FACS	Becton-Dickinson Fluorescence Activated Cell Sorting
β-HCG	Beta-Human Chorionic Gonadotropin
BMT	Bone Marrow Transplant
BMTU	Bone Marrow Transplant Unit
BSA	Bovine Serum Albumin
BSN	Bachelors of Science in Nursing
BTSC	Brain Tumor Stem Cells
BTIP	Brain Tumor Immunotherapy Program
BU	Busulfan
BUN	Blood Urea Nitrogen

C	Chemotherapy
Ca	Calcium
Cc	Cubic Centimeters
C3D	Cancer Clinical Database
CA	California
CaBIG	Cancer Biomedical Informatics Grid
cDNA	Complimentary Deoxyribonucleic Acid
CDPP	Cisplatin
CEA	Carcinoembryonic Antigen
caAEARS	Cancer Adverse Event Reporting System
CD133	Cell Differentiation 133
CEF	Cytomegalovirus, Epstein Barr virus, Flu virus
CDE	Common Data Elements
CFA	Complete Freund's Adjuvant
CFC	Cytokine Flow Cytometry
CLIA	Clinical Laboratory Improvement Act
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CNC	Clinical Neurologic Change
CNS	Central Nervous System
CO_2	Carbon Dioxide
Con-A	Concanavalin A
Co-PI	Co-Primary Investigator
CPC	Cancer Protocol Committee
Cr	Serum Creatinine
CR	Complete Response
CRF	Conformance Review Checklist
CSF	Cerebro Spinal Fluid
CSI	Craniospinal Irradiation
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T-Lymphocyte
CTLA4	Cytotoxic T-Lymphocyte Antigen 4
CTX	Cyclophosphamide
Cy/Flu	Cyclophosphamide and Fludarabine
DC	Dendritic Cell
dL	Deciliter
DLT	Dose Limiting Toxicity
DLCO	Diffusion Capacity of Carbon Dioxide
DMSO	Dimethyl Sulfoxide
DMZ	Data Management Zone
DNA	Deoxyribonucleic Acid
DNase	Deoxyribonuclease
D. Sci.	Doctor of Science
DSMB	Data Safety Monitoring Board

dT	Diphtheria and Tetanus
DTH	Delayed-type Hypersensitivity
DTRI	Duke Translational Research Institute
DUMC	Duke University Medical Center
EAE	Experimental Autoimmune Encephalomyelitis
EBRT	External Beam Radiation Therapy
ECHO	Echocardiography
EDTA	Ethylenediaminetetraacetic Acid
EKG	Electrocardiogram
EGFR	Epidermal Growth Factor Receptor
EGFRvIII-KLH	EGFRvIII conjugated to Keyhole Limpet Hemocyanin
ELISA	Enzyme-Linked ImmunoSorbent Assay
ELISPOT	Enzyme-linked Immunospot
ERADICATe	**E**valuation of **R**ecovery from **D**rug-**I**nduced lymphopenia using **C**ytomegalovirus-specific T-cell **A**doptive **T**ransfer
FACS	Fluorescence Activated Cell Sorting
FACT-Br	Functional Assessment of Cancer Therapy for Brain Tumors
FACT-accredited	Foundation for the Accreditation of Cellular Therapy
FDA	Food and Drug Administration
FEC	Forced Expiratory Capacity
FEV	Forced Expiratory Volume
FEV1	Forced Expiratory Volume at 1 second
FITC	Fluorescein Isothiocyanate
FOXP3	Forkhead Box P3
FTP	File Transfer Protocol
GADPH	Glyceraldehyde-3-phosphate dehydrogenase
GBM	Glioblastoma Multiforme
GFP	Green Fluorescent Protein
GFR	Glomerular Filtration Rate
GLP	Good Laboratory Practice
G-CSF	Granulocyte Colony Stimulating Factor
G-Tube	Gastrostomy Tube
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
H&E	Hematoxylin and Eosin
HABS	Human Monoclonal Antibodies
HB	Hepatitis B
HbsAg	Hepatitis B Surface Antigen
HD	High Dose
HDC	High Dose Chemotherapy
hCD133	Human Cell Differentiation 133
HER2	Human Epidermal growth factor Receptor 2
hGADPH	Human glyceraldehyde-3-phosphate dehydrogenase
hHPRT	Human Hypoxanthine Phosphoribosyltransferase
HIPPA	The Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus

HPRT	Hypoxanthine Phosphoribosyltransferase
HLA	Human Leukocyte Antigens
HMO	Health Management Organization
HSC	Hematopoietic Stem Cells
HVTN	HIV Vaccine Trials Network
IBC	Institutional Biosafety Committee
ICCC	International Classification of Childhood Cancer
IFN	Interferon
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IL-1β	Interleukin 1B
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-12	Interleukin-12
IL-10	Interleukin-10
IL-13	Interleukin-13
IL-15	Interleukin-15
IND	Investigational New Drug
IP10	Human interferon-inducible protein 10
IRB	Institutional Review Board
IV	Intravenous
K	Potassium
Kg	Kilogram
KLH	Keyhole Limpet Hemocyanin
KLEB	Poorly Differentiated Endometrial Cell Line
KPS	Karnofsky Performance Status
L	Liters
LIMS	Laboratory Information Management System
LMD	Leptomeningeal Disease
LPS	Lansky Performance Score
M^2	Meters Squared
MA	Myeloablative
MAb	Monoclonal Antibody
MAbs aCD28	Monoclonal Antibodies anti-CD28
MB	Medulloblastoma
MAD	Maximally Achievable Dose
MAGE	Melanoma Antigens
M.D.	Medical Doctor
MEL	Melphalan
METS	Metastasis
Mcg	Micrograms
MG	Malignant Glioma
Mg	Magnesium

Mg	Milligrams
MHC	Major Histocompatibility Complex
MIN	Minutes
Ml	Milliliters
mIU/ml	Million International Units/ Milliliter
MMSE	Mini-Mental Status Examination
MRCP	Member of the Royal College of Physicians
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MTD	Maximal Tolerated Dose
MTIC	Maximal Tolerated Inhibitory Concentration
Na	Sodium
NA	Non-adherent
NC	North Carolina
NCC	Nucleated Cell Count
NDC	National Documentation Center
NEJM	New England Journal of Medicine
NEPSY	Neuropsychological Assessment
NCI	National Cancer Institute
NCI CTC	National Cancer Institute Common Toxicity Criteria
NG-Tube	Nasogastric Tube
NJ	New Jersey
NIAID	National Institute for Allergy and Infectious Diseases
NIH	National Institutes of Health
NINDS	National Institutes of Neurological Diseases and Strokes
NK	Natural Killer
NMA	Non-Myeloablative
NR	Not Recorded
NSC	Cancer Chemotherapy National Service Center
NY	New York
OS	Overall Survival
OVA	Ovalbumin
PALS	Pediatric Advanced Life Support
PBLs	Peripheral Blood Lymphocytes
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PBSC	Peripheral Blood Stem Cell
PBSCT	Peripheral Blood Stem Cell Transplant
PBTC	Pediatric Brian Tumor Consortium
PBTFI	Pediatric Brain Tumor Foundation Institute
PCP	Pneumocystis Carinii Pneumonia
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PE	Phycoerythrin
PEPvIII	Peptide variant III

PFS	Progression Free Survival
PFS-12	Progression Free Survival, 12 Months
PHA	Phytohemagglutinin
Ph.D.	Doctorate of Philosophy
PHI	Private Health Information
PI	Principal Investigator
PNETS	Primitive Neuro Ectodermal Tumors
PO	Per Os (by mouth)
polyAAA	Poly Adenosine Tail
PR	Partial Response
PRTBC	Preston Robert Tisch Brain Tumor Center
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QA/QC	Quality Assessment/ Quality Control
Qdot	Quantum dot
QOL ®	Quality of Life Registered
RAC	Recombinant DNA Advisory Committee
RE-MATCH	**Re**current **M**edulloblastoma and Primitive Neuroectodermal Tumor **A**doptive **T** Cell Therapy during Recovery from Myeloablative **C**hemotherapy and **H**ematopoietic Stem Cell Transplantation
reMB/PNETS	Recurrent Medulloblastoma/Primitive Neuro Ectodermal Tumors
RECIST	Response Evaluation Criteria in Solid Tumors
Re-START	REcurrent GBM Stem cell Tumor Amplified RNA immunotherapy Trial
RDC	Remote Data Capture
RN	Registered Nurse
RNA	Ribonucleic Acid
RPA	Ribonuclease Protection Assay
RPMI	Roswell Park Memorial Institute
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
Rx	Treatment
S	Surgery
SAE	Serious Adverse Event
SEB	Staphylococcal Enterotoxin B
SEER	Surveillance, Epidemiology, and End Results
SD	Standard Deviation
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SH	Subtractive Hybridization
SIADH	Syndrome of Inappropriate Antidiuretic Hormone
SOP	Standard Operating Procedure
SOX2	Sex Determining Region Y Box 2

SPORE	Specialized Programs of Research Excellence
SPICE	Specialized Program for Incredibly Complex Evaluations
SQ	Subcutaneous
TAA-40	Top 40 Tumor Associated Antigens
TAA	Tumor Associated Antigens
TBI	Total Body Irradiation
TCR	T-cell Receptor
Td	Tetanus and Diphtheria Toxoids
TERT	Telomerase
TGF-β1-2	Transforming Growth Factor Beta1-2
TILs	Tumor Infiltrating Lymphocytes
TLR	Toll Like Receptor
TM	Trademark
TMZ	Temozolomide
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor- Alpha
T_{regs}	Regulatory T-cells
TRP-2	Tyrosinase Related Protein 2
TTP	Time to Progression
TTRNA	Total Tumor mRNA
UF	University of Florida
µg	Micrograms
µl	Micro liters
US	United States
USP	United States Pharmacopeia
UV	Ultraviolet
Vβ	V Beta
VCR	Vincristine
VICTORI	Dose-Finding and Safety Study of Autologous, Tumor-Specific Antigen-Pulsed Dendritic Cell Immunotherapy for Malignant Brain Tumors
VP	VePesid®
VP-16	Etoposide
WA	Washington

STUDY SYNOPSIS

Title	**A**doptive **C**ellular **T**herapy following Dose-**I**ntensified Tem**o**zolomide in **N**ewly-diagnosed Pediatric High-grade Gliomas (**ACTION** trial)
Study Drug	Total tumor mRNA-pulsed autologous Dendritic Cells (DCs) (TTRNA-DCs) and tumor-specific *ex vivo* expanded autologous lymphocyte transfer (TTRNA-xALT) with or without autologous hematopoietic stem cells (HSCs)
Primary Objective	To determine the safety of adoptive cellular therapy in pediatric patients with HGG receiving dose intensified TMZ and DC+xALT therapy with and without HSCs.
Secondary Objectives	1) Examine feasibility of completing treatment in enrolled subjects 2) Comparison of baseline to post-immunotherapy functional anti-tumor immune responses 3) Analysis of progression-free survival and overall survival after treatment with DC + xALT therapy with and without HSCs
Rationale	Pilot clinical trial of adoptive immunotherapy for pediatric high-grade gliomas. DC vaccination and adoptive T cell strategies targeting unfractionated tumor antigens in children with MBs and other brain tumors have been shown to be feasible and safe with and without HSC transfer. The feasibility and safety of ACT using amplified tumor RNA-pulsed DCs to expand tumor-specific lymphocytes has been established in patients with recurrent MB/PNETs during our recently completed phase I trial (Re-MATCH trial, FDA IND BB-14058; PI: Duane A. Mitchell). We have demonstrated in preclinical studies that HSCs may act synergistically to enhance the efficacy of adoptive cellular therapy. Therefore, we will enroll subjects with a diagnosis of pediatric High-grade Gliomas (HGG) to either receive DCs plus ex vivo expanded autologous lymphocyte transfer (DC/xALT) (Group A) or DCs/xALT plus autologous HSCs (Group B). This phase 1 study design will be performed in 12-18 subjects (6-12 Group A subjects followed by 6 Group B subjects) using a fixed dose of autologous T cells and DC vaccines. The primary objective will be to demonstrate the safety of the DC + xALT + HSCs in pediatric subjects with HGGs. We will also determine feasibility of successfully completing treatment in enrolled subjects and estimate the effect size and variation on immunologic and clinical response in order to determine the sample size required to appropriately power a phase II study design. Although standard treatment of primary HGG includes maximal surgical resection, and external beam radiotherapy, various adjuvant chemotherapy regimens including high-dose chemotherapy and peripheral blood stem cell transplant (HDC+PBSCT) have been utilized; however, outcomes have remained poor despite this intensive therapy. While chemotherapy induces a profound lymphopenia that would be predicted to prevent the induction of an effective immune response to anti-tumor vaccination, recent studies have shown, somewhat counterintuitively, that vaccination during recovery from profound but transient lymphopenia or the adoptive transfer of tumor-specific lymphocytes into lymphodepleted hosts leads to dramatic in vivo T cell expansion and potent immunologic and clinical responses to immunotherapy. Therefore, we expect that tumor-specific lymphocytes, expanded ex vivo with the use of TTRNA-pulsed DCs may provide a source of lymphocytes that preferentially expand in this lymphopenic environment and serve as a source of responder cells to subsequent DC vaccination.

	We and others have successfully employed the use of DCs loaded with total tumor RNA as an innovative strategy to induce cellular immune responses against the repertoire of, as yet, largely uncharacterized antigens present in malignant brain tumors. Tumor cells from HGGs are often limited and cannot be reliably isolated or propagated in sufficient quantity to serve directly as a source of antigen for use in human vaccination protocols. We have, however, been able to reproducibly amplify the RNA content from as few as 500 isolated tumor cells using RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) and in vitro transcription from amplified cDNA (complimentary Deoxyribonucleic Acid) templates to generate RNA libraries in sufficient quality and quantity for clinical-scale immunotherapy trials. We have established the feasibility and safety of adoptive cellular therapy (ACT) using TTRNA-pulsed DCs as a platform for expansion of tumor-specific lymphocytes and delivered after lymphodepletive conditioning in pediatric patients with recurrent medulloblastoma (MB) and primitive neuroectodermal tumors (PNETs) in an ongoing phase I/II trial (Re-MATCH protocol, FDA IND BB-14,058, UF IRB 128-2013; PI: Duane A. Mitchell, MD, PhD). ACT and TTRNA-pulsed DCs will be given in conjunction with the adjuvants GM-CSF and tetanus-diphtheria toxoid vaccine (Td), which we have demonstrated can potentiate immunologic response to DC vaccination. GM-CSF is a powerful adjuvant capable of stimulating macrophage function, inducing proliferation and maturation of DCs, and is able to enhance T-lymphocyte stimulatory function. Intradermal administration of GM-CSF enhances the immunization efficacy at the site of administration in a dose dependent fashion. Significant anti-tumor immunity has been demonstrated in preclinical murine studies in which irradiated, stably transfected tumor cell lines secrete GM-CSF. Tetanus-diphtheria toxoid (Td) is a routinely used vaccine in the normal human population that we have shown in pilot studies may function as a potent adjuvant to enhance DC trafficking to vaccine-site draining lymph nodes (VDLNs). Our previous studies have shown that successful DC migration to VDLNs may be a requisite for clinical activity of RNA- pulsed DCs and administration of Td prior to vaccination may improve the effectiveness of DC vaccines in patients with GBM. The main objectives of this clinical trial are to assess the safety, immunologic effects, and estimate the clinical efficacy of DC vaccination during recovery from cycles of dose-intensified temozolomide (TMZ) followed by adoptive transfer of ex vivo expanded tumor-specific lymphocytes with and without HSCs in pediatric subjects with newly-diagnosed HGGs. The in vivo expansion, persistence, and function of tumor-specific lymphocytes will be followed serially in treated subjects using TCR sequencing and functional immunologic analysis.

Inclusion Criteria	**Screening Eligibility** • Age 3-21 years of age • Has undergone or will undergo surgical resection of suspected HGG **Post-Surgical Resection Eligibility** • Histologically confirmed WHO Grade III or IV malignant glioma • Karnofsky Performance Status (KPS) of ≥ 60% (KPS for ≥ 16 years of age) or Lansky performance Score (LPS) of ≥ 60 (LPS for < 16 years of age) assessed within 2 weeks prior to registration • Bone Marrow: - ANC (Absolute neutrophil count) ≥ 1000/µl (unsupported) - Platelets ≥ 100,000/µl (unsupported for at least 3 days) - Hemoglobin > 8 g/dL (may be supported) • Renal: - Serum creatinine ≤ upper limit of institutional normal • Hepatic: - Bilirubin ≤ 1.5 times upper limit of institutional normal for age. - SGPT (ALT) ≤ 3 times upper limit of institutional normal for age. - SGOT (AST) ≤ 3 times upper limit of institutional normal for age. • Signed informed consent according to institutional guidelines. • Patient or patient guardian consent to PBSC harvest following registration. • Subjects of childbearing or child-fathering potential must be willing to use medically acceptable forms of birth control while being treated on study and for a minimum of six months after receiving study therapy (last date of DC or ALT/HSC administration). • Subjects with post-surgical neurological deficits should have deficits that are stable for a minimum of 1 week prior to leukapheresis.
Exclusion Criteria	• Pregnant or need to breast feed during the study period (Negative serum pregnancy test required). • Known autoimmune or immunosuppressive disease or human immunodeficiency virus infection. • Subjects with significant renal, cardiac (congestive cardiac failure, myocardial infarction, myocarditis), pulmonary, hepatic or other organ dysfunction. • Severe or unstable concurrent medical conditions.

	• Prior allergic reaction to TMZ, GM-CSF, or Td • Subjects who are unwilling or unable to receive treatment and undergo follow-up evaluations at the enrolled National Pediatric Cancer Foundation (NPCF) treatment site.
Study Design	This multi-institutional clinical trial will consist of three parts: Surgery, Chemoradiation, and adjuvant TMZ with Immunotherapy (Five TMZ cycles with TTRNA-DCs and TMZ Cycle 6 followed by DCs + xALT +/- HSCs). Potentially eligible subjects can be screened and provide screening consent for tumor tissue collection at any NPCF site. If potential subjects are referred from outside physicians, and it is not feasible for the subject to travel to a NPCF site, consent may be obtained by designated staff from a NPCF treating center via telephone from the subject/LAR in accordance with institutional policies and procedures. Once screening consent is obtained, the Coordinating Center will interact with site to obtain tumor tissue in a manner suitable for RNA extraction per UF SOP. If standard-of-care sample collection occurred within 12 weeks prior to enrollment and frozen sample remains, a subject may enroll using previously collected samples. Samples must additionally have been snap frozen within 20 minutes of collection and kept in a -70/-80 freezer. Once it has been determined that an adequate amount of tumor tissue was collected and a pathological diagnosis of HGG has been confirmed, the subject must be evaluated at a NPCF treatment site (UF Health Shands Children's Hospital, Childrens of Alabama or Children's National Medical Center). The NPCF treatment site will confirm eligibility, obtain consent for the treatment phase of the study, and administer all subsequent therapy. Up to 24 subjects will be screened to treat 12-18 evaluable subjects. Since HSCs may act synergistically to enhance the efficacy of cellular therapeutics in pediatric patients, subjects will be grouped into cohorts that receive either TTRNA-DC and TTRNA-xALT alone (Group A) or TTRNA-DC and TTRNA-xALT with HSCs (Group B). All enrolled subjects will undergo a mobilized leukapheresis prior to chemoradiation for collection of PBSCs and PBMCs for generation of DCs. G-CSF mobilization of CD34+ HSCs will occur for both groups and leukapheresis for autologous peripheral blood stem cells (PBSCs) may be collected for up to 3 days for Group B subjects to obtain a recommended dose of > 2 x 10^6 CD34+ HSCs/kg. If unable to reach the recommended dose, all available stem cells will be infused. After chemoradiation subjects will receive the first cycle of dose-intensified TMZ followed by three biweekly TTRNA-DC vaccines with GM-CSF. Fourteen days (+/- 3 days) after the third biweekly DC vaccine, subjects will undergo a second leukapheresis (non-mobilized) to collect PBMCs for generation of DCs and xALT. Subjects will then receive an additional five cycles of dose-intensified TMZ with concurrent monthly DC vaccinations on days 22-24 of each cycle. During the 6^{th} TMZ cycle, subjects in Group A will receive TTRNA-DCs and TTRNA-xALT 48-96 hrs after completion of 21-day TMZ. Group B subjects will receive HSCs 24-72 hrs after completion of TMZ and then TTRNA-DCs and TTRNA-xALT will be administered12-36 hrs after HSC infusion. Both groups will receive an additional two bi-weekly TTRNA-DC vaccines during Cycle 6. If a subject cannot tolerate diTMZ, the subject will remain on study and evaluable for the primary endpoint. Immunologic endpoint analyses would describe any differences observed in subjects that did not received diTMZ but not be powered to detect any differences.

