Re-MATCH:

Recurrent **M**edulloblastoma and Primitive Neuroectodermal Tumor
Adoptive **T** Cell Therapy during Recovery from Myeloablative
Chemotherapy and **H**ematopoietic Stem Cell Transplantation

Food and Drug Administration Investigational New Drug Application 14,058
University of Florida Institutional Review Board Protocol 201500502
(Previously 128-2013)

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Summary of Protocol Changes

Version Date	Sections Revised	Purpose
20130913 1230 RB	N/A	- Initial UF Protocol Release
20140410 0800 RB	Entire Document	- Re-formatted for improved readability - Corrected typographical errors
	Title Page	- Added IRB number - Deleted non-UF investigators
	List of Possible Abbreviations	- Added Myeloablative (MA)
	2	- Clarified how subjects will be analyzed
	Study Synopsis	- Clarified the primary and secondary objectives - Limited inclusion to first localized recurrence - Clarified the study design - Added language to outline the timing for non-mobilized leukapheresis - Clarified use and timing of GM-CSF and G-CSF
	3	- Updated titles and work location s - Added Jianping Huang, MD, PhD
	4	- Updated locations and amended the list of supervising personnel
	5	- Updated to reflect completion of reviews and approvals - Updated to reflect new study completion time frame
	6	- Removed reference to the Phase I Aims since that portion of the study was completed at Duke University - Updated Phase I study data - Limited inclusion to first localized recurrence
	7	- Clarified the primary and secondary objectives
	8	- Clarified the primary efficacy endpoint and how subjects will be analyzed - Added language to outline the timing for non-mobilized leukapheresis - Clarified use and timing of GM-CSF and G-CSF
	9	- Limited inclusion to first localized recurrence
	10	- Limited inclusion to first localized recurrence - Clarified eligibility criteria for cardiac parameters - Deleted reference to Phase I of the study
	11	- Deleted reference to Duke personnel
	14	- Deleted requirement for signed and dated eligibility checklist before enrollment

Summary of Protocol Changes

Version Date	Sections Revised	Purpose
	15	- Added language to outline the timing for non-mobilized leukapheresis - Amended language for PBSC mobilization and leukapheresis to outline the collection goals for leukapheresis and TTRNA and DC vaccine - Clarified eligibility criteria for cardiac parameters
	17	- Clarified types of events requiring prompt reporting
	19	- Amended language for PBSC mobilization and leukapheresis to outline the collection goals for leukapheresis and TTRNA and DC vaccine
	20	- Updated shipping address for pathology slides - Clarified which infectious disease markers are required
	Study Road Map	- Revised and expanded to include all study requirements referenced in other sections of the protocol
	21	- Added language to indicate that samples collected at Duke from Phase I subjects will be analyzed at UF with Phase II samples - Clarified the starting point for survival analysis - Deleted reference to Duke follow-up track record
	22	- Removed all reference to Duke's Safety Oversight Committee and Regulatory Officer and added language related to the use and role of an independent external monitor
	24	- Deleted reference to Duke's Phase I RE-START protocol - Amended monthly PI meeting to conference calls
	26	- Removed reference to Regulatory Officer and added language related to the use of an independent external monitor
20140808 0830 RB	2.1	- Clarified dose of TTRNA-DCs as $1x10^7$ cells.
	6.1	- Replaced "our institution" with "Duke University" since Dr. Mitchell relocated to the University of Florida.
	6.2	- Corrected grammatical error. - Deleted reference to IFNγ since this cytokine is no longer used.
	6.4	- Added section identifier for reference in the table of contents. - Formatting & grammatical changes. - Modified section to indicate that the Phase I study is complete.
	8 15.3.1 15.3.2	- Changed TTRNA-xALT infusion duration and vital sign monitoring. - Clarified dose of TTRNA-DCs as $1x10^7$ cells.
	15.2.1	- Corrected typographical error.

Summary of Protocol Changes

Version Date	Sections Revised	Purpose
	16.2	- Modified section to reflect that the preparation, delivery, and administration of TTRNA-xALT will be according to Standard Operating Procedures and that subject identifiers will be double-verified prior to administration. - Changed TTRNA-xALT infusion duration and vital sign monitoring.
	17.3 29.1	- Corrected grammatical error. - Modified section to reflect use of the most current CTCAE, version 4. - Modified adverse event descriptions to be consistent with CTCAE version 4.
	19	- Modified title to reflect addition of "External Sites" section.
	19.1	- Added new section to describe how samples will be collected/ prepared/shipped between external sites and the University of Florida.
	19.2 19.3 19.5	- Modified the language in these sections (TTRNA Isolation, Amplification of RNA, and Procedure for TTRNA-DC and TTRNA-xALT Generation) to be consistent with recent changes to the Chemistry, Manufacturing, and Control section of the IND.
	20.2 STUDY ROAD MAP	- Modified language to indicate that the Type and Screen is only required if clinically indicated. - Modified language to indicate that oral Tums are only required if standard of care.
20140916 1130 RB	2.1	- Corrected KPS to be greater than or equal to 50% to be consistent throughout document.
	10.2 11.2 13 20.1	- Added language to allow potential enrollment of subjects who have had tumor tissue snap frozen at outside institutions.
	15.2.1 15.3.2	- Changed tissue transport to 'aseptic' to be consistent with IND.
20141013 1245 RB	10.1	- Added the ability to obtain Tissue Consent by telephone.
20141014 1240 RB	2.1 10.2	- Added carriers of Hepatitis B and/or Hepatitis C virus to exclusion criteria.
	4	- Updated the name of the University of Florida study location.
	17.4 17.4.1 17.4.2 17.4.3	- Added new section identifiers for reference in the Table of Contents.
	17.4.3	- Defined the adverse event reporting windows.

Summary of Protocol Changes

Version Date	Sections Revised	Purpose
	20.2	- Deleted reference to the Td manufacturer and injection location. - Deleted reference to Hepatitis B vaccination and titers.
	STUDY ROAD MAP	- Deleted reference to Hepatitis B vaccination and titers. - Moved non-mobilized leukapheresis assessments before pre-induction or salvage chemo assessments to align with study activities.
	23 23.1 23.2 23.3 23.4	- Added new section identifiers for reference in the Table of Contents.
	23.1	- Added the University of Florida Health Cancer Center DSMB.
	25	- Moved the first paragraph to Section 23.2. - Deleted the second paragraph since content is covered in Section 17.4.2. - Revised the third paragraph and moved it to Section 23.2
	25.2	- Revised the fourth paragraph and moved it to Section 23.1.
	26	- Deleted section since content is covered in Section 23.
	29 29.1	- Moved language to Sections 17.4.1 and 17.4.2.
20141218 1430 RB	2.1 8 15.4 16.1 20.2 STUDY ROAD MAP	- To serve as a baseline measure, a new time point for the collection of peripheral blood for immunologic monitoring was added prior to non-mobilized leukapheresis.
	15.2.2 15.3.3 19.4	- Two new, "Leukapheresis for Peripheral Blood Mononuclear Cells (PBMCs)", sections were added to outline specific parameters for PBMC collection. The "Procedure for PBSC Mobilization and Leukapheresis" and "Procedure Leukapheresis" sections were re-named and amended to provide each institution the flexibility to follow their local standards and to provide guidelines for PBSC mobilization and apheresis duration. The "Leukapheresis and PBSC Mobilization" section was deleted since the content was already covered in other protocol sections.
	2.1 8 10.2 15.2.3 15.3.4	- Language was added to clarify that focal boost radiotherapy for local control is allowed.

Summary of Protocol Changes

Version Date	Sections Revised	Purpose
	19.5	- Language was amended for clarity and to correlate with minor changes to the manufacturing processes.
	Entire Document 30 30.1	- Reference to study-specific documents (Standard Operating Procedures, Batch Records, and Source Document Worksheets) was added throughout the entire protocol. A new "Appendices" section was added that lists each document number and title referenced in the narrative sections.
20150306 0715 RB	2.1 10.1 11.2	- Language was amended to clearly define subjects who have received and failed definitive standard radiotherapy.
	19.4	- 'Leukapheresis' was replaced with 'PBMCs', 'DMSO' was replaced with 'Cryoserv', 'production' was replaced with 'stimulation', and 'cell' was replaced with 'DC' to more clearly outline the manufacturing process. 'Ph' was amended to "PH'.
	8 15.3.1	- The instructions for peripheral blood stem cell administration were amended to allow each institution the flexibility to infuse the cells in accordance with local guidelines.
	2.1 8 15.4 16.1 20.2 STUDY ROAD MAP	- Select immune monitoring time points were amended to allow for flexibility in collection time. The requirement for bimonthly immune monitoring samples was revised to 'if feasible' to accommodate subjects who may not be seen at the main institution.
	8 15.3.1 15.3.2 16.2	- The terms 'up to' were added to the acetaminophen and benadryl pre-medication language to allow for dosing flexibility,
20150622 0930 RB	8 15.3.1 15.3.2	- To provide institutions the flexibility to follow local guidelines, IV was added as a route of administration for Sargramostim and Filgrastim.
	20.2 STUDY ROAD MAP	- To enhance DC trafficking, Td booster will be administered to all subjects regardless of vaccination history.
	23.2	- At the request of the Medical Monitor, written reports will be generated biannually to correlate with DSMB reports.
20151015 0730 RB	Title Page	- As a result of the conversion to the electronic myIRB system, the new IRB number was added.
	2.1 10.1 15.3.2	- To minimize variability, Group B subjects with an ANC < 1000/µL and/or platelet count < 100,000/µL at time of non-myeloablative work-up will receive vaccine therapy but will not receive non-myeloablative conditioning.

Summary of Protocol Changes

Version Date	Sections Revised	Purpose
	2.1 8 15.1 15.2.1 15.3.2	- The language regarding DCs and/or xALT release criteria or targeted dose was amended for clarity.
	2.1 8 15.3.2	- The infusion of PBSCs 72 hours following fludarabine, if successfully collected, was added for Group B patients to potentially enhance the immune response.
	2,2	- The schema was updated to state that the pheresis collection is sent to UF.
	4	- The names of the external study sites were added.
	5	- The grant end date was updated.
	6.4	- Language was amended for clarity.
	15.2.3 15.3.4	- The storage recommendations for collected PBSCs were removed to allow for institutional variation.
	15.3.5	- Reference to the collection of back-up PBSCs was removed since the cells, if successfully collected, will be administered to all Group B subjects.
	16.2	- Language was amended to incorporate the infusion of the TTRNA-xALT product in accordance with SOP-UFBTIP-132.
	17.4.3	- Language was amended to delineate what adverse events will be recorded.
	19.3	- Language was amended to update the sterility testing location and type of testing.
	19.4	- Language was amended to align with the Chemistry, Manufacturing, and Control language.
	STUDY ROAD MAP	- PBSCs were added to the Consolidation or NMA Chemo time point to correlate with the narrative sections of the protocol.
	30.1	- Reference to the SOP for the “Administration of ALT” was added.
20160303 1200 RB	3 3.1 3.2 4 8 21.1 21.6 25 25.1	- Key study personnel updated throughout protocol to reflect current individuals with assigned study duties.
	17.4.3	- The duration of adverse event follow-up was modified for clarity.

Summary of Protocol Changes

Version Date	Sections Revised	Purpose
	2.1 8 15.4 16.1 20.2 STUDY ROAD MAP	- A plus or minus 1 day window was added to the day 4 assessments to provide scheduling flexibility.
20160505 1749 DAM	2.1 9 10.1 11.2	- Language regarding disease status eligibility was clarified.
20160908 1515 KW	31	- Single Patient-Specific Protocol (UF Site Only) was added as Addendum A to protocol
20170220 1100 KW	Overall Document	- Table of Contents updated for pagination - Footer updated with new version date. - Additional editorial, typographical and formatting changes have been made throughout the body of the protocol, along with minor changes for document consistency, all of which are tracked but not individually listed.
	2.1 10.1	- Performance status age range modified to align with NIH criteria.
	2.1 10.1	- Language regarding additional eligibility requirements updated to clarify applicability to both consolidation and NMA chemotherapy.
	2 2.1 8 10.1 15.3.5	- Description of Group B regimen in these sections revised for consistency with other sections of the protocol.
	2.1 8	- Language regarding localized relapse and disseminated disease clarified for consistent interpretation across sites.
	2.1 15.2.3 15.3.4	- Minimum number of stem cells for harvest in Group A subjects specified in this section for clarity and consistency.
	2.1 8 15.3.2 15.3.4	- Goal for stem cell harvest in Group B subjects added to this section for clarity and consistency.
	2.1 8	- For subject safety and to minimize variability, Group B subjects with no peripheral blood stem cells (PBSCs) for rescue will have NMA conditioning withheld and move to immunotherapy from salvage chemotherapy if all other eligibility has been met.

	2.1 8 15.4	- Timeframes and windows for follow-up visits clarified.
	2.2-2.4	- Study schema updated to more accurately reflect existing protocol procedures.
	3.2 4.0 25.1 25.2	- Duke and Stanford removed from study due to underperformance and/or inability to complete all requirements to activate trial at respective sites.
	5	- Projected time frame to complete study modified.
	10.2 15.1 15.2.1	- Maximum steroid dose amended to more closely align with exclusion criteria in brain tumor protocols.
	15.3.2	- Timing of organ performance assessment clarified. - Pulmonary function parameters that were inadvertently deleted from this section in prior protocol revision re-inserted.
	15.3.2 19.4	- Modified to allow subjects to receive all available T cells if target dose is not met. This provision exists in the ICF.
	17.4.2 17.4.3	- Regulatory definitions and reporting requirements for adverse events clarified. - Timeframe for following adverse events added for clarity.
	17.5	- Requirements for concomitant medication collection and documentation added for clarity.
	20.2 (Roadmap)	- Pre-Induction or Salvage Chemo testing done as part of standard care for these patients removed. - Timeframe for pre-immunotherapy Td booster and labs clarified.
20180628	2.1 8.0 15.3 20.2 Study Road Map	- Modify administration of GM-CSF to maintain consistent manufacturing across all of our adoptive cellular therapy trials. For the remaining subjects in this trial, we will embed the GM-CSF with the DC vaccines rather than administering intravenously. - Remove GM-CSF as part of Group A and Group B consolidation of NMA chemo since it will be embedded with DC vaccine.
	2.1 8.0 15.3	- Provide a window for G-CSF administration to allow for management per institutional guidelines and treating Transplant physician discretion.
	3	- Remove Timothy Driscoll since Duke is no longer participating in this trial.
	3.1 25	- Remove David Pincus and Barbara Frentzen who left the University of Florida and are no longer affiliated with the trial.
	3.2	- Remove Stanford personnel since Stanford is no longer a participating site.

	4	- Remove David Pincus who left UF and is no longer affiliated with the trial.
	5	- Update projected time frame to complete trial.
	16.1	- Add provision for continued DC vaccination for subjects who have additional DC vaccines available (beyond planned 3 vaccines) and will not receive T-cell infusion either because of the inability to manufacture a viable product or failure of the product to meet release criteria.
	20.2 and Study Road Map	- Clarify procedures related to pre-leukapheresis laboratory assessments to allow for institutions to follow local standards (i.e., some institutions perform basic metabolic panels while others perform comprehensive metabolic panels).
	20.2	- Clarify Td administration to reflect standard dosing and ensure consistency in administration across sites.

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
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

TABLE of CONTENTS

1. STUDY TITLE

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

2. DESCRIPTION OF CLINICAL TRIAL

This is a dual-arm, prospective **Phase II clinical trial** of adoptive cellular therapy targeting recurrent medulloblastoma (MB) and recurrent supratentorial primitive neuroectodermal tumors (PNETs). Group A will consist of patients undergoing hematopoietic recovery from high-dose chemotherapy and autologous peripheral blood stem cell transplant. Group B will consist of patients undergoing non-myeloablative salvage chemotherapy and autologous peripheral blood stem cell transplant The 12-month progression-free survival (PFS) rate of all patients will be compared collectively to a historical cohort, as patients did not have statistically different survival outcomes in the historical data set whether they received myeloablative (MA) or non-myeloablative (NMA) chemotherapy for recurrent disease. The PFS and OS in each arm will be analyzed descriptively to explore whether therapeutic outcomes may differ on the two treatment arms in this study.

2.1 Study Synopsis

Title:	Re-MATCH: Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor Adoptive T Cell Therapy during Recovery from Myeloablative Chemotherapy and Hematopoietic Stem Cell Transplantation
Study Drug:	Total tumor mRNA-pulsed autologous Dendritic Cells (DCs) (TTRNA-DCs) and tumor-specific *ex vivo* expanded autologous lymphocyte transfer (TTRNA-xALT)
Rationale:	Vaccination and adoptive T cell strategies targeting unfractionated tumor antigens in humans in other contexts have been safe and effective. DC vaccinations targeting tumor antigens in children with MB and PNETs and other brain tumors have been shown to be feasible and safe. The prognosis for patients with recurrent MB/PNETs (reMB/PNET), particularly for those who have failed prior definitive focal +/- craniospinal irradiation, is dismal. High Dose Chemotherapy (HDC) + PBSCT have been used as a standard of care in recent years in recurrent disease but are seldom curative. MA and NMA chemotherapy induces a profound lymphopenia that would be predicted to prevent the induction of effective immune response to anti-tumor vaccination. However, 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. The use of TTRNA-pulsed DCs to expand tumor-specific lymphocytes *ex vivo* 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 MB/PNETs 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 also demonstrated that subtractive hybridization of RNA from normal brain can be used to enrich for antigens expressed exclusively within malignant brain tumor cells and possibly increase the specificity and safety profile of total tumor RNA directed immunotherapy.
Primary Objective:	**Phase II:** 1. To determine if DC + xALT therapy extends progression-free survival compared to historical benchmarks during recovery from HDC + PBSCT (Group A) or salvage chemotherapy (Group B) in pediatric patients with reMB/PNETs.
Secondary Objectives:	1. To determine the objective radiographic response rate of MA and NMA chemotherapy coupled with DC + xALT therapy in patients with residual disease 2. To determine if magnitude and persistence of anti-tumor humoral or cellular immunity correlates with clinical outcome 3. To evaluate changes in cytokine profile and Toll-Like Receptor (TLR) activation status in pediatric patients with reMB/PNETs after HDC **(Group A)** or salvage chemotherapy **(Group B**) and during DC + xALT therapy 4. To characterize the immunologic phenotype of lymphocyte subsets (naïve, effector, memory, regulatory) and NK cells in patients with reMB/PNETs at diagnosis and during experimental therapy 5. To identify potential tumor specific antigens as vaccine candidates through characterizing the frequency of expression of the top 40 tumor associated antigens (TAA_{40}) identified in common malignancies in recurrent MB/PNETs 6. To determine the progression-free survival and overall survival rate in pediatric patients with recurrent MB/PNETs receiving DC + xALT therapy after HDC plus PBSCT **(Group A)** compared to a historical benchmark 7. To determine the progression-free survival and overall survival rate in pediatric patients with recurrent MB/PNETs receiving DC + xALT therapy after salvage chemotherapy **(Group B)** compared to a historical benchmark
Inclusion Criteria:	**Screening:** • Age ≤ 30 years of age. • Suspected first recurrence/progression of MB/PNET since completion of definitive focal +/- craniospinal irradiation. Disease progression prior to receiving definitive focal +/- craniospinal irradiation will not disqualify patients from enrollment if they have subsequently failed definitive radiotherapy and are at first recurrence/progression at time of enrollment. Patients who are unable to receive radiation therapy due to genetic disorders that put them at significant risk for radiation-induced secondary malignancies (i.e. Gorlin's syndrome or NF1 mutation) are eligible for enrollment at first disease recurrence/progression. **Re-MATCH protocol:** • Patients must have histologically confirmed reMB/PNET that is a first relapse/progression after completion of definitive focal +/- craniospinal irradiation. Patients with a first relapse/progression who are unable to receive radiation therapy due to genetic disorders that put them at significant risk for radiation-induced secondary malignancies (ie. Gorlin's syndrome or NF1 mutation) are eligible for enrollment. • Patients with neurological deficits should have deficits that are stable for a minimum of 1 week prior to registration. • Karnofsky Performance Status (KPS) of ≥ 50% (KPS for > 16 years of age) or Lansky performance Score (LPS) of ≥ 50 (LPS for ≤ 16 years of age) assessed within 2 weeks prior to registration and documented in the medical record or on **SDW-UFBTIP-004**. Patients who are

	unable to walk because of paralysis but who are up in a wheel chair will be considered ambulatory for the purposes of the performance score. • Bone Marrow: - ANC (Absolute neutrophil count) ≥ 1000/µl (unsupported)* - Platelets ≥ 100,000/µl (unsupported)* - Hemoglobin > 8 g/dL (may be supported) *Patients on Group B with ANC < 1000/µL and/or platelet count < 100,000/µL prior to NMA will be allowed to get vaccine and ALT infusion; however, NMA conditioning will be withheld. • Renal: -Serum creatinine ≤ upper limit of institutional normal • Hepatic: - Bilirubin ≤ 1.5 times upper limit of normal for age. - SGPT (Serum Glutamic Oxaloacetic Transaminase) (ALT) ≤ 3 times institutional upper limit of normal for age. - SGOT (AST) ≤ 3 times institutional upper limit of normal for age. • Patients of childbearing or child-fathering potential must be willing to use a medically acceptable form of birth control, which includes abstinence, while being treated on this study. • Patient or patient guardian consent to PBSC and/or bone marrow harvest following registration if PBSC or bone marrow (CD34 count of at least $2x10^6$/kg) has not been previously stored and available for use. • Signed informed consent according to institutional guidelines must be obtained prior to registration. **Additional Eligibility Requirements Prior to Initiation of Consolidation or NMAChemotherapy:** • Renal - GFR of at least 70 ml/min/1.73m^2 (Group A only). • Cardiac: - ECHO cardiogram with an ejection fraction of > 45% or Multiple Gated Acquisition (MUGA) scan within normal limits (Group A only). Patients in Group B will be eligible if the ejection fraction is < or equal to 45% but will receive Fludarabine only during NMA conditioning prior to vaccine + ALT infusion. - EKG without clinically significant arrhythmias (Group A only). • Pulmonary - DLCO, FEV1, FEC (diffusion capacity) > 50% of predicted (corrected for hemoglobin and alveolar volume). (Group A only) - Crying Vital Capacity if too young to perform pulmonary function tests (Group A only). - Chest X-ray without significant abnormalities and a pulse oximetry reading of ≥ 93% in room air at rest and following a brisk 6-minute exercise resulting in a target heart rate of at least 100/min. These two tests would be required for any patient in Group A who cannot co-operate for the DLCO test due to neurologic deficits and for all patients in Group B. Patients in Group B who do not meet this requirement will still be eligible but will receive Fludarabine only during NMA conditioning.
Exclusion Criteria:	• Pregnant or need to breast feed during the study period (Negative serum pregnancy test required). • Active infection requiring treatment or an unexplained febrile (> 101.5° F) illness.