ACTION

	After tumor progression, subjects will be followed for survival status only. Subjects will be followed until death due to any cause. As part of standard care for these subjects, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure, consent will be obtained separately. If tissue is obtained, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and antigen expression profile.

1. BACKGROUND

1.1 Study Disease(s)

Pediatric brain tumors are now the leading cause of cancer-related deaths in children in the United States due to the advances in the treatment of more common hematologic malignancies. Standard and intensive multi-modality therapies employed in the treatment of pediatric brain cancers encompass maximal surgical resection, brain and spinal irradiation, and adjuvant chemotherapeutic regimens including high-dose chemotherapy coupled with autologous hematopoietic stem cell (HDC+HSC) rescue. While these regimens can cure up to 70% of children with medulloblastoma, the most common pediatric brain tumor, the prognosis for children with high-grade gliomas (HGGs) remains abysmally poor even after HDC+HSC rescue.[6,7] Therefore, there is a clear and urgent need for the development of targeted therapeutics that do not add significant toxicity to current treatment regimens for HGGs. Despite considerable advancements and promising clinical results observed in immunotherapy trials at our center and others directed against adult malignant brain tumors, efforts in the immunologic treatment of pediatric brain tumors have been limited to relatively few notable studies. This is often due, at least in part, to the limited amount of viable tumor tissue available for tumor cell- based vaccine preparations, and the lack of identification of consistently expressed tumor-specific antigens within these cancers.

1.2 Investigational Agent(s)

The use of total tumor RNA-loaded dendritic cells (DCs) was pioneered as a novel platform for inducing potent immunologic responses against the variety of uncharacterized and patient- specific antigens present within malignant tumor cells. **We have demonstrated that sufficient RNA for clinical vaccine preparations can be amplified with high fidelity using existing molecular technologies from as few as 500 isolated pediatric and adult brain tumor cells,** thus allowing vaccine preparation from surgical biopsies and even microdissected archival tumor specimens. We are currently exploring adoptive cellular therapy using amplified tumor RNA- pulsed DCs and autologous lymphocyte transfer (DC + xALT therapy) in pediatric patients with recurrent MB and PNETs (Re-MATCH protocol, FDA IND BB-14,058, UF IRB 128-2013; PI: Duane A. Mitchell, MD, PhD). In this proposal, we aim to extend evaluation of this novel platform to the treatment of pediatric HGGs.

Immunotherapy administered during recovery from HDC and salvage chemotherapy may have tremendous advantages, as **adoptive cellular therapy** following **lymphodepletive conditioning regimens** has **emerged as the most effective treatment strategy** for advanced and refractory melanoma. Remarkable objective clinical responses in up to 75% of treated refractory melanoma patients have been observed, including **durable complete regressions of metastatic lesions within the CNS**. Although the mechanisms by which lymphodepletion enhances immunotherapy in humans are not well elucidated, elegant studies in murine tumor models have highlighted some key catalysts for induction of potent anti-tumor immunity. **Depletion of immunosuppressive** T_{regs}**, increased bioavailability of inflammatory**

cytokines and homeostatic proliferative cytokines (most notably IL-7 and IL-15) after removal of host lymphocytes and NK cells that compete with transferred tumor-specific T cells, and increased **toll-receptor agonistic signals** during myeloablative therapy play significant roles.

1.3 Summary of Background and Supporting Data

Adoptive cellular therapy, involving the *ex vivo* expansion and intravenous transfer of autologous tumor-specific lymphocytes after non-myeloablative (NMA) and myeloablative (MA) conditioning regimens, has emerged as a remarkably effective treatment modality for advanced malignant disease.[8] This success has been most notably in the setting of metastatic melanoma; with objective clinical responses observed in 30-50% of patients treated with adoptive cellular therapy after NMA conditioning and in up to 75% of patients after receiving MA regimens[9,10] Importantly, these responses have also included a greater than 40% durable complete response rate (>3 years) of lesions within the central nervous system (CNS), demonstrating that the brain is not refractory to effective treatment by cellular immunotherapy approaches.[11] Therefore, despite its somewhat complex and highly-specialized nature, **adoptive cellular therapy has demonstrated significant curative potential in patients with advanced, invasive, and refractory malignant disease**. A major limitation in the extension of these successes to cancers other than melanoma, however, has been the inability to reliably expand polyclonal tumor-infiltrating lymphocytes (TILs) from the majority of other solid tumors. To circumvent these limitations, genetically engineered T cells or chimeric antigen receptor (CAR) modified T cells have emerged as a favorable treatment strategy.[12,13] However, this approach is significantly hampered by the very few tumor-specific antigens and receptors identified to date, and toxicities that have emerged when engineered T cells with high-affinity receptors have targeted antigens with shared expression in normal tissues.[14]

Through prior phase I and II clinical trials (FDA IND BB-9939, 12839, 13240, 13560, 14058; PI: Duane A. Mitchell, M.D., Ph.D.), we have gained considerable experience in evaluating the safety and potential efficacy of cellular immunotherapy targeting tumor-specific antigens in adult patients with glioblastoma.[15-19] These trials have shown the capacity to induce potent tumor specific immune responses in vaccinated patients, elicit complete radiographic responses in patients with residual disease, and achieve patient survival that significantly exceeds that of historical controls **(Figs. 1-2)**. The advent of myelosuppressive alkylator-based chemotherapy with TMZ as standard-of-care treatment in GBM patients would be predicted to curtail the capacity to induce effective immune responses in these patients. However, somewhat counter-intuitively, we have shown TMZ to function as a potent adjuvant for enhancing responses to vaccination in humans **(Figs. 2-3)** and to antigen-specific vaccines and adoptive T cell therapy in experimental mice **(Figs. 4-6)**.[16] These observations are consistent with recent reports demonstrating that treatment-induced lymphopenia creates a homeostatic environment in which *ex vivo* activated and adoptively transferred or vaccine-induce tumor specific lymphocytes can gain a proliferative advantage over host T cells and achieve markedly high precursor frequencies.

The identification and validation of tumor-specific antigens in pediatric brain tumors is limited at present but we have shown that DCs pulsed with the total antigenic content of tumor cells in the form of RNA can induce potent anti-tumor immunity against the largely uncharacterized antigens present in murine and human tumors. We have shown that despite increased levels of immunosuppressive CD4+CD25+CD127-FOXP3+ T_{regs} in patients with GBM, DC vaccination coupled with adoptive transfer of naïve autologous lymphocytes (DC + nALT therapy) during hematopoietic recovery from chemotherapy-induced lymphopenia can safely elicit cellular and humoral immune responses in patients with GBM and achieve promising clinical responses (ATTAC Protocol, **A**nti-**T**umor Immunotherapy **T**argeted **A**gainst **C**ytomegalovirus in Patients with Newly Diagnosed Glioblastoma Multiforme during Recovery from Therapeutic Temozolomide-induced Lymphopenia; FDA IND-BB-12839, Duke IRB 3877; PI: Duane A. Mitchell).[19]

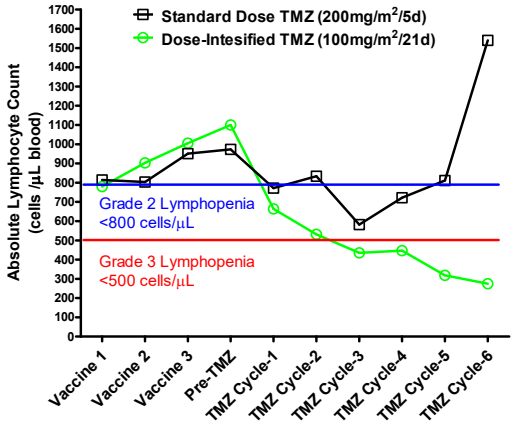


Fig. 1: Lymphocyte counts of patients with newly-diagnosed GBM receiving standard dose adjuvant TMZ (200 mg/m^2/5d) and dose-intensified TMZ (100 mg/m^2/21d). Standard dose TMZ induces Grade 2 lymphopenia in 100% of patients (<800 cells/uL; blue line) and transient Grade 3 lymphopenia in 40% of patients. Sustained Grade 3 lymphopenia (<500 cells/uL; red line) was induced by dose-intensified TMZ cycle #3 in all patients.

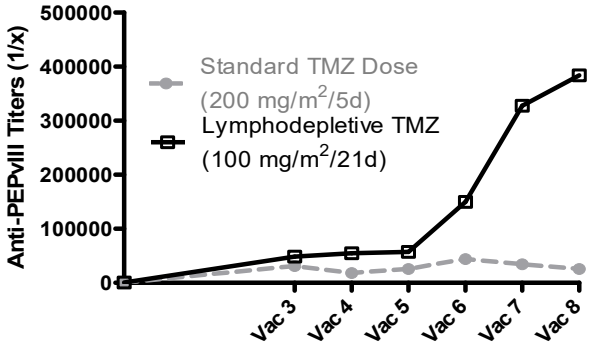


Fig. 2: Antibody titers in two representative patients during standard-dose TMZ (200 mg/m^2/5d) and dose-intensified TMZ (100 mg/m^2/21d) are shown. All patients receiving the 21 day dose regimen developed EGFRvIII-specific antibodies with titers detectable at greater than 1:100,000 dilution. Humoral and cellular immune responses have been dramatically better in patients receiving the higher TMZ dose.

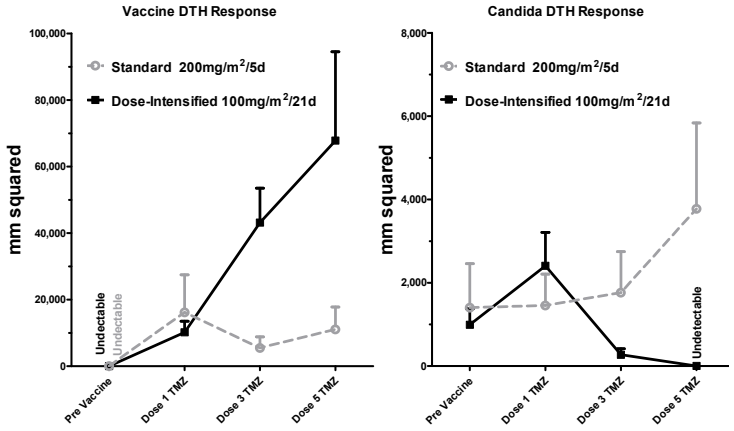


Fig. 3: DTH responses to EGFRvIII were undetectable prior to vaccination in patients receiving non-lymphodepletive TMZ (200 mg/m^2/5d) and lymphodepletive TMZ (100 mg/m^2/21d) cycles and increased during vaccination in all treated patients. DTH response to vaccine was progressively greater in patients receiving lymphodepletive TMZ *(left; p=0.0037, Two-way ANOVA, repeated measures for interaction)*, while pre-existing DTH responses against *Candida* were maintained during non-lymphodepletive TMZ but abolished by the third cycle of TMZ in all patients receiving lymphodepletive TMZ *(right; p=0.035, Two-way ANOVA, repeated measures for interaction)*. Results demonstrate a differential response to TMZ cycles of vaccine-induced immunity compared to pre-existent *Candida* immunity.

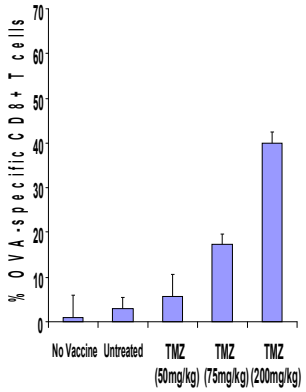


Fig. 4: Mice were given increasing doses of TMZ (50 mg/kg -200 mg/kg x 1) prior to transfer of OT-I OVA-specific T cells as responder cells (1x10 cells/mouse) and a single vaccination with OVA RNA-loaded DCs. Expansion of OVA-specific T cells was monitored in peripheral blood using an OVA-specific tetramer and anti- CD8 monoclonal antibody. Acute immunologic responses showed a dose-dependent increase in mice pre-treated with TMZ. Five day course of lymphodepletive TMZ (60mg/kg/5d) also leads to a significant enhancement of vaccine response to OVA RNA-loaded DCs compared to untreated animals (not shown).

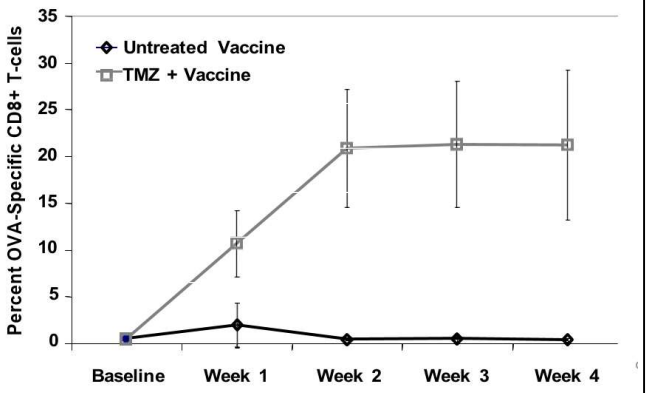


Fig. 5: Untreated mice revealed a normal acute phase expansion of OVA-specific T cells in response to RNA pulsed DCs that peaked at 7-10 days followed by a contraction phase of OVA-specific T cells in which 90-95% of cells undergo apoptosis. TMZ-pretreated animals (60 mg/kg x 5 days), however, did not exhibit contraction of the stimulated T cells even a month after primary stimulation and maintained elevated levels of effector/memory OVA-specific T cells (IFNg+CD44hi/CD43low) in the blood.

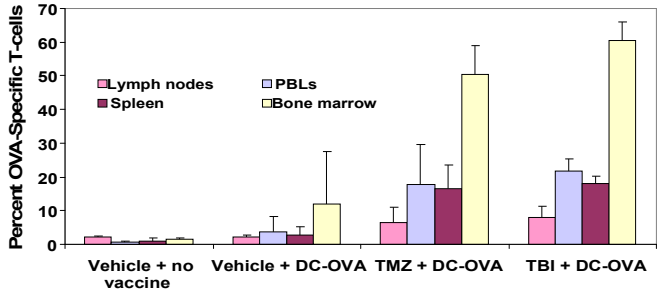


Fig. 6: C57BL/6 mice (n=5) were treated with TMZ (60 mg/kg/5d) or vehicle or given TBI (500 rad). 48 hours later mice were vaccinated with DC electroporated with OVA RNA (5 x 10^5 DC i.d.). Peripheral blood was sampled bi-weekly for precursor frequency of OVA-specific T cells and after 12 weeks, lymph nodes, bone marrow, spleen and peripheral blood were examined for memory OVA T cells ($CD44^{hi}$ x $CD43^{lo}$ x OVA tetramer ^{pos}). Results demonstrated that TMZ pre-treatment dramatically enhanced the expansion of OVA-specific memory T cells in all compartments (P<0.005).

DC therapy led to an overall increase in antigen-specific T cells producing IFNγ or expressing cytolytic function (CD107 expression) in treated patients, but was unable to increase antigen-specific polyfunctional immune responses (T cells simultaneously secreting IFNγ, IL-2, TNFα, and expressing surface CD107) which have recently been shown to be important in mediating anti-viral and anti-tumor immunity **(Figs. 7-8)**.[20-24] However, polyfunctional immune responses were restored in these patients cells using *ex vivo* activation with RNA-pulsed DCs **(Figs. 7-8)** demonstrating that autologous lymphocyte transfer of *ex vivo* activated T cells (xALT) may be a mechanism for overcoming functional deficits that persist *in vivo* in patients with malignant brain tumors.

Based on these observations, we have initiated a clinical trial exploring DC + xALT therapy during recovery from NMA and MA conditioning in children with reMB/PNET (Re-MATCH Protocol, FDA IND-BB 14058, UF IRB 128-2013; PI: Duane A. Mitchell). We have successfully expanded T cells to clinical-scale (>3 x 10^7 cells/Kg, dose level 2 of 3) using tumor RNA-pulsed DCs from 10 out of 11 subjects in our phase I portion of the trial.

The capacity to amplify the tumor RNA for pulsing dendritic cells has been demonstrated in prior published studies from freshly resected tumor specimens and micro-dissected archival histologic tumor sections for loading of DCs from a variety of tumor types including colorectal tumors **(Fig. 9),** renal carcinomas, melanomas, and pediatric and adult brain tumors **(Fig 10)**. We have successfully amplified tumor RNA to clinical scale (>1 mg of polyAAA+ RNA) from as few as 500 sorted glioma tumor cells, including CD133+ putative 'brain tumor stem cells' (BTSC). We have demonstrated the use of unfractionated tumor RNA in an EAE-susceptible preclinical glioma model to be efficacious and without toxicity,[25,26] and clinical studies using tumor RNA pulsed DCs in pediatric patients with malignant brain tumors have been well-tolerated and shown to be capable of inducing anti-tumor immunity.[27-29] We anticipate, however, that with increasingly potent anti-tumor immunity the risk of induction of intolerable autoimmunity will likely also increase. We have therefore explored substractive hybridization (SH) of normal brain antigens from total tumor RNA preparations as a means to enrich amplified tumor RNA for tumor-specific transcripts. This provides a potential added safety measure should use of unfractionated total tumor RNA preparations prove to be toxic in treated patients.

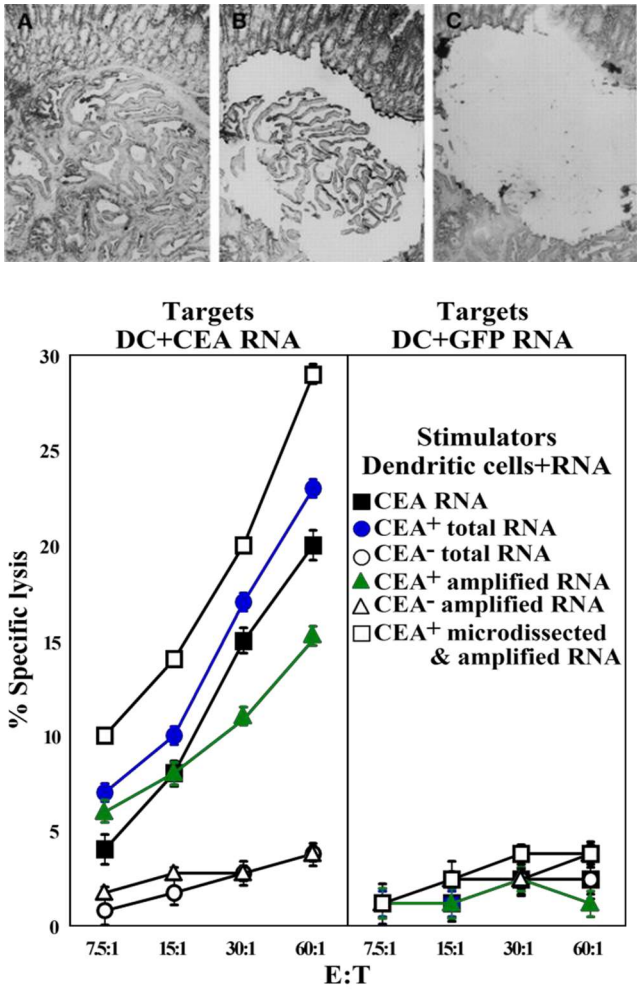


Fig. 7: Induction of cytotoxic T cell responses in vitro using DC pulsed with amplified tumor RNA: Microdissection of a colorectal metastatic nodule from a frozen section. A liver metastasis was resected for curative intent from a patient with CEA- positive colorectal cancer. Frozen sections were cut at 20 nm in a cryostat mounted on glass slides and stained with H&E. A single tumor nodule can be seen in A, surrounded by normal tissue.
Microdissection was performed using an inverted microscope and an attached mechanical micromanipulator for manipulating a glass capillary tube, which is used as a cutting tool. Under x40-x400 magnification, tumor cells are separated from benign tissue by first removing the normal cells adjacent to the tumor nodule (B) and then collecting the tumor tissue in the middle (C). Thirty-five tumor nodules were isolated and pooled from two slides, and RNA was extracted using phenol/chloroform and ethanol precipitation. RNA yield, determined by UV absorption, was 2.24 µg. The integrity of the RNA was confirmed by agarose gel electrophoresis under denaturing conditions and ethidium bromide staining (data not shown).

Fig. 8: Bottom panel: Induction of a primary, CEA-specific CTL response in vitro using DCs transfected with RNA amplified from microdissected colorectal tumor cells. PBMCs from a healthy volunteer were stimulated with autologous DCs transfected with various RNA preparations and tested for the presence of CEA- specific CTL. DCs transfected with in vitro synthesized CEA RNA or GFP RNA were used as specific and nonspecific targets, respectively. For stimulations, DCs were transfected with in vitro transcribed CEA RNA, RNA isolated from a CEA+ cell line (SW1463), a CEA- cell line (KLEB), RNA amplified from SW1463 and KLEB cells, and RNA amplified from the microdissected tumor cells. These results are representative of two experiments; bars, SD. Figure taken from (Boczkowski and Nair et al., Cancer Res. 2000; 60(4):1028-34)

1.4 Pre-clinical Experience

Pre-Clinical Results Against Intracerebral Tumors

In our laboratories and those of others, systemic immunization using DCs co-cultured with uncharacterized tumor homogenate,[30] whole tumor RNA,[31] unidentified peptides eluted from tumor cells by gentle acid washing,[32] or a distinct peptide encompassing the tumor-specific EGFRvIII (Epidermal Growth Factor Receptor v. III) mutation[33] have been shown to induce humoral and cell-mediated systemic immune responses and to prolong the survival of mice with intracranial brain tumors. We have used a strain of mice (VMDk) that is susceptible to experimental autoimmune encephalitis (EAE) to demonstrate the safety and efficacy of total tumor RNA pulsed DCs in mediating potent antitumor immune responses and regression of established tumor that has prolonged survival in treated animals.[25,26] Preclinical efficacy and toxicity data in this strain of mice has been used in support of several FDA and IRB approved IND applications investigating DC based immunotherapy in patients with malignant brain tumors (FDA INDs 9944, 12839, 13240, 13630 and Duke IRB protocols 4040, 3877, 0580, 0581, 0947, 3108, and 6677; PI: Duane A. Mitchell, M.D., Ph.D.).

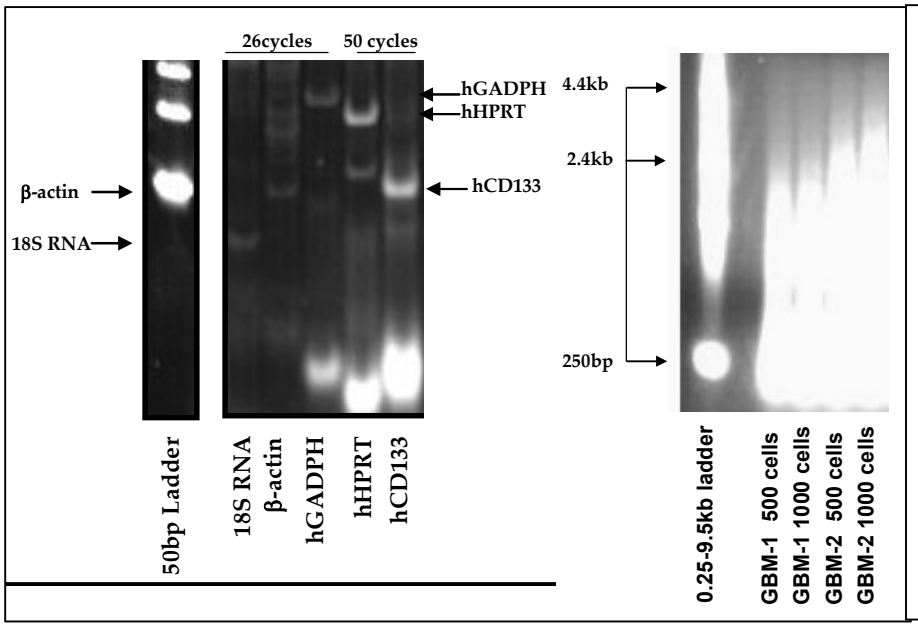


Fig. 9: Amplification of tumor RNA to clinical scale from freshly resected glioma specimens. RNA was extracted from 500 and 1000 isolated tumor cells from a resected pediatric glioma and subjected to reverse transcriptase cDNA synthesis using oligo dT primers and a cap-switch 5' for 2nd strand cDNA synthesis. Full-length cDNA libraries were amplified by PCR using a 3' oligo dT primer and 5' cap-switch oligo containing a T7 polymerase initiate site. Presence of housekeeping genes (18S RNA, b-actin, GAPDH, and HPRT) was analyzed using 5' primers (exon 1) as well as presence of the brain tumor stem cell marker CD133. *in vitro* transcribed mRNA (right panel) showed wide range of RNA transcripts (250bp to > 4.0 Kb).

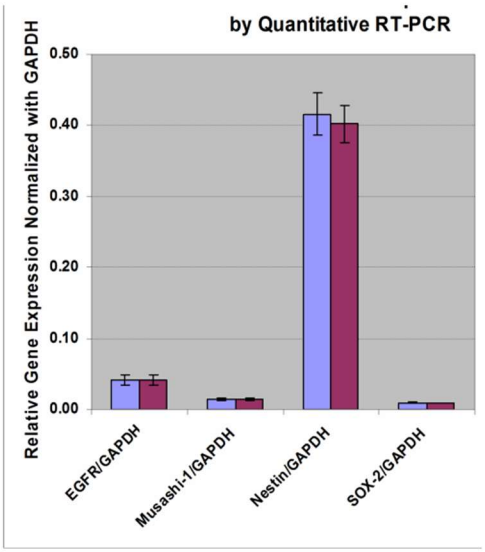


Fig. 10: High fidelity maintenance of gene expression in total tumor RNA after amplification. In order to test the fidelity of RNA amplification, real-time comparative PCR was conducted on tumor RNA from a resected glioblastoma specimen using four separate RNA extractions from aliquoted tumor cells and four separate amplification reactions. The expression levels of tumor associated transcripts (EGFR, Musashi-1, nestin, SOX-2) were normalized against GAPDH. Experimental results demonstrated a high degree of reproducibility in maintenance of gene expression profile after amplification (30 PCR cycles). We have found that 20-25 cycles of cDNA library amplification is sufficient to produce clinical-scale quantities of tumor RNA from 5 out of 5 brain tumor specimens evaluated (4 adult GBM and 1 pediatric malignant glioma).

1.5 Clinical Experience

1.5.1 Lymphopenia and Homeostatic Proliferation

We have gained considerable experience in the induction of anti-tumor immunity in adult patients with newly-diagnosed GBM receiving cycles of TMZ as adjuvant chemotherapy. Prolonged therapy with TMZ is also associated with an increased incidence of Pneumocystis carinii pneumonia, and as a result, patients treated with TMZ are widely believed to be profoundly immunosuppressed.[34,35] Our preliminary data, however, demonstrates that while TMZ administration abolishes pre-existing immunity to recall antigens in patients with GBM, it does not prevent induction of *de novo* protective immunity against tumor antigens during vaccination.

We believe that the potent and specific anti-tumor immune responses generated in our patients are the result of a direct competitive advantage enjoyed by B- and T-cells recognizing unique antigens contained within a vaccine during the hematopoietic recovery from the profound lymphopenia induced by myeloreductive chemotherapy. Although counterintuitive, our murine models, and those of others, confirm that vaccine-induced immune responses can be significantly enhanced when given during the recovery from lymphodepletive treatment regimens.

Additionally, our preliminary data demonstrate that in mice and humans, increasing lymphodepletive doses of TMZ led to progressively higher antibody titers and antigen-specific T cell frequencies in response to vaccination that can ultimately exceed a precursor frequency of 80% and are capable of inducing superior antitumor responses. Similarly, as we dose-intensified the chemotherapy treatment regimen in patients with GBM and reached Grade III lymphopenia routinely, tumor-specific immunological responses were dramatically enhanced compared to less myelosuppressive chemotherapy doses. While vaccine-induced responses were dramatically enhanced during lymphodepleting chemotherapy administration in patients with GBM, immunologic recall responses, for example to *Candida*, were rapidly abolished during treatment cycles in these patients, demonstrating that the immunosuppressive effects of chemotherapy can be dramatically overridden by repetitive vaccination in patients with malignant brain tumors.

Although this proposal argues that the profound lymphopenia induced by escalating doses of myeloablative chemotherapy may actually enhance promising immunotherapeutic approaches like the one described above in adult patients with GBM, there is already a strong rationale for use of high-dose alkylator based therapy against solid tumors. The steep dose- response relationships demonstrated by other alkylating agents such as carmustine, cyclophosphamide, or melphalan suggests that efforts to maximize drug levels may result in improvement in therapeutic outcome.[36] In fact, response rates can exceed 65% in patients with MG with carmustine when dose escalation is supported by hematopoietic stem cells (HSCs).[37,38] This rationale has supported the routine use of HDC+HSC in pediatric patients with recurrent and high-risk brain tumors. Thus immunotherapy targeting HGGs conducted in children during hematopoietic recovery from diTMZ in order to enhance the efficacy of a novel immunotherapeutic strategy. Interestingly, an independent benefit on the immune response might be conferred by the HSC transplant itself. HSC support has been shown in a murine model to cause an independent and robust expansion of tumor-specific effector $CD8^+$ T cells after adoptive transfer into myeloablated hosts.[39] Our preclinical studies have demonstrated that HSCs can enhance T cell trafficking to intracranial tumors and facilitate immunologic tumor rejection (Flores et al. Oncoimmunology 2015).

ACTION

Following periods of lymphopenia, there is a homeostatic proliferation of the host's remaining lymphocytes which is **designed to recover normal lymphocyte counts.**[40] Probably as a result of a surge in cytokines (IL-7, IL-15) in response to lymphopenia, lymphocytes undergoing homeostatic proliferation enjoy a **reduced activation threshold**[40,41] and differentiate **directly into effector memory T cells capable of rapid and intense response to antigen.**[42] Still, lymphocytes must encounter their cognate antigen and compete for limiting amounts of these homeostatic cytokines to proliferate even under these conditions.[40] Thus, B- or T cells specific for antigens that predominate during this recovery period, like those provided in the form of a vaccine, have a **competitive advantage and become disproportionately over-represented in the recovering lymphocyte** population, both in murine models[43,44] and in humans.[45] These skewed homeostatic responses have been shown to enhance antitumor immunity[43,44,46] but can also increase the risk of autoimmunity.[47,48]

Leveraging this principle, Rosenberg and colleagues used intentional non-myeloablative lymphodepletion to enhance the preferential expansion and maintenance of adoptively transferred, tumor-specific T cells. This has resulted in dramatic clinical responses[49-53] along with some autoimmune toxicity in patients with advanced malignant melanoma.[54,55] These studies have shown that under these conditions, transferred T cells can expand dramatically in the lymphopenic host to constitute up to 90% of the host's T cell repertoire and can be maintained for months following transfer.[54,56] These studies demonstrated that clinical regression of systemic disease correlates with the frequency of tumor-specific T cells achieved in the peripheral blood and persistence of these cells *in vivo.*[49-53,57] Subsequent studies in murine models demonstrated this anti-tumor effect was also significantly enhanced by myeloablative conditioning regimens coupled with autologous HSC support. Interestingly, HSCs were found to confer an enhancing effect on the *in vivo* expansion and persistence of tumor-specific lymphocytes transferred simultaneously with HSCs independent of the effects of lymphopenia.[39] Pursuant to these findings, Rosenberg and colleagues proceeded to evaluate myeloablative conditioning regimens coupled with PBSCT in patients with malignant melanoma receiving adoptive cellular therapy. Adoptive lymphocyte transfer was also conducted in the peri- transplant period within 24hrs of PBSCT demonstrating feasibility and safety in humans of this approach.[10] As demonstrated in murine studies, this conditioning regimen enhanced anti-tumor responses in patients with refractory metastatic disease, resulting in increased objective clinical responses from 30-50% of patients with nonmyeloablative regimens to over 70% in patients receiving myeloablative conditioning coupled with PBSCT.

The mechanisms by which lymphodepletion leads to an enhancement of immune responses in humans are not well elucidated but elegant murine studies have implicated the following important processes:

- Increased in production of homeostatic cytokines such as IL-7 and IL-15 that drive lymphocyte proliferation;[58]
- Decreased competition with adoptively transferred tumor-specific lymphocytes through removal of "cytokine sinks" consisting of host lymphocytes and NK cells that decrease the bioavailability of growth factors;[59]
- Removal of CD4+CD25+FOXP3+ Tregs that attenuate anti-tumor immunity;[60]
- Increased toll-like receptor agonistic signals and inflammatory cytokines through release of gut microbial antigens such as endotoxin during damage to gut endothelium by myeloablative therapy;[61]
- Direct enhancing effects of hematopoietic stem cell transplant on the *in vivo* expansion and function of adoptively transferred lymphocytes.[62]

ACTION

The source of anti-tumor lymphocytes for human studies in melanoma have been polyclonal populations of autologous CD4+ and CD8+ tumor-infiltrating lymphocytes (TILs) expanded from resected melanoma deposits. While TILs are a readily expandable source of lymphocytes from accessible melanoma lesions, no published studies have demonstrated capacity to reliably expand TILs with anti-tumor activity from malignant brain tumors. We have demonstrated in several published studies, however, the capacity to induce anti-tumor lymphocytes *in vitro* and *in vivo* using tumor RNA-pulsed DCs from a variety of human and murine tumors, including malignant brain tumors.[25,26,31,63-71]

1.5.2 RNA-Loaded Dendritic Cells

In this proposal, we will investigate the potential of DCs loaded with a broad range of antigens derived directly from HGG tumor tissue as a strategy to effectively expand tumor-specific lymphocytes *in vitro* for adoptive transfer after non-myeloablative chemotherapy coupled with HSCs. Continued expansion of anti-tumor T cells *in vivo* will be attempted with intradermal total tumor RNA-loaded DC vaccines throughout hematopoietic recovery in enrolled subjects.

The use of RNA to encode tumor antigens for DCs was pioneered in Dr. Nair's and Gilboa's laboratory, but the ability of mRNA-loaded DCs to stimulate potent anti-tumor immunity has been independently confirmed in murine and human systems.[66,72-76] In fact, there is accumulating evidence that RNA transfection represents a superior method for loading antigens onto DCs.[74,77] This novel and innovative approach to DC antigen loading has multiple conceptual advantages over other forms of antigen delivery as well. RNA-based antigen loading does not require knowledge of Major Histocompatability Complex (MHC) restriction, and responses are not restricted to single MHC haplotypes or to a narrow B- or T-cell repertoire. This diversity increases the likelihood of inducing effective and sustained anti-tumor immune responses by simultaneous activation of both CTLs and helper T cells.[78-80] Furthermore, in direct comparisons, mRNA-loaded DCs have been found to be better stimulators of antigen-specific T cells than other approaches.[77] Finally, RNA also carries a significant safety advantage, not possessed by other nucleic acid or viral vectors, in that it cannot be integrated permanently into the host genome.

1.6 DC + ALT Therapy

1.6.1 RNA loaded DC vaccines and amplified tumor RNA (preclinical and clinical studies)

We have shown use of RNA-pulsed DCs to be a versatile platform for activating tumor-specific T cells in vitro and in vivo in several murine and human systems. Several clinical trials have been conducted by our team demonstrating the feasibility and safety of tumor RNA- pulsed DCs in human patients. While specific tumor-associated antigens such as carcinoembryonic antigen (CEA), telomerase (TERT), melanoma antigens (MAGE, TRP-2), and human CMV pp65 have all been utilized as RNA-encoded antigens in our DC trials, studies have demonstrated that the majority of endogenous anti-tumor immune responses in patients with malignancy are against unidentified, patient-specific antigens.[81] The use of total tumor RNA pulsed DCs to expand tumor-specific lymphocytes allows for these patient-specific antigens to be targeted, however, sufficient tumor tissue for clinical-scale vaccination is not always readily available. Our laboratory has utilized amplification of total tumor RNA with reverse-transcriptase primed PCR to generate cDNA library templates encoding for the antigenic content of tumor cells from as few as 500 starting tumor cells. Through inclusion of a T7 RNA polymerase binding site in the 5' primer used for amplification, total tumor RNA can be readily generated through in vitro transcription after cDNA

amplification. Using such techniques we have been able to generate sufficient RNA for clinical DC vaccine preparations from colorectal tumors, renal carcinoma, and pediatric and adult brain tumor specimens using excess tumor material harvested during surgical resection.[65,70,82]

1.6.2 Amplified Total Tumor RNA from human malignant brain tumors

While expansion of tumor cells using in vitro culture is feasible, primary brain tumor cells are often difficult to propagate and gene expression microarray analysis has demonstrated that the majority of tumor specific genes expressed in vivo are not recapitulated within in vitro propagated tumor cells. Furthermore, we have demonstrated in murine intracranial glioma models that a significant shift in brain tumor gene expression is induced in response to host anti- tumor immunity.[83] This strongly suggests that the antigenic content of tumor cells propagated in vitro will be significantly different than in vivo propagated tumors, and thus the relevance of in vitro propagated tumors as an antigenic source for immunotherapy is questionable. This observation prompted us to investigate the capacity to amplify the RNA content of tumor cells isolated directly from surgically resected MG specimens in order to utilize an antigenic source more representative of the antigens expressed within patients' tumor cells in vivo. Our laboratory has successfully amplified tumor RNA from murine and human carcinomas and demonstrated the capacity to elicit effective cytotoxic anti-tumor responses using DC pulsed with amplified tumor RNA.[65,82] Based on current clinical protocols at our institution utilizing total tumor RNA pulsed DCs,[84] we have determined a production requirement for clinical use of up to 750ug of amplified tumor mRNA per patient. We have successfully amplified tumor mRNA to clinical scale (over 1mg) from as few as 500 astrocytoma cells from resected human glioma specimens from adult and pediatric brain tumors Verification of full-length cDNA templates was conducted using real-time PCR analysis of housekeeping genes (β-actin, HRPT, GADPH, 18S RNA) and stem cell markers (nestin, SOX2, musashi-1, EGFR), 5' gene specific primers spanning exon- intron splice junctions, gel electrophoresis demonstrating mRNAs spanning from 0.2Kb to over 5Kb in length, and Northern blot analysis demonstrating full-length β-actin mRNA in amplified tumor samples. Enrichment of tumor antigens was performed using subtractive hybridization of excess pooled normal brain RNA from tumor RNA prior to amplification and in vitro RNA synthesis and verification of enrichment of tumor-associated genes by comparative real-time PCR.

1.7 Autoimmune Encephalomyelitis

While DC vaccinations administered to patients with GBM have been safe in our clinical trial experience and others,[85-87] they are also quite capable of initiating significant autoimmune responses in mice and humans.[88,85] In light of the expression of normal and fetal brain antigens on brain tumor tissue[89-92] and human glioma cell lines,[93] active immunization of patients with malignant brain tumors risks inducing an uncontrolled autoimmune response against normal CNS antigens that is similar to experimental autoimmune encephalomyelitis (EAE). The susceptibility of humans to the induction of EAE was demonstrated accidentally when patients were vaccinated against rabies with spinal cords from rabbits that were infected with the rabies virus.[94-98] Lethal EAE has also been induced in non-human primates after vaccination with unrefined human GBM tissue.[99] It is likely, then, that with increasingly potent immunotherapies that target antigens shared by tumor and normal tissue, that the risk for autoimmunity against normal brain tissue will increase.

To focus the immunologic response against tumor-specific antigens and potentially reduce the risk for autoimmunity, subtractive hybridization can be conducted to generate a template for mRNA synthesis that consists of cDNAs expressed preferentially in malignant tumor cells. Such approaches can also aid in future

efforts in antigen discovery should the targeting of tumor cells prove advantageous. This is important because most well-characterized tumor antigens are over- expressed normal proteins which have triggered immunologic tolerance to some degree. This compromises their effectiveness as tumor rejection antigens and poses a risk of autoimmunity if effectively targeted.[81,100] Conversely, tumor-specific antigens derived from mutations in somatic genes are less prejudiced by central tolerance and less likely to be associated with autoimmunity. Some studies also suggest that the autonomous immune response to human tumors is dominated by such neoantigens.[101] The stringency of SH techniques including suppression subtractive PCR amplification techniques can be utilized to enrich cDNA libraries for uniquely expressed and mutated transcripts from tumor cells using normal brain RNA as a subtractor substrate.[102] Such methods are under active development in our laboratory and may be utilized to enhance either the safety profile or tumor-specific immune response in subsequent clinical studies.

1.8 Rationale

Despite brain tumors being the leading cause of cancer deaths in children and the considerable advances in immunologic targeting of adult brain tumors, studies examining immunotherapy for pediatric brain tumors are markedly lacking except for a notable few reports. Efforts in targeting pediatric brain tumors are hindered somewhat by the relatively small number of clinical cases compared to adult cancers, the lack of syngeneic murine models of common pediatric brain tumors in which immunotherapy efforts can be evaluated in preclinical studies, and the significant regulatory hurdles in conducting experimental therapies in the pediatric setting.

Despite these limitations, the use of NMA TMZ chemotherapy in pediatric patients with HGGs affords an ideal setting in which to leverage the recent findings by our laboratory and others in the benefits of treatment-induced lymphopenia in enhancing immune responses to tumor immunotherapy.

Three notable studies have demonstrated the safety and potential efficacy of immunotherapy in children with malignant brain tumors.

Ashley and colleagues conducted a phase I study of 9 pediatric patients with recurrent brain tumors using monocyte-derived DCs pulsed with tumor RNA isolated from resected tumor specimens.[27] DCs were derived from monocytes after 7 days of culture with IL-4 and granulocyte-macrophage colony-stimulating factor (GM-CSF), incubated with RNA for 45 minutes in serum-free media, washed and then cyropreserved until administration. DC vaccines were administered intradermally and intravenously in treated patients. The feasibility and safety of tumor RNA pulsed DC vaccines was demonstrated in this study with 7 of the 9 enrolled patients receiving RNA-pulsed DC vaccines. 1 patient demonstrated a partial response to therapy as assessed by MRI and 2 patients showed disease stabilization. Tumor-specific immune responses were demonstrated after vaccination in 2 of 7 patients.