	• Known immunosuppressive disease, human immunodeficiency virus infection, or carriers of Hepatitis B or Hepatitis C virus. • Patients with active renal, cardiac (congestive cardiac failure, myocardial infarction, myocarditis), or pulmonary disease. • Patients receiving concomitant immunosuppressive agents for medical condition. • Patients who need definitive radiotherapy for treatment of reMB/PNET. Focal boost radiotherapy may be delivered prior to immunotherapy if required for local control. • Patients receiving any other concurrent anticancer or investigational drug therapy. • Patients with any clinically significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction). • Patients with inability to return for follow-up visits or obtain follow-up studies required to assess toxicity to therapy.
Study Design:	This prospective Phase II multi-institutional clinical trial will evaluate the progression-free survival of autologous tumor-specific T cell immunotherapy (TTRNA-xALT) in conjunction with TTRNA-loaded dendritic cell vaccine in patients with first recurrence of medulloblastoma (reMB)/supratentorial primitive neuroectodermal tumors (PNETs) after completion of definitive radiation therapy. Up to 42 patients will be enrolled to treat 35 evaluable patients with reMB/PNET with DC + xALT therapy. Following surgical resection, biopsy, or cytology examination with confirmatory pathologic diagnosis, patients will be enrolled into HDC **(Group A)** or NMA salvage chemotherapy **(Group B)** based on eligibility for HDC. Patients with localized relapse and have not failed HDC+PBSCT previously will be enrolled into Group A as HDC+PBSCT is considered an acceptable standard-of-care in the relapsed disease setting. Patients with disseminated disease, have previously failed HDC+PBSCT, or are otherwise considered poor candidates for HDC based on overall health status, but otherwise meet eligibility criteria, will be enrolled into Group B and will receive a salvage chemotherapy regimen followed by non-myeloablative cyclophosphamide/fludarabine lymphodepletive conditioning and PBSCT prior to DC + xALT therapy. For purposes of this protocol, localized relapse will be defined as recurrence in the same CNS location **as the original tumor or a single location outside of the primary site**. Disseminated disease will be defined as new lesions with little evidence of being contiguous with prior resection cavity, or intraventricular spread, or dissemination along any anatomically definable subcortical white matter tracts. ***Group A***: Patients enrolled into Group A will receive Induction chemotherapy that will include cyclophosphamide, etoposide, and temozolomide. Leukapheresis for collection of autologous peripheral blood stem cells (PBSCs) and peripheral blood mononuclear cells (PBMCs) for DC and T cell generation will occur prior to and during induction cycles of chemotherapy and will continue until adequate quantities of nucleated cells are obtained. The leukapheresis products will be aliquoted for future autologous PBSC rescue during Consolidation and used to generate the TTRNA-xALT and multiple doses of the TTRNA-DCs for future use during Post-transplant Immunotherapy. After completion of Induction, eligible patients will proceed to Consolidation with high dose chemotherapy consisting of carboplatin, etoposide, and thiotepa, followed by autologous PBSC rescue. The following day, Post-transplant Immunotherapy begins with infusion of the TTRNA-xALT and injection of the first TTRNA-DC vaccine embedded with GM-CSF 150 ug followed by G-CSF (5 micrograms/kg/day beginning no later than day 6 until count recovery). Patients will receive TTRNA-xALT at $3x10^7$ cells/kg at vaccine #1 in combination with the first

of three biweekly fixed doses of TTRNA-DC ($1x10^7$ cells) with GM-CSF. Vaccines #2 and #3 will consist of intradermal DC with GM-CSF vaccination only.

Enrolled patients with reMB/PNETs will receive 3-4 cycles of induction therapy consisting of two four week cycles of intravenous cyclophosphamide (2 gm/m^2/day for 2 days as Cycles 1 and 2) followed by two four week cycles of oral etoposide 30 mg/m^2/day for 14 days plus oral temozolomide (TMZ) (150 mg/m^2/d for 5 days as Cycles 3 and 4). Patients will undergo a non-mobilized leukapheresis prior to induction chemotherapy for harvest of peripheral blood mononuclear cells (PBMCs) for TTRNA-xALT and TTRNA-DC generation. Afterwards, mobilized peripheral blood stem cells (PBSC) will be collected after each induction chemotherapy cycle until enough peripheral blood stem cells (PBSCs) are harvested for autologous stem cell rescue (minimum of $2x10^6$ CD34+ PBSCs/kg) Patients who have radiographic or histologic evidence of progression will be replaced. 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. For the purpose of this study, progression will be defined as a new lesion confirmed by biopsy or resection, positive cerebrospinal fluid (CSF) cytology, or radiographic progression as defined by protocol criteria. Following recovery from the 3rd or 4th cycle of induction chemotherapy (ANC $\geq$ 1000 cells/μL), patients will undergo disease restaging and organ function evaluation. Patients will be admitted to the Pediatric Bone Marrow Transplant Unit (BMTU) and receive Carboplatin (either 500 mg/m^2 or a dose calculated based on Calvert's formula and a AUC (Area under the concentration time curve) 7 mg/ml/min, whichever is less) on days -8, -7, and -6, followed by thiotepa 300 mg/m^2 and etoposide 250 mg/m^2 daily on days -5, -4, and -3. Three days after HDC (Day 0), patients will receive PBSC rescue. DC + xALT therapy will begin 24 hours after PBSCT. These patients will receive TTRNA-xALT ($3x10^7$/kg i.v.) and TTRNA-DCs ($1x10^7$ cells i.d.) following the transplant as vaccine #1. Patients will receive 3 biweekly TTRNA-DC vaccines with GM-CSF as long as clinically stable. Patients will also receive G-CSF 5 microgram/kg/day beginning no later than day 6 until ANC recovers to >1000 cells/μL.

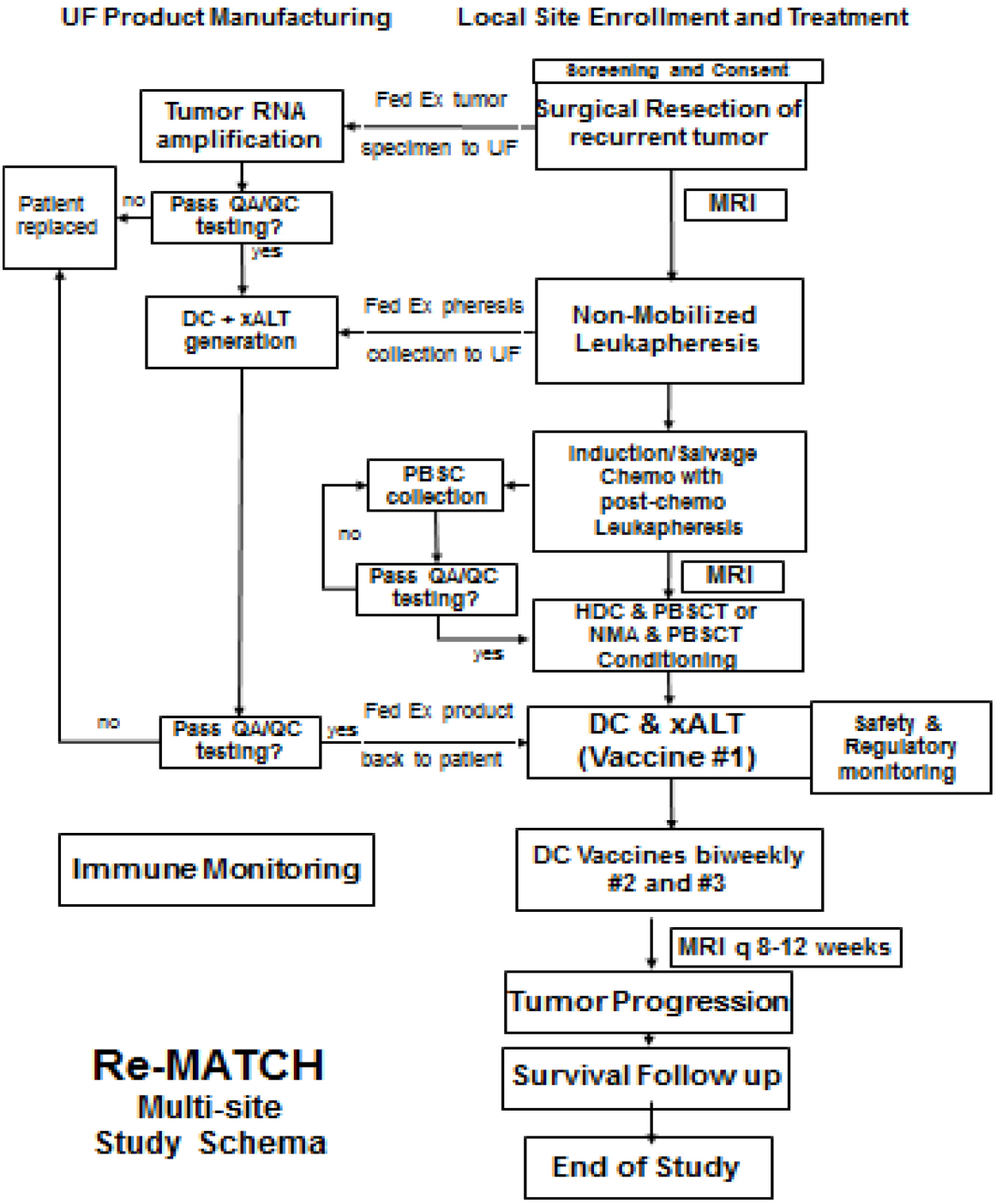
NOTE: Patients who are no longer candidates for HDC will be eligible to transfer from Group A to Group B.

Group B:
Patients enrolled into Group B will receive NMA salvage chemotherapy that will include cycles of etoposide, and temozolomide followed by cyclophosphamide and fludarabine. Enrolled patients with reMB/PNETs will receive salvage chemotherapy consisting of 2-4 cycles of oral etoposide 30 mg/m^2/day for 14 days plus oral temozolomide (TMZ) 150 mg/m^2/d for 5 days monthly until Immunotherapy preparation is complete. Patients will undergo a non-mobilized leukapheresis prior to salvage chemotherapy for harvest of peripheral blood mononuclear cells (PBMCs) for TTRNA-xALT and TTRNA-DC generation. Afterwards, mobilized leukapheresis will be attempted after each salvage chemotherapy cycle until enough peripheral blood stem cells (PBSCs) are harvested for autologous stem cell infusion (goal of $2x10^6$ CD34+ PBSCs/kg). Subjects who do not mobilize any peripheral blood stem cells or do not undergo leukapheresis, will continue with immunotherapy without NMAchemotherapy. 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 assessment. Patients who progress prior to receiving immunotherapy may remain on study as long as they continue to meet the other eligibility criteria. Patients may receive the first 3 vaccines as long as they are clinically stable

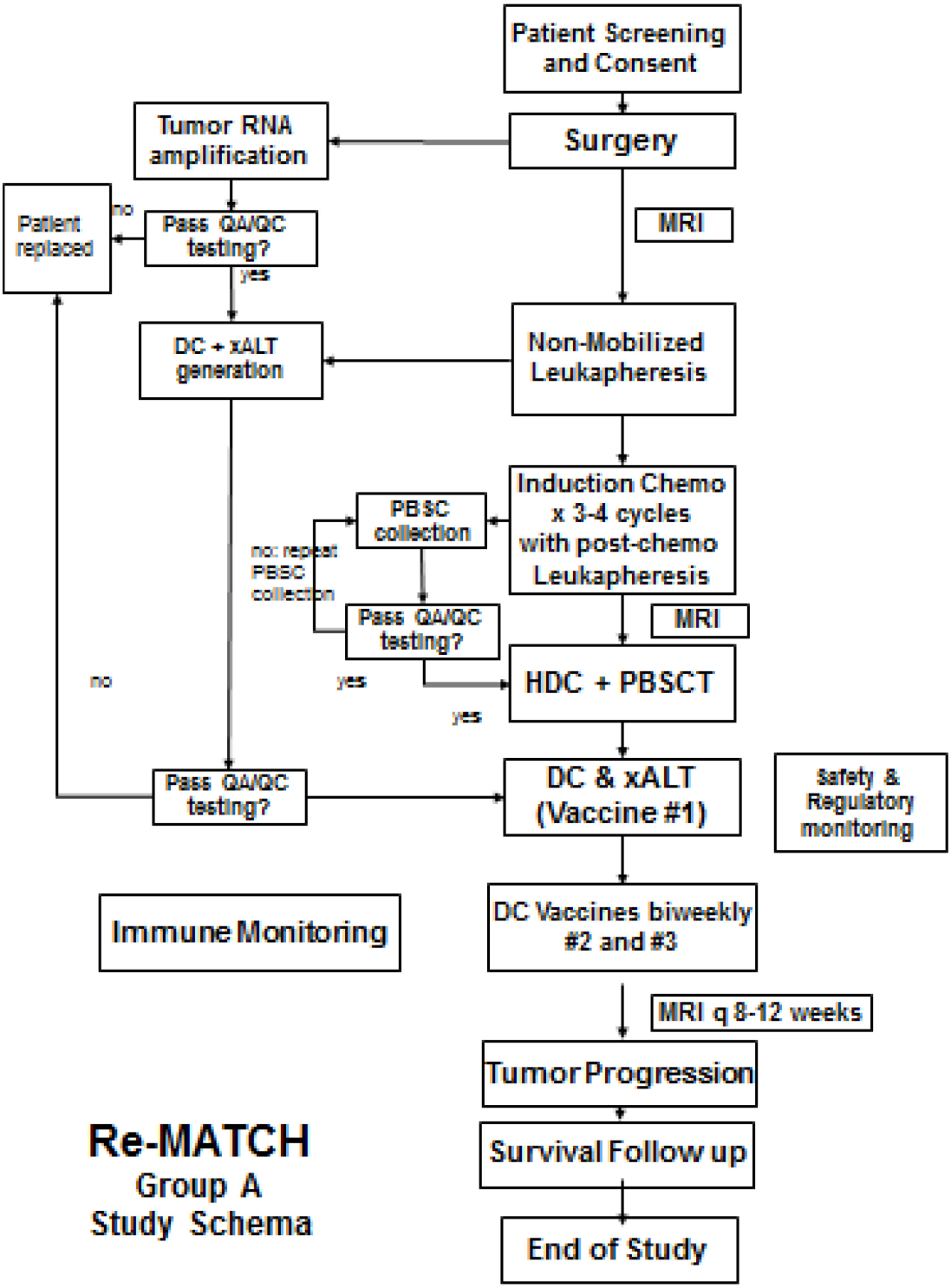
	and remain eligible. Subsequently, for the purpose of this study, progression will be a new lesion confirmed by biopsy or resection, positive cerebrospinal fluid (CSF) cytology (when previously negative), or radiographic progression as defined by protocol criteria. Following surgery and leukapheresis, adequate organ function testing will be repeated prior to initiation of cyclophosphamide and fludarabine conditioning. Drug administration and dosage for the lymphodepletive conditioning may be adjusted based upon hematologic performance and organ function testing results as per protocol. After completion of NMA salvage chemotherapy and starting 11 days before Immunotherapy, patients will receive 2 days of cyclophosphamide I.V. at $1g/m^2$, followed by 5 days of fludarabine I.V. at 25 mg/m^2. Approximately 72 hours following fludarabine, PBSCs, if available, will be infused. Approximately 96 hours following the final dose of fludarabine, Immunotherapy begins with infusion of TTRNA-xALT and injection of the first TTRNA-DC vaccine. Patients will receive G-CSF after DC + xALT therapy until ANC recovers to >1000 cells/μL. Patients will receive TTRNA-xALT at $3x10^7$ cells/kg with the 3 total biweekly fixed doses of TTRNA-DC ($1x10^7$ cells i.d.) with GM-CSF 150 ug. The NMA recovery will incorporate G-CSF at 5 microgram/kg/day beginning no later than day 6 until ANC recovers to >1000 cells/μL. *NOTE: Patients on Group B with ANC <1000/μL and/or platelet count <100,000/μL prior to NMA will be allowed to get vaccine and ALT infusion; however, NMA conditioning will be withheld.* *Based on prior chemotherapy history and/or hematologic performance, patients enrolled on Group B receiving salvage chemotherapy may need to have adjustment to the planned chemotherapy regimen at the discretion of the treating physician(s). Focal boost radiotherapy may be delivered prior to immunotherapy if required for local control. As no salvage chemotherapy regimens in the recurrent setting of MB or PNETs have been found to be curative or achieve significantly prolonged disease-free survival, we do not anticipate any clinical impact of adjustments to the planned regimen in assessing PFS endpoint. All chemotherapy regimens will be recorded and any deviation from the prescribed regimen above noted.* The primary efficacy endpoint will be 12-month progression-free survival (PFS-12) rate after treatment with DC + xALT therapy as a surrogate for overall survival. Gururangan et al. reported that PFS-12 for patients with recurrent disease after initiating treatment with HDC + PBSCT or standard salvage therapy was 33% (95% confidence interval of 10%, 59%) and two-year PFS of 0%. The population of patients that will be treated in the current protocol in Group A is similar to the subset of patients treated on the Gururangan regimen. Due to the complexity and expense of adoptive cellular therapy treatment, if the true PFS-12 associated with DC + xALT therapy is 33%, there would be limited interest in further investigation of this treatment regimen without significant modification to improve efficacy. However, if the true PFS-12 were 55% or greater, there would be interest in studying this treatment regimen within the context of larger confirmatory Phase II or III clinical trials. Within Group B, a good historical benchmark for this patient population receiving a standardized salvage regimen is not readily available. However, previously published studies have shown that patients unable to receive HDC and receive salvage regimens for recurrent disease generally have poorer prognosis than patients in Group A with localized disease relapse receiving HDC + PBSCT. These differences, however, did not result in a statistically significant difference in survival outcomes between patients receiving HDC+PBSCT vs salvage chemotherapy. Therefore, we will conservatively use the benchmark described above for analysis

	of all patients collectively for the primary 12-month PFS endpoint. With that assumption, the overall sample size justification described above is for evaluable patients on both treatment arms. Therefore, the study is designed to differentiate between a PFS-12 rate of 33% (null hypothesis) and 55% (alternative hypothesis). With a total of 35 patients enrolled in the Phase II trial, this assessment has 90% power assuming a type I error rate of 0.10. There is also 97% power to differentiate between PFS-12 rates of 33% and 60%. PFS and OS for patients enrolled on Group A and Group B will also be analyzed separately as a descriptive analysis to explore the possibility that treatment outcomes may differ significantly on the two treatment arms in this study. ***Immune Monitoring:*** Peripheral blood will be drawn prior to non-mobilized leukapheresis, prior to immunotherapy, 1 day post Vaccine #1, 4 (+/- 1) days post Vaccine #1, weekly post Vaccine #1 for six weeks, and bimonthly, if feasible, until progression for T cell kinetics. Blood will be processed in accordance with SOP-UFBTIP-126. DCs will be given intradermally and divided equally to both inguinal regions. Vaccines #2 and #3 will occur at 2 week intervals following the first dose. Patients will be followed bi-monthly (+/- one month) for the first year post-immunotherapy, then every 3 months (+/- one month) until tumor progression and death due to any cause. As part of standard care for these patients, 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.