A subsequent study by the same group in 11 pediatric patients with newly-diagnosed, Stage 4 neuroblastoma, evaluated tumor RNA-pulsed DC vaccines after HDC+PBSCT.[28] The patients received two courses of carboplatin induction therapy followed by standard chemotherapy, surgery, radiation, and HDC + stem cell rescue. DC vaccines were administered intravenously and intradermally beginning 6 weeks after stem cell transplantation and were prepared as described in the previous study. The investigators noted immunologic impairment in recall responses and proliferative responses in patients at 6 weeks post HDC+PBCT. Seven of 11 enrolled patients were successfully treated during this study. The safety and feasibility of the total tumor RNA DC vaccines after HDC + PBSCT was demonstrated by this study but no clinical or radiographic responses were observed.

ACTION

Importantly, Ashley and colleagues demonstrated the safety and feasibility of total tumor RNA- pulsed DCs in pediatric patients with recurrent and primary brain tumors in these studies. Recent advances, however, in our understanding of DC biology and their use in anti-tumor immunotherapy have made even the limited clinical responses observed in the first study somewhat encouraging. These significant differences in the previously published reports and the methods we will use in this study are as follows:

Ashley and colleagues used immature DCs in their study which have now been shown to be vastly inferior in the induction of immunologic responses. Our DCs are matured with a combination of IL-1β, TNFα, IFNγ, and IL-6.

Route of injection comparisons have shown that intravenous injection of DCs in humans is in many cases tolerogenic and the preferred route for induction of immunity is intradermal. Our vaccines will be administered through intradermal injection only.

We have shown electroporation of RNA into DCs to be 100 to 1000 times more efficient than co-incubation of DCs with "naked RNA." The labile nature of RNA results in degradation with a half-life of seconds to minutes, thus electroporation to deliver RNA directly into DCs is far superior in mediating gene expression and anti-tumor immunity.

We and others have shown that the early recovery period after lymphodepletion is the most effective period for enhancing immunity to vaccination and adoptive T cell transfer and thus we will administer DC+xALT therapy during the post-transplant period (24 hours after PBSCT) as has been done in studies at the NCI using myeloablative therapy and adoptive cellular therapy in patients with melanoma.

Ashley and colleagues administered 3 to 5 vaccines to their patients, while in this trial, we will continually
vaccinate patients throughout the recovery period of lymphopenia and normalization of T cell function as we have shown capacity to increase immunologic responses dramatically over time in vaccinated patients with newly-diagnosed GBM. In fact, significant anti-tumor immune responses have often occurred in our patients only after repetitive monthly vaccinations.

We believe these advances will significantly enhance the potential clinical impact of our proposed therapeutic approach. Admittedly, as more potent vaccination platforms are advanced, the risks of inducing intolerable autoimmunity will also likely increase, and thus careful evaluation of the safety of these potent vaccination platforms will be necessary. Our proposed phase I study will establish the safety of DC + xALT therapy using amplified total tumor RNA pulsed DC vaccines to expand tumor-specific lymphocytes in vitro for adoptive transfer and maintenance in vivo with DC vaccination.

Improving survival in pediatric patients with HGG will require combined therapy with resection, chemo-radiotherapy, followed by adjuvant chemotherapy (Temozolomide) and potentially novel approaches including immunotherapy. Based on the preclinical and clinical experience with adoptive cellular therapy summarized above, we believe there is the potential to induce immune responses (anti-tumor immunity) in children with brain tumors (high-grade gliomas) utilizing the immunotherapy strategy proposed in this trial. We have developed the use of total tumor RNA (TTRNA) loaded DCs as an effective platform for induction of tumor-specific lymphocytes and will use this approach to expand lymphocytes specific for HGG in these children undergoing primary resection, radiation and adjuvant chemotherapy. Administering DC vaccine following temozolomide as standard treatment in children with HGG is also expected to augment tumor-specific immune responses during the hematopoietic recovery phase of each cycle of chemotherapy. In order to maintain T cell expansion and function, serial vaccinations with TTRNA-DCs will be administered. Based on durable remissions achieved using adoptive cellular therapy

after lymphodepletive regimens in adults with advanced stage melanoma, we believe this treatment platform may also improve outcomes for children with HGG.

1.9 Re-MATCH phase 1 clinical trial of adoptive cellular therapy in recurrent medulloblastoma (reMB) and PNETs

Nine subjects with reMB/PNET were treated during the phase 1 trial. Seven were evaluable for safety evaluation. Of the two determined non-evaluable, one subject failed to generate T cells and was treated with DC vaccine only and one subject suffered disease progression prior to the DLT evaluation period. There were no immunotherapy related DLTs. A single patient experienced a grade III asymptomatic elevation in liver alkaline phosphatase detected during routine clinical follow-up at nine months post immunotherapy treatment. The etiology was not determined and resolved spontaneously without medical intervention. This AE was ruled as possibly related to study drug since no attributable cause was determined. Overall, the treatment was deemed feasible and safe for pediatric patients with recurrent MB/PNET. A maximum achievable dose of T cells (3 x 10^7 cells/Kg) was reached in treated subjects.

Recent analysis of the progression-free survival outcomes for patients enrolled on the phase 1 trial and treated with chemotherapy followed by adoptive cellular therapy compared to a historical cohort of patients with recurrent medulloblastoma treated with chemotherapy only demonstrated promising clinical efficacy for the cellular therapy regimen. Median progression-free survival on Re-MATCH phase 1 patients is 21 months vs 10.5 months historical control (P=0.07) and 12-month PFS is 67% vs 33%, 24-month PFS is 33% vs 0%. A single-arm phase 2 trial is underway using the maximal achievable dose of T cells to assess PFS in 35 subjects.

1.10 Improved T cell Expansion

Under support of a research grant from the Pediatric Cancer Foundation, we have explored the capacity to enhance the expansion of antigen-specific T cells using the RNA-pulsed DC platform through employing a rapid expansion protocol (REP) developed at the National Cancer Institute for expansion of melanoma-reactive tumor-infiltrating lymphocytes. The REP employs irradiated allogeneic PBMCs as feeder cells and low-dose anti-CD3 monoclonal antibodies (OKT3) and IL-2 for the rapid expansion of activated lymphocytes in culture. We have validated the capacity to expand lymphocytes stimulated by RNA-pulsed DCs 200-500 fold using the REP protocol and reliably achieve greater than 1 x 10^10 T cells with an input of 1 x 10^8 activated lymphocytes. We have additionally demonstrated that the utilization of IL-7 and IL-21 during co-culture of T cells with RNA-pulsed DCs prior to REP leads to a greater generation of central memory antigen-reactive T cells which have been shown to be superior in anti-tumor efficacy. Further investigation in our lab demonstrated that IL-21 alone can promote memory cell generation that was equal to that of the combination of IL-7 and IL-21, thus IL-21 will be used alone for initial T cell stimulation. This study will employ xALT utilizing our improved stimulation and expansion protocol at a target dose of 3 x 10^8 T cells/Kg.

1.11 Study Design

Primary and Secondary Objectives -The primary purpose of this study is to explore the safety of DC vaccines and ACT in patients with primary HGGs. Secondary objectives will include feasibility assessment as a proportion of enrolled subjects in which immunotherapy is successfully delivered, and immunologic responses to estimate the mean difference and the variation in INF gamma secretion between baseline and 2 weeks post-completion of immunotherapy in Group A and Group B. OS and PFS will be secondary clinical outcomes measures. We do not expect the sample size of the current study to be large enough to allow definitive conclusions, but we will use a t-test to compare gamma secretion differences (baseline – 2

weeks post-immunotherapy) between Group A and B, and Kaplan-Meier methods to compare progression-free and overall survival.

Enrolled subjects will be pediatric patients with newly-diagnosed Grade III or Grade IV HGG's. This study will establish the safety, feasibility, and immunologic effects of DC + xALT therapy with or without HSC transfer in pediatric subjects receiving dose-intensified adjuvant TMZ.

This multi-institutional clinical trial will consist of three parts: **Surgery**, **Chemoradiation,** and adjuvant **dose-intensified TMZ with Immunotherapy** (five TMZ cycles with TTRNA-DC and TMZ, cycle 6 followed by DCs and xALT +/- HSCs). Potentially eligible subjects will be enrolled on a screening consent for the sterile collection of tumor material in a manner suitable for RNA extraction, amplification, and loading of autologous DCs. Following surgical resection with confirmatory pathologic diagnosis, subjects will be enrolled in the trial after informed consent has been obtained.

Potential subjects can be screened and provide consent for tumor tissue collection at any NPCF site. If potential subjects are referred from outside physicians, and it is not feasible for the subject to travel to a NPCF site, tissue consent may be obtained by designated staff from a NPCF treating center via telephone from the subject/LAR in accordance with institutional policies and procedures. Once screening consent is obtained, the Coordinating Center will interact with site to obtain tumor tissue in a manner suitable for RNA extraction per UF SOP. If standard-of-care sample collection occurred within 12 weeks prior to enrollment and frozen sample remains, a subject may enroll using previously collected samples. Samples must additionally have been snap frozen within 20 minutes of collection and kept in a -70/-80 freezer.

Once it has been determined that an adequate amount of tumor tissue was collected and a pathological diagnosis of HGG has been confirmed, the subject must be evaluated at a NPCF treatment site (UF Health Shands Children's Hospital, Childrens of Alabama or Children's National Medical Center). The NPCF treatment site will confirm eligibility, obtain consent for the treatment phase of the study, and administer all subsequent therapy.

Up to 24 subjects will be screened to treat 12-18 evaluable subjects, six subjects into Group A followed by six subjects into Group B. The first six evaluable subjects enrolled will be for Group A evaluating the safety of TTRNA- DC vaccines and TTRNA-xALT at a target dose of 3 x 10^8/kg. If 2 of 6 patients experience immunotherapy related DLT following administration of 3 x10^8/kg x-ALT, an additional 6 patients will be enrolled on Group A to receive x-ALT at a dose of 3 x10^7/kg. The trial will stop if 2 of 6 patients develop DLT at this dose level. Once MTD is identified in Group A patients, 6 patients will be treated in Group B using this MTD for x-ALT infusion.

1.12 On-Study Tests and Procedures

Timepoint	Date	Study Requirements
Screening		Tissue screening consent
Surgery		Resection
		Tumor tissue for extraction of total tumor RNA
		MRI brain (and spine if applicable) with & without contrast post-surgery
Post-Surgical Resection		Inclusion & exclusion criteria
		Treatment consent
		Medical history, physical & neurological exam

ACTION

		Performance Status: KPS or LPS
		CBC w/ differential, PT/INR, PTT, comprehensive metabolic panel
		β-HCG pregnancy test (serum)
Mobilized Leukapheresis		CBC w/ differential, CD4/CD8 ratio, comprehensive metabolic panel, research labs within 24 hrs prior to leukapheresis
		β-HCG pregnancy test (serum or urine)
		Infectious disease testing to include, at a minimum: HIV, Hepatitis B, Hepatitis C & CMV
		Medical history, physical & neurological exam
Chemoradiation		TMZ 90mg/m2 daily for up to 49 days. Taken 1 hr prior to radiation and each morning on non-xRT days
Prior toTMZ Cycle 1 and on day of Dendritic Cell Vaccine		Medical history, physical & neurological exam
		CBC w/ differential, CD4/CD 8 ratio, comprehensive metabolic panel
		β-HCG pregnancy test (serum or urine)
		Within 24 hours prior: Research labs
		Performance Status: KPS or LPS
Non-Mobilized Leukapheresis		CBC w/ differential, CD4/CD 8 ratio, comprehensive metabolic panel, research labs within 24hrs prior to leukapheresis
		β-HCG pregnancy test (serum or urine)
		Infectious disease testing
		Medical history, physical & neurological exam
TMZ Cycles 2-5 and on day of Dendritic Cell Vaccine		Medical history, physical & neurological exam
		Within 24 hours prior to vaccine and within 1 week of starting TMZ cycles: CBC w/ differential, CD4/CD 8 ratio, comprehensive metabolic panel
		β-HCG pregnancy test (serum or urine)
		Within 24 hours prior: Research labs
		Performance Status: KPS or LPS
		Brain (and spine if applicable) MRI with and without contrast (within 2 weeks prior to C1, C2 and C4)
TMZ Cycle 6 and on day of DC Vaccine and xALT – Group A and B		Medical history, physical & neurological exam

ACTION

		Within 24 hours prior to vaccine and within 1 week of starting TMZ cycle: CBC w/ differential, CD4/CD 8 ratio, comprehensive metabolic panel
		β-HCG pregnancy test (serum or urine)
		Within 24 hours prior: Research labs
		Performance Status: KPS or LPS
		Brain (and spine if applicable) MRI with and without contrast (within 2 weeks prior to C6)
Post ALT infusion		Day 1, Day 4 (+/- 1 day): CBC w/ differential, CD4/CD 8 ratio, comprehensive metabolic panel and research labs (if feasible)
TMZ Cycle 6 – Group B Only		HSC infusion (12-36-hours prior to DC vaccine and xALT)
Bi-Monthly follow-up +/- 1 month for one year and then per PI discretion (minimum of every 6 months) until tumor progression or death due to any cause		Medical history, physical & neurological exam
		Contrast-enhanced MRI scan
		CBC w/ diff, CD4/CD 8 ratio, research labs (if feasible)
		Brain (and spine if applicable) MRI with and without contrast

1.13 Correlative Studies Background

Our preclinical studies have demonstrated that adoptive cellular therapy in tumor-bearing hosts results in the in vivo expansion and persistence of specific TCR V beta families that are associated with tumor rejection. Additionally, the transfer of HSCs leads to a greater expansion and altered diversity of T cells in the treated hosts. We will examine the TCR V beta gene usage in ex vivo expanded lymphocytes prior to adoptive transfer as well as in the peripheral blood of treated subjects prior to leukapheresis, during cycles of TMZ and each DC vaccination, and prior to DC + xALT therapy and at follow-up visits until progression to determine if clonal expansion of T cells are observed during immunotherapy and if HSC transfer alters the kinetics of expansion and the diversity of TCRs observed in treated subjects.

2. PATIENT SELECTION

2.1 Inclusion Criteria Screening

- Age 3-21 years of age.
- Has undergone or will undergo surgical resection of suspected HGG

Post-Surgical Resection

- Histologically confirmed WHO Grade III or IV malignant glioma.

ACTION

- Karnofsky Performance Status (KPS) of ≥ 60% (KPS for ≥ 16 years of age) or Lansky performance Score (LPS) of ≥ 60 (LPS for < 16 years of age) assessed within 2 weeks prior to registration.
- Bone Marrow:
  - ANC (Absolute neutrophil count) ≥ 1000/µl (unsupported - no growth factors within 3 days)
  - Platelets ≥ 100,000/µl (unsupported- defined as no platelet transfusion within 3 days)
  - Hemoglobin > 8 g/dL (may be supported)
- Renal:
  - Serum creatinine ≤ upper limit of institutional normal
- Hepatic:
  - Bilirubin ≤ 1.5 times upper limit of institutional normal for age.
  - SGPT (ALT) ≤ 3 times upper limit of institutional normal for age.
  - SGOT (AST) ≤ 3 times upper limit of institutional normal for age.
- Signed informed consent according to institutional guidelines.
- Patient or patient guardian consent to PBSC harvest following registration
- Subjects of childbearing or child-fathering potential must be willing to use medically acceptable forms of birth control while being treated on study and for a minimum of six months after receiving study therapy (last date of DC or ALT/HSC administration).

  Pregnancy: The effects of Temozolomide on the developing human fetus are unknown. For this reason and because antineoplastic agents as well as other therapeutic agents used in this trial are known to be teratogenic, women of child- bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of *DRUG* administration.
- Subjects with post-surgical neurological deficits should have deficits that are stable for a minimum of 1 week prior to leukapheresis.

2.2 Exclusion Criteria

- Pregnant or need to breast feed during the study period (Negative serum pregnancy test required).
- Known autoimmune or immunosuppressive disease or human immunodeficiency virus infection.
- Subjects with significant renal, cardiac (congestive cardiac failure, myocardial infarction, myocarditis), pulmonary, hepatic or other organ dysfunction.
- Severe or unstable concurrent medical conditions.
- Patients scheduled to receive any other concurrent anticancer or investigational drug therapy.
- Prior allergic reaction to TMZ, GM-CSF, or Td
- Patients who are unwilling or unable to receive treatment and undergo follow-up evaluations at a Sunshine Project Consortium treatment site.

2.3 Inclusion of Women and Minorities

Our brain tumor studies are open to both males and females, and varying ethnicities and races. Patients, regardless of gender, race or ethnicity, meeting eligibility criteria may be enrolled in this trial if consent to participate is provided.

2.4 Consent/Assent Procedures

ACTION

Provisions for seeking, obtaining and documenting consent for adult subjects and assent for children and permission of their parents or guardians will be in accordance with the determinations and requirements of the IRB per 21 CFR 50. The treatment portion of this clinical trial involves greater than minimal risk but holds out the prospect of direct benefit to individual subjects (21 CFR 50.52) as described in section 1.8. In addition to parental permission, the assent of minor subjects will be obtained, when feasible, as determined by the IRB. Potential subjects can be screened and provide consent for tumor tissue collection at any NPCF site. If potential subjects are referred from outside physicians and it is not feasible for the subject to travel to a NPCF site, tissue consent may be obtained by designated staff from a NPCF treating center via telephone from the subject/LAR in accordance with institutional policies and procedures. Once screening consent is obtained, the Coordinating Center will interact with site to obtain tumor tissue in a manner suitable for RNA extraction per UF SOP. If standard-of-care sample collection occurred within 12 weeks prior to enrollment and frozen sample remains, a subject may enroll using previously collected samples. Samples must additionally have been snap frozen within 20 minutes of collection and kept in a -70/-80 freezer.

Once it has been determined that an adequate amount of tumor tissue was collected and a pathological diagnosis of HGG has been confirmed, the subject must be evaluated at a NPCF treatment site (UF Health Shands Children's Hospital, Children's Hospital Los Angeles, or Nationwide Children's Hospital). The Sunshine Project Consortium treatment site will confirm eligibility, obtain consent for the treatment phase of the study, and administer all subsequent trial-related therapy.

A multi-disciplinary team at the site (at minimum a radiation oncologist or medical oncologist) will evaluate potential subjects. During their visit, a physician-investigator and/or a research coordinator will explain the study to the subject and/or parents/guardian, using the IRB-approved consent document to guide the discussion. The informed consent document will be provided to the subject, who will be given time to read over and ask questions of the study team. If the subject agrees to participate, a signed consent form will be obtained prior to initiation of any trial-related interventions and a copy of the signed consent will be provided to the subject. Those subjects that turn 18 while on-study will be re-consented.

3. REGISTRATION PROCEDURES

3.1 Site and Investigator Registration with Moffitt Cancer Center

Only those members of the Sunshine Project Consortium registered with Moffitt Cancer Center may participate and enroll subjects on this trial. Sunshine Project Consortium Member sites are defined as those centers who have established financial agreements with Moffitt Cancer Center and who have designated a local Principal Investigator who will assume the responsibility of conduct of the trial according to Moffitt Clinical Research Network (MCRN) procedure and Federal and State regulations.

All subjects to be enrolled on this trial must be registered with the MCRN office and the Sunshine Project Coordinator prior to being enrolled at the local level. Subjects may not be treated with any agent without registration with Moffitt staff and an associated Sequence number confirmed in the Moffitt Cancer Center electronic database OnCore.

3.2 Patient Registration

When a potential subject presents to a participating site, the local investigator will confirm candidacy for screening by reviewing known inclusion and exclusion criteria. The participating site must notify the

ACTION

coordinating center to confirm slots are available for participation, and then discuss the protocol with the potential subject. Before a subject can be enrolled, it is the responsibility of the local investigator to verify, sign, and date a completed Moffitt Cancer Center Eligibility Checklist and Moffitt Cancer Center Registration sheet.

The local investigator will confirm all screening eligibility criteria are met. A signed copy of the Checklist and Registration sheet must be e-mailed, with all supporting source documentation and signed consent form, to the Sunshine Project Coordinator **prior** to a patient being enrolled and initiation of trial-related procedures.

The source documentation and registration sheets must be sent to the Sunshine Project Coordinator at Moffitt Cancer Center via email at SunshineProject@moffitt.org..
Note: No study therapy may be initiated before confirmation that the subject is eligible for screening.

3.3 General Guidelines

Following registration, subject information must be placed in the OnCore system within 24 hours of receipt of Subject Sequence Number (OnCore CRF pages: demographics, consent, eligibility, on-study, and treatment).

4. TREATMENT PLAN

Chemo-Radiation on this protocol can be administered on an outpatient basis or per institutional guidelines. Reported adverse events and potential risks are described in Section 7. No investigational or commercial agents or therapies other than those described as acceptable within the protocol may be administered with the intent to treat the patient's tumor.

4.1 Agent Administration

Drug Name	Route	Dose	Schedule	Instructions for administration
Temozolomide (TMZ) during radiation	PO	90 mg/m^2/day	Day 1 of xRT- max of 49 days	PO 1 hour prior to radiation and in AM on non-xRT days.
Dose-intensified (DI) Temozolomide	PO	See dose escalation table	Days 1-21 of 28 (+7) day cycle	PO in AM
TTRNA-loaded DCs (10 vaccines-V1-V10)	Intra-dermal (ID)	1x10^{7}cells	V1: Cycle 1 Day 22-24 V2: Cycle 1 Day 36-38 V3: Cycle 1 Day 50-52 V4: Cycle 2 Day 22-24 V5: Cycle 3 Day 22-24 V6: Cycle 4 Day 22-24 V7: Cycle 5 Day 22-24 V8: Cycle 6 Day 23-25 V9: Cycle 6 Day 37-39 V10: Cycle 6 Day 51-53	Each immunization will be administered according to **SOP-UFBTIP-128**. DC immunizations will be divided equally between both inguinal regions. A total volume of 200 µL per side will be delivered, with no more than 0.1 ml in each injection site intradermal after preparation of skin with ELMA anesthetic cream. May be administered up to 72 hours after dose-intensified TMZ. If dose-intensified TMZ is delayed within the allowable +7 day window, the vaccine administration day should be adjusted accordingly.

ACTION

*Adoptive Tumor-specific T cells (xALT)	IV	Target dose of $3x10^8$cells/kg or all available cells	Day 23-25 **Cycle 6 only**	xALT will be administered according to **SOP-UFBTIP-2132**. Cells will be given IV over 10-60 minutes through venous catheter. Pre-medicate 30–60 minutes prior to infusion with acetaminophen (10-15 mg/kg/dose) and Benadryl (1 mg/kg) to reduce infusion-related reactions. Vital signs will be assessed prior to and immediately following infusion, then every 15 minutes for 1 hour after completion of infusion.
Hematopoietic Stem Cells **(Group B Only)**	IV	Target dose of $2x10^6$ CD34+ cells/kg or all available cells	Day 22-24 **Cycle 6 only**	IV through venous catheter. Premedication, administration and monitoring will be done in accordance with institutional policies.
Sargramostim (GM-CSF)	Intra-dermal (ID)	150ug Embedded within each vaccination of TTRNA-loaded DCs	Concurrent with each DC vaccine (V1-V10) as above	
Tetanus/diphtheria (Td) vaccination	V1: IM V3, V6, V8 DC site pre-treatment: ID	V1: 5 Lf V3, V6, V8 DC site pre-treatment: 1 Lf	V1: 5 Lf V3, V6, V8 DC site pre-treatment: 1 Lf	Td vaccine #1 to be given IM. Td given as vaccine site pre-treatment will be given ID 4-24 hours prior to DC vaccine #3, #6, and #8
Filgrastim (G-CSF)	IV/SQ	10 micrograms/kg/day (max dose of 480 mcg/day)	Prior to mobilized leukapheresis and PRN until count recovery following NMA chemo (Group B) per PI and institutional policy.	

***** If 2 of 6 patients experience immunotherapy related DLT following administration of 3 $x10^8$cells/kg x-ALT, an additional 6 patients will be enrolled on Group A to receive x-ALT at a dose of 3 $x10^7$cells/kg. The trial will stop if 2 of 6 patients develop DLT at this dose level. Once MTD is identified in Group A patients, 6 patients will be treated in Group B using this MTD for x-ALT infusion.

4.1.1 Radiation Guidelines

Standard external beam RT will be administered concomitantly with TMZ. Institutional practices for administration of external beam RT for subjects with HGG may be followed. Otherwise the following guidelines should be used:

Target volume definition:

- Gross Tumor Volume (GTV): The GTV includes all gross residual tumor and/or the tumor bed as defined by MR imaging and operative report. The GTV in many cases will involve a contracted or collapsed tumor bed. Tissue defects resulting from surgical approaches will not be included as part of the GTV when not previously involved by tumor.

ACTION

- Clinical Target Volume (CTV): The CTV is meant to treat subclinical microscopic disease and will be an anatomically constrained 5-10 mm margin on the GTV, with additional expansion as necessary to encompass areas of T2/FLAIR change suspicious for tumor involvement prior to surgery. The CTV is limited to the confines of the bony calvarium, falx and tentorium where applicable and extends up to but not beyond neuroanatomic structures through which tumor extension or invasion is certain not to have occurred. When the GTV approaches the boundary of an anatomic compartment, the CTV will extend up to and include the boundary.

- Planning target volume 1 (PTV1): CTV + 3 mm

- Planning target volume 2 (PTV2): GTV + 3 mm

Total dose:
PTV1: 50.4 Gy
PTV2: 59.4 Gy (Note: 54 Gy in infants who have undergone a gross total resection)

Dose per fraction:
1.8 Gy/fx daily, 5 days per week. PTV1 and PTV2 should be delivered in sequential phases over 33 fractions.

Temozolomide during radiation

TMZ should be administered continuously from day 1 of radiotherapy to the last day of radiation at a daily oral dose of 90 mg/m^2 radiation for a maximum of 49 days.

If radiotherapy has to be temporarily interrupted for technical or medical reasons unrelated to the temozolomide administration, then treatment with daily temozolomide should continue. If radiotherapy has to be permanently interrupted, then treatment with daily temozolomide should stop.

TMZ administration and any modifications during radiation should be at the discretion of the treating physician or radiation oncologist. The total number of days and total dose as well as any missed doses will be recorded in the data management system.

4.1.2 Dose Escalation for Temozolomide Cycles 1-6 following radiation

Dose Level	Dose	Timing
-2	50 mg/m2/day	If dose level -1 not tolerated
-1	75mg/m^2/day	If Dose level 0 not tolerated
0	85mg/m^2/day	Starting dose C1 and C2
+1	100mg/m^2/day	One time dose escalation for C3-6

Cycle 1: TMZ will be started at a dose of 85mg/m^2/day for 21 days of a 28 (+7) day cycle.

Cycle 2: TMZ will be given at a dose of 85mg/m^2/day if ANC ≥ 1000 and platelets ≥ 100,000 within 4 weeks of last dose TMZ in C1. If counts do not meet criteria to start C2 within 4 weeks, wait for recovery and then decrease one dose level to -1 for C2 and continue treatment.

ACTION

Cycle 3: If dose level 0 of TMZ is well tolerated in previous cycles, increase TMZ to 100mg/m^2/day. If dose has been reduced to -1 in C2, continue the rest of cycles at 75 mg/m^2/day. If counts do not meet criteria to start C3 within 4 weeks, wait for full recovery and then decrease one dose level to -2 for C3 and continue treatment. TMZ may be held at current dose and not escalated or stopped if Principal Investigator deems necessary in view of patient safety.

Cycles 4 - 6: Continue at same dose as tolerated in previous cycles. If severe myelosuppression is experienced during these 3 cycles, dose reductions will be instituted as stated above. DI TMZ will be complete with the cycle preceding the ALT infusion (Cycle 6).

Prior to chemoradiation, enrolled subjects will undergo a mobilized leukapheresis for collection of PBSCs and PBMCs for generation of DCs. G-CSF mobilization of CD34+ HSCs will occur for both groups and leukapheresis for autologous peripheral blood stem cells (PBSCs) may be collected for up to 3 days for Group B subjects to obtain a recommended dose of > 2 x 10^6 CD34+ HSCs/kg. If unable to reach the recommended dose, all available stem cells will be infused.

Chemoradiotherapy should begin within 5 weeks +/- 1 week of surgery. Standard external beam RT will be administered concomitant with TMZ. Institutional practices for administration of external beam RT for subjects with HGG may be followed. Otherwise the guidelines from section 4.1.1 should be used. Temozolomide 90mg/m^2/day will be given continuously during radiation for up to 49 days to account for delays in radiation treatment. Modifications to dosing may be necessary due to ongoing clinical and laboratory assessment and tolerability of concomitant TMZ. These modifications may be made at treating investigator's discretion, but must be reported in database and justification for dose-modification documented.

Five weeks (+/- 1) after the completion of chemoradiotherapy, the first cycle of adjuvant TMZ will be given at a dosage of 85 mg/m^2/day orally for 21 days followed by three biweekly TTRNA-DC vaccines ($1x10^7$ cells) with GM-CSF(150µg per injection embedded within the DC vaccine) beginning on Days 22-24 of cycle 1. Study vaccine will be given intradermally at day 22-24 after the first TMZ cycle and divided equally between both inguinal regions in accordance with SOP-UFBTIP-128. All subjects will receive Td booster IM (5 Lf) at Vaccine #1 regardless of booster history.

Vaccine #2 and #3 will occur at 2 week intervals. Fourteen days (+/- 3 days) after the third biweekly DC vaccine, subjects will undergo a second leukapheresis (non-mobilized) to collect PBMCs for generation of further TTRNA-DC vaccines and for the expansion of vaccine-stimulated T cells using TTRNA-pulsed DCs (TTRNA-xALT). The collection goal for TTRNA- xALT and TTRNA-pulsed Dendritic Cell vaccine is a minimum of $5x10^9$ mononuclear cells.

4.1.3 Criteria for Starting Subsequent Cycles

A cycle may be repeated every 28 days if platelets are greater than ≥100,000 (unsupported for at least 3 days) and ANC is ≥1,000 and the patient has at least stable disease. All other non-hematologic toxicities must have resolved to baseline to move onto cycles 2 and beyond.

The second cycle of TMZ beginning after the second leukapheresis will also be administered at a dose of 85 mg/m^2/day orally for 21 days of a 28 (+7) day cycle. If no dose modifications are required, starting with cycle 3, subjects will be escalated to a dose of 100 mg/m^2/day of TMZ for 21 days of 28 (+7) day cycle. TMZ cycles 2-5 will be followed by a single monthly TTRNA-DC vaccine given on day 22-24 (+ 7).

ACTION

During Cycle 6, both Groups A and B will receive an intravenous infusion of the TTRNA-xALT ($3x10^8$ cells/kg) along with the DC vaccine between days 23-25 and an additional two biweekly TTRNA-DC vaccines. All DC vaccines will be given intradermal and divided equally to both inguinal regions. Autologous HSCs will be administered intravenously 12-36 hours prior to DC vaccines/xALT to Group B only.

Since HSCs may act synergistically to enhance the efficacy of cellular therapeutics in pediatric patients, subjects will be grouped into cohorts that receive either TTRNA-xALT alone (Group A) or TTRNA-xALT and HSCs (Group B). Subjects in Group A will receive only TTRNA-DCs and TTRNA-xALT between day 23-25 after completion of 21-day TMZ during the 6^{th} cycle. During the 6^{th} TMZ cycle, subjects in Group B will receive HSCs between days 22-24 after completion of TMZ. TTRNA-DCs and TTRNA-xALT will be administered 12-36 hrs after HSC infusion. Both groups will receive an additional two bi-weekly TTRNA-DC vaccines during Cycle 6. In addition to receiving a full Td booster vaccine at Vaccine #1, all subjects will undergo vaccine site pretreatment with a one-fifth dose of Td (1 Lf) intradermally 4-24 hours prior to Vaccine #3, #6, and #8. While receiving TMZ, subjects should receive PCP prophylaxis with pentamidine. The subjects will then be observed with serial MRIs for follow up until progression. After immunotherapy is completed, subjects should not receive any additional anti-tumor treatment until documented tumor progression.

If either DCs or xALT are generated but do not meet release criteria, the subject may remain on study and receive the qualified product (either DCs or xALT) but will be replaced for the purposes of safety assessment. Subjects that are unable to tolerate TMZ will continue on study and receive cellular therapy per protocol. If either DCs, xALT or PBSC are generated but do not meet targeted dose, all available cells will be administered and dose documented. Subjects who progress prior to receiving DC vaccination can receive immunotherapy treatment if cellular products have been generated and meet release criteria. For subjects that meet iRANO criteria for progression after the initiation of study drug, the subject will be replaced for evaluability and safety but may continue to receive cellular therapy if cellular products have been generated and meet release criteria. Once progression is determined, the subject will be managed by salvage therapy under the direction of the Protocol Chair until immunotherapy treatment is available for administration.

4.1.4 Immune Monitoring

Peripheral blood will be drawn within 24 hours of leukapheresis, within 24 hours prior to the start of each TMZ cycle, if feasible, within 24 hours prior to each vaccination (1-10), 1 day post xALT infusion, and 4 days +/1 day post xALT infusion (if feasible). Research blood will be drawn at each bi-monthly follow-up visit, if feasible.

Will conduct assays to test the ability to expand T cells against tumor antigens, exploring immunoreactivity against neoantigens and tumor-associated antigens (TAAs) *in vitro*. Assays will be conducted to test whether T cell responses are driven predominately by neoantigens or TAAa through analysis of RNAs encoding the individual antigens in T cell stimulation assays.

4.1.5 Follow-up

Progression free survival. Subjects will be followed bi-monthly (+/- 1 month) for one year and then per PI discretion (minimum of every 6 months) until tumor progression or death due to any cause. Subjects will undergo medical history, neurological and physical exam, MRI, and CBC with differential, CD4/CD8 ratio, and research labs.

ACTION

Upon progression, subjects will be followed for overall survival only. This contact for follow-up will be made every 3 to 6 months and may be via phone or in-person.

Subjects will be followed until death due to any cause. As part of standard care for these subjects, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure, consent will be obtained separately. If tissue is obtained, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and antigen expression profile in recurrent lesions.

4.2 General Concomitant Medication and Supportive Care Guidelines

There is a potential for interaction of immunotherapy with other concomitantly administered drugs. The following medications will be recorded in the data management system with start date, stop date, and indication:

Steroids
Anticonvulsants
Any medication used in the treatment of a VACCINE/STUDY DRUG related AE/SAE
Any medication deemed to possibly be interactive with vaccine or study drug by PI or designee.

The site investigator will determine if medications given while on treatment constitute significant drug-drug interactions that require reporting of this medication to the Principal Investigator.

4.2.1 Treatment of pseudo-progression

For signs and symptoms of pseudo-progression, treatment with bevacizumab is recommended to minimize swelling; would caution use of corticosteroids, as these agents may induce lymphopenia post-immunotherapy. Treatment options should be discussed with the Protocol Chair prior to initiation of therapy.

Treatment of pseudoprogression or inflammation

bevacizumab	7.5 mg/kg q 3 weeks.

4.2.2 PCP Prophylaxis

pentamidine	300 mg IH or 4 mg/kg IV monthly starting with chemoradiation

4.2.3 Neurosurgical Procedures

In uncertain cases where there is significant clinical decline and possible pseudo-progression is suspected, if biopsy or resection is feasible, pathological assessment should be strongly considered.

4.3 Duration of Therapy

The duration of treatment following the initial non-mobilized leukapheresis is shown in the table below. Subjects may have up to 4 weeks between cycles for ANC and platelets to recover or for unacceptable toxicity recovery therefore treatment may last 9 - 14 months.

ACTION

Length of Treatment

Weeks 1-6	Chemoradiation
Weeks 7-12	Rest
Weeks 13-15	C1 TMZ
Weeks 16-21	DC Vaccine 1,2,3 and 2nd non-mobilized leukapheresis
Weeks 22-25	C2 + DC 4
Weeks 26-29	C3 + DC 5
Weeks 30-33	C4 + DC 6
Weeks 34-37	C5 + DC 7
Weeks 38-40	C6 TMZ
Weeks 41-46	DC 8 + xALT +/- HSC, DC 9, DC 10

4.3.1 Subject Withdrawal

Subjects may withdraw at any time or be dropped from the study at the discretion of investigator should any untoward effects occur. In addition, a patient may be withdrawn by the investigator if he/she violates the study plan or for administrative and/or other safety reasons. The investigator or study coordinator must notify Moffitt immediately when a patient has been discontinued/withdrawn due to a serious adverse experience. All trial treatment-related toxicities and SAEs at the time of discontinuation/withdrawal should be followed until resolution or stabilization. Subjects who discontinue/withdrawal from the study will continue to be followed for disease progression.

Subjects who discontinue study participation for reasons unrelated to the study (e.g., personal reasons, or adverse events after registration but prior to receiving study therapy) may be replaced as required for the study to meet its objective. The replacement will generally receive the same treatment or treatment sequence (as appropriate) as the allocation number replaced.

Subjects may be removed from the study if any of the following criteria applies:

- Unacceptable treatment related toxicity, NCI CTC AE version 4.0 Grade 3 or 4 that fails to recover to baseline or < Grade 3 in the absence of treatment within 4 weeks
- Grade 3 neurologic toxicity that does not recover in 5 days.
- Any toxicity or other issue that causes a delay of study drug administration by more than 4
- weeks
- Intercurrent illness that prevents further administration of treatment
- An adverse event which requires discontinuation of the trial medication or results in inability to continue to comply with trial procedures
- Subject decides to withdraw from the study
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator
- Subject non-compliance with treatment or trial requirements: defined as any deviation from the protocol without prior agreement of the principal investigator
- Investigator non-compliance: defined as any significant medical or non-medical deviation from the protocol without agreement of the principal investigator
- Loss to follow-up

4.3.2 Subject Death

ACTION

Any deaths that occur within the trial treatment period, beginning with leukapheresis through 30 days after administration of the last dose of trial drug, regardless of relatedness, must be reported to the Moffitt Coordinating Center via the OnCore system for the purposes of serious adverse event (SAE) reporting. Deaths will be reviewed and reported in accordance with protocol section 7.2.5. Any deaths outside of these reporting timeframes thought to be related or possibly related to trial drug must also be reported.

4.3.3 Off Treatment Criteria

For the purposes of this trial, "Off treatment" is defined as the day the last dose of investigational agent is administered.

4.3.4 Off Study Criteria

A subject is considered "Off study" if the subject withdraws consent, or if the subject dies. The "off study" date is considered the last day of Follow Up.

4.4 Duration of Follow Up

Duration of Follow Up is defined as follows:

Subjects will be followed until death due to any cause. MRI and clinical evaluation for assessment of disease progression will be conducted bi-monthly +/- 1 month for one year and then per PI discretion (minimum of every 6 months) until tumor progression or death due to any cause. Radiographic disease progression will be assessed. As part of standard care for these subjects, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure, consent will be obtained separately. If tissue is obtained, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and antigen expression profile in recurrent lesions.

Upon progression, subjects will be followed for overall survival only.

Follow-up Requirements for Early Withdrawal:

Subjects treated on this study that are withdrawn by the PI for any of the aforementioned reasons will continue to be followed for survival by the study coordinator until death or are lost to follow up. Subjects that are withdrawn prior to any protocol treatment for any of the aforementioned reasons as well as for failure of T cells to qualify or inability to undergo leukapheresis will be considered eligibility failures and thus will not be followed for survival.

4.5 Criteria for Removal from Study

Subjects will be removed from study when any of the criteria listed in Section 4.3.1 applies. The reason and date for the removal from treatment and study must be captured in the OnCore eCRF.

4.6 Criteria for Evaluable Subjects

Evaluable subjects are those that have received 10 vaccines with a minimum of 2 weeks follow up. Once potential subject signs the informed consent form, that subject will be considered "on study". Rationale for taking subject off protocol treatment will be documented.

ACTION

4.7 Replacement

All subjects enrolled on the study, even if taken off study prior to treatment, will be included in the data analysis on an intent to treat basis. However, subjects will be replaced for safety assessments for the following reasons:

- If either DCs and/or xALT are generated but do not meet release criteria or targeted dose then the patient may remain on study and receive the qualified product but will be replaced for the purposes of safety assessment.
- Subjects requiring an increase in corticosteroids, with the exception of nasal or inhaled steroid, such that at the time of first vaccination they require a dose above physiologic levels, may remain in the study but will be replaced for assessment of safety as increased steroid usage may mask risks of autoimmune toxicity. For the purposes of this study, physiologic dose will be defined as > 4 mg of dexamethasone/day.
- Subjects receiving less than 10 vaccines without toxicity will be replaced for safety assessments.
- If subject cannot tolerate DI TMZ the subject will remain on study and evaluable for primary endpoint. Immunologic endpoint analyses would describe any differences observed in subjects that did not received DI TMZ but not be powered to detect any differences.

Subjects receiving or requiring any other concurrent anticancer or investigational drug therapy will be excluded and replaced.

If a subject withdraws from the study before receiving immunotherapy, then that subject will be replaced. PFS and OS will be reported for all subjects on an intent-to-treat basis.