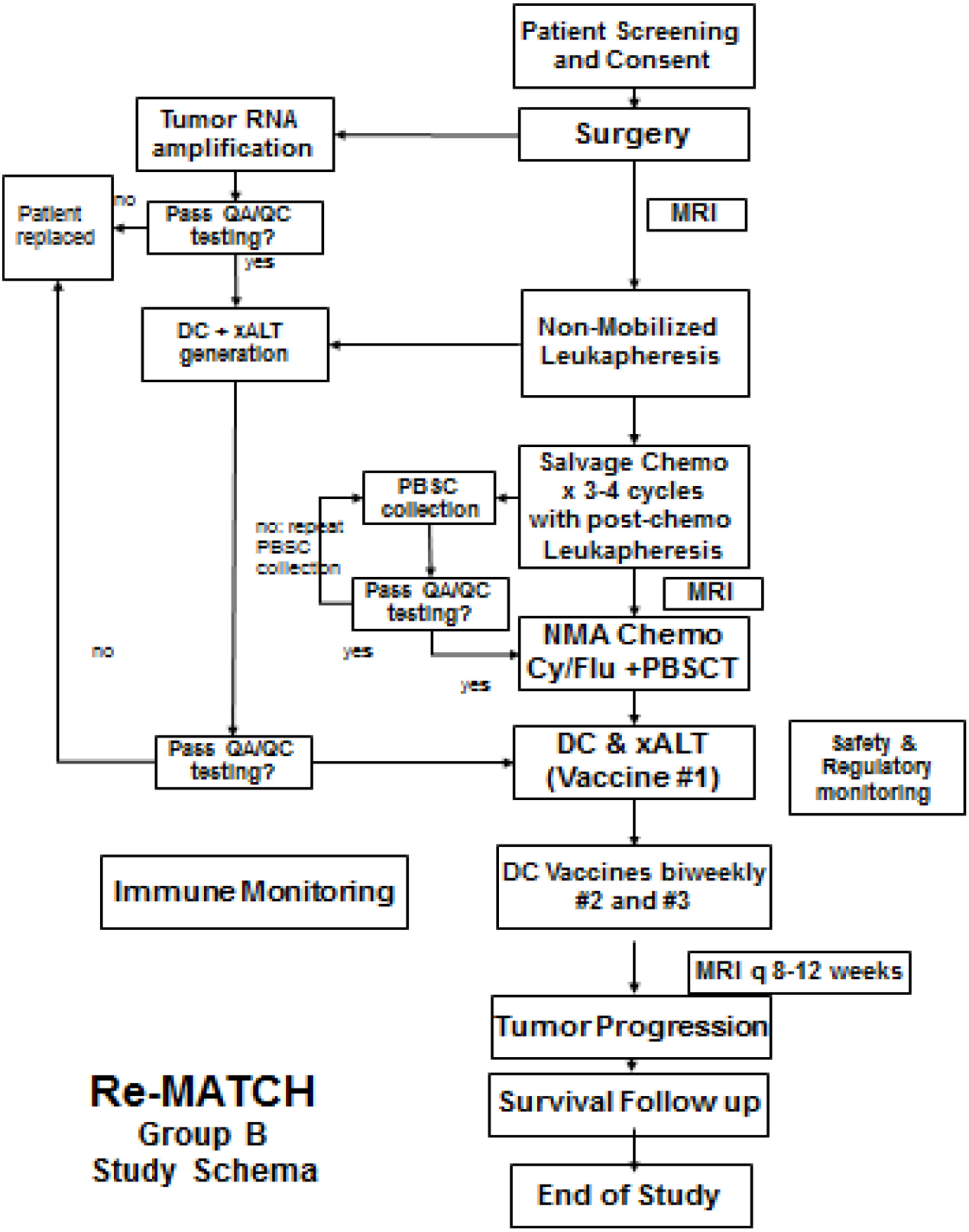
2.2 Multi-Site Study Schema



2.3 Study Schema for Group A



2.4 Study Schema for Group B



3. PRINCIPAL INVESTIGATOR (PI) AND STUDY STAFF

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3.1 KEY STUDY PERSONNEL

Duane A. Mitchell, M.D., Ph.D. (Principal Investigator) **is the Phyllis Kottler Friedman Professor of Neurosurgery, Associate Professor of Neuroscience, Pathology, Immunology, and Laboratory Medicine., Co-Director of the Preston A. Wells, Jr. Center for Brain Tumor Therapy, and Director of the University of Florida Brain Tumor Immunotherapy Program.** Dr. Mitchell has considerable translational research experience involving the immunologic treatment of cancer. Dr. Mitchell is a graduate of the prestigious NIH-sponsored Medical Scientist Training Program (MD/PhD Program) and received his Ph.D. under the guidance of Dr. Eli Gilboa, a world-renowned tumor immunologist and pioneer of the RNA transfected DC platform that will

serve as the antigen presentation platform in this investigation. He has devoted his research efforts on the development of immunotherapeutic approaches to the treatment of malignant brain tumors, and has served as PI on seven FDA and IRB approved Phase I/II clinical trials investigating immunotherapy in patients with malignant brain tumors. He is the Chair of the Immunotherapy Working Group for the NCI Pediatric Brain Tumor Consortium and a panel member of the Immunotherapy Working Group for the NCI Brain Malignancy Steering Committee. The study outlined in this proposal will be conducted under FDA IND-BB-14058 covering the use of adoptive T cell therapy and amplified total tumor RNA-pulsed DCs in pediatric patients with recurrent MB/PNETs. He will be responsible for supervising all aspects of the study; including writing and disseminating all study results and reports. He will also be ultimately responsible for ensuring the regulatory compliant execution of this trial and coordinating the multidisciplinary interactions of the investigators and administrators involved with this study. Overall coordination of the multi-site phase II trial will be assisted by Dr. Gerald Grant, Stanford University (Neurosurgery Chair) and Sri Gururangan, University of Florida (Neuro-Oncology Chair). A Leadership Plan for this collaboration with Dr. Mitchell functioning as lead PI and Immunotherapy Chair has been developed and is outlined below (Section 21.1 Leadership Plan).

William Slayton, M.D. is Professor, Division Chief, and Program Director of Pediatric Hematology/Oncology at the University of Florida with over 20 years of experience in the care of pediatric patients. Dr. Slayton has an active clinical protocol involving the use of high-dose chemotherapy followed by autologous stem cell rescue in children with CNS tumors and extensive experience in treating children with CNS tumors using intensive chemotherapy that will be utilized as the backbone for the clinical trial design investigating the safety and immunologic effects of adoptive lymphocyte transfer and DC vaccination during hematopoietic recovery from high-dose chemotherapy. http://hemonc.pediatrics.med.ufl.edu/faculty-and-staff/attending-physicians/dr-william-slayton/

John R. Wingard, M.D. is the Price Eminent Scholar and Professor of Medicine, Deputy Director for Research of the UF Health Cancer Center, and Director of the Bone Marrow Transplant Program for the University of Florida. Dr. Wingard is a renowned expert in bone and stem cell transplantation as well as active clinical researcher. He also oversees the pediatric and adult stem cell transplantation programs and in such capacity coordinates the care of patients receiving high-dose chemotherapy and stem cell transplantation at the University of Florida including children with CNS tumors. Dr. Wingard has extensive experience in the collection and dissemination of autologous and allogeneic cellular products to patients at multiple FACT-accredited institutions and will assist Dr. Mitchell in the multi-site coordination of collection of leukapheresis specimens, peripheral blood stem cell products, bar-coded storage and dissemination of cellular products, and batch records for the cellular products generated on this protocol. The UF stem and bone marrow transplant program maintains state-of-the-art facilities and technologies for autologous cell product delivery to pediatric and adult patients. http://hemonc.medicine.ufl.edu/about-us/meet-the-team/john-r-wingard-md/

David R. Nelson, M.D. is Professor of Medicine, Molecular Genetics and Microbiology, Director, UF Clinical and Translational Science Institute, and Associate Dean for Clinical Research. He has met with Dr. Mitchell several times to outline the many facets of this protocol that can leverage the extensive clinical research infrastructure supported by the NIH-funded CTSI at the University of Florida. Dr. Nelson will oversee the coordination of the multi-site collection and registration of clinical data into a clinical database, coordinate the availability of the clinical research hospital and infusion center for this protocol, as well as coordinate the interaction between clinical research sites. Additionally, the CTSI maintains extensive biorepository and tissue distribution capabilities as one of only 12 CAP-certified biorepositories in the United States. The CTSI-supported biorepository and distribution capabilities will be leveraged to coordinate the collection of tumor tissues and

leukapheresis specimens from participating centers and disseminate autologous vaccine products to subjects enrolled at sites outside of the UF health network.
https://www.ctsi.ufl.edu/
http://biorepository.pathology.ufl.edu/
http://gastroliver.medicine.ufl.edu/hepatology/faculty-staff/david-r-nelson-m-d/

Scott Rivkees, M.D. (Other Significant Contributor) **is Professor and Chairman of Pediatrics and Chair of the Child Health Research Institute at the University of Florida**. Dr. Rivkees has met several times with Dr. Mitchell to coordinate the Re-MATCH protocol within the extensive UF pediatric health system. Dr. Rivkees currently oversees the active partnership between the UF and Orlando Regional Medical Center (ORMC) including Arnold Palmer Children's Hospital. ORMC extends the patient network of the UF health system by over 2 million subjects, and Dr. Rivkees is attending physician at both UF/Shands medical center and Arnold Palmer Children's with active on-site rotating work between both centers. He will facilitate the interactions between Dr. Mitchell and his clinical research team with the pediatric oncology and clinical research teams both at UF and ORMC to enhance recruitment and enrollment of subjects served by the Florida health system. He will also ensure that all physical and intellectual resources available within the Department of Pediatrics at the University of Florida are available to Dr. Mitchell in support of this protocol.
http://research.pediatrics.med.ufl.edu/researchers/research-faculty/scott-rivkees/

Brian D. Cleaver, Ph.D. is Director of the Powell Gene Therapy Center (PGTC) and Human Applications Laboratory at the University of Florida. Dr. Cleaver has extensive experience (>10 years) in the FDA-regulated cGMP manufacturing of cellular and gene vector products for use in clinical trials. He has directed GMP manufacturing of products for use at single-site, multi-site, and even international clinical studies and is an expert in the regulatory compliant manufacturing of GMP materials. Dr. Cleaver and his staff within the PGTC will be responsible for the generation of cellular products used in this protocol and the regulatory oversight, record keeping, and release of qualified cellular products for this study.
http://www.gtc.ufl.edu/core/gtc-apps.htm
http://urology.ufl.edu/about-us/faculty-staff-directory/brian-d-cleaver-phd/

Anthony T. Yachnis, M.D. is Professor of Pathology and Medical Director of Anatomical Pathology at the University of Florida. He is a board-certified neuropathologist with over twenty years of clinical experience. In such capacity, he reviews and performs confirmatory diagnosis on all brain tumor specimens collected at University of Florida (>600 annually) and will ensure the pathologic eligibility criteria for patients enrolled in this study are met. He will also evaluate the histochemical analysis of lymphocytic infiltrates in tumor specimens, and perform all neurotoxicity evaluations. Dr. Yachnis will interact with the study team in the coordination of collection of tumor materials collected at the UF site and will perform the confirmatory diagnostic evaluation of specimens collected from external sites.
http://pathlabs.ufl.edu/team/pathologists/yachnis-a

William A. Friedman, M.D. (Other Significant Contributor) **is Professor and Chairman of Neurosurgery at the University of Florida and Co-Director of the Preston A. Wells, Jr. Center for Brain Tumor Therapy.** Dr. Friedman is a highly experienced neurosurgeon specializing in the care of patients with malignant brain tumors. As Co-Director of the Preston Wells Center with Dr. Mitchell, his major interests are in the advancement of novel therapeutics for pediatric and adult patients with malignant brain tumors. Dr. Friedman's role will be to facilitate the utilization of the vast financial, physical, and environmental resources within the Department of Neurosurgery and Preston Wells Brain Tumor Center in support of this protocol. He will meet regularly with Dr.

Mitchell in the coordination of this study and ensure that all institutional resources are leveraged appropriately. Dr. Friedman is also actively overseeing the partnership with the Orlando Regional Medical Center's neurosurgical practices and thus will be able to assist in the recruitment and enrollment of subjects from the ORMC network. Additionally, Dr. Friedman will ensure that the clinical research and regulatory staff of the department are available in support of this protocol including requisite CRC, regulatory, administrative, and nursing support. Dr. Mitchell and Friedman are coordinately responsible for staffing any additional specialized needs in support of the research mission of the Preston A. Wells, Jr. Center for Brain Tumor Therapy. In such capacity, up to $20M in unrestricted funds are available to Drs. Mitchell and Friedman to support clinical research activities. The capacity to support unforeseen budget shortfalls from the clinical trial award supporting this protocol is an additional administrative role that Dr. Friedman would facilitate in collaboration with Dr. Mitchell.
http://neurosurgery.ufl.edu/faculty-staff/our-faculty/william-a-friedman-md/
http://neurosurgery.ufl.edu/faculty-staff/clinical-research-staff/
http://news.medinfo.ufl.edu/articles/lead-story/10-million-wells-foundation-gift-will-enable-uf-to-speed-brain-tumor-remedies/

Jianping Huang, M.D., Ph.D. is an Associate Research Professor and Director of Clinical Laboratory Operations for the UF Brain Tumor Immunotherapy Program. She previously functioned as Head of Molecular Immunology at the Laboratory for Tumor Immunology at the National Cancer Institute and has extensive experience in adoptive cellular therapy of patients with cancer and in the immunologic monitoring of patients enrolled in cancer immunotherapy trials. She will oversee the interactions between the UFBTIP laboratory and GMP facility and function as the primary supervisor of the research staff involved in cellular product manufacturing and QA/QC testing. She will also oversee the immunologic monitoring of subjects enrolled on this protocol.

Elias J. Sayour, M.D. is a pediatric oncologist who conducted his research fellowship on the immunotherapy of malignant brain tumors. Dr. Sayour will function as an integrative co-investigator at the University of Florida. He will assist in the enrollment, evaluation, and medical management of patients enrolled, and in the evaluation of clinical data and laboratory analysis on acquired tissue and blood specimens.

Jeffrey Drake is a research associate with considerable experience in molecular biology techniques and cell culture. He will be responsible for working with the cGMP facility and staff in the generation of clinical-grade RNA and the generation of autologous dendritic cells and tumor-specific lymphocytes.

Marcia B. Hodik, RN, BSHS, CCRC is a registered nurse with over 20 years of pediatric nursing experience. She has functioned as a study coordinator for Gastroenterology and Hepatology. She currently holds certification as a Certified Clinical Research Coordinator and is the president of the local ACRP Chapter. She will be responsible for screening and enrolling patients, ensuring protocol compliance and collecting and recording study data.

Paul Kubilis, MS is a biostatistician dedicated full-time to research efforts within the Department of Neurosurgery. He is committed to delivering understandable and useful results that help investigators achieve their research goals. He has extensive knowledge in study design, data analysis and presentation of data for publication. He will collaborate with Dr. Mitchell on data analyses and manuscript preparation.