5. DOSING DELAYS/DOSE MODIFICATIONS

5.1 Definition of Dose-Limiting Toxicity (DLT)

A DLT will be defined as any i**mmunotherapy-related**: 1) Grade III or greater non-neurologic toxicity; 2) Grade III neurologic toxicity that does not improve to Grade II or better within 5 days; or 3) Grade IV neurologic toxicity. If neurologic toxicity returns to baseline prior to next vaccine, the next vaccine can be administered on schedule. Vaccine can be withheld for up to four weeks if neurologic symptoms have improved to grade II or below within 5 days (thus not a DLT) but have not returned to baseline by time of next scheduled vaccine. If the event cannot be reversed within 5 days of its onset (improving to Grade II or better), a DLT will be declared for that patient and no further vaccinations or study-related procedures will be performed. If the event is reversed, but Grade III neurologic toxicity is again seen with subsequent vaccinations, all further vaccinations and study-related procedures will be withheld and a DLT will be declared. Medical therapy may be used to reverse any toxicity if necessary, but any episode of a toxicity requiring surgical intervention will still be considered a DLT even if reversible by surgery. All patients meeting DLT criteria will no longer receive immunotherapy and be taken off study.

Cerebral edema toxicity exception:

NCI CTCAE criteria categorize all cerebral edema as grade 4. Cerebral edema normally presents in patients with malignant gliomas as part of the disease process and can be exacerbated by standard of care chemotherapy and radiation. Furthermore, an effective anti-tumor immune response may involve

ACTION

inflammatory response and edema in infiltrative tumor cells. Therefore cerebral edema toxicity, although ranked grade IV by NCI CTCAE criteria, will be not be considered a DLT if patient is stable or improved clinically. If a cerebral edema is observed in a patient in clinical decline, the event will be considered a DLT if it is clearly attributable to the investigational drug and patient does not show improvement within 5 days of clinical management.

Grade III or greater toxicities associated with DC-based immunotherapy have been rare. Adoptive T cell therapy using tumor-infiltrating lymphocytes and high-dose IL-2 in patients with melanoma and treatment with anti-CLTA-4 monoclonal antibody blockade have been the immunotherapy regimens most often associated with treatment related toxicities. To take the most conservative approach to assessing possible toxicities associated with this treatment, investigators should be vigilant for any similar toxicity associated with this therapy, in addition to autoimmune toxicity specific to the CNS. Possible immune-mediated disorders that have been observed in patients who have received immunotherapy in early phase trials have involved the skin (vitiligo and cutaneous leukocytoclastic vasculitis), the thyroid gland (autoimmune thyroiditis), the liver (autoimmune hepatitis) and the pituitary (hypophysitis). Abnormal lab results, which may be immune-mediated, include elevations of serum lipase and amylase and liver function tests. If a patient has an AE that is thought to be possibly related to autoimmune antibodies (e.g., thyroiditis, hepatitis, thrombocytopenia) the PI will send a blood sample for appropriate autoimmune antibody testing. If specific autoantibodies are present, the serum sample taken at baseline will be tested for the presence of those autoantibodies.

Continuation of DC + xALT therapy in the presence of immune-mediated events may proceed only after discussion with the Protocol Chair, DSMB and patient on a case-by-case basis with consideration to risk-benefit analysis. The IRB will be notified promptly and review the event and determine whether the problem does or does not represent an unanticipated risk to the study participants. If review determines that unanticipated increased risk did occur a revised consent form stating such a risk will be drafted, approved by the IRB and prior to continuing therapy will require the patient to be re-consented with the revised consent.

5.2 Dose Modification

If 2 of 6 patients experience immunotherapy related DLT following administration of 3 $x10^8$/kg x-ALT, an additional 6 patients will be enrolled on group A to receive x-ALT at a dose of 3 $x10^7$/kg. The trial will stop if 2 of 6 patients develop DLT at this dose level. Once MTD is identified in group A patients, 6 patients will be treated in group B using this MTD for x-ALT infusion. There is no dose modification for DC vaccine in this study.

5.3 Adverse Events and Management

Hematological AE related to Temozolomide on Day 1 of Each Cycle (within the prior 48 hours before Day 1)

AE	Delay
ANC< 1.5 x 10^9/L and/or Platelet count < 100 x 10^9/L	Delay up to 4 weeks until all resolved. If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on hematologic and non-hematologic AEs in table below are applicable.

Worst Treatment-Related Hematologic AE during the Previous Cycles

ACTION

Worst AE		Platelets	
		≥100 x 10^9/L	**< 100 x 10^9/L**
ANC	**≥ 1.5 x 10^9/L**	Dose unchanged	Reduce by 1 dose level
	≥1 & <1.5 x 10^9/L	Dose unchanged	Reduce by 1 dose level
	< 1 x 10^9/L	Reduce by 1 dose level	Reduce by 1 dose level

Non-Hematologic Temozolomide related AE (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) On the day 1 of Each Cycle (within the prior 72 hours)

Grade	Delay
2-3	Delay up to 4 weeks until all resolved (to grade ≤ 1). If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on hematologic and non-hematologic AEs are applicable.
4	Stop (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy)

Summary of Dose Modification or Discontinuation During Post-Radiation Temozolomide Worst Non-Hematologic AE (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) During the Previous Cycles

Grade	Dose Modification
0-2	No dose modifications for non-hematologic AEs. Dose escalations (only for cycle 3) or reductions based on ANC and platelet counts are applicable.
3	Reduce by one dose level (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy). Dose modifications (escalations or reductions) based on ANC and platelet counts are not applicable. No further escalation is possible. If the same non-hematologic grade 3 AE recurs (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) after reduction for that AE, then stop.
4	Stop (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy). Dose modifications (escalations or reductions) based on ANC and platelet counts are not applicable.

In the case of hypersensitivity reactions, treatment measures deemed medically appropriate will be initiated and the PI notified of the event. The following treatment recommendations may be applicable and can be adopted at the judgment of the supervising pediatrician:

CTCAE (Common Terminology Criteria for Adverse Events) v.4.0 Grade I Allergy (transient flushing or rash, drug fever <38°C):

- Supervise at the bedside.

CTCAE v.4.0 Grade II Allergy (urticaria, drug fever=38°C, and/or asymptomatic bronchospasm):

ACTION

- Interrupt the infusion of xALT and disconnect infusion tubing from patient. Do not inject any remaining DCs.
- Administer IV antihistamines (diphenhydramine 1mg/kg and ranitidine 3 mg/kg).
- After recovery of symptoms, resume the infusion at half the initial infusion rate. If no further symptoms appear, complete the administration of the xALT. If symptoms reappear, stop infusion and discontinue patient from the study.

CTCAE v.4.0 Grade III or IV Allergy (symptomatic bronchospasm requiring parenteral medication(s) with or without urticaria; allergy-related edema/angioedema; hypotension; anaphylaxis):

- Stop the infusion of xALT and disconnect infusion tubing from patient. Do not inject any remaining DCs
- Administer epinephrine (1:10,000) in 3.5 to 5 mL IV boluses (no more than 6 doses).
- Administer IV antihistamine (diphenhydramine 1mg/kg (up to 50 mg) IV push).
- If wheezing persists: 0.35 mL of inhaled albuterol or other bronchodilators.
- Consider methylprednisolone (1-2mg/kg or 30-60 mg IV push), which may prevent recurrent or ongoing reactions.
- Anaphylaxis will be treated promptly with standard of care treatment procedures.
- Patient must be taken off treatment.

All toxicities should be graded according to the Common Terminology Criteria for Adverse Events (version 4.0).

The CTCAE version 4.0 is identified and located on the CTEP website at **http://ctep.cancer.gov.**
All appropriate treatment areas should have access to a copy of the CTCAE version 4.0.

6. SAFETY CONSIDERATIONS

6.1 Allergic Reactions to DC Immunization or TTRNA-xALT

Injection of antigen presenting cells may result in an allergic reaction, which could include redness and swelling at the injection site, itching, hives, low blood pressure, difficulty breathing, or in the most extreme circumstances, death. In addition, if the immune system becomes overly activated, potential discomforts may include pain, redness and swelling at the injection site. The risks associated with the injection of autologous lymphocytes for immunotherapy in humans are currently unknown. The best current assessment of potential risks of TTRNA-xALT can be gathered from the extensive experience gained at our institution and others in autologous PBSCT using mobilized progenitors. We have experienced no grade III or IV toxicities associated with infusion of autologous lymphocytes in patients who have received mobilized PBSCT. A recent review describing management of toxicities associated with this procedure describes only complications associated with the consolidation and conditioning regimens utilized in stem cell transplantation, as well as the post-transplant period where patients exhibit profound neutropenia. Subjects may experience an allergic reaction to preserved cells or other transfusion associated side effects such as pain at the injection site, mild swelling or edema, hypotension, or shortness of breath.

6.2 Cerebral Edema

Cerebral edema may be secondary to the disease process itself, the surgical procedure, necrosis from previous radiation, or inflammation due to immune infiltration of the brain or destruction of tumor cells.

ACTION

Symptoms may include, but are not limited to, severe headache, confusion, lethargy, unresponsiveness, coma, or focal neurological deficits. Subjects will be monitored throughout the course of the study and those subjects with any signs or symptoms of cerebral edema may need their steroid doses increased, treatment with an osmotic diuretic, or surgical decompression.

Edema that fails to respond to aggressive therapy may lead to permanent neurological impairment. The probability of this risk can be predicted to some degree based upon tumor size, location, pre-operative neurological impairment, and post-operative course prior to DC injections. Subjects will be monitored throughout the course of the study.

6.3 Infection

The DC injections or the TTRNA-xALT may include the risk of infection due to potential contamination of the DCs or leukocyte product in the laboratory. This may result in localized redness, swelling, or induration at the injection site. In the most extreme situation, this may lead to systemic bacterial/fungal sepsis and possibly death. The probability of this risk is relatively low, given the small injection volume (1 mL divided between >2 intradermal locations) and the fact that the DCs will be strictly tested for sterility prior to each injection. The risk of infection due to potential contamination of the DCs in the laboratory will be minimized by biosafety quality assurance and testing. All cell cultures will be handled under sterile conditions in a core tissue culture facility dedicated to the processing of human cells. Prior to injection into subjects, DCs must pass sterility tests in thiglycolate broth, tryptic soy blood agar, and inhibitory Sabouraud agar. Following injections, subjects will be monitored throughout the course of the study for any signs and symptoms of infection.

6.4 Delayed Autoimmune Diseases

It is possible that delayed autoimmune disease(s) may develop as a result of injection with DCs or TTRNA-xALT. This means that the immune system may be stimulated to attack natural tissue in the body. Animal studies have reported the development of autoimmunity in the context of vaccination and recovery from lymphopenia. However, our current experience with DC vaccination in glioma patients has not demonstrated evidence of autoimmunity in treated patients. It therefore, is unknown what the risk of delayed autoimmune disease is for this study.

6.5 Leukapheresis

Autologous hematopoietic stem cells (HSCs) for administration to Group B patients will be processed and stored under FACT-accredited processes by the clinical bone marrow transplant team at the participating treatment center. The mobilized pheresis will be collected by the clinical Bone Marrow Transplant team and stored at ambient temperature until processing following FACT-accredited guidelines for hematopoietic stem cell harvest and processing. Subjects will undergo pheresis collection until a target dose of 2 x 10^6 CD34+ cells/Kg has been collected or maximal dose achieved on first day of collection. HSCs will be processed and stored by BMT clinical team following institutional clinical SOPs and stored according to FACT -accredited guidelines. Following pheresis for PBSCs, subjects will undergo leukapheresis to collect PBMCs for DC generation, which will proceed to target of 5 x 10^9 mononuclear cells or until completion of second day of pheresis. The second pheresis collection will be transported to the cGMP laboratory for DC generation following SOP-UFBTIP-121 (for cells collected at UF Health) or SOP-UFBTIP-127 (for cells collected at external sites).

ACTION

As with any donation of blood, a variety of minor reactions may occur with leukapheresis, which include fainting, dizziness, or nausea. Uncommon but serious complications may also result, which include bleeding, infection, an adverse reaction to the anticoagulant or replacement fluids, hypocalcemia, hypotension, shock, convulsions, air emboli, heart failure, or the inability to transfuse blood back into the patient. These risks are reduced by the fact that the procedure will be performed by qualified staff at a specialized clinical hemapheresis unit. Subjects will be carefully monitored throughout the procedure by trained nursing and medical staff and managed per institutional policy. Blood will be routinely screened for *HIV*, hepatitis, and syphilis to minimize the risk of transmitting infection.

6.5.1 Central Venous Catheter Placement

Patients may require placement of a central venous catheter, using conscious sedation or anesthesia, for the leukapheresis procedure. Separate operative/procedural consent will be obtained from the surgeon/proceduralist placing the central line. The surgeon/proceduralist may use fluoroscopy to place the central venous catheter and ensure that it is in a satisfactory position. Fluoroscopy involves low dose radiation and is low risk. Risk of central line placement include (but not limited to): bruising, bleeding, injury to the vessel which may be permanent, possible injury to adjacent structures/organs, pneumothorax, infection and postoperative pain/discomfort. Removal of the central line is low risk but does include possible thrombosis or stenosis of the vessel. This may prevent future use of this vessel for additional leukapheresis. Risks of conscious sedation include pain from the initial needle stick when placing the IV. Possible bruising from where the needle went into the skin may occur, as well as rarely, an allergic reaction to the medication. Vomiting or inhaling food contents, drowsiness, amnesia, hypotension, respiratory depression or rarely, death, may result from conscious sedation. Risks of anesthesia may include complications such as breathing difficulties, low blood pressure, or an irregular heartbeat, which can rarely lead to death.

7. ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (See section 7.3).

Additionally, certain adverse events must be reported in an expedited manner for more timely monitoring of patient safety and care. The following sections provide information about expedited reporting. The characteristics of an observed AE will determine whether the event requires expedited reporting via a Medwatch form and OnCore SAE eCRF to the Coordinating center **in addition** to routine reporting.

7.1 Adverse Event Characteristics

Characteristics of an Adverse Event must be characterized as follows:

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the investigational study treatment.

ACTION

- Probable – The AE *is likely related* to the investigational study treatment.
- Possible – The AE *may be related* to the investigational study treatment.
- Unlikely – The AE *is doubtfully related* to the investigational study treatment.
- Unrelated – The AE *is clearly NOT related* to the investigational study treatment.

7.2 Expedited Adverse Event Reporting (Serious Adverse Event)

7.2.1 Determination of Reporting Requirements:

Reporting requirements should include the following considerations: 1) whether the patient has received the investigational or commercial agent; 2) the characteristics of the adverse event including the grade (severity), the relationship to the study immunotherapy (attribution), and the prior experience (expectedness) of the adverse event. 3) the reporting window: This study will include two adverse event collection periods: leukapheresis and immunotherapy (DC + xALT +/- HSC). The leukapheresis collection periods will begin at initiation of leukapheresis and continue until 24 hours after the completion of the procedure. The immunotherapy collection period will begin with first DC vaccine and continue through 30 days after administration of the last dose of trial drug or subject death, whichever comes first.

Events occurring outside the reporting windows that have a possible relationship to vaccine therapy must also be reported. All AEs must be recorded in the subject's medical and/or record and case report form.

7.2.2 Steps to Determine if an Adverse Event is to be reported in an Expedited Manner

Step 1: Identify the type of event: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Step 2: Grade the event using the NCI CTCAE version 4.0.

Step 3: Determine whether the adverse event is related to the protocol therapy (investigational or commercial). Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite. All AE's, regardless of grade, deemed by the PI as possibly, probably or definitely related to Immunotherapy should be reported to the MCRN Coordinating Office and Protocol Chairs (Immunotherapy and Neuro-Oncology) within 24 hours of knowledge of the event via e-mail.

Step 4: Determine the prior experience of the adverse event. Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is NOT listed in the investigational brochure or package insert for the investigational agent.

If any of the following criteria are met, the Adverse Event should be reported in an expedited manner and will qualify as a Serious Adverse Event on this trial.

A Serious Adverse Event is defined as any untoward medical occurrence that, at any dose, meets any of the following criteria:

- Results in death.
- Is life-threatening: The term 'life-threatening' in the definition refers to an event in which the patient was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

ACTION

- Requires inpatient hospitalization or prolongation of existing hospitalization: A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:
  - The admission results in a hospital stay of less than 24 hours.
  - The admission is for observation or minor treatments such as hydration and released in 24 hrs.
  - The admission is pre-planned (e.g. elective or scheduled surgery arranged prior to the start of the study).
  - The admission is not associated with an AE. (e.g. social hospitalization for purposes of respite care).
- Disability or Permanent Damage
- Congenital Anomaly/Birth Defect
- Other Serious (Important Medical Events)

Please see the FDA website: http://www.fda.gov/Safety/MedWatch/HowToReport/ucm053087.htm for more information regarding the definition of a Serious Adverse Event

7.2.3 Serious Adverse Event Reporting

Serious Adverse Event (SAE) reporting for this study must follow a two-step reporting process to comply with Moffitt AE reporting guidelines (Policy standard CTO.65).

Step 1: All SAEs will be reported to the MCRN Coordinating office within 24 hours of learning of the event via the OnCore eCRF. In addition, an email notification should be sent to the Sunshine Project coordinator (affiliate.research@moffitt.org) indicating that an SAE has been entered in the system.

Step 2: Within 24 hours of learning of the event, the reporting investigator should download a Medwatch form, Form FDA 3500A – Mandatory Reporting from the FDA website http://www.fda.gov/safety/medwatch/howtoreport/downloadforms/default.htm

- Fill out required information and
- Email or fax the completed Medwatch form to Sunshineproject@moffitt.org.

A follow up Medwatch form will be required to update all information captured on the original form.

Serious Adverse Event Reporting at Site Level:

All SAE's should be reported to the treating institutions IRB or Ethics board per local IRB policy. All SAEs must also be reported to the Moffitt Cancer Center Coordinating Center for review per standard operating procedures. The Protocol Chair and IND holder, Dr. Mitchell, Preston A. Wells, Jr. Center for Brain Tumor Therapy at University of Florida, will establish FDA reporting system in collaboration with Moffitt Coordinating Center.

Supporting source documentation for serious adverse events will be monitored and the following should be available to support reported information:

- Laboratory reports
- Emergency room notes
- Hospital discharge summary
- Death certificate (if available)
- Autopsy report (if available)

ACTION

REMINDER: All Deaths occurring during the trial treatment period beginning with leukapheresis through 30 days post administration of the trial drug must be reported as a Serious Adverse Event.

7.2.4 Distribution of Adverse Event and Serious Adverse Event Reports

Routine Adverse Events for each patient will be discussed on the bi-weekly calls held by the Coordinating Center. The calls are a consistent bi-weekly check-in with PIs and site coordinators to ensure the continued safety of all subjects receiving treatment, and for the distribution of information regarding dosing and drug toxicity seen in real time with enrolled subjects.

The Sunshine Project Coordinators are responsible for the distribution of Serious Adverse Event reports (via Medwatch forms) to all enrolling sites participating on the trial. Sites are responsible for the reporting of those Serious Adverse Event reports to the local PI and IRB according to the site's reporting policy.

7.2.5 Expedited Reporting Guidelines - Death

A death on study requires <u>both</u> routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.

If a death occurs within the trial treatment AE reporting periods, enrollment of new subjects will be suspended until review by the Protocol Chair/IND sponsor has been completed and the FDA notified. Causes of death definitely not related to study participation (i.e., motor vehicle accident) will result in notification of FDA but will not result in suspension of new subject enrollment or continued treatment for enrolled subjects. Given the high degree of expected mortality in patients with HGG, treatment for enrolled subjects may continue on schedule unless the cause of death is assessed to be possibly, probably or definitely related to the trial drug. If attribution of death is determined by the Protocol Chair/IND Sponsor or FDA to be possibly, probably or definitely related to trial drug, then continued treatment of all enrolled subjects with trial drug will be suspended until the review is completed and recommendations for study continuation have been issued and approved by FDA. Any death attributed to trial drug, regardless of the timeline with respect to last treatment, will result in suspension of enrollment and suspension of continued treatment for enrolled subjects until review by FDA is complete and recommendations for study continuation issued and approved. The IRB will be notified of subject deaths per local policy. Reporting to and review by the Moffitt Protocol Monitoring Committee and Clinical Trials Oversight Committee will be in accordance with standard procedures. Deaths or life-threatening adverse events will be reported to FDA by the IND sponsor as soon as possible but no later than 7 calendar days after the sponsor's initial receipt of the information (21 CFR 312.32).

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

7.3 Routine Adverse Event Reporting

All adverse events (AE) will be monitored throughout the course of the study. The National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v 4.0 will be utilized for AE reporting.

ACTION

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner and must be collected at every assigned or unintended study visit and/or contact with the patient (phone call, email, etc.)

An adverse experience is defined as:

- Any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational drug, whether or not related to the investigational drug.

An adverse event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional overdose, drug abuse, or drug withdrawal.

- Any worsening of a pre-existing condition or illness is considered an adverse event.

CTCAE Grade 1 or 2 adverse events will be recorded if the attribution is at least possibly related to the DC or xALT vaccines.

CTCAE Grade 3 or higher adverse events will be recorded regardless of relatedness to trial drug or protocol procedures.