3.2 KEY STUDY PERSONNEL EXTERNAL SITES

CHILDREN'S NATIONAL MEDICAL CENTER, WASHINGTON, D.C.
111 MICHIGAN AVE., N.W.
WASHINGTON, D.C. 20010
http://www.childrensnational.org/

Roger J. Packer, M.D. is Professor of Neurology & Pediatrics, Senior Vice President of the Center for Neuroscience, and Director of the Brain Tumor Institute at Children's National Medical Center in Washington, D.C. Dr. Packer is a renowned pediatric neuro-oncologist that leads the brain tumor program at CNMC, one of the nation's leading pediatric medical centers. He has been a colleague and associate of Dr. Mitchell's for several years through interactions within the NCI-funded Pediatric Brain Tumor Consortium (PBTC) and has a strong interest in the implementation of novel therapeutics for children with malignant brain tumors. He will supervise the implementation of the Re-MATCH protocol within CNMC and ensure that the vast expertise, infrastructure, and resources that the center maintains in support of the treatment of pediatric patients is appropriated in support of this protocol. Dr. Packer will be available to assist Dr. Eugene Hwang in the routine care of patients enrolled on this protocol as well.
http://www.childrensnational.org/research/faculty/bios/cnr/packer_r.aspx

Anthony Sandler, M.D. is the Diane and Norman Bernstein Professor of Pediatric Surgery, Senior Vice President and Surgeon-in-Chief of the Joseph E. Robert Center for Surgical Care, and Principal Investigator of the Immunology Initiative in the Sheik Zayed Institute at Children's National Medical Center. Dr. Sandler's primary interest is in the immunologic treatment of pediatric brain tumors. The Shiek Zayad Institute at CNMC was established with a $150 million philanthropic gift to advance novel therapeutics for pediatric patients. Dr. Sandler has hosted Dr. Mitchell as a visitor to the Shiek Zayad Institute to tour the outstanding basic and clinical research facilities established at CNMC. In addition to his strong scientific and clinical interests in immunotherapy, Dr. Sandler is an experienced pediatric neurosurgeon. He will oversee the surgical obtainment and transfer of tumor specimens of suitable quality for vaccine preparation. He will also be responsible for the routine pre- and post-operative care of subjects enrolled on the Re-MATCH protocol.
http://innovationinstitute.childrensnational.org/about-us/team/1001

Eugene I. Hwang, M.D. is a pediatric neuro-oncologist and Director of the Pediatric Neuro-Oncology Fellowship Program at Children's National Medical Center. Dr. Hwang received his pediatric neuro-oncology fellowship training at Duke University and worked with Dr. Mitchell during the development of the Re-MATCH protocol. He has a strong interest in pediatric immunotherapy and is collaborating with Dr. Mitchell on novel initiatives within the Pediatric Brain Tumor Consortium. Dr. Hwang will play an active role in the care of patients enrolled on this protocol and in the integration of the Re-MATCH protocol with the outstanding clinical research team at CNMC. He will also oversee the collection and transport of leukapheresis products to the GMP facility at University of Florida for vaccine and T cell preparation and be directly responsible for vaccine administration for subjects enrolled at CNMC.
http://www.childrensnational.org/findadoctor/profiles/eugene-hwang-4385.aspx

CHILDREN'S HOSPITAL LOS ANGELES
4650 SUNSET BLVD
LOS ANGELES, CA 90027
http://www.chla.org

Girish Dhall, M.D. is Clinical Director of Neuro-Oncology at Children's Hospital Los Angeles. He is an experienced pediatric hematology/oncologist who will oversee the management of subjects enrolled on the protocol at CHLA. Dr. Dhall will also direct the collection and dissemination of leukapheresis products for

vaccine and T cell preparation to the University of Florida and the administration of vaccine products to subjects enrolled at CHLA.
http://www.chla.org/site/apps/kb/cs/contactdisplay.asp?c=ipINKTOAJsG&b=3832751&sid=ewJXIjPWJfLYKdP1KzG&r=1

Mark Krieger, M.D. is Division Chief of Pediatric Neurosurgery and Director of the Neurosurgery Fellowship Program at CHLA. Dr. Krieger is a highly-experienced pediatric neurosurgeon at one of the top ranked pediatric hospitals in the nation. He will oversee the protocol compliant collection and dissemination of tumor materials suitable for vaccine preparation at the CHLA site and in his duties as Division Chief will ensure the proper pre- and post operative management of subjects enrolled on this protocol. Dr. Krieger will also ensure coordination of information with the Neurosurgical Chair of the protocol, Dr. Gerald Grant at Stanford University.
http://www.chla.org/site/apps/kb/cs/contactdisplay.asp?c=ipINKTOAJsG&b=3832751&sid=dvKTKhPVIiI5IfMQJrF&r=1

Clinical Research Staff:

ANJELIKA M. DECHKOVSKAIA, M.D., Senior Biological Scientist, UF Brain Tumor Immunotherapy Program, Department of Neurosurgery, University of Florida

CATHERINE FLORES, Ph.D., Assistant Professor, UF Brain Tumor Immunotherapy Program, Department of Neurosurgery, University of Florida

4. STUDY LOCATION(S)

UNIVERSITY OF FLORIDA HEALTH

The sites for the routine and experimental care for patients enrolled on this study will be as follows:

- Neurosurgical Care: Resections performed at UFHealth Shands Hospital, Pediatric Neurosurgeons
- Consolidation chemotherapy and peripheral blood stem cell mobilization: UFHealth Shands Children's Hospital, Supervising Physicians: William Slayton, MD, and John R. Wingard, MD
- Leukapheresis and Stem Cell Harvest: UFHealth Bone Marrow and Stem Cell Transplantation Clinics, Supervising Physician: John R. Wingard, M.D.
- Dendritic cell generation, tumor RNA isolation and amplification, autologous T cell expansions: UF Brain Tumor Immunotherapy Program, Supervising Physician: Duane A. Mitchell, M.D., Ph.D.
- High-dose chemotherapy and peripheral blood stem cell transplantation: UFHealth Shands Children's Hospital Bone and Stem Cell Transplantation Unit, Supervising Physicians: John R. Wingard M.D., and William Slayton, MD
- Autologous lymphocyte transfer and dendritic cell vaccination (inpatient): UFHealth Shands Children's Hospital Bone and Stem Cell Transplantation Unit, Supervising Physicians: John R. Wingard, M.D., and William Slayton, MD
- Dendritic cell vaccination and follow-up visits (outpatient): UFHealth Pediatric Hematology/Oncology Clinic, Supervising Physicians: Duane A. Mitchell, M.D., Ph.D., William Slayton, MD, and John R. Wingard, MD
- Immunologic monitoring and correlative assays: UF Brain Tumor Immunotherapy Program, Principal Investigator: Duane A. Mitchell, M.D., Ph.D.

EXTERNAL SITES

- Children's National Medical Center

- Children's Hospital Los Angeles

5. TIME REQUIRED TO COMPLETE THE STUDY

This protocol will be conducted under an FDA approved IND (IND BB-14058) covering the use of amplified tumor RNA pulsed DCs and adoptive lymphocyte transfer in patients with malignant brain tumors. University of Florida IRB and Institutional Biosafety Committee (IBC) reviews are completed and full-regulatory approval by these agencies is finalized. National Recombinant DNA Advisory Committee (RAC) review has been completed and a letter of approval/permission to proceed has been received. The projected time frame for the phase I/II study is December 1, 2010 through March 31, 2019.

6. BACKGROUND

6.1 Significance and Overall Rationale

Malignant brain tumors now represent the second **most frequent cause of cancer death in children.** Despite aggressive and highly toxic multi-modality therapy including surgery, craniospinal radiation, and HDC + PBSCT, **almost half of the children diagnosed with medulloblastoma and primitive neuroectodermal tumors (MB/PNETs), still die from recurrent disease**. Furthermore, survivors are often left with severe and lifelong treatment-associated cognitive and motor deficits. The development of more effective and less toxic tumor-specific therapies is paramount in improving clinical outcomes for children affected by MB/PNETs. **Immunotherapy targeting tumor-specific antigens** expressed within brain tumors is a treatment modality potentially capable of meeting this clear and urgent need.

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.

The use of total tumor RNA-loaded dendritic cells (DCs) was pioneered by Drs. Nair and Gilboa at Duke University, 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 adult patients with recurrent GBM (FDA IND BB-13630; Duke IRB protocol 6677; PI: Duane A. Mitchell, M.D., Ph.D.). In this proposal, we aim to extend evaluation of this novel platform to the treatment of recurrent MB/PNETs (reMB/PNETs) during hematopoietic recovery from HDC + PBSCT and NMA salvage chemotherapy.

Immunotherapy administered during recovery from HDC and NMA salvage therapy 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.

Our **HYPOTHESIS** is that DC + xALT therapy targeting reMB/PNETs during recovery from myeloablative (MA) and non-myeloablative (NMA) chemotherapy will induce potent tumor-specific immune responses that mediate the safe eradication of invasive malignant disease. In this effort, we aim to leverage the myelosuppressive toxicity of standard-of-care HDC and NMA salvage therapy in order to enhance the potential efficacy of a novel immunotherapy employing the use of amplified tumor RNA-pulsed DCs. Toward that end, our **SPECIFIC AIMS** are to:

1. Conduct a Phase II clinical trial of DC + xALT therapy during hematopoietic recovery from NMA salvage chemotherapy and HDC + PBSCT in pediatric patients with reMB/PNETs with the following sub-aims:
   - **a.** Determine the impact of DC +xALT therapy on progression-free survival (PFS) and overall survival (OS) of pediatric patients with reMB/PNETs compared to historical cohort;
   - **b.** Investigate the correlation between clinical outcome and the frequency and persistence of tumor-specific T cells in the peripheral blood of treated patients;
   - **c.** Determine the impact of induction therapy and HDC + PBSCT (Group A) or salvage chemotherapy (Group B) on circulating levels of homeostatic and inflammatory cytokines, toll-like receptor activation status, and on the functional recovery of lymphocyte and NK cellular subsets in children with re-MB/PNETs receiving DC + xALT therapy;
   - **d.** Characterize the frequency of expression of the TAA-40 identified in common malignancies in first and second recurrent MB/PNETs in order to identify potential candidates for subsequent evaluation in antigen-specific immunotherapy approaches.

6.2 Immunotherapy for Pediatric Brain Tumors

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 MB/PNETs are markedly lacking except for a few notable 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 HDC + PBSCT and NMA chemotherapy in pediatric patients with reMB/PNETs 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[1]. 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[2]. 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 six 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. 7 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.

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, and IL-6.
- Route of injection comparisons have shown that intravenous injection of DCs in humans is in many cases tolerigenic 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+ALT therapy during the peri-transplant period (24 hrs 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 to5 vaccines to their patients, while we will continually vaccinate patients throughout the recovery period of lymphopenia and normalization of T cell function (up to 1 year post HDC + PBSCT) 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 **(Figs. 5-6)**.

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.

6.3 Rationale for Current Study

Adoptive cellular therapy after lymphodepletive regimens has emerged as a potent and effective means of inducing durable remissions of advanced stage refractory melanoma and therefore holds significant promise in the treatment of other solid tumors. Recurrent MB/PNETs are the most common pediatric brain tumors and brain cancer is currently the leading cause of cancer deaths in children. The use of HDC + PBSCT and NMA chemotherapy as standard therapy in patients with reMB/PNETs provides an ideal opportunity to administer adoptive cellular therapy during the hematopoietic recovery phase from HDC and NMA chemotherapy. We have developed the use of total tumor RNA loaded DCs as an effective platform for induction of tumor-specific lymphocytes and will use TTRNA-pulsed DCs to expand lymphocytes specific for reMB/PNETs from children undergoing secondary resection and HDC + PBSCT (Group A) or NMA salvage chemotherapy (Group B). In order to maintain T cell expansion and functional administration concurrent and monthly vaccinations with TTRNA-pulsed autologous DCs will be administered.

6.4 Re-MATCH Phase I Study

21 subjects were enrolled on the protocol using a screening consent for tumor collection. Eleven of these subjects were removed from the study (ineligible histology, tumor progression, inability to obtain sufficient PBMCs for DC/xALT generation) prior to immunotherapy. Ten subjects were treated with DC+ALT therapy and nine were evaluable for the maximal tolerated dose (MTD) and dose-limiting toxicities (DLTs). One patient's T cells did not grow and was treated with DC vaccination only and thus not evaluable for phase I safety endpoints.

Therefore, ten were treated with DCs/xALT with nine of these patients evaluable for DLTs and MTD. For Group A, we treated a single subject that reached the safety endpoint at dose cohort #1 ($3x10^6$ T cells/Kg). For Group B, we treated three subjects that reached the safety endpoint at dose cohort #1, and 5 subjects at dose cohort #2 ($3x10^7$ T cells/Kg). A maximal achievable dose of T cells was obtained at $3x10^7$ T cells/Kg. Of the nine patients who were evaluable for DLTs and MTD (Group A, N=1; Group B, N=8), no DLTs were associated with adoptive cellular therapy as defined at two weeks after the third dendritic cell (DC) vaccination. The phase I study was closed with a defined safe dose of $3x10^7$ T cells/Kg i.v. and $1x10^7$ DCs i.d.

7. OBJECTIVES/SPECIFIC AIMS/STUDY QUESTIONS

7.1 Hypotheses

- Our **MAIN HYPOTHESIS** is that:
  - Adoptive transfer of tumor-specific lymphocytes coupled with TTRNA-pulsed DC vaccinations that target reMB/PNETs will lead to the safe eradication of residual tumor cells during hematopoietic recovery from myeloablative and non-myeloablative chemotherapy and autologous PBSCT.
- **SECONDARY HYPOTHESES** that will be explored in this study are that:
  - Treatment of pediatric patients with reMB/PNETs using TTRNA-xALT and TTRNA-DCs will enhance polyfunctional anti-tumor T cell responses
  - Treatment of pediatric patients with reMB/PNETs using TTRNA-xALT and TTRNA-DCs extends progression free survival (PFS) compared to a historical cohort.
  - A correlation exists between persistence of anti-tumor immunity and clinical outcome in patients with reMB/PNETs
  - Myeloablative and NMA chemotherapy will lead to increases in homeostatic and inflammatory cytokines, decreases in circulating CD4+CD25+FOXP3+ T_{regs}, as well as increases in TLR activation in patients with reMB/PNETs

7.2 Study Objectives

- PRIMARY OBJECTIVES for the PHASE II Trial:
  - To determine if DC + xALT therapy extends progression-free survival compared to historical benchmarks during recovery from HDC + PBSCT **(Group A)** or salvage chemotherapy (Group B) in pediatric patients with reMB/PNETs
- SECONDARY OBJECTIVES
  - To determine the objective radiographic response rate of MA and NMA chemotherapy coupled with DC + xALT therapy in patients with residual disease
  - To determine if magnitude and persistence of anti-tumor humoral or cellular immunity correlates with clinical outcome
  - To evaluate changes in cytokine profile and Toll-Like Receptor (TLR) activation status in pediatric patients with reMB/PNETs after HDC **(Group A)** or salvage chemotherapy **(Group B)** and during DC + xALT therapy

- To characterize the immunologic phenotype of lymphocyte subsets (naïve, effector, memory, regulatory) and NK cells in patients with reMB/PNETs at diagnosis and during experimental therapy
- To identify potential tumor specific antigens as vaccine candidates through characterizing the frequency of expression of the top 40 tumor associated antigens (TAA_{40}) identified in common malignancies in recurrent MB/PNETs
- To determine the progression-free survival and overall survival rate in pediatric patients with recurrent MB/PNETs receiving DC + xALT therapy after HDC plus PBSCT **(Group A)** compared to a historical benchmark
- To determine the progression-free survival and overall survival rate in pediatric patients with recurrent MB/PNETs receiving DC + xALT therapy after salvage chemotherapy **(Group B)** compared to a historical benchmark

8. STUDY DESIGN

This will be a dual-arm prospective multi-institutional Phase II clinical trial where the 12-month progression-free survival rate in each group will be compared to a historical benchmark.

Using guidance provided by the FDA for tumor collection and transfer on an existing protocol (FDA IND 13630, Duke IRB 6677; PI: Duane A. Mitchell, M.D., Ph.D.), we have received IRB and FDA approval to use identical processes for the transfer of tumor materials for the Re-MATCH protocol. To date, we have successfully transferred 3 specimens from outside institutions for RNA amplification. All attempts to amplify RNA sterilely have been successful whether tumor specimens were collected locally or transferred via overnight shipping. Additionally, we have received strong letters of support and commitment from collaborating institutions to open the phase 2 trial as a multi-institutional study. This would greatly facilitate the enrollment of the targeted number of evaluable subjects in a timely manner. Guidelines from the FDA have been obtained on centralized cellular manufacturing for the treatment of subjects at outside institutions.

As no study drug is administered during either the Induction phase or the Salvage chemotherapy phase of the trial, the regimens for both Group A and Group B patients and the mobilization and collection of PBSCs may be administered at UF or other Foundation for the Accreditation of Cellular Therapy (FACT)-accredited medical institutions under the care of a board-certified/board-eligible pediatric oncologist. PBSC products collected and cryopreserved at FACT-accredited institutions shall be shipped in a dry shipper to the UF Stem Cell Laboratory per SOPs prior to Consolidation (Group A) or NMA conditioning with cyclophosphamide and fludarabine (Group B). Dr. Sri Gururangan (Neuro-oncology Chair) will be responsible for providing a roadmap of the induction and salvage chemotherapy regimens to outside oncologists and acquiring documentation of all prescribed medications and any chemotherapy or procedure related toxicities during these periods (see attached cover letter and check sheet). Standard medication regimens may need to be adjusted due to hematologic or other toxicities and may be managed at the discretion of the treating pediatric oncologist. Focal boost radiotherapy may be delivered prior to immunotherapy if required for local control. All deviations from intended regimens during induction or salvage therapy will be documented, but not considered a protocol deviation, as is within routine standard of practice for this patient population

Phase II:

This study will assess the clinical impact of DC + xALT therapy in patients with first localized reMB/PNETs. Up to 42 patients will be enrolled to treat 35 patients with reMB/PNET with DC + xALT therapy. Following surgical resection, biopsy, or cytology examination with confirmatory pathologic diagnosis, patients will be enrolled into HDC **(Group A)** or NMA salvage chemotherapy **(Group B)** based on eligibility for HDC. Patients with localized relapse and have not failed HDC+PBSCT previously will be enrolled into Group A as HDC+PBSCT is considered standard-of-care in the relapsed disease setting. Patients with

disseminated disease, have previously failed HDC+PBSCT, or are otherwise considered poor candidates for HDC based on overall health status but otherwise meet eligibility criteria will be enrolled into Group B and will receive a salvage chemotherapy regimen followed by non-myeloablative cyclophosphamide/fludarabine lymphodepletive conditioning with PBSCT prior to DC + xALT therapy. For purposes of this protocol, localized relapse will be defined as recurrence in the same CNS location **as the original tumor or a single location outside of the primary site**. Disseminated disease will be defined as new lesions with little evidence of being contiguous with prior resection cavity, or intraventricular spread, or dissemination along any anatomically definable subcortical white matter tracts.

The primary efficacy endpoint will be 12-month progression-free survival (PFS) from HDC + PBSCT or NMA salvage chemotherapy, after treatment with TTRNA-xALT and TTRNA-DCs. In Group A, the progression-free survival experience among patients who initiated HDC after having previously received definitive radiotherapy is reported by Gururangan et al., (2008). The 12-month PFS (PFS-12) rate is 33% (95% confidence interval: 10%, 59%). The population of patients that will be treated in the current protocol is similar to the subset of patients treated on the myeloablative regimen reported by Gururangan et al., but will also include recurrent central PNETs in addition to MB. For patients receiving NMA chemotherapy, there is no good historical benchmark. Considering that patients in Group B tend to have poorer prognoses than Group A, we will conservatively use the benchmark from Group A for Group B.

Adoptive cellular therapy during recovery from myeloablative therapy has shown extraordinary promise in treatment of refractory melanoma (greater than 70% objective response rate)[3], but is a complex and labor intense individualized cancer therapy requiring a high-level of technical expertise and infrastructural support. Therefore, we would continue to refine this regimen until strong suggestion of significant improvement in treatment outcome were observed before proceeding to large-scale confirmatory trials. With this rationale in mind, in the current study, if the true proportion of patients with reMB/PNETs who have failed prior definitive radiotherapy experienced a PFS-12 with TTRNA-xALT and TTRNA-DCs treatment of 55% or more there would be definite interest in continuing the treatment regimen without further modification in larger confirmatory phase II or III studies. If the true PFS-12 associated with DC + xALT therapy is 33%, there would be limited interest in further investigation of this treatment approach without significant modification. Therefore, the study is designed to differentiate between a PFS-12 rate of 33% (null hypothesis) and 55% (alternative hypothesis). With **35 patients treated in the Phase II study**; this assessment has 90% power assuming a type I error rate of 0.10. There is also 97% power to differentiate between PFS-12 rates of 33% and 60%.

The PFS and OS of patients with rePNETs following definitive radiotherapy does not differ significantly from reMBs following definitive RT [4,5] and therefore all patients will be analyzed collectively for primary and secondary endpoint evaluations in this study. Though this study is not powered for histologic tumor subgroup analyses, we will analyze PFS of patients with reMBs and rePNETs separately as a <u>hypothesis driven investigation only</u> to test the notion that no appreciable difference in treatment outcome for patients with reMB and other PNETs after DC + xALT therapy will be observed. Analysis of patients with reMB separately will also allow us to confirm that any comparisons with the historical control cohort made with analysis of all patients are consistent when analyzing patients with MB.

Patients will be followed bi-monthly (+/- one month) for the first year post-immunotherapy, then every 3 months (+/- one month) until tumor progression and death due to any cause. As part of standard care for these patients, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure consent will be obtained separately. However, if tissue is obtained, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and antigen expression profile in secondary recurrent tumors compared to original recurrent tumor RNA used in TTRNA-DC preparation by gene expression microarray.

Children and young adults with reMB/PNETs will be identified from the patient population referred for treatment of recurrent disease. Our Centers are major referral centers for the treatment of pediatric brain tumors for the United States and abroad. Additionally, patients will be identified through referral by network resources

such as the Pediatric Brain Tumor Consortium (PBTC) (see letter of support from Dr. Larry Kun, Director PBTC) and the Pediatric Brain Tumor Foundation of the United States (PBTFUS) (see letter of support from Mike Traynor, President, PBTFUS).

Group A Phase II:

Patients in Group A with reMB/PNETs will start Induction therapy with attempted gross total resection for tumor debulking and to provide tumor tissue that is required for histologic diagnosis and the extraction of total tumor RNA. Approximately two weeks later, subjects will undergo a non-mobilized leukapheresis for peripheral blood mononuclear cells (PBMCs) for TTRNA-xALT and TTRNA-DC generation and then start induction chemotherapy that consists of three to four 28-day cycles as presented below:

Leukapheresis after each induction cycle until collection of a minimum $2x10^6$ CD34+ cells/kg				
	Drug	**Dosage**	**Route**	**Days Given**
Cycle 1 & 2	Cyclophosphamide	$2g/m^2$/day	IV over 1 hour	1, 2
	Mesna	$2g/m^2$/day	IV	1, 2
	Filgrastim	10mcg/kg/day	SQ or IV	3 - 28*
Cycle 3 & 4	Temozolomide	$150mg/m^2$/day	By mouth	1 - 5
	Etoposide	$30mg/m^2$/day	By mouth	1 - 14

*administered until bone marrow recovery and leukapheresis complete

Patients will undergo a non-mobilized leukapheresis prior to induction for harvest of peripheral blood mononuclear cells (PBMCs) for TTRNA-xALT and TTRNA-DC generation. The target PBMC count based on the desired dose of TTRNA-xALT and sufficient TTRNA-DCs for T cell expansion and in vivo vaccination will be collected. Afterwards, mobilized Peripheral blood stem cells (PBSC) will be collected after each induction cycle until the target CD34(+) PBSC yield needed for Consolidation ($2x10^6$ CD34+ cells per Kg at a minimum) is obtained. Absolute nucleated cell count collected will be adjusted as needed on a patient specific basis. Upon recovery from the 3rd or 4th cycle of induction chemotherapy, patients will undergo disease restaging and organ function evaluation. Patients will be replaced if they have disease progression by radiographic or histological findings or inadequate organ function. Patients without tumor progression and suitable organ function (defined below in Section 10) will be admitted to the PBMT unit to begin Consolidation as presented below:

Drug Name	**Route**	**Dose**	**Schedule**	**Instructions for administration**
Carboplatin	IV	The lesser of $500mg/m^2$ or dosing = AUC 7 mg/ml-min per Calvert Formula; dilute to 4 mg/ml in Normal Saline	Days -8, -7,-6	IV over 4 hours through central venous catheter

Etoposide	IV	250mg/m^2; dilute to 0.3 mg/ml in Normal Saline	Days -5,-4,-3	IV over 3 hours through central venous catheter
Thiotepa	IV	300mg/m^2; dilute to 1 mg/ml in Normal Saline	Days -5,-4,-3	IV over 3 hours through central venous catheter
Peripheral Blood Stem Cells	IV	**Minimum: $2x10^6$ CD34+ cells/kg**	Day 0	IV through central venous catheter per institutional guidelines
TTRNA-xALT	IV	$3x10^7$/kg	Day 1	IV over 10-30 minutes through central venous catheter. Pre-medicate 30-60 minutes prior to infusion with acetaminophen (up to 10-15 mg/kg/dose) and Benadryl (up to 1 mg/kg). Vital signs will be assessed prior to and post infusion then every 15 minutes for 1 hour.
TTRNA-DCs	Intradermal	$1x10^7$ cells	Days 1, 15, 29	Each immunization will be divided equally to both inguinal regions. A total volume of 200 µL per side will be delivered intradermally after preparation of skin with EMLA anesthetic cream.
Sargramostim (GM-CSF)	Intradermal	150 ug	1, 15, 29	Embedded with DC Vaccine
Filgrastim (G-CSF)	SQ or IV	5 micrograms/ kg/day	Beginning no later than Day 6 post-transplant-count recovery	Per institutional guidelines

Three days after completion of the myeloablative therapy (Day 0), patients will be given their autologous PBSC transplant. Immunotherapy will commence 24 hours after autologous PBSC transplant with infusion of TTRNA-xALT via an intravenous catheter. The equally-divided TTRNA-DC vaccine embedded with GM-CSF will be injected intradermally into the groin bilaterally which drain to the inguinal lymph nodes (upper medial thigh). Peripheral blood will be drawn prior to non-mobilized leukapheresis, prior to immunotherapy, 1 day post Vaccine #1, 4 (+/- 1) days post Vaccine #1, weekly post Vaccine #1 for six weeks, and bimonthly, if feasible, until progression for T cell kinetics. Blood will be processed in accordance with **SOP-UFBTIP-126**. The subsequent DC vaccines #2 and #3 will occur at 2 week intervals following the first vaccine. Patients will receive

at least 3 biweekly vaccines as long as clinically stable. Patients will receive G-CSF at 5 microgram/kg/day beginning no later than day 6 post-transplant until ANC recovers to >1000 cells/μL.

NOTE: Patients who are no longer candidates for HDC will be eligible to transfer from Group A to Group B.

Group B Phase II:

Patients on Group B will receive salvage chemotherapy consisting of cycles of oral etoposide for 14 days plus oral temozolomide (TMZ) for 5 days monthly until preparation of immunotherapeutic vaccines has been completed as presented below:

Drug Name	**Route**	**Dose**	**Schedule**
Temozolomide	PO	150 mg/m^2/day	Days 1-5
Etoposide	PO	30 mg/m^2/day	Days 1-14
Filgrastim (G-CSF)	SQ or IV	10 micrograms/ kg/day	Starting Day 15 *administered each cycle until enough CD34 cells are harvested (goal of 2x10^6/kg) for infusion

Patients will undergo a non-mobilized leukapheresis prior to salvage chemotherapy for harvest of peripheral blood mononuclear cells (PBMCs) for TTRNA-xALT and TTRNA-DC generation. Afterwards, mobilized leukapheresis will be attempted after each salvage chemotherapy cycle until enough peripheral blood stem cells (PBSCs) are harvested for autologous stem cell infusion (goal of 2x10^6 CD34+ PBSCs/kg). Subjects who do not mobilize any peripheral blood stem cells or do not undergo leukapheresis, will continue with immunotherapy without NMA chemotherapy. 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 assessment. If only DCs are to be administered, then lymphodepleting conditioning with cyclophosphamide/fludarabine will be withheld and DCs administered when the patient has recovered detectable peripheral blood lymphocyte counts as the rationale for lymphodepletion is for enhancing the expansion of adoptively transferred T cells (xALT). Patients who progress prior to immunotherapy may remain on study as long as they continue to meet the other eligibility requirements. Patients may receive the first 3 vaccines as long as they are stable and remain eligible. Subsequently, for the purpose of this study, progression will be defined as a new lesion confirmed by biopsy or resection, positive cerebrospinal fluid (CSF) cytology (when previously negative), or radiographic progression as defined by protocol criteria. Following surgery and leukapheresis, adequate organ function testing will be repeated prior to initiation of cyclophosphamide and fludarabine conditioning. Drug administration and dosage for the lymphodepletive conditioning may be adjusted based upon hematologic performance and organ function testing results.

After completion of salvage chemotherapy and starting 11 days before immunotherapy, patients will receive NMA chemotherapy which includes 2 days of cyclophosphamide IV followed by 5 days of fludarabine IV. Approximately 72 hours following fludarabine, PBSCs, if available, will be infused. Approximately 96 hours following the final dose of fludarabine, Immunotherapy begins with infusion of TTRNA-xALT (3x10^7/kg) and injection of the first TTRNA-DC (1x10^{7}cells) vaccine. The NMA recovery will incorporate G-CSF at 5 microgram/kg/day beginning no later than Day 16 until ANC recovers to >1000 cells as presented below:

Drug Name	Route	Dose	Schedule	Instructions for administration
Cyclophosphamide	IV	1000 mg/m^2/day	Days 1 & 2	Dose reduce or omit based on cardiac and/or pulmonary function
Fludarabine	IV	25 mg/m^2/day	Days 3-7	Dose reduce or omit based on creatinine clearance
Peripheral Blood Stem Cells, if available	IV	A minimum of 2x10^6 CD34+ cells/kg or all available cells if < 2x10^6 CD34+ cells/kg	Day 10	IV through central venous catheter per institutional guidelines
TTRNA-xALT	IV	3x10^7/kg	Day 11	IV over 10-30 minutes through central venous catheter. Pre-medicate 30-60 minutes prior to infusion with acetaminophen (up to 10-15 mg/kg/dose) and Benadryl (up to 1 mg/kg) to reduce infusion-related reactions. Vital signs will be assessed prior to and post infusion then every 15 minutes for 1 hour.
TTRNA-DCs	Intra-dermal	1x10^7 cells	Days 11, 25, 39	Each immunization will be divided equally to both inguinal regions. A total volume of 200 µL per side will be delivered intradermally after preparation of skin with EMLA anesthetic cream.
Sargramostim (GM-CSF)	Intradermal	150 ug	Days 11, 25, 39	Embedded in DC vaccine
Filgrastim (G-CSF)	SQ or IV	5 micrograms/ kg/day	Beginning no later than day 16- until count recovery	Per institutional guidelines

Based on prior chemotherapy history, patients enrolled on Group B receiving salvage chemotherapy may need to have adjustment in their chemotherapy regimen at the discretion of the treating physician. Focal boost radiotherapy may be delivered prior to immunotherapy if required for local control. As no salvage chemotherapy regimens in the recurrent setting have been found to be curative or achieve significantly prolonged survival, we do not anticipate any clinical impact of adjustments to the planned regimen. All chemotherapy regimens will be recorded and any deviation from the prescribed regimen above noted.

9. STUDY POPULATION

The target population for this study is children and young adults (age 0-30 years) with recurrent MB, pineoblastoma, and cerebral PNETs who suffer first disease recurrence following standard radiotherapy +/- craniospinal irradiation and who are candidates for HDC plus autologous stem cell rescue or NMA salvage chemotherapy. Patients with disease recurrence prior to completion of standard radiotherapy +/- craniospinal irradiation will remain eligible as long as are first recurrence since completion of radiotherapy. Patients who are

unable to receive radiotherapy due to genetic syndromes that put them at significant risk for secondary malignancies (i.e. Gorlin's syndrome or NF1 mutation) are eligible at first recurrence/progression as these patients have failed definitive treatment.

10. INCLUSION CRITERA

10.1 Inclusion Criteria

Age:

The patient must be ≤ 30 years of age at the time of enrollment.

Disease Status:

The patient must have a histologically confirmed first recurrence/progression of reMB/PNET (as confirmed by the study pathologist or his designate) after completion of definitive focal +/- craniospinal irradiation, or must be a patient with first recurrence/disease progression who is unable to receive radiation therapy due to genetic disorders that put them at significant risk for radiation-induced secondary malignancies (ie. Gorlin's syndrome or NF1 mutation).

Performance Status:

The patient must have a KPS of ≥ 50% (KPS for > 16 years of age) or Lansky performance Score (LPS) of ≥ 50 (LPS for ≤ 16 years of age) assessed within 2 weeks prior to registration and documented in the medical record or on **SDW-UFBTIP-004**. Patients who are unable to walk because of paralysis but who are up in a wheel chair will be considered ambulatory for the purposes of the performance score.

Laboratory Analysis and Organ Function Requirements:

Bone Marrow function:

- Absolute neutrophil count ≥ 1000/µl (unsupported)*****
- Platelets ≥ 100,000/µl (unsupported)*****
- Hemoglobin > 8 g/dL (may be supported)

Patients on Group B with ANC <1000/µL and/or platelet count <100,000/µL prior to NMA will be allowed to get vaccine and ALT infusion; however, NMA conditioning will be withheld.

Renal:

-Serum creatinine ≤ upper limit of institutional normal.

Hepatic:

- Bilirubin ≤ 1.5 times upper limit of normal for age.
- SGPT (ALT) ≤ 3 times institutional upper limit of normal for age.
- SGOT (AST) ≤ 3 times institutional upper limit of normal for age.

Ability to tolerate PBSC Collection:

- No known contraindications to PBSC collection. Examples of contraindications might be a weight or size less than the collecting institution finds feasible, or a physical condition that would limit the ability of the child to undergo apheresis catheter placement (if necessary) and/or the apheresis procedure.

Consent:

The patient guardian or patient (if > 18 yrs of age) must consent to all elements of this protocol including surgical resection, the use of resected tumor specimens for RNA preparation, induction chemotherapy and PBSC collection during leukapheresis, high-dose chemotherapy with autologous stem cell rescue (Group A), NMA salvage chemotherapy with autologous stem cell rescue (Group B), and experimental immunotherapy consisting of DC + xALT therapy. Signed informed consent according to institutional guidelines must be obtained prior to

registration. Patients who turn 18 while on the study will be re-consented at that time using an adult consent form. If subjects are referred from outside physicians and it is not feasible for the subject to travel to a participating site, Tissue Consent can be obtained by telephone from the subject/LAR in accordance with institutional policy, procedure, and/or directive.

Additional Eligibility Requirements Prior to Initiation of Consolidation or NMA Chemotherapy:

Renal:

- GFR of at least 70 ml/min/1.73m^2 (Group A only).

Cardiac:

- EKG without clinically significant arrhythmias (Group A only)
- ECHO cardiogram with an ejection fraction of > 45% or MUGA scan within normal limits (Group A only). Patients in Group B will be eligible if the ejection fraction is < or equal to 45% but will receive Fludarabine only during NMA conditioning prior to vaccine + ALT infusions.

Pulmonary:

- DLCO, FEV1, FEC (diffusion capacity) > 50% of predicted (corrected for hemoglobin and alveolar volume). (Group A only)
- Crying Vital Capacity if too young to perform pulmonary function tests. (Group A only)
- Chest X-ray without significant abnormalities and a pulse oximetry reading of ≥ 93% in room air at rest and following a brisk 6-minute exercise resulting in a target heart rate of at least 100/min. These two tests would be required for any patient in Group A who cannot co-operate for the DLCO test due to neurologic deficits and for all patients in Group B. Patients in Group B who do not meet this requirement will still be eligible but will receive Fludarabine only during NMA conditioning.

10.2 Exclusion Criteria

Disease Status:

Patients with clinically significant increased intracranial pressure (e.g., impending herniation), uncontrolled seizures, or requirement for immediate palliative treatment will also be excluded from this study.

Concurrent Therapy:

Each patient will need to be scheduled for definitive resection or, in select cases, have had surgical resection and tumor tissue snap frozen and available for TTRNA to be eligible for enrollment. Patients receiving any other concurrent anticancer or investigational drug therapy will be excluded and replaced. Focal boost radiotherapy may be delivered prior to immunotherapy if required for local control.

Pregnancy:

Females who are pregnant or need to breast feed during the study period will be excluded. A negative β-HCG test will be required within 2 weeks of enrollment. Patients found to be pregnant will be excluded. Male and female enrolled patients must be willing to use a medically acceptable form of birth control, which includes abstinence, while being treated on this study.

Corticosteroids:

Patients 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.

Medical Conditions:

Patients with the following conditions will be excluded to avoid confounding results:

- Patients with an active infection requiring treatment or an unexplained febrile (>101.5º F) illness.
- Patients with known immunosuppressive disease, human immunodeficiency virus infection, or carriers of Hepatitis B or Hepatitis C virus.
- Patients with active renal, cardiac (congestive cardiac failure, myocardial infarction, myocarditis), or pulmonary disease.
- Patients receiving concomitant medications that may interfere with study outcome (e.g., immunosuppressive agents).
- Patients with any clinically significant unrelated systemic illness (serious infections or significant cardiac, pulmonary, hepatic or other organ dysfunction) that would compromise the patient's ability to tolerate protocol therapy or would likely interfere with the study procedures or results.

Patients with the inability to return for follow-up visits or obtain follow-up studies required to assess toxicity to therapy

10.3 Inclusion of Women and Minorities

Our brain tumor studies are open to patients of all ages, both sexes, and all minorities. In addition, certain diseases that we treat occur preferentially in one gender or minority than another. For example, carcinoma of the breast is a frequent cause of metastatic brain tumors and, of course, is much more common in women than in men. Although the lung cancer incidence is rising in women, the incidence is still higher in men and it is also a frequent cause of metastatic brain tumors. Primary brain tumors and metastatic brain tumors from some very common tumors such as melanoma are an infrequent disease in African-American populations. Our accrual statistics reflect the lower incidence in the African-American population. We will continue to actively recruit women and minorities into this study using methods that have shown previous success in recruiting the participation of these subgroups.

We routinely send letters to our primary referring physicians notifying them of our interest in including minorities and women in all our brain tumor clinical trials.

11. DESCRIPTION OF THE RECRUITMENT PROCESS

11.1 Recruitment

The following table shows the incidence rates by ICCC group and race/ethnicity for both sexes, 1992-1999 as provided by the Central Brain Tumor Registry of the United States. A breakdown by sex was not available but the observation has been that in all race/ethnicity groups the incidence is slightly higher in males.

	Number per 1,000,000
WHITE	28.7
AFRICAN AMERICAN	24.1
NATIVE AMERICAN/ALASKA NATIVE	14.1
ASIAN/PAC ISLANDER	22.0
HISPANIC	21.7

*SEER Cancer Incidence Public-Use Data, 1992-1999.

Our demographics of all brain tumor patients treated at the Preston A. Wells, Jr., Center for Brain Tumor Therapy at the University of Florida does not differ significantly from the demographic break down of brain tumor incidences nationwide, demonstrating that a representative spectrum of patients affected by this disease are routinely seen at our Center.

11.2 Screening and Enrollment

Potentially eligible patients with reMB/PNET scheduled to undergo definitive surgical resection will be identified through direct referral to the multi-institutional sites. In select cases, patients at outside institutions who have had surgical resection and tumor tissue snap frozen and available for TTRNA will be consider for enrollment. PHI will be reviewed by the PI(s) and/or Study Coordinator but not recorded in order to pre-screen patients for eligibility for this trial. The pre-screening PHI will consist of a review of the medical record to confirm presentation of first recurrence of an initial diagnosis of MB or central PNET after receipt of previous definitive focal +/- craniospinal irradiation for diagnosis of MB/PNET in patients $\leq$ 30 years of age at time of enrollment. Of note, patients with recurrent MB/PNET who have failed prior radiation therapy or are not able to receive radiation therapy due to genetic disorders that put them at significant risk for radiation-induced secondary malignancies (ie. Gorlin's syndrome or NF1 mutation) will still be eligible to receive treatment The guardian(s) of pediatric patients or young adult patients potentially eligible for this trial will be approached by PI(s) and/or Study Coordinator about interest in the study and to give informed consent to participation in this clinical trial. At the time of consent the PI(s) of the various treatment teams will be available to answer questions and provide any clarification needed regarding the content of the consent document or clinical study details. The Study Coordinator will also be present to obtain informed consent and provide a copy of the signed documentation to the guardian or adult patient.

Given the history of safety for immunotherapy in adult patients we don't anticipate significant toxicity with this treatment regimen. All patients 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. We anticipate that a minimum of 80% of patients enrolled will proceed to HDC + PBSCT and receive DC + xALT therapy without prior tumor progression and therefore anticipate targeted enrollment using multi-institutional study sites. The target distribution for patients that will be treated with DC + xALT therapy based on the SEER Cancer Incidence Public-Use Data is shown in the following Table:

	Patient Numbers	**White**		**African-American**		**Hispanic**		**Asian**		**Other**	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Phase I	9	4	3	1	0	0	0	0	0	1	0
Phase II	35	17	12	1	1	1	0	0	1	1	1
TOTAL	44	21	15	2	1	1	0	0	1	2	1

Actual accrual will be actively reviewed by the PI (Dr. Duane A. Mitchell) and the Medical Monitor and compared with this table. If the expected accrual diversity is not met or if accrual overall is slower than anticipated (assessed every 3 months), we will initiate a targeted recruitment program through techniques already established within our multi-institutional sites, including the placement of advertisements in newspapers or on radio stations to increase public awareness overall and specifically catering to the underrepresented population(s) and providing educational seminars for patients and staff at community institutions such as churches and medical clinics that preferentially serve the underrepresented population(s). The PRTBTC has a strong referral network and we will

also utilize resources such as the Pediatric Brain Tumor Consortium and the Pediatric Brain Tumor Foundation of the United States to increase publicity and recruitment for this trial should accrual fall below expectation.