Laboratory abnormalities and changes in vital signs or test results are considered clinically significant and are adverse events if it results in discontinuation from the study, necessitates change in therapeutic medical intervention, or is considered a dose limiting toxicity.

The investigator will evaluate all adverse experiences as to their severity and relationship to the trial drug or protocol procedures.

Adverse Events will be reported in routine study data submissions via the clinical data management system. All recorded toxicities and adverse events will be followed until resolution, or return to baseline during the 30 days following the last dose of investigational drug or subject death whichever comes first. If the subject has progression of disease and begins another line of therapy or another therapeutic trial the adverse events collection will be discontinued.

Please note: All adverse events must be entered into the clinical trial management system within required timelines as set forth in the current MCC standards and policies.

7.4 Secondary Malignancy

A *secondary malignancy* is defined as a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm. Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

ACTION

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

8. PHARMACEUTICAL INFORMATION

8.1 Names, Classification, and Mechanism of Action

The active immunotherapy components are TTRNA-DCs and TTRNA-xALT.

TTRNA-DCs are autologous dendritic cells loaded with total tumor messenger ribonucleic acid (mRNA) (TTRNA) derived from malignant tumor.

TTRNA-xALT is autologous lymphocytes stimulated ex vivo against tumor antigens using TTRNA-DCs for autologous lymphocyte transfer.

The classification is a biologic.

8.2 Packaging and Labeling

All products will be appropriately labeled with subject identifiers, contents, lot number, manufacture date, and the "Caution: New Drug-Limited by Federal Law to Investigational Use" statement.

8.3 Dispensing and Preparation

Study personnel at external sites will be provided with SOP on the transport of tumor tissue and leukapheresis product as well as the preparation and administration of experimental therapeutic agents. Tumor tissue and leukapheresis product will be collected, prepared, and shipped to the University of Florida in accordance with **SOP-UFBTIP-120** or **SOP-UFBTIP-130** for tumor tissue and **SOP-UFBTIP-127** for leukapheresis product. External sites will be supplied with kits that will include all materials necessary to prepare and transport tumor tissue and leukapheresis product, including shipping labels.

TTRNA DC and TTRNA-xALT products prepared by the University of Florida will be shipped to external sites in a liquid nitrogen dry-shipper under controlled conditions in accordance with **SOP-UFBTIP-129**. External sites will store products in accordance with standard operating procedures/written instructions until time of formulation. When the subject is present and both the subject and site staff are ready for administration, products will be formulated in accordance with **SOP-UFBTIP-124 (Procedure for DC Vaccine Preparation) and SOP-UFBTIP-125 (Procedure for Preparation of TTRNA-ALT for Adoptive Transfer)**, and administered in accordance with **SOP-UFBTIP-128 (DC Vaccine) and SOP-UFBTIP-2132 (ALT infusion)**.

Products will be labeled with, at a minimum, the subject's name, medical record number, study ID code and Lot number. Individuals transporting samples must be certified to ship biological products, diagnostic or infectious materials. All packaging must comply with the Department of Transportation (DOT), the Federal Aviation Authority (FAA), and the International Air Transport Association (IATA) dangerous goods regulations.

The final TTRNA-DC product is a subject-specific single-dose syringe containing a target dose of $1x10^7$ cells formulated in 400 µL of preservative free saline. The administered volume is 0.4 mL.

ACTION

The final targeted dose of TTRNA-xALT product is a subject-specific single-dose syringe or infusion bag containing a total dose goal of $3x10^8$ cells/kg formulated as $2.5x10^7$ cells/mL in preservative free saline with 1% HSA. The administered volume is dependent on the cell dose required for each subject.

8.4 Commercial Agent(s) Non-investigational

Temozolomide: Temozolomide (Temodar™) NSC # 362856 Source and Pharmacology: An orally administered alkylating agent, a second generation imadazotetrazine. A prodrug of MTIC, temozolomide spontaneously decomposes to MTIC at physiologic pH. Temozolomide exerts its effect by cross-linking DNA. This is likely a site specific alkylation at the O6 -position of guanine with some effect at the N7 position. Temozolomide reaches its peak concentration in 1 hour. Food reduces the rate and extent of absorption. It has an elimination half-life of 1.13hr (intraperitoneally) and 1.29hr (orally) with an oral bioavailability of 0.98. Total apparent body clearance is 100ml/min/m2 and plasma elimination half-life is ~100 minutes.

Risks and side effects related to Temozolomide include those which are:		
Likely In 100 people receiving Temozolomide, more than 20 and up to 100 may have:	**Less Likely** In 100 people receiving Temozolomide, from 4 to 20 may have:	**Rare, but serious** In 100 people receiving Temozolomide, 3 or fewer may have:
• Constipation, nausea, vomiting, diarrhea • Dizziness • Muscle weakness, paralysis, difficulty walking • Trouble with memory • Tiredness • Difficulty sleeping • Hair loss	• Headache, seizure • Infection, especially when white blood cell count is low • Anemia which may cause tiredness • Bruising, bleeding • Damage to the bone marrow (irreversible) which may cause infection, bleeding, may require blood transfusions	• Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat • Cancer of bone marrow caused by chemotherapy • Rash • Severe skin rash with blisters and can involve inside of mouth and other parts of the body • Liver damage which may cause yellowing of eyes and skin, swelling and may result in liver failure.

ACTION

8.5 GCSF, GMCSF, and Td Vaccine Risks

Risks and side effects related to the Filgrastim (G-CSF) include those which are:		
Likely *("Likely" refers to a side effect that is expected to occur in more than 20% of patients.)*	***Less Likely*** *("Less likely" refers to a side effect that is expected to occur in 20% or fewer patients.)*	***Rare, but Serious*** *(These possible risks have been reported in rare occurrences, typically less than 2% of patients. They may be serious if they occur.)*
	• Local irritation (skin) at injection site • Ache or pain inside the bones, increased levels of liver enzymes and uric acid in the blood, low number of platelets in the blood	• Allergic reaction, low fever • Enlargement or rupture of the spleen • Worsening of pre-existing skin rashes • Temporary hair loss • Inflammation of a blood vessel in the skin

ACTION

Risks and side effects related to Sargramostim (GM-CSF) include those which are:		
Likely:	**Less Likely:**	**Rare, but Serious:**
• Headache • Bone pain • Muscle and joint pains • Fever and Chills • Rash and itchiness • A feeling of discomfort or not feeling well and/or tiredness	• Stomach or abdominal pain or cramps • Weakness • Loss of appetite • Nausea and/or vomiting • Diarrhea • Excessive sweating • Inflammation of a vein through which the drug was given • Redness and pain at the injection site • Weight gain • Fewer platelets in the blood. A low number of platelets causes you to bruise and bleed more easily • Increase in the blood of certain enzymes or bilirubin (a substance that comes from the liver breaking down waste products) which could indicate liver irritation or damage • Elevation in the blood of creatinine which normally is removed from the blood by the kidney and could indicate kidney damage • Fluid build-up in the tissues usually of the lower legs	• Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, and a rapid heart beat • A severe reaction which can cause shortness of breath, a low blood pressure, a rapid heart rate, fever, a feeling of warmth and back pain which may occur only with the first dose and not with further doses • An abnormally rapid heat beat • Leakage of fluid into the lungs which may result in shortness of breath and difficulty breathing and/or leakage of fluid into body tissues with puffiness of legs, arms or abdomen, weight gain and a drop in blood pressure • Inflammation of the lungs which may lead to pain and shortness of breath • A build-up of fluid around the heart which may be painful

ACTION

Risks and side effects related to the Td (Tetanus, Diphtheria)Vaccine include those which are:		
Mild Problems *(Did not interfere with activities)*	***Moderate Problems*** *(Interfered with activities, but did not require medical attention)*	***Severe Problems*** *(Unable to perform usual activities; required medical attention)*
• Pain where the shot was given (about 8 people in 10) • Redness or swelling where the shot was given (about 1 person in 4) • Mild fever (rare) • Headache (about 1 person in 4) • Tiredness (about 1 person in 4)	• Fever over 102°F (rare)	• Swelling, severe pain, bleeding and/or redness in the arm where the shot was given (rare).

Risks Associated with Non-investigational Agents

Complete information on FDA-approved drug products included in this clinical trial can be found on the Drugs@FDA website. Each drug product label will include:

- description of the drug
- clinical pharmacology
- indications (uses for the drug)
- contraindications (who should not take the drug)
- warnings
- precautions
- adverse events (side effect)
- drug abuse and dependence
- dosage and administration
- use in pregnancy, use in nursing mothers
- use in children and older patients
- how the drug is supplied
- safety Information for the patient

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Immune Monitoring:

Peripheral blood will be drawn within 24 hrs of leukapheresis, within 24 hours prior to the start of each TMZ cycle if feasible, within 24 hours prior to each vaccination (1-10), Day 1 post xALT infusion, and 4 days +/1 day post xALT infusion (if feasible). Research blood will be drawn at each bi-monthly follow-up visit, if feasible, until progression for T cell kinetics, to test the ability to expand T cells against tumor antigens, exploring immunoreactivity against neoantigens and tumor-associated antigens (TAAs) *in vitro*. Assays will be conducted to test whether T cell responses are driven predominately by neoantigens or TAAa through analysis of RNAs encoding the individual antigens in T cell stimulation assays. Blood will be processed in accordance with **SOP-UFBTIP-126**.

ACTION

Peripheral blood for immune monitoring will require approximately 3 ml of whole blood in SST tube and 8-10 ml in 2 ACD-A tubes. Research blood may remain at room temperature and must be processed within 6 hours of collection.

10. STUDY CALENDAR

Study Requirements	Surgery	Enrollment	Mobilized Leukapheresis	Chemo-radiation[17]	Non -Mobilized Leukapheresis	Adjuvant TMZ Cycles			DC Vaccine	Bi Monthly Follow-Up[21]
						#1	#2-#5	#6		
Inclusion & exclusion criteria		X								
Informed consent to collect tumor	X									
Consent to treatment		X								
Brain MRI (and spine if applicable)	X[1]					X[7]	X[7]	X[7]		X[7]
Performance Status		X				X	X	X	X	
Serum/urine pregnancy test		X[4]	X		X	X[4]	X [4]	X[4]		
Tumor tissue for extraction of total tumor RNA	X[2]									
CBC with differential & CD4+/CD8+ T cell ratio		X	X		X	X[8]	X[8]	X[8]	X	X[8]
PT/INR, PTT		X								
Comprehensive metabolic panel		X	X		X	X[8]	X[8]	X[8]	X	X[8]
Infectious disease testing			X[3]		X[3]					
Leukapheresis			X[19]		X[19]					
Temozolomide				X[5]		X[9]	X[9]	X[9]		
External Beam RT				X[6]						
History, physical & neurological exam		X	X		X	X[10]	X[10]	X[10]		X[10]
TTRNA -DCs						X[11]	X[11]	X[11]	X[11]	
TTRNA-xALT								X[13]		
HSC infusion								X[12]		
Peripheral blood for immune monitoring			X[14]		X[14]				X[14]	X[14]
Td booster									X[15]	
Td Skin Prep									X[16]	
Concomitant medications			X[20]		X	X	X	X	X	
Adverse event assessment			X[18]		X[18]	X[18]	X[18]	X[1,8]	X[18]	X[18]

ACTION

Footnotes for Schedule of Study Assessments:

1 A diagnostic contrast-enhanced MRI of the brain (and spine if applicable) must be performed pre- and postoperatively within 28 days prior to study registration.

2 Potentially eligible subjects will be enrolled on a screening consent for the sterile collection of tumor material in a manner suitable for RNA extraction, amplification, and loading of autologous DCs.

3 Pre-leukapheresis infectious disease testing per institutional policy to include at a minimum HIV, Hepatitis B, Hepatitis C, and CMV.

4 Negative serum pregnancy test at enrollment and negative serum/urine pregnancy test within 72 hours prior to starting each TMZ cycle.

5 Administer TMZ continuously from Day 1 of RT to the last day of RT at a daily oral dose of 90 mg/m^2 for a maximum of 49 days.

6 Per section 4.1.1 or institutional guidelines for HGG.

7 MRI imaging within 2 week prior to C1, C2, C4, C6, then bi-monthly +/- 1 month.

8 CBC with differential and CD4+/CD8+ T cell ratio at each vaccine visit and, if clinically indicated, at routine clinic visits.

9 Cycle #1 should be started 5 (+/- 1) weeks after completing chemoradiation. Administer once per day for 21 consecutive days (days 1-21) for a 28 (+7) day cycle. The starting dose for the Cycle 1 and 2 will be 85mg/m^2/day, with a single dose escalation to 100 mg/m^2/day in subsequent cycles if no dose modifying adverse events are noted.

10 Within 14 days prior to initial vaccine therapy and prior to subsequent TMZ cycles.

11 Administer vaccine #1 intradermal at day 22-24 (+7) after the first TMZ cycle. Vaccines #2 and #3 will occur at 2 (+/- 1 day) week intervals. Monthly vaccines #4-7 will be given on day 22-24 (+7) of each TMZ cycle. Vaccine #8 will be given on day 23-25 of TMZ cycle 6. Vaccines #9 and #10 will occur at 2 (+/- 1 day) week intervals until a total of 10 vaccines have been administered or until tumor progression (whichever comes first).

12 Peripheral blood stem cell infusion to be completed on day 22-24 of TMZ cycle 6 (Group B only). Autologous HSCs will be administered 12-36 hrs prior to DC vaccine/xALT.

13 TTRNA-xALT on day 23-25 of TMZ cycle 6.

14 2 tubes (ACD-A) and 1 tube (SST) Peripheral blood will be drawn within 24 hrs of leukapheresis, within 24 hours prior to the start of each TMZ cycle if feasible, within 24 hours prior to each vaccination (1-10), Day 1 post xALT infusion, and 4 days +/1 day post xALT infusion (if feasible). Research blood will be drawn at each bi-monthly follow-up visit, if feasible. Peripheral blood will be processed in accordance with **SOP-UFBTIP-126**.

15 All subjects will receive IM Td booster (5 Lf) at Vaccine #1.

16 All subjects will undergo vaccine site pretreatment with Td (1 Lf) 4-24 hours prior to Vaccine #3, #6, and #8.

17 Within 7 weeks of surgery.

18 CTCAE Grade 1 or 2 adverse events will be recorded if the attribution is at least possibly related to the DC or xALT vaccines. CTCAE Grade 3 or higher adverse events will be recorded regardless of relatedness.

Adverse events will be collected during two periods: leukapheresis and immunotherapy (DC + xALT +/- HSC) as described in Section 10.1.

19 Enrolled subjects will undergo a mobilized leukapheresis prior to chemoradiation for collection of PBMCs for generation of DCs and HSCs for Group B subjects. Fourteen days (+/- 3 days) after the third biweekly DC vaccine, subjects will undergo a second leukapheresis (non-mobilized) to collect PBMCs for generation of DCs and xALT.

20 Concomitant medications should be recorded from Day 1 Cycle 1 through 30 days post last dose of Immunotherapy

21 Subjects will undergo bi-monthly follow-up +/- 1 month for one year and then at the PI discretion (at least every 6 months) until tumor progression or death due to any cause

ACTION

10.1 Study Calendar Descriptions

Demographics

Subject's gender, race, ethnicity and date of birth will be collected at screening.

Medical History

Medical history findings (i.e. previous diagnoses, diseases or surgeries) and treatments considered relevant to the study (including planned interventions) will be collected prior to the first dose of study drug.

Physical Measurements

The screening examination should include physical measurements (height, weight, performance status). Weight and performance status should be collected at each study visit.

Concomitant Medications

All concomitant medications (including first dose / last dose and if medication was given for Immunotherapy related AE) must be recorded from 4 weeks prior to immunotherapy through 30 days after the last dose of immunotherapy.

Physical Exam

In addition to physical measurements, signs and symptoms should be assessed at each study visit by the appropriate staff.

Performance Status

Lansky or Karnofsky status will be evaluated and documented at screening and visits per study calendar.

Adverse Events

Adverse events Grade 3 and higher and all adverse events regardless of grade that are at least possibly related to DC vaccine or xALT will be collected during two periods: leukapheresis and immunotherapy (DC + xALT +/-HSC).

The leukapheresis collection periods will begin at initiation of leukapheresis and continue until 24 hours after the completion of the procedure.

The immunotherapy collection period will begin with first DC vaccine and continue through 30 days after administration of the last dose of trial drug or subject death, whichever comes first.

Events occurring outside the reporting windows, regardless of grade, that have a possible relationship to vaccine therapy must also be reported. All AEs Grade 3 or higher must be recorded in the subject's medical and/or research record and case report form.

Adverse events will be graded according to the NCI-CTCAE version 4.0.

ACTION

10.2 Laboratory Evaluations

Safety labs, including chemistry, hematology and coagulation tests will be measured by the local lab for clinical assessment.

- Hematology: A complete blood count (CBC) with differential will include the following tests: hemoglobin, hematocrit, white cell count (WBC) with differential as percentage or absolute value, red cell count (RBC), reticulocyte count and platelet count.

- Coagulation: Coagulation tests are as follows: Prothrombin time (PT), activated partial thromboplastin time (PTT), and international normalized ratio (INR).

- Blood Chemistry: A blood chemistry will include the following: albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

- Pregnancy Test: Women of child bearing potential are required to have a serum pregnancy test (B-HCG) performed prior enrollment and serum or urine test prior to the start of study drug.

The definition of a woman of childbearing potential is defined as: a woman is considered of childbearing potential (WOCBP) i.e., fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy.

10.3 Radiologic Evaluations

MRI is the best currently available and reproducible method to measure lesions selected for response assessment. Measurability of lesions on MRI scan is based on the assumption that MRI slice thickness is 5 mm or less. When MRI scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. In the interest of consistent measurements, the same method of evaluation used at baseline must be utilized throughout the subjects' time on study.

Study evaluations will take place in accordance with the Study Calendar in section 10. All radiographic evaluations must be performed within 28 days of start of therapy utilizing MRI. Unusual circumstances related to >28 day old scans not involving target lesions may be discussed with the Protocol Chair. Tumor response will be assessed by the investigator using the iRANO (immunotherapy Response Assessment in Neuro-oncology).

11. MEASUREMENT OF EFFECT

11.1 Immunologic confounders of radiographic response

Inflammatory responses induced by immunologic treatment may confound radiographic assessment of tumor progression versus treatment response. PFS and OS are not primary endpoints of this study and iRANO criteria will be applied to assess progression defined below. However, if treatment response vs progression is unclear after administration of i mmunotherapy but criteria for radiographic progression are met, then subjects may remain on study without change in therapy and subsequent evaluation by MRI can be reassessed. If continued neurologic and/or radiographic evaluation determines tumor progression then the date of PFS will be back-dated to the original MRI evaluation meeting criteria.

ACTION

11.2 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 8-12 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 8 -12 (not less than 4) weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the Immunotherapy Response Assessment in Neuro-Oncology (iRANO) criteria as proposed by "Immunotherapy response assessment in neuro-oncology: a report of the RANO working Group" *Lancet Oncology* 2015 Nov;16: e534-42.

11.2.1 Definitions

Evaluable for toxicity. All subjects will be evaluable for toxicity from the time of their first treatment with DC vaccine.

Evaluable for objective response. For those subjects who have had a complete resection or measurable disease present at baseline (post-surgery), have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. (Note: Subjects who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.).

Of note, patients who need increased corticosteroid use within 2 weeks of MRI assessment relative to the dose taken at the time of the previous assessment, cannot be classified as having a complete response, partial response, or stable disease and should be classified as non-evaluable at that timepoint. Conversely, patients who decrease corticosteroid use within 2 weeks of MRI assessment relative to the dose taken at the time of the previous assessment cannot be classified as having progressive disease and should be classified as non-evaluable.[103]

11.3 General Methodology for Determining Tumor Measurements

All radiographic responses will be based on MRI scans of the brain and spine (if applicable) obtained with and without contrast. Tumor size will be based on the product of the maximal diameters in 2 different planes. Response will be assessed as a percentage change in tumor size from baseline. Tumor measurements in the follow-up scans will be compared to those of the pre-treatment (baseline - post surgery) scan to document response. Determination of progressive disease will be based on comparison of the current tumor dimensions with the smallest measurements of the tumor recorded since the start of treatment. **However, based on the iRANO criteria, if the initial radiological progression is within the first 6 months, this will serve as the new reference scan if treatment is continued.** The assessment of steroid dose as "stable or tapering" will be based on the dose the patient was receiving on the day of the follow-up scan with that on the day of the pre-treatment scan. Residual disease will be defined as contiguous contrast enhancement ≥ 1 cm^2 (product of measurement obtained in 2 perpendicular planes (i.e., axial and coronal)).