12. SAMPLE SIZE JUSTIFICATION

Progression-free survival in Group A will be measured from the initiation of HDC for comparison to our historical experience of HDC + PBSCT in this patient population. The progression-free survival experience among patients with recurrent disease who initiated HDC after having previously received definitive radiotherapy is reported by Gururangan (2008). The 12-month PFS rate is 33% (95% confidence interval: 10%, 59%) with a two-year PFS of 0%.

Due to the complexity and expense of adoptive cellular therapy treatment, if the true PFS-12 associated with DC + xALT therapy is 33%, there would be limited interest in further investigation of this treatment regimen without significant modification to improve efficacy. However, if the true PFS-12 were 55% or greater, there would be interest in studying this treatment regimen within the context of larger confirmatory Phase II or III clinical trials. Therefore, the study is designed to differentiate between a PFS-12 rate of 33% (null hypothesis) and 55% (alternative hypothesis). With 35 patients enrolled in the Phase II trial, this assessment has 90% power assuming a type I error rate of 0.10. There is also 97% power to differentiate between PFS-12 rates of 33% and 60%.

In Group B, progression-free survival will be measured from the time of proximal tumor recurrence before adoptive cellular therapy. Patients with progression prior to immunotherapy may remain on study as long as they continue to meet other eligibility requirements. Currently, no good historical benchmark exists for this patient population. The Group A benchmark described above will be used conservatively for patients in Group B given that they are generally considered to have poorer prognosis than Group A. Given this assumption, the sample size justification described above is also applicable to Group B.

13. DESCRIPTION OF INFORMED CONSENT PROCESS

Study site personnel will identify potentially eligible subjects who are scheduled for craniotomy due to recurrent MB/PNET and initially screen eligibility and interest in possible protocol participation. In select cases, patients at outside institutions who have had surgical resection and tumor tissue snap frozen and available for TTRNA will be screened for eligibility and possible protocol participation. Dr. Mitchell (or his designee) of the UF Brain Tumor Immunotherapy Program and Department of Neurosurgery will discuss study participation with potential adult subjects or parents of potential minor subjects for education regarding this research study. The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the patient or the patient's parents or guardian if the patient is a child, and a signed informed consent and assent will be obtained according to institutional guidelines. Those patients that turn 18 while on study will be re-consented using an adult consent form.

14. VOLUNTEER SCREENING PROCEDURES

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial must only be done after obtaining written informed consent. Documentation of the informed consent for screening will be maintained in the patient's research chart. Studies or procedures that were performed for clinical indications (not exclusively to determine eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

15. STUDY PROCEDURES/STUDY INTERVENTIONS

15.1 Overview

In this Phase II trial, 35 to 42 children and young adults with reMB/PNETs will be treated with DC + xALT therapy at the MTD ($3x10^7$ T cells/kg) determined from the Phase I trial in order to estimate the clinical efficacy of this treatment. Patients unable to undergo surgical resection, dependent on corticosteroid supplements above physiologic levels at time of first vaccine (> 4 mg decadron per day), whose final pathology is not recurrent MB or PNET, unable to generate mRNA from resected tumor specimen, fail induction chemotherapy regimen due to tumor progression, or are unable to tolerate HDC after induction therapy will be replaced. 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 assessment.

A study schema outlining the stages of this clinical protocol covering both Groups is provided in **Sections 2.2, 2.3, and 2.4**.

15.2 Chemotherapy for Group A

15.2.1 INDUCTION THERAPY

During Induction therapy, surgery and anti-cancer drugs (chemotherapy) are used to remove and to kill as much tumor as possible. Also, peripheral blood stem cells (PBSCs) are collected during the Induction phase of therapy by leukapheresis as described below.

Surgery: Maximal debulking surgery will be performed to minimize tumor burden and confirm diagnosis of recurrent tumor. All tumors will be confirmed as recurrent MB/PNET by a board-eligible/board-certified neuropathologist. Tumor tissue in excess of what is needed to confirm pathologic diagnosis will be transported under aseptic precautions to Dr. Mitchell's laboratory for tumor RNA preparation. Tumor tissue may also be obtained through biopsy or CSF (cytology).

Patients will undergo a non-mobilized leukapheresis prior to induction for harvest of peripheral blood mononuclear cells (PBMCs) for TTRNA-xALT and TTRNA-DC generation. The target PBMC count based on the desired dose of TTRNA-xALT and sufficient TTRNA-DCs for T cell expansion and in vivo vaccination will be collected.

Approximately two weeks but no more than four weeks post-surgical resection, patients will continue induction treatment with 3-4 cycles of chemotherapy at 28-day intervals. Cycles 1 & 2 will consist of intravenous cyclophosphamide. Cycles 3 & 4 will consist of oral etoposide plus oral temozolomide (TMZ). After each induction cycle, patients will receive G-CSF in order to mobilize PBSCs for later stem cell support following high-dose chemotherapy. G-CSF will be given daily starting 24 hours following the last dose of chemotherapy and until completion of the leukapheresis procedure. Peripheral blood stem cells (PBSC) will be collected after each induction cycle until the target CD34(+) PBSC yield needed for Consolidation ($2x10^6$ CD34+ cells per Kg at a minimum) is obtained. Absolute nucleated cell count collected will be adjusted as needed on a patient specific basis. The drugs will be given on the following schedule:

Leukapheresis after each induction cycle until collection of a minimum $2x10^6$ CD34+ cells/kg				
	Drug	**Dosage**	**Route**	**Days Given**
Cycle 1 & 2	Cyclophosphamide	$2g/m^2$/day	IV over 1 hour	1, 2
	Mesna	$2g/m^2$/day	IV	1, 2

	Filgrastim	10mcg/kg/day	SQ or IV	3 - 28*
Cycle 3 & 4	Temozolomide	150mg/m^2/day	By mouth	1 - 5
	Etoposide	30mg/m^2/day	By mouth	1 - 14

Upon recovery from the 3rd or 4th cycle of induction chemotherapy (ANC > 1000 cells/μL), patients will undergo disease restaging and organ function evaluation. Patients dependent on steroid supplements above physiologic levels at time of Vaccine #1 (> 4 mg decadron per day), have radiographic or histologic evidence of progression will be replaced. 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 assessment. For the purpose of this study, progression will be defined as a new lesion confirmed by biopsy or resection, positive cerebrospinal fluid (CSF) cytology, or a new metastatic lesion (if previously negative).

15.2.2 LEUKAPHERESIS FOR PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS)

- **Intravenous Access**: PBMCs will be obtained by placing two large gauge catheters into the antecubital veins bilaterally or by using a dialysis-type central venous catheter that will allow for large volume apheresis.
- **PBMC Mobilization**: Patients will not receive mobilization prior to leukapheresis.
- **Apheresis Machine**: The choice of apheresis machine is at the discretion of the institution.
- **Blood Priming**: Blood priming of the apheresis machine is at the discretion of the institution.
- **Apheresis Procedure**: The apheresis machine settings, procedure duration, and volume of whole blood processed shall be in accordance with **SOP-UFBTIP-131**.
- **PBMC Collection Goal:** The collection goal for TTRNA-xALT and TTRNA-pulsed Dendritic Cell vaccine is a minimum of 5x10^9 mononuclear cells.
- **Product Testing**: An aliquot of cryopreserved TTRNA-xALT and TTRNA-pulsed Dendritic Cells will be thawed and prepared for QC testing and must meet batch release criteria.

15.2.3 PBSC MOBILIZATION AND LEUKAPHERESIS

- Leukapheresis products previously collected and cryopreserved may be used for this protocol as long as the cells meet all standard release criteria and transfer agreements for use of the products, if applicable, are executed.
- **Intravenous Access**: PBSCs will be collected using a dialysis-type catheter that will allow for large volume apheresis.
- **PBSC Mobilization**: Patients will begin G-CSF (5mcg/kg/day) 24-36 hours after completion of chemotherapy. When the WBC count starts recovering (WBC > 1,000 post nadir or in accordance with institutional standards), increase the G-CSF dose to 10mcg/kg/day. PBSC collection will begin when the circulating CD34
- cell count is > 10 cells/μL or in accordance with institutional standards. G-CSF will continue until PBSC collection is complete. It is recommended that G-CSF be administered at night (approximately 2200) on the days(s) prior to scheduled leukapheresis. If the post-G-CSF WBC is > 60,000, decrease the G-CSF dose to 5mcg/kg.
- **Apheresis Machine**: The choice of apheresis machine is at the discretion of the institution.
- **Blood Priming**: Blood priming of the apheresis machine is at the discretion of the institution.
- **Apheresis Procedure**: The apheresis procedure will be performed in accordance with institutional standards.
- **PBSC Collection Goal:** The PBSC collection goal is 2x10^6 CD34+ cells per Kg at a minimum.

- **PBSC Product Testing:** Each PBSC collection will be cultured for bacterial and fungal contamination, nucleated cell count and differential, and CD34+ cell enumeration.

15.2.4 GUIDELINES FOR INDUCTION THERAPY AND PBSC COLLECTION

As no study drug is administered during the Induction phase of the trial, the Induction regimen for Group A patients and mobilization and collection of PBSCs for stem cell rescue may be administered at UF or other Foundation for the Accreditation of Cellular Therapy (FACT)-accredited medical institutions under the care of a board-eligible/board-certified pediatric oncologist, with the exception of the first leukapheresis for collection of PBMCs for DC and xALT generation, which must occur at the main institution. Subsequent PBSC mobilization and leukapheresis may be conducted according to institutional guidelines. PBSC products collected and cryopreserved at FACT-accredited institutions shall be shipped in a dry shipper to the main institution prior to Consolidation. Dr. Sri Gururangan (Neuro-Oncology Chair) will be responsible for providing a roadmap of the induction and salvage chemotherapy regimens to outside oncologists and acquiring documentation of all prescribed medications and any chemotherapy or procedure related toxicities during these periods. Standard medication regimens may need to be adjusted due to hematologic or other toxicities and may be managed at the discretion of the treating pediatric oncologist. Focal boost radiotherapy may be delivered prior to immunotherapy if required for local control. All deviations from intended regimens during induction or salvage therapy will be documented, but not considered a protocol deviation, as is within routine standard of practice for this patient population. All patients must return to the main institution for high-dose Consolidation therapy (Group A) and subsequent immunotherapy.

15.3 Consolidation

15.3.1 PRE-TRANSPLANT EVALUATION

- Adequate collection of autologous PBSCs for transplant
- Successful production of vaccine
- Medical history
- Physical examination
- Bone Marrow function:
  - Absolute neutrophil count > 1000/µl (unsupported).
  - Platelets > 100,000/µl (unsupported).
  - Hemoglobin > 8 g/dL (may be supported).
- Renal:
  - Serum creatinine ≤ upper limit of institutional normal
  - GFR of at least 70 ml/min/1.73m^2.
- Hepatic:
  - Bilirubin ≤ 1.5 times upper limit of normal for age.
  - SGPT (ALT) ≤ 3 times institutional upper limit of normal for age.
  - SGOT (AST) ≤ 3 times institutional upper limit of normal for age.
- Adequate cardiac function defined as:
  - ECHO Cardiogram with ejection fraction of > 45% by radionuclide angiogram or MUGA scan within normal limits
  - EKG without clinically significant arrhythmias.
- Pulmonary:
  - DLCO, FEV1, FEC (diffusion capacity) > 50% of predicted (corrected for hemoglobin and alveolar volume)
  - Crying Vital Capacity if too young to perform pulmonary function tests

- Chest X-ray and pulse oximetry reading of ≥ 93% in room air at rest and following a brisk 6-minute exercise resulting in a target heart rate of at least 100/min if patient cannot perform well on pulmonary function test due to neurologic deficits.

- Negative serum pregnancy test for females of child-bearing potential.

Eligible patients will be admitted to the Pediatric Blood and Marrow Unit (BMTU) to receive the myeloablative regimen of carboplatin, etoposide, and thiotepa, with autologous PBSC rescue.
(Gururangan S, et al. Neuro-Oncol 2008; 10(5):745-751).

Drug Name	Route	Dose	Schedule	Instructions for administration
Carboplatin	IV	The lesser of 500mg/m^2 or dosing = AUC 7 mg/ml-min per Calvert Formula; dilute to 4 mg/ml in Normal Saline	Days -8, -7,-6	IV over 4 hours through central venous catheter
Etoposide	IV	250mg/m^2; dilute to 0.3 mg/ml in Normal Saline	Days -5,-4,-3	IV over 3 hours through central venous catheter
Thiotepa	IV	300mg/m^2; dilute to 1 mg/ml in Normal Saline	Days -5,-4,-3	IV over 3 hours through central venous catheter
Peripheral Blood Stem Cells	IV	**Minimum: $2x10^6$ CD34+ cells/kg**	Day 0	IV through central venous catheter per institutional guidelines
TTRNA-xALT	IV	$3x10^7$/kg	Day 1	IV over 10-30 minutes through central venous catheter. Pre-medicate 30-60 minutes prior to infusion with acetaminophen (up to 10-15 mg/kg/dose) and Benadryl (up to 1 mg/kg) to reduce infusion-related reactions. . Vital signs will be assessed prior to and post infusion then every 15 minutes for 1 hour.
TTRNA-DCs	intradermal	$1x10^7$ cells	Days 1, 15, 29	Each immunization will be divided equally to both inguinal regions. A total volume of 200 µL per side will be delivered intradermally after preparation of skin with EMLA anesthetic cream.
Sargramostim (GM-CSF)	Intradermal	150 ug	Days 1,15, 29	Per institutional guidelines
Filgrastim (G-CSF)	SQ or IV	5 micrograms/ kg/day	Beginning no later than Day 6 post-transplant-count recovery	Per institutional guidelines

Note: The modified Calvert Formula calculates the ACTUAL mg of carboplatin to be given

Carboplatin Dosing per modified Calvert formula[6] and using AUC of 7: dose in mg/m^2= 7 [0.93 x GFR (ml/min/m^2) + 15]

15.3.2 NMA SALVAGE CHEMOTHERAPY FOR GROUP B

During NMA salvage chemotherapy, surgery and anti-cancer drugs (chemotherapy) are used to remove and to kill as much tumor as possible. Also, peripheral blood stem cells (PBSCs) are collected during the salvage phase of therapy by leukapheresis as described below.

Surgery: Maximal debulking surgery will be performed to minimize tumor burden and confirm diagnosis of recurrent tumor. All tumors will be confirmed as recurrent MB/PNET by a board-eligible/board-certified neuropathologist. Tumor tissue in excess of what is needed to confirm pathologic diagnosis will be transported under aseptic precautions to Dr. Mitchell's laboratory for tumor RNA preparation. Tumor tissue may also be obtained through biopsy or CSF (cytology).

Patients will undergo a non-mobilized leukapheresis prior to salvage chemotherapy for harvest of peripheral blood mononuclear cells (PBMCs) for TTRNA-xALT and TTRNA-DC generation. The target PBMC count based on the desired dose of TTRNA-xALT and sufficient TTRNA-DCs for T cell expansion and in vivo vaccination will be collected.

Following standard salvage chemotherapy adequate organ function testing will be repeated prior to initiation of NMA chemotherapy (cyclophosphamide and fludarabine conditioning) as described below:

- Bone Marrow function:
  - Absolute neutrophil count > 1000/µl (unsupported)*
  - Platelets > 100,000/µl (unsupported)*
  - Hemoglobin > 8 g/dL (may be supported)

  * Patients on Group B with ANC <1000/µL and/or platelet count <100,000/µL prior to NMA will be allowed to get vaccine and ALT infusion; however, NMA conditioning will be withheld.
- Renal:
  - Serum creatinine ≤ upper limit of institutional normal and/or GFR > 70 ml/min/1.73m^2
    - If GFR ≤ 70 ml/min/1.73 m^2, Fludarabine dose adjusted for GFR < 70 ml/min/1.73m^2 per drug guidelines
- Hepatic:
  - Bilirubin ≤ 1.5 times upper limit of normal for age.
  - SGPT (ALT) ≤ 3 times institutional upper limit of normal for age.
  - SGOT (AST) ≤ 3 times institutional upper limit of normal for age.
- Adequate cardiac function defined as:
  - ECHO cardiogram with an ejection fraction of > 45% or MUGA scan within normal limits
    - Patients in Group B will be eligible if the ejection fraction is ≤ 45% but will receive Fludarabine only during NMA conditioning prior to vaccine + ALT infusion.
- Pulmonary:

  -- DLCO, FEV1, FEC (diffusion capacity) > 50% of predicted (corrected for hemoglobin and alveolar volume) or
  - Chest X-ray and pulse oximetry reading of ≥ 93% in room air at rest and following a brisk 6-minute exercise resulting in a target heart rate of at least 100/min.
    - Patients in Group B who do not meet this requirement will still be eligible but will receive Fludarabine only during NMA conditioning.
- Negative serum pregnancy test for females of child-bearing potential.

Of note, cyclophosphamide will be withheld if a patient does not meet the cardiac performance criteria and lymphodepletive conditioning will proceed with fludarabine only. Additionally, the fludarabine dose may be modified for GFR < 70 ml/min/1.73m^2. Refer to Section 18.8 Fludarabine Phosphate for dose modification guidelines.

NMA salvage chemotherapy will consist of 28-day cycles until Immunotherapy preparation is complete. The drugs will be given on the following schedule:

Drug Name	**Route**	**Dose**	**Schedule**
Temozolomide	PO	150 mg/m^2/day	Days 1-5
Etoposide	PO	30 mg/m^2/day	Days 1-14
Filgrastim (G-CSF)	IV or SQ	10 micrograms/ kg/day	Starting Day 15 *administered each cycle until enough CD34 cells are harvested (goal of 2x10^6/kg) for infusion

After completion of NMA salvage chemotherapy and starting 11 days before Immunotherapy, patients will receive 2 days of cyclophosphamide IV at 1 g/m^2, followed by 5 days of fludarabine IV at 25 mg/m^2 (dose may be modified for GFR < 70 ml/min/1.73m^2. Refer to Section 18.8 for dose modification guidelines). Approximately 72 hours following fludarabine, PBSCs, if available, will be infused. Approximately 96 hours following the final dose of fludarabine, Immunotherapy will begin with infusion of the TTRNA-xALT and injection of the first TTRNA-DC vaccine. Patients will receive 3 biweekly vaccines as long as clinically stable.

Drug Name	Route	Dose	Schedule	Instructions for administration
Cyclophosphamide	IV	1000 mg/m^2/day	Days 1 & 2	Dose reduce or omit based on cardiac and/or pulmonary function
Fludarabine	IV	25 mg/m^2/day	Days 3-7	Dose reduce or omit based on creatinine clearance
Peripheral Blood Stem Cells, if available	IV	A minimum of 2x10^6 CD34+ cells/kg or all available cells if < 2x10^6 CD34+ cells/kg	Day 10	IV through central venous catheter per institutional guidelines
TTRNA-xALT	IV	3x10^{7}cells/kg or all available cells	Day 11	IV over 10-30 minutes through central venous catheter. Pre-medicate 30-60 minutes prior to infusion with acetaminophen (up to 10-15 mg/kg/dose) and Benadryl (up to 1 mg/kg) to reduce infusion-related reactions. Vital signs will be assessed prior to and post infusion then every 15 minutes for 1 hour.
TTRNA-DCs	Intra-dermal	1x10^7 cells	Days 11, 25, 39	Each immunization will be divided equally to both inguinal regions. A total volume of 200 µL per side will be delivered intradermally after preparation of skin with EMLA anesthetic cream.
Sargramostim (GM-CSF)	Intradermal	150 ug	Days 11, 25, 39	Embedded in DC vaccine
Filgrastim (G-CSF)	SQ or IV	5 micrograms/ kg/day	Beginning no later than Day 16- count recovery	Per institutional guidelines

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 assessment.

15.3.3 LEUKAPHERESIS FOR PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMCS)

- **Intravenous Access**: PBMCs will be obtained by placing two large gauge catheters into the antecubital veins bilaterally or by using a dialysis-type central venous catheter that will allow for large volume apheresis.
- **PBMC Mobilization**: Patients will not receive mobilization prior to leukapheresis.
- **Apheresis Machine**: The choice of apheresis machine is at the discretion of the institution.
- **Blood Priming**: Blood priming of the apheresis machine is at the discretion of the institution.

- **Apheresis Procedure**: The apheresis machine settings, procedure duration, and volume of whole blood processed shall be in accordance with **SOP-UFBTIP-131**.
- **PBMC Collection Goal:** The collection goal for TTRNA-xALT and TTRNA-pulsed Dendritic Cell vaccine is a minimum of $5x10^9$ mononuclear cells.
- **Product Testing**: An aliquot of cryopreserved TTRNA-xALT and TTRNA-pulsed Dendritic Cells will be thawed and prepared for QC testing and must meet batch release criteria.

15.3.4 PBSC MOBILIZATION AND LEUKAPHERESIS

- Leukapheresis products previously collected and cryopreserved may be used for this protocol as long as the cells meet all standard release criteria and transfer agreements for use of the products, if applicable, are executed.
- **Intravenous Access**: PBSCs will be collected using a dialysis-type catheter that will allow for large volume apheresis.
- **PBSC Mobilization**: Patients will begin G-CSF (5mcg/kg/day) 24-36 hours after completion of chemotherapy. When the WBC count starts recovering (WBC > 1,000 post nadir or in accordance with institutional standards), increase the G-CSF dose to 10mcg/kg/day. PBSC collection will begin when the circulating CD34 cell count is > 10 cells/μL or in accordance with institutional standards. G-CSF will continue until PBSC collection is complete. It is recommended that G-CSF be administered at night (approximately 2200) on the days(s) prior to scheduled leukapheresis. If the post-G-CSF WBC is > 60,000, decrease the G-CSF dose to 5mcg/kg.
- **Apheresis Machine**: The choice of apheresis machine is at the discretion of the institution.
- **Blood Priming:** Blood priming of the apheresis machine is at the discretion of the institution.
- **Apheresis Procedure**: The apheresis procedure will be performed in accordance with institutional standards.
- **PBSC Collection Goal:** The PBSC collection goal is 2 $x10^6$ CD 34+ cells/kg.
- **PBSC Product Testing:** Each PBSC collection will be cultured for bacterial and fungal contamination, nucleated cell count and differential, and CD34+ cell enumeration.

15.3.5 GUIDELINES FOR SALVAGE CHEMOTHERAPY AND PBSC COLLECTION

As no study drug is administered during the salvage chemotherapy phase of the trial and administration of salvage chemotherapy is a standard-of-care for patients not amenable to HDC, the salvage regimen for Group B patients and mobilization and collection of PBSCs may be administered at UF or other Foundation for the Accreditation of Cellular Therapy (FACT)-accredited medical institutions under the care of a board-eligible/board-certified pediatric oncologist, with the exception of the first leukapheresis for collection of PBMCs for DC and xALT generation, which must occur at the main institution. Subsequent PBSC mobilization and leukapheresis may be conducted according to institutional guidelines. PBSC products collected and cryopreserved at FACT-accredited institutions shall be shipped in a dry shipper to the Stem Cell Laboratory under per SOPs prior to NMA conditioning with cyclophosphamide and fludarabine. Dr. Sri Gururangan (Neuro-Oncology Chair) will be responsible for providing a roadmap of the induction and salvage chemotherapy regimens to outside oncologists and acquiring documentation of all prescribed medications and any chemotherapy or procedure related toxicities during these periods. Standard medication regimens may need to be adjusted due to hematologic or other toxicities and may be managed at the discretion of the treating pediatric oncologist. Focal boost radiotherapy may be delivered prior to immunotherapy if required for local control. All deviations from intended regimens during induction or salvage therapy will be documented, but not considered a protocol deviation, as is within routine standard of practice for this patient population. All patients must return to the main institution for NMA conditioning regimen with cyclophosphamide and fludarabine (Group B) with PBSCT, and subsequent immunotherapy.

15.4 Post-transplant Immunotherapy

All patients will receive TTRNA-xALT and TTRNA-DCs as Vaccine #1. Peripheral blood will be drawn prior to non-mobilized leukapheresis, prior to immunotherapy, 1 day post Vaccine #1, 4 (+/- 1) days post Vaccine #1, weekly post Vaccine #1 for six weeks, and bimonthly, if feasible, until progression for T-cell kinetics. Blood will be processed in accordance with **SOP-UFBTIP-126**. DCs will be given intradermally and divided equally to both inguinal regions. Vaccines #2 and #3 will occur at 2-week intervals following the first dose. Patients will receive at 3 biweekly vaccines as long as clinically stable. Studies in patients receiving myeloablative and NMA chemotherapy have shown that T cell function normalizes (without intervening immunotherapy) between 6-12 months in most patients.

Patients will be followed bi-monthly (+/- one month) for first year post immunotherapy, then every 3 months (+/- one month) until death due to any cause. As part of standard care for these patients, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure consent will be obtained separately. However, if tissue is obtained, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and antigenic profile changes from the initially resected tumor specimen used in vaccine preparation.

16. EXPERIMENTAL STUDY PROCEDURES (DC + XALT THERAPY)

16.1 DC Vaccination

Following myeloablative (Group A) and NMA chemotherapy (Group B), patients will receive intravenous TTRNA-xALT at $3x10^7$cells/kg and intradermal TTRNA-DCs as vaccine #1. Peripheral blood will be drawn prior to non-mobilized leukapheresis, prior to immunotherapy, 1 day post Vaccine #1, 4 (+/- 1) days post Vaccine #1, weekly post Vaccine #1 for six weeks, and bimonthly, if feasible, until progression for T cell kinetics. Blood will be processed in accordance with SOP-UFBTIP-126. DCs will be given intradermally and divided equally to both inguinal regions in accordance with SOP-UFBTIP-128. Vaccines #2 and #3 will occur at 2 week intervals following the first dose. Each immunization will be divided equally to both inguinal regions. A total volume of 200 µL per side will be delivered intradermally after preparation of skin with EMLA anesthetic cream. Details of the procedure will be recorded on SDW-UFBTIP-002. Patients will be monitored in the clinic for thirty minutes post-immunization for the development of any adverse effects. The immunizations will be supervised by a nurse or physician that has completed a Pediatric Advanced Life Support (PALS) course. A cardiac resuscitation cart will be available in the immediate vicinity when performing these immunizations in case of severe allergic reactions. The three vaccines will each be given 2 ± 1 week(s) apart.

Subjects who will not receive xALT either due to failure of the product to meet release criteria or inability to manufacture a viable product, may receive additional DC vaccination(s) if more than 3 doses were initially generated. Subsequent DC vaccination will begin 4 weeks (+/- 5 days) after vaccine number 3 and continue every 4 weeks (+/- 5 days) until all doses have been exhausted, the subject meets a withdrawal endpoint, or the treating physician elects to stop further vaccination. These subjects will be evaluated for toxicity and laboratory immune measures; however, they will be replaced for the purposes of efficacy assessments.

Patients will be monitored clinically with routine physical and neurologic examinations at every visit and with a contrasted-enhanced CT or MRI every 8 ± 4 weeks. Peripheral blood will be obtained bimonthly, if feasible, until progression as well as outlined in the study flow sheet below for immunologic monitoring.

16.2 TTRNA-xALT Administration

Patients will receive a single dose of TTRNA-xALT consisting of autologous lymphocytes enriched for tumor-specific lymphocytes after *in vitro* stimulation with TTRNA-pulsed DCs on the same day as the first DC vaccination. No further TTRNA-xALT administrations will be given. TTRNA-xALT infusions will be

administered at the main site and the patients will be monitored by an RN during and for 1 hour following the procedure. If any patient develops signs of infection or is febrile prior to the infusion it will be postponed pending clinical work-up. The TTRNA-xALT product will be prepared, delivered, and administered according to Standard Operating Procedures. Subject identifiers will be double-verified prior to TTRNA-xALT administration as is standard BMT transfusion procedure.

The patient will be pre-medicated 30-60 minutes prior to infusion with acetaminophen (up to 10-15 mg/kg/dose) and Benadryl (up to 1 mg/kg) to reduce infusion-related reactions. All patients will have an intravenous catheter placed during PBSCT or one will be placed for the procedure. The TTRNA-xALT will be infused in accordance with SOP-UFBTIP-132. Vital signs will be assessed prior to and post infusion then every 15 minutes for 1 hour. The infusion will last 10-30 minutes depending on volume to be infused. The Infusion and any adverse experiences will be documented on SDW-UFBTIP-003. All other institutional transfusion and cell infusion policies will be followed.

17. SAFETY, TOXICITY, AND ADVERSE EVENTS

17.1 Toxicity associated with Standard Therapy (HDC + PBSCT)

HDC + PBSCT is an aggressive chemotherapeutic regimen that is standard therapy in this patient population at our Center and worldwide. The significant toxicities associated with this treatment are universal, with 99% of all patients experiencing Grade 3 or greater treatment related toxicity within the first 100 days post HDC. In order to assess any potential toxicity's associated with DC + xALT therapy we have classified toxicity evaluation into three possible categories:

1) Non-overlapping toxicities
2) Immunotherapy related mortality
3) Chemotherapy related toxicity

Toxicity (Grade 3, 4)	Gururangan et al. 1998 n = 20	Broniscer et al. 2004 n = 17	Sung et al. 2007 n = 25
Microbiologically documented Infections	4(20%)	9 (53%)	4 (16%)
Presence of high fever (≥ 38.5ºC)	20(100%)	NR	14 (56%)
Vomiting	NR	NR	10 (40%)
Oropharyngeal mucositis	20 (100%)	17 (100%)	10 (40%)
Diarrhea	NR	NR	12(48%)
Elevation of liver enzyme	NR	NR	8(32%)
Veno-occlusive disease	NR	NR	2(8%)
Hyponatremia	NR	NR	5(20%)
Hypokalemia	NR	NR	5(20%)
Treatment related mortality	2(10%)	2(12%)	4(16%)
Intracerebral hemorrhage	1(5%)	NR	NR
Subgaleal hematoma	1(5%)	NR	NR
Hepatotoxicity	NR	5(29%)	NR
Nephrotoxicity	NR	2(12%)	NR
Seizures	NR	2(12%)	NR

For ease of surveying common toxicities associated with this treatment regimen (or similar high-dose chemotherapy regimens) in patients with reMB/PNETs a table of toxicities experienced in three representative

clinical trials including one of our own is included above. Toxicities external to this list will be considered non-overlapping toxicity.

17.2 Immunotherapy Related Dose-limiting Toxicity

17.2.1 Phase II Toxicity Monitoring

The stopping rules described below for monitoring dose-limiting toxicity and treatment-related mortality during the Phase II trial are guidelines for determining whether accrual should be formally suspended and the data carefully reviewed.

17.2.2 Monitoring of Non-Overlapping Vaccine Related Toxicity

Dose-limiting toxicity rates of 5% or less are considered desirable while rates of 20% or greater are considered undesirable. The statistical hypothesis that will be tested will differentiate between a 5% (alternative hypothesis) and 20% (null hypothesis) rate of unacceptable toxicity. Stopping rules based upon boundaries proposed by Pocock will be used to monitor this study. If the following thresholds for DLT ratios are satisfied (≥3/5, ≥4/15, ≥4/25, ≥5/35), then the study will be suspended and the data reviewed carefully. The type I and type II errors associated with this testing/monitoring are 0.025 and 0.019, respectively.

17.2.3 Monitoring of Vaccine Related Mortality

Accrual will be automatically terminated in the following situations: (1) The occurrence of any vaccine-related death, or (2) The occurrence of any death more than 100 days post-transplant that is not due to tumor progression (or other readily identifiable unrelated cause of death (i.e., motor vehicle accident). A review by the DSMB and IRB along with discussion with the PI will ensue to determine whether accrual to this trial would continue.

17.2.4 Monitoring of Chemotherapy-Related Mortality

During the 100 day period post-transplant, there is concern that the administration of vaccines will exacerbate chemotherapy-related mortality. Based upon our experience, a mortality rate of 3-5% is expected with the administration of HDC followed by transplantation without vaccination. If the true rate of chemotherapy-related mortality with the novel treatment regimen during the 100 day period was 10% or greater the regimen would be considered unacceptable for further clinical development due to excessive toxicity unless significant modifications were made to mitigate the rate of chemotherapy-related mortality. If that percentage were 2.5% or less, the treatment regimen would have an acceptable toxicity mortality rate. Monitoring and decision rules have been developed to differentiate between a 2.5% (alternative hypothesis) and 10% (null hypothesis) rate of chemotherapy-related mortality. Boundaries defined by Pocock will be used to monitor this study. If the following thresholds are satisfied (≥2/5, ≥2/15, ≥2/25, ≥2/35), the study will be terminated due to excessive toxicity. The type I error or probability of concluding that the treatment regimen is safe from the perspective of chemotherapy-related mortality when it is truly unsafe is 0.05. The type II error rate, or probability of concluding that there is excessive treatment-related mortality when the treatment is safe, is 0.12.

These monitoring rules were generated using S+ SeqTrial (S+ SeqTrial 2 User's Manual, Insightful Corporation, Seattle, WA) using the alternative variance method.

17.3 Management of Toxicities

A DLT will be defined as a drug-related Grade IV toxicity or any non-neurologic toxicity ≥ Grade III of any duration. A Grade III neurologic toxicity will only be declared a DLT if not reversible within 48 hours. Only patients receiving at least 3 vaccines without toxicity will be considered as safe for MTD assessment. Patients receiving less than 3 vaccines without toxicity will be replaced for safety assessments. If toxicity is encountered before the 3rd vaccine, this will still be considered a DLT. Although not considered a DLT, any patient with ≥ Grade II urticaria will not receive further vaccines and will be withdrawn from the study. Any Grade IV toxicity

or any life-threatening event not attributable to a concomitant medication, co-morbid event, or disease progression, even if reversible, will be considered a DLT. If biopsy cannot be obtained, any new radiographic or clinical changes not attributable to tumor progression that produce a Grade IV or life-threatening toxicity will be considered at DLT. Life-threatening events, as described above, even if considered a Grade III toxicity, will still be considered a DLT even if reversible and in such cases no further vaccinations will be given.

If a Grade III NCI CTC toxicity is seen that is not attributable to a concomitant medication, co-morbid event, or disease progression that has been documented radiographically or clinically, the next immunization for that patient will be withheld for up to 2 months or until the NCI CTC toxicity improves to a Grade II or until the KPS score returns to within 10 points of baseline. Grade III neurologic toxicities will be declared irreversible if they cannot be reversed within 48 hours of their onset. If the event cannot be reversed within 48 hours of its onset, 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 NCI CTC 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. This study will be halted if a total of 2 patients experience a study-related DLT.

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, we will 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 (eg, thyroiditis, hepatitis, thrombocytopenia) the PI will send a blood sample for appropriate autoimmune antibody testing. If specific autoantibodies are present, the serum sample taken for storage at baseline will be tested for the presence of those autoantibodies. Further immunotherapy treatment in that patient will be held until the etiology of the event is established. Continuation of DC + xALT therapy in the presence of Grade III or greater immune-mediated events should be done by the PI only after discussion with the patient, medical monitor, and DSMB on a case-by-case basis with consideration to risk-benefit analysis. The IRB will be notified promptly and will 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 continued therapy will require the patient to be re-consented with the revised consent.

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 allergic reaction (transient flushing or rash, drug fever <38°C; intervention not indicated):

-Supervise at the bedside.

CTCAE v.4.0 Grade II allergic reaction (intervention or infusion interruption indicated; responds promptly to symptomatic treatment; prophylactic medications indicated for $\leq$ 24 hours):

-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 allergic reaction (prolonged; recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae; life-threatening consequences; urgent intervention indicated):

-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 study.

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

17.4 Adverse Event Reporting and Documentation

17.4.1 ADVERSE EVENTS

An "AE" will be defined as any adverse change from the subject's pre-treatment baseline condition, including any clinical or laboratory test abnormality that occurs during the course of research after treatment has started. AEs will be categorized and graded in accordance with the NCI CTC (Version 4.0).

17.4.2 SERIOUS ADVERSE EVENTS

A "SAE" will be defined as an undesirable sign, symptom or medical condition which results in any of the following outcomes: 1) fatal or life threatening; 2) inpatient hospitalization or a prolongation of existing hospitalization; 3) a persistent or significant disability/incapacity; 4) a congenital anomaly or a birth defect and/or; 5) medically significant event such that it may jeopardize the subject, and may require medical or surgical intervention to prevent one of the outcomes listed above.

SAEs which are serious should be reported immediately to the PI, Dr. Duane A. Mitchell, M.D., Ph.D. or his designee and to the local IRB per standard reporting procedures. Fatal or life-threatening, unexpected AEs where there is evidence to suggest a causal relationship between the drug and the adverse event will be reported by the sponsor to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the sponsor followed by as complete a report as possible within 8 additional calendar days. Serious, unexpected AEs that are related or possibly related to the drug but are not fatal or life-threatening will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the sponsor. Site Investigators will be notified in accordance with 21 CFR 312.32.

SAEs that are considered serious, unexpected, and related or possibly related to the research (as defined by 21CRF312.32[a]) will be discussed with the Medical Monitor. At the time of the annual progress report to the UF IRB, a summary of the overall toxicity experience will be provided.

17.4.3 ADVERSE EVENT DOCUMENTATION

This study will include two adverse event collection periods: non-mobilized leukapheresis and Consolidation or NMA Chemotherapy. The non-mobilized leukapheresis collection period will begin at initiation of leukapheresis

and continue until 24 hours after the completion of the procedure. The Consolidation or NMA Chemotherapy period will begin at initiation of chemotherapy and continue until 100 days after either the infusion of peripheral blood stem cells or completion of chemotherapy for subjects who did not receive PBSCs. All AEs thought to be related to vaccine therapy (any grade) and all grade 2 or greater AEs will be collected. After the 100 day reporting period, AEs that are stable and not related to vaccine therapy will not be followed until resolution. If the subject has progression of disease and /or begins another line of therapy or another therapeutic trial the adverse events collection will be discontinued. 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 record and case report form, if applicable.

A summary of recorded AEs (not just those considered related to the DC + xALT therapy) will be kept which will categorize the event by organ system, relationship to treatment, its grade of severity, and resolution. The PI will periodically review the collective AEs with the intention of identifying any trends or patterns in toxicity. If any such trends are identified, depending on their severity and frequency, a protocol amendment will be considered.

17.5 Concomitant Medication

The following medications will be recorded in the data management system:

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

Start date, stop date, and indication (reporting windows are those of adverse events) will be documented.

18. DESCRIPTION OF PROTOCOL DRUGS OR DEVICES

18.1 Preparation and Administration of Standard of Care Therapeutic Agents

18.2 Cyclophosphamide

Source and Pharmacology

Cyclophosphamide ((Cytoxan) NSC #26271) is an alkylating agent related to nitrogen mustard. Cyclophosphamide is inactive until it is metabolized by P-450 isoenzymes (CYP2B6, CYP2C9 and CYP3A4) in the liver to active compounds. The initial product is 4-hydroxycyclophosphamide (4-HC) which is in equilibrium with aldophosphamide which spontaneously releases acrolein to produce phosphoramide mustard. Phosphoramide mustard, which is an active bifunctional alkylating species, is 10 times more potent in vitro than is 4-HC and has been shown to produce interstrand DNA cross-link analogous to those produced by mechlorethamine. Approximately 70% of a dose of cyclophosphamide is excreted in the urine as the inactive carboxyphosphamide and 5-25% as unchanged drug. Cyclophosphamide is well absorbed orally with a bioavailability greater than 75%. The plasma half-life ranges from 4.1 to 16 hours after IV administration and 1.3 to 6.8 hours after oral administration.