ACTION

11.4 Tumor Response Criteria

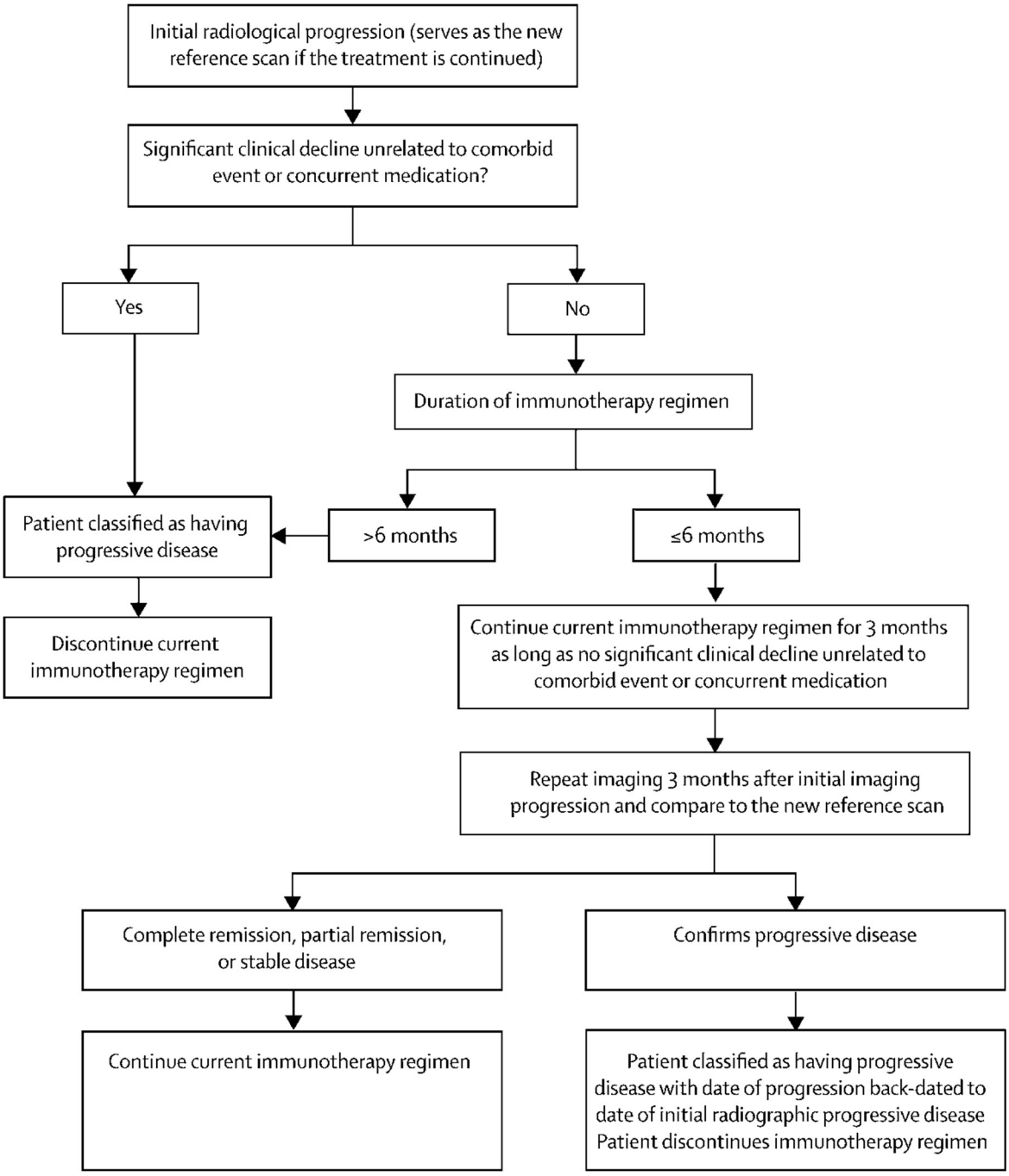
iRANO criteria for Malignant glioma

Complete response	Disappearance of all enhancing disease for ≥ 4 weeks; no new lesions; stable or improved T2/FLAIR; no more than physiological steroids; clinically stable or improved
Partial response	≥ 50% decrease in the sum of biperpendicular diameters of enhancing disease for ≥ 4 weeks; no new lesions' stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved
Stable disease	Does not qualify for complete response, partial response, or progressive disease; no new lesions; stable or improved T2/FLAIR; stable or decreased steroid dose; clinically stable or improved
Progressive disease	≥ 25% increase in the sum of biperpendicular diameters of enhancing disease; or new lesions; or substantial worsened T2/FLAIR; or substantial clinical decline

The iRANO criteria integrate into the existing RANO criteria for malignant glioma, low-grade glioma, and brain metastases by providing recommendations for the interpretation of progressive imaging changes. Specifically, iRANO recommends confirmation of disease progression on follow-up imaging 3 months after initial radiographic progression if there is no new or substantially worsened neurological deficits that are not due to comorbid events or concurrent medication, and it is 6 months or less from starting immunotherapy. If follow-up imaging confirms disease progression, the date of actual progression should be back-dated to the date of initial radiographic progression. The appearance of new lesions 6 months or less from the initiation of immunotherapy alone does not define progressive disease. FLAIR=fluid-attenuated inversion recovery. iRANO=immunotherapy Response Assessment in Neuro-Oncology. N/A=not applicable.

The algorithm below indicates the iRANO treatment algorithm for the assessment of progressive imaging findings in patients with neuro-oncological malignancies undergoing immunotherapy:

ACTION


Initial radiological progression (serves as the new reference scan if the treatment is continued)
Significant clinical decline unrelated to comorbid event or concurrent medication?
Yes
No
Duration of immunotherapy regimen
Patient classified as having progressive disease
>6 months
≤6 months
Discontinue current immunotherapy regimen
Continue current immunotherapy regimen for 3 months as long as no significant clinical decline unrelated to comorbid event or concurrent medication
Repeat imaging 3 months after initial imaging progression and compare to the new reference scan
Complete remission, partial remission, or stable disease
Confirms progressive disease
Continue current immunotherapy regimen
Patient classified as having progressive disease with date of progression back-dated to date of initial radiographic progressive disease
Patient discontinues immunotherapy regimen

ACTION

Progressive Disease (PD)

Progressive neurologic abnormalities or worsening neurologic status not explained by causes unrelated to tumor progression (e.g., anticonvulsant or corticosteroid toxicity wean, electrolyte disturbances, sepsis, hyperglycemia, hydrocephalus etc.), OR ≥25% increase in the bi-dimensional measurement of enhancing disease, OR the appearance of a new tumor lesion, OR substantial worsened T2/FLAIR. Increasing doses of corticosteroids required to maintain stable neurological status should be strongly considered as a sign of clinical progression unless in the context of recent wean or transient neurologic change.

Patients with early progressive imaging findings, including patients who develop new lesions but who do not have substantial neurological decline, confirmation of radiographic progression by follow-up imaging should be sought 3 months after initial radiographic evidence of progressive disease to decrease the likelihood of prematurely declaring progressive disease in patients with pseudoprogression or delayed response. Imaging within the 3-month follow-up can be done as medically appropriate at the discretion of the treating clinician.

Patients who develop substantial new or worsened neurological deficits not due to comorbid events or a change in co-administered medication at any time within the 3-month follow-up window should be designated as non-responsive to treatment and should discontinue immunotherapy. For these patients, the date of actual tumour progression should also be back-dated to the date when radiographic progressive disease was initially identified.[103]

Pseudoprogression

For subjects showing possible radiographic evidence of tumor progression on MRI (on required MRI studies performed after courses 3, 6 and 9), during the first 6 months after immunotherapy, the treating physician in consultation with the Protocol Chair will have the option of allowing the patient to remain on study or holding protocol therapy, and repeating disease reassessment in 3 months. Symptomatic patients should be treated with BVZ (7.5 mg/kg q 3 weeks) and MRI repeated in 3 months. If SD or resolution of neuroimaging changes and clinical stability or improvement, patient should continue DC vaccine along with BVZ. If the repeat MRI(s) or clinical status shows continue to worsen, patients should be taken off protocol therapy. Clinical worsening or new/worsening changes on neuroimaging more than 6 months following start of vaccine therapy should be considered true progression and patient should discontinue protocol therapy and be replaced. If follow-up imaging or clinical evaluation confirms disease progression, rather than pseudoprogression, then the time of progression will be the date of the initial MRI demonstrating progression, not the follow up scan(s).

11.4.1 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence. The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

11.4.2 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

ACTION

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: Definition and Reporting Requirements).

12.1 Data Reporting

Data collection for this study will be done exclusively through OnCore. All members of the study team are granted access to the trial in OnCore by the MCRN External Research Regulatory Coordinator. See instructions in Moffitt Clinical Research Network Handbook for OnCore access.

Regulatory documents and case report forms will be monitored by the Sunshine Project. Monitoring will be performed regularly for accuracy, completeness, and source verification of data entry, validation of appropriate informed consent process, reporting of SAEs, and adherence to the protocol, Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

Study documents should be retained for a minimum of 10 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 10 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when there is no longer a need for these documents to be retained. Permission must be acquired from the State of Florida for document destruction after the 10-year minimum record-retention period described above has elapsed.

12.1.1 Method for Data Reporting and Monitoring

Research Monitoring Core
Data will be captured in OnCore, Moffitt's Clinical Trials Database. Regulatory documents and case report forms will be reviewed routinely for accuracy, completeness and source verification of data entry, validation of appropriate informed consent process, adherence to study procedures, and reporting of SAEs and protocol deviations according to Moffitt's Monitoring Policies. Monitoring Visits will include a review of data entry to source documentation, regulatory documents, and when appropriate, investigational pharmacy or clinical laboratory.

Audit and Inspection
Inspections by regulatory health authority representatives i.e. FDA and IEC(s)/IRB(s) are possible. Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP), Form FDA 1572 and in the US Code of Federal Regulations.

Investigators must enter study data into Moffitt's electronic database, OnCore. The investigator will permit study related audit visit by a sponsor or it's representatives, IRB/EC review, and regulatory inspection(s), providing direct access to the facilities where the study took place, to source documents, to CRF's and to other study documents.

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

ACTION

Subject (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

Records pertaining to this trial must be retained for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. Research records should also be retained in accordance with institutional policy.

Sunshine Project Clinical Trials Oversight Committee (CTOC)

The CTOC is a group of pediatric oncologists who specialize in phase 1 clinical trials. This group of physicians review and evaluate the conduct of each trial the Sunshine Project conducts. Every quarter, the CTOC meets to ensure all clinical research conducted or coordinated by NPCF is scientifically well designed, responsibly managed, appropriately reported, and protects the rights and welfare of human participants.

The CTOC provides an independent review of the interim results of each trial and offers objective guidance regarding unforeseen events that may occur during the course of a study. The CTOC will help to evaluate and ensure events that may have an impact on patient safety are appropriately addressed and managed on an on-going basis. At the quarterly meetings, discussions will include adverse events, accrual, and protocol deviations. Adverse event and deviation collection begins when a patient is first placed on trial.

All events are classified using the NCI Common Toxicity Criteria and are summarized and presented to all members at each safety meeting. The CTOC will also help to evaluate and ensure events that may have an impact on patient safety are appropriately addressed and managed. The CTOC will make recommendations regarding the conduct and safety of the trial and a public report will be made available to all participating member sites. The CTOC will be convened in the event of safety concerns brought to the attention of investigators or the study chair.

Protocol Monitoring Committee

The Moffitt Cancer Center reviews investigator initiated trials that take place at Moffitt through the Protocol Support Office (PSO) at Protocol Monitoring Committee meetings. The PSO reviews all deviations, AEs, and SAEs on all Sunshine trials. Reports for phase I trials are created and reviewed when a trial increases a dose level or when a maximized tolerated dose is met. Because Moffitt acts as the main coordinating center for the Sunshine Trials, all Sunshine Project trials will undergo review by the PSO when a dose level is increased.

The PMC meets once a month, and reviews and evaluates safety and/or efficacy data for all physician authored clinical intervention trials. The PMC ensures the safety of subjects and the validity and integrity of data. PMC reviews SAEs, deviations, Interim analysis, interim and final reports from the external Data Monitoring Committee (DMC) as well as audits both internally and externally. The PMC can make the following determinations, Accepted, Acceptable with Corrective Action and Tabled.

Scientific Review Committee (SRC)

The two Therapeutic boards of the SRC meet every other week one on the first Wednesday and the second one meets on the third Thursday of every month.

Each SRC conducts a formal internal peer review of all clinical protocols and general scientific oversight of interventional clinical research. Protocols are reviewed for scientific merit, adequate study design,

ACTION

safety, availability of targeted study population, and feasibility of timely completion of all proposed research projects to be conducted by its assigned programs at the Cancer Center. The SRC is responsible for evaluating the risk/benefit assessment and corresponding data and safety monitoring plan as part of the scientific review and approval process.

Sunshine Project Bi-Weekly Teleconference
Starting with the first patient enrollment, a safety call consisting of principle investigators, a biostatistician, and study coordinators will occur biweekly. In addition, all AE's will be collected and shared with drug suppliers for input prior to escalating or de-escalating to the next dose level.

Moffitt Cancer Center Scientific Review Committee and Institutional Review Board All Sunshine
Project trials undergo an initial review at the center's Scientific Review Committee and Central Institutional Review Board.

12.1.2 Responsibility for Data Submission

It is the responsibility of the PI(s) at the site to ensure that all study team members at the site understand the procedures for data submission, and that protocol specified data is entered accurately and in a timely manner into the OnCore system.

All eCRF entries into OnCore will be verified via source documentation during on-site or remote monitoring visits.

12.2 Moffitt Cancer Center Guidelines

This protocol will adhere to the policies and requirements of the Moffitt Cancer Center Guidelines.

The Moffitt Cancer Center is responsible for distributing all IND Action Letters or Safety Reports received to all participating institutions for submission to their individual IRBs for action as required.

12.2.1 Suspension/Termination

The PMC and/or the IRB may vote to suspend or terminate approval of a research study not being conducted in accordance with the IRB, the Cancer Center and/or regulatory requirements or that has been associated with unexpected problems or serious harm to subjects. The PMC/IRB will notify the PI in writing of such suspension or terminations. It is the responsibility of the PMC/IRB Chairperson to ensure prompt written notification of any suspensions or terminations of PMC/IRB approval to the relevant Federal Agencies, including OHRP, FDA, the study sponsor/funding source and if applicable, the Affiliate Program.

12.2.2 Trial Discontinuation

For reasonable cause the Investigator and/or sponsor may terminate this study prematurely. Conditions that may warrant termination include, but are not limited to: the discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the study or if the accrual goals are met. A written notification of termination will be issued.

12.2.3 Protocol Modifications

No modifications will be made to the protocol without the agreement of the investigators. Changes that significantly affect the safety of the subjects, the scope of the investigation, or the scientific quality of the

study will require Scientific Review Committee and Institutional Review Board approval prior to implementation, except where the modification is necessary to eliminate apparent immediate hazard to human subjects. Any departures from the protocol must be fully documented in the case report form and the source documentation.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

13.1.1 Analysis Sets

The populations that may be used for analysis are defined as:

All subjects who receive any protocol treatment will be included in safety analyses.

- If either DCs and/or xALT do not meet release criteria or targeted dose then the patient may remain on study and receive the qualified product but will be replaced for the purposes of safety and efficacy assessment.
- Subjects requiring an increase in corticosteroids, with the exception of nasal or inhaled steroid, such that at the time of first vaccination they require a dose above physiologic levels, may remain in the study but will be replaced for assessment of safety as increased steroid usage may mask risks of autoimmune toxicity. For the purposes of this study, physiologic dose will be defined as > 4 mg of dexamethasone/day.
- Subjects receiving less than 10 vaccines without toxicity will be replaced for safety assessments.

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

13.1.2 Patient Demographics and other Baseline Characteristics

Summaries of clinical and socio-demographic characteristics will be generated for all subjects as well as patient subgroups defined by assigned treatment sequence. Categorical descriptors will be summarized using frequency distributions; whereas, interval variables will be summarized using percentiles, as well as means and standard deviations.

13.1.3 Treatments

The number of subjects who receive ACT treatment will be summarized.

13.1.4 Primary Objective

The primary purpose of this study is to explore the safety, feasibility and tolerability of DC vaccines and ACT in subjects with primary HGGs.

13.1.5 Secondary Objectives

Secondary objectives will be to estimate the mean difference and the variation in INF gamma secretion between baseline and 2 weeks post-completion of immunotherapy in Group A and Group B. OS and PFS will be secondary clinical outcomes measures If the effect of HSCs appears promising, we will use this information to determine the sample size required to appropriately power a phase II study. We do not

expect the sample size of the current study to be large enough to allow definitive conclusions, but we will use a t-test to compare gamma secretion differences (baseline – 2 weeks post-immunotherapy) between the two groups, and Kaplan-Meier methods to compare progression-free and overall survival.

13.1.6 Interim Analysis

No interim efficacy analysis is planned.

The safety of ACT using DC+xALT and MTD of tumor-specific lymphocytes was established in our ongoing phase I/II trial (Re-MATCH). Should differential dose-limiting toxicity be observed in subjects enrolled on this trial, a redesign to assess the MTD and DLT will be performed following our current phase I trial design in subjects with reMB/PNETs.

13.1.7 Safety endpoint statistical analysis and stopping criteria

No interim efficacy analysis is planned.

Any deaths within the treatment period will be reported as described in 7.2.5.

If 2 of 6 patients experience immunotherapy related DLT following administration of 3 $x10^8$/kg x-ALT, an additional 6 patients will be enrolled on group A to receive x-ALT at a dose of 3 $x10^7$/kg. The trial will stop if 2 of 6 patients develop DLT at this dose level. Once MTD is identified in group A patients, 6 patients will be treated in group B using this MTD for x-ALT infusion.

An overall toxicity rate within the 12 subjects will also be monitored to ensure that unacceptable toxicity is not observed during the treatment of both cohorts. We will use a dynamic sequential testing method (Kramar and Bascoul-Mollevi, 2009) to determine if the sequential proportion of enrolled and treated subjects who have experienced DLTs that have exceeded a maximum acceptable rate 10.0% This method employs a concave alpha-spending function that allows a greater chance of detecting an unacceptably high toxicity rate earlier in the study while maintaining a nominal one-sided Type I error rate of 10% over the entire sequence of tests performed(Kramar A and C Bascoul-Mollevi, 2009. Trials based on sequential monitoring of serious adverse events. Med Decis Making 29:343-350). Thus, the trial will be stopped if any of the following occur after dose modification of xALT to the lower dose of 3 x 10^7/kg:

1. 2 of the first 2, 3, 4, 5,6 subjects treated experience DLTs
2. 3 of the first 7 subjects treated experience DLTs
3. 4 subjects experience DLTs at any point during the trial

13.1.8 Handling of missing values, censoring, and discontinuations

If a patient drops out of the study before receiving immunotherapy, then that patient will be replaced. PFS and OS will be reported for all subjects on an intent to treat basis.

13.2 Sample Size/Accrual Rate

This pilot study design will be performed in 12-18 subjects (6-12 Group A subjects and 6 Group B subjects) where we will demonstrate feasibility and safety in pediatric subjects with HGGs and estimate the effect of size and variation in order to determine the sample size required to appropriately power a phase II study design.

ACTION

13.3 Stratification Factors

Up to twenty-four subjects will be enrolled to treat 12-18 evaluable subjects, 6-12 subjects into Group A followed by 6 subjects into Group B.

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15. APPENDIX A

Performance Scales

Lansky Performance Scale (subjects < 16 yo)		**Karnofsky Performance Scale (subjects ≥ 16 yo)**	
Percent		Percent	
100	Fully active.	100	Normal, no complaints, no evidence of disease.
90	Minor restriction in physically strenuous play.	90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Restricted in strenuous play, tires more easily, otherwise active.	80	Normal activity with effort; some signs or symptoms of disease.
70	Both greater restrictions of, and less time spent in active play.	70	Cares for self, unable to carry on normal activity or to do active work.
60	Ambulatory up to 50% of time, limited active play with assistance/supervision.	60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Considerable assistance required for any active play, fully able to engage in quiet play	50	Requires considerable assistance and frequent medical care.
40	Able to initiate quite activities.	40	Disabled, requires special care and assistance.
30	Needs considerable assistance for quiet activity.	30	Severely disabled, hospitalization indicated. Death not imminent.
20	Limited to very passive activity initiated by others (e.g. TV).	20	Very sick, hospitalization indicated. Death not imminent.
10	Completely disabled, not even passive play.	10	Moribund, fatal processes progressing rapidly.
0	Dead.	0	Dead.

16. APPENDIX B

TEMOZOLOMIDE PILL DIARY				
Name: _____ **Study ID:** _____				**Dose:** _____ **Cycle:** _____
Course:	**Date**	**Time**	**Dose**	**Comments**
Day 1				
Day 2				
Day 3				
Day 4				
Day 5				
Day 6				
Day 7				
Day 8				
Day 9				
Day 10				
Day 11				
Day 12				
Day 13				
Day 14				
Day 15				
Day 16				
Day 17				
Day 18				
Day 19				
Day 20				
Day 21				