Formulation and Stability

Cyclophosphamide for Injection is available as powder for injection or lyophilized powder for injection in 500 mg, 1 gm and 2 gm vials. The powder for injection contains 82 mg sodium bicarbonate/100 mg cyclophosphamide and the lyophilized powder for injection contains 75 mg mannitol/100 mg cyclophosphamide. Storage at or below 25ºC (77ºF) is recommended. The product will withstand brief exposures to temperatures up

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Anorexia, nausea & vomiting (acute and delayed)	abdominal discomfort, Diarrhea	Transient blurred vision, nasal stuffiness with rapid administration, arrhythmias (rapid infusion), skin rash, anaphylaxis, SIADH
Prompt: Within 2-3 weeks, prior to the next course	Leukopenia, alopecia, Immune suppression	Thrombocytopenia, Anemia, Hemorrhagic cystitis (L),	Cardiac toxicity with high dose (acute – CHF hemorrhagic myocarditis, myocardial necrosis) (L), hyperpigmentation, nail changes, impaired wound healing, infection secondary to immune suppression
Delayed: Any time later during therapy, excluding the above conditions	Gonadal dysfunction: azoospermia or oligospermia (prolonged or permanent)[1] (L)	amenorrhea[1]	gonadal dysfunction : ovarian failure[1] (L) Interstitial pneumonitis, pulmonary fibrosis[2](L),
Late: Any time after completion of treatment			Secondary malignancy (ALL, ANLL, AML), bladder carcinoma (long term use > 2 years), bladder fibrosis
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of cyclophosphamide (alone or in combination with other antineoplastic agents) have been noted in humans. Toxicities include: chromosomal abnormalities, multiple anomalies, pancytopenia, and low birth weight. Cyclophosphamide is excreted into breast milk. Cyclophosphamide is contraindicated during breast feeding because of reported cases of neutropenia in breast fed infants and the potential for serious adverse effects.		

[1] *Dependent on dose, age, gender and degree of pubertal development at time of treatment*

to 30º C (86ºF).

Supplier

Commercially available from various manufacturers. See package insert for more detailed information.

18.3 Mesna

Source and Pharmacology

[(sodium 2-mercaptoethane sulfonate, UCB 3983, Mesnex®) NSC #113891] was developed as a prophylactic agent to reduce the risk of hemorrhagic cystitis induced by ifosfamide. Mesna is rapidly oxidized to its major metabolite, mesna disulfide (dimesna). Mesna disulfide remains in the intravascular compartment and is rapidly eliminated by the kidneys. In the kidney, the mesna disulfide is reduced to the free thiol compound, mesna, which reacts chemically with the urotoxic ifosfamide metabolites (acrolein and 4-hydroxy-ifosfamide) resulting in their detoxification. The first step in the detoxification process is the binding of mesna to 4-hydroxy ifosfamide forming a nonurotoxic 4- sulfoethylthioifosfamide. Mesna also binds to the double bonds of acrolein and to other urotoxic metabolites. In multiple human xenograft or rodent tumor model studies, mesna in combination with ifosfamide (at dose ratios of up to 20-fold as single or multiple courses) failed to demonstrate interference with antitumor efficacy. After an 800 mg dose the half-life for Mesna and DiMesna are 0.36 hours and 1.17 hours, respectively. Approximately 32% and 33% of the administered dose was eliminated in the urine in 24 hours as mesna and dimesna, respectively. The majority of the dose recovered was eliminated within 4 hours. Mesna tablets have an oral bioavailability of 45-79% and a urinary bioavailability which ranged from 45-79% of intravenously administered mesna. The oral bioavailability is unaffected by food. When compared to intravenously administered mesna, the intravenous plus oral dosing regimen increases systemic exposures (150%) and provides more sustained excretion of mesna in the urine over a 24-hour period

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Bad taste with oral use	Nausea, vomiting, stomach pain, fatigue, headache	Facial flushing, fever, pain in arms, legs, and joints, rash, transient hypotension, tachycardia, dizziness, anxiety, confusion, periorbital swelling, anaphylaxis, coughing
Prompt: Within 2-3 weeks, prior to the next course		Diarrhea	
Unknown Frequency and Timing:	Fetal toxicities and teratogenic effects of mesna have not been noted in animals fed 10 times the recommended human doses. There are however no adequate and well-controlled studies in pregnant women. It is not known if mesna or dimesna is excreted into human milk.		

Formulation and Stability

Mesna for injection is available as 100mg/ml 10ml multidose vials which contain 0.25 mg/mL edetate disodium and sodium hydroxide for pH adjustment. Mesna Injection multidose vials also contain 10.4 mg/ml of benzyl alcohol as a preservative. Store product at controlled room temperature 15-25°C (68-77ºF). Mesna is not light-sensitive, but is oxidized to DiMesna when exposed to oxygen. Mesna as benzyl alcohol-preserved vials may be stored and used for 8 days.
Mesna non-preserved ampoules are no longer provided by Bristol-Myer Squibb Company.

Supplier

Commercially available from various manufacturers. See package insert for further information.

18.4 Etoposide

Source and Pharmacology

Etoposide ((VePesid®, Etopophos®,VP-16) NSC #141540 (112005)) is asemisynthetic derivative of podophyllotoxin that forms a complex with topoisomerase II and DNA which results in single and double strand DNA breaks. Its main effect appears to be in the S and G2 phase of the cell cycle. The initial t1/2 is 1.5 hours and the mean terminal half-life is 4 to 11 hours. It is primarily excreted in the urine. In children, approximately 55% of the dose is excreted in the urine as etoposide in 24 hours. The mean renal clearance of etoposide is 7 to 10 mL/min/m2 or about 35% of the total body clearance over a dose range of 80 to 600 mg/m2. Etoposide, therefore, is cleared by both renal and non renal processes, i.e., metabolism and biliary excretion. The effect of renal disease on plasma etoposide clearance is not known. Biliary excretion appears to be a minor route of etoposide elimination. Only 6% or less of an intravenous dose is recovered in the bile as etoposide. Metabolism accounts for most of the non renal clearance of etoposide. The maximum plasma concentration and area under the concentration time curve (AUC) exhibit a high degree of patient variability. Etoposide is highly bound to plasma proteins (~94%), primarily serum albumin. Pharmacodynamic studies have shown that etoposide systemic exposure is related to toxicity. Preliminary data suggests that systemic exposure for unbound etoposide correlates better than total (bound and unbound) etoposide. There is poor diffusion into the CSF < 5%. Cmax and AUC values for orally administered etoposide capsules consistently fall in the same range as the Cmax and AUC values for an intravenous dose of one-half the size of the oral dose. The overall mean value of oral capsule bioavailability is approximately 50% (range 25-75%). Etoposide phosphate is a water soluble ester of etoposide which is rapidly and completely converted to etoposide in plasma. Pharmacokinetic and pharmacodynamic data indicate that etoposide phosphate is bioequivalent to etoposide when it is administered in molar equivalent doses. Etoposide for Injection is available in sterile multiple dose vials. The pH of the clear, nearly colorless to yellow liquid is 3 to 4. Each mL contains 20 mg etoposide, 2 mg citric acid, 30mg benzyl alcohol, 80 mg modified polysorbate 80/tween 80, 650 mg polyethylene glycol 300, and 30.5 percent (v/v) alcohol. Vial headspace contains nitrogen. Unopened vials of Etoposide are stable until expiration date on package at room temperature (25°C). Etoposide phosphate for injection is available for intravenous infusion as a sterile lyophilized powder in single-dose vials containing etoposide phosphate equivalent to 100 mg etoposide,
32.7 mg sodium citrate USP, and 300 mg dextran 40. Etoposide phosphate must be stored under refrigeration 2°-8°C (36°- 46°F). Unopened vials of etoposide phosphate are stable until the expiration date on the package.
3.6.2 **Supplier**

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting	Anorexia	Transient hypotension during infusion; anaphylaxis (chills, fever, tachycardia, dyspnea, bronchospasm, hypotension)
Prompt: Within 2-3 weeks, prior to next course	Myelosuppression (anemia, leukopenia), alopecia	thrombocytopenia, diarrhea, abdominal pain, asthenia, malaise, rashes and urticaria	Peripheral neuropathy, mucositis, hepatotoxicity, chest pain, thrombophlebitis, congestive heart failure, Stevens-Johnson Syndrome, exfoliative dermatitis
Delayed: Any time later during therapy			Dystonia, ovarian failure, amenorrhea, anovulatory cycles, hypomenorrhea, onycholysis of nails
Late: Any time after completion of treatment			Secondary malignancy (preleukemic or leukemic syndromes)
Unknown Frequency and Timing: Fetal toxicities and teratogenic effects of etoposide have been noted in animals at 1/20th of the human dose. It is unknown whether the drug is excreted in breast milk.			

18.5 Temozolomide (Temodar®)

Chemical Name

3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-*as*-tetrazine-8-carboxamide1

TMZ is commercially available from Schering-Plough Research Institute under the trade name Temodar®.

TMZ undergoes rapid nonenzymatic conversion at physiologic pH to the reactive compound, Maximally Tolerated Inhibitory Concentration (MTIC). The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs primarily at the O6 and N7 positions of guanine. TMZ is available in capsules that are white in color with colored imprints indicating the different doses: 5 mg (green imprint), 20 mg (brown imprint), 100 mg (blue imprint) and 250 mg (black imprint). Capsules should be stored at 25ºC (77ºF); excursions permitted to 15-30ºC (59-86ºF). The commercial product has an expiration date on the bottle. TMZ will be administered orally, 30 minutes following the completion of each O6-Benzylguanine infusion. If a patient has emesis within 10 minutes of receiving a dose, the dose should be repeated as soon as the patient is able to tolerate re-dosing. If a patient cannot tolerate re-dosing within 2 hours of completion of the O6-Benzylguanine infusion, or has repeated emesis, no further doses of TMZ will be given on that day. For patients unable to swallow intact capsules, capsules may be opened and mixed with apple juice or applesauce, or administered via Gastronomy Tube (G-Tube) or Nasogastric Tube (NG-tube.) If mixing capsule contents with apple juice or apple sauce is necessary, mix the contents immediately prior to administration. Care must be taken to avoid inhaling the powder or having the powder come in contact with skin or mucous membranes. If opening of capsules is necessary, it should be performed by hospital staff trained in handling chemotherapeutics. Chemotherapy precautions should be utilized in preparation. Gloves should be worn and all mixing media must be discarded as chemotherapy waste. TMZ is available from commercial sources by the treating hospital pharmacy.

Known AEs of Temozolomide

Allergy/Immunology: sinusitis
Blood/Bone Marrow: decreased WBC, lymphopenia, neutropenia, thrombocytopenia, anemia
Cardiovascular: peripheral edema
Constitutional Symptoms: fatigue, asthenia, fever, weight gain
Dermatology/Skin: rash, pruritis
Endocrine: adrenal hypercorticism
Gastrointestinal: nausea, vomiting, constipation, diarrhea, anorexia
Infection: viral infection, urinary tract infection
Musculoskeletal/Soft Tissue: myalgia
Neurology: convulsions, hemiparesis, dizziness, abnormal coordination, amnesia, insomnia, paresthesia, somnolence, paresis, ataxia, dysphasia, abnormal gait, confusion, anxiety, depression
Ocular/Visual: diplopia, blurred vision, visual deficit or changes
Pain: headache, back pain, abdominal pain, breast pain (female)
Pulmonary/Upper Respiratory: upper respiratory tract infection, pharyngitis, coughing
Renal/Genitourinary: urinary incontinence, micturition increased frequency

18.6 Carboplatin

Source and Pharmacology

The mechanism of action of carboplatin (Paraplatin®) NSC #241240 (092006) would appear to be similar to that of cisplatin. It binds to replicating DNA causing single strand breaks and interstrand cross-links with DNA. Data suggests that other factors also contribute to cytotoxicity. The α t1/2 is 1.1 to 2 hours and the β t1/2 is 2.6 to 5.9 hours. Carboplatin is not protein bound. The major route of elimination of carboplatin is renal excretion. Patients with creatinine clearances of approximately 60 mL/min or greater excrete 65% of the dose in the urine within 12 hours and 71% of the dose within 24 hours. In patients with creatinine clearances below 60 mL/min the total body and renal clearances of carboplatin decrease as the creatinine clearance decreases. Carboplatin dosages will require adjustment dependent on the glomerular filtration rate.

Formulation and Stability

Carboplatin is available in 50 mg, 150 mg, 450 mg and 600 mg vials.

Aqueous Solution: Carboplatin aqueous solution is supplied as a sterile, pyrogenfree, 10mg/mL aqueous solution of carboplatin in multidose vials. Unopened vials of carboplatin aqueous solution are stable to the date indicated on the package when stored at 25° C (77° F); excursions permitted from 15°-30° C (59°-86° F). Protect from light. Carboplatin aqueous solution multidose vials maintain microbial, chemical, and physical stability for up to 14 days at 25º C following multiple needle entries.

Powder for Injection: Carboplatin powder for injection is a sterile lyophilized white powder in single dose vials containing equal parts by weight of carboplatin and mannitol. Unopened vials of carboplatin are stable to the date indicated on the package when stored at 15°-30° C (59°-86° F). Protect from light.

Supplier

Commercially available from various manufacturers. See package insert for more detailed information.

Toxicities

	Common Happens to 21-100 out of every 100 children	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting	Hypersensitivity reactions[2] (anaphylaxis, bronchospasm, hypotension), constipation, diarrhea	Metallic taste, rash, mucositis
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression[1] (anemia, neutropenia, leucopenia, thrombocytopenia), Electrolyte abnormalities (↓ Na, K, Ca, Mg)	↑ LFT's (Alk Phos, AST), abdominal pain, nephrotoxicity (↓ GFR, ↑ Cr and BUN), asthenia	↑ bilirubin
Delayed: Any time later during therapy		ototoxicity (tinnitus, hearing loss)	Peripheral neuropathy with mild paresthesias, diminished sense of vibration, light touch, pinprick, and joint position, alopecia; temporary loss of vision to light and colors
Late: Any time after completion of treatment			Secondary leukemia
Unknown Frequency and Timing	Fetal toxicities and teratogenic effects of carboplatin have been noted in animals and may cause fetal harm when administered to pregnant women. It is unknown whether the drug is excreted in breast milk.		

[1] *Thrombocytopenia is more severe or dose limiting.*

[2] *Hypersensitivity reactions are seen more frequently with repeated courses of therapy (after six courses in adults).*

(L) Toxicity may also occur later.

18.7 Thiotepa

Source and Pharmacology

Thiotepa (Tespa, Thiophosphamide, Triethylenethiophosphoramide Tspa, WR- 45312) NSC #6396 (032006) is a cytotoxic agent of the polyfunctional type, related chemically and pharmacologically to nitrogen mustard. The radiomimetic action of thiotepa is believed to occur through the release of ethylenimine radicals which, like irradiation, disrupt the bonds of DNA. One of the principal bond disruptions is initiated by alkylation of guanine at the N-7 position, which severs the linkage between the purine base and the sugar and liberates alkylated guanines. Thiotepa is desulfurated by cytochrome P-450 enzymes such as 2B1 and 2C11 which catalyze the conversion of thiotepa to tepa. Tepa is less toxic than thiotepa and has been demonstrated to produce alkali-labile sites in DNA, rather than cross-links. These findings indicate that tepa reacts differently from thiotepa and produces monofunctional alkylation of DNA. A second metabolite of thiotepa, a mercapturic acid conjugate, is formed via glutathione conjugation. Monochloro tepa is the third metabolite found in the urine.

Following short intravenous infusion (less than 5 minutes), peak concentrations of thiotepa were measured within 5 minutes. At steady state, the volume of distribution was independent of dose and ranged from 0.3 to 1.6 liters per kilogram (l/kg). Approximately 4.2% of the original dose is eliminated in the urine within 24 hours as tepa. The elimination half-life of thiotepa ranges from 2.3 to 2.4 hours. The half-life of tepa ranged from 3.0 to 21.1 hours in one study.

Formulation and Stability

Thiotepa for Injection USP, for single use only, is available in vials containing 15 mg of nonpyrogenic, sterile lyophilized powder. Store in a refrigerator at 2° to 8°C (36° to 46°F).**PROTECT FROM LIGHT AT ALL TIMES**.

Supplier

Commercially available from various manufacturers. See package insert for further information.

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, anorexia, fatigue, Weakness	Pain at the injection site, Dizziness, headache, blurred vision, abdominal pain, Contact dermatitis, rash	Anaphylaxis, laryngeal edema, wheezing, hives
Prompt: Within 2-3 weeks, prior to next course	Myelosuppression; At higher doses in conditioning regimens for BMT: mucositis, esophagitis	At higher doses in conditioning regimens for BMT: inappropriate behavior, confusion, somnolence, increased liver transaminases, increased bilirubin, hyperpigmentation of the skin (bronzing effect)	Febrile reaction, Conjunctivitis, dysuria, urinary retention
Delayed: Anytime later during therapy, excluding the above conditions	gonadal dysfunction/infertility, azoospermia, amenorrhea		Alopecia, Secondary Malignancy
Unknown Frequency and Timing:	Fetal and teratogenic toxicities: Carcinogenic and teratogenic effects of thiotepa have been noted in animal models at doses ≤ to those used in humans. It is not known if thiotepa is excreted into human breast milk.		

18.8 Fludarabine phosphate

Source and Pharmacology

9-b-Barabinofuanosyl-2-fluoroadenine.

Fludarabine (FAMP, Fludara®) is a synthetic purine nucleoside. While the exact mechanism of action of fludarabine has not been fully elucidated, fludarabine appears to inhibit alpha-DNA polymerase, ribonucleotide reductase, and DNA primase by competing with the physiologic substrate, deoxyadenosine triphosphate, resulting in inhibition of DNA synthesis. Additionally, the drug may inhibit DNA chain elongation and interfere with RNA and protein synthesis by decreasing the incorporation of uridine and leucine.

Pharmacokinetics - After administration, fludarabine phosphate is rapidly dephosphorylated to fludarabine triphosphate, the cytotoxic form of the drug. Areas under the concentration-time curve for fludarabine appear to be dose related. The elimination profile of fludarabine has been reported as either biphasic or triphasic, with terminal elimination half-lives for fludarabine and fludarabine triphosphate of 8-10 and 15 hours, respectively. Fludarabine appears to be eliminated through the kidneys.

Formulation and Stability

Fludarabine is supplied in a 50mg powder form, and reconstituted with 2mL of sterile water. The appropriate dose of drug is then withdrawn and added to 100 or 125mL of D5W or 0.9% sodium chloride solution. Fludarabine contains no preservatives and should be used within 8 hours after reconstitution.

Supplier

Commercially available from various manufacturers. See package insert for further information.

Dosage Modification Rules

Patients with renal impairment dosing:

CrCl > 70 ml/min: No dosage adjustment required.

CrCl 30-70 ml/min: Reduce dose by 20%.

CrCl < 30 ml/min: Fludarabine should not be administered intravenously to these patients.

Toxicity

Toxicity from fludarabine includes the following:

(a) hematologic-myelosuppression with anemia, neutropenia and thrombocytopenia;

(b) nervous system-weakness, pain, malaise, paresthesias, visual and hearing disturbances, encephalopathy;

(c) gastrointestinal-nausea, vomiting, GI bleeding, mucositis, and anorexia;

(d) integumentary-rash, and pruritis;

(e) other-interstitial pneumonitis, cough, fever, myalgia, tumor lysis, and elevated LFTs.

Fludarabine

Likely *("Likely" refers to a side effect that is expected to occur in more than 20% of subjects.)*	**Less Likely** *("Less likely" refers to a side effect that is expected to occur in 20% or fewer subjects.)*	**Rare, but Serious** *(These possible risks have been reported in rare occurrences, typically less than 2% of subjects. They may be serious if they occur.)*
• Decreased white blood cell count with increased risk of infection • Decreased platelet count with increased risk of bleeding • Tiredness • Nausea • Vomiting	• Pneumonia • Diarrhea	• Numbness and tingling in hands and/or feet related to irritation of nerves of the hand and/or feet • Changes in vision • Agitation/nervousness • Confusion • Cough • Difficulty breathing

		• Weakness • Severe brain injury and death

19. PREPARATION, ADMINISTRATION, AND TRANSPORT OF EXPERIMENTAL THERAPEUTIC AGENTS (DC + XALT THERAPY)

19.1 External Sites

Study personnel at external sites will receive training on the transport of tumor tissue and leukapheresis product as well as the preparation and administration of experimental therapeutic agents. Documentation of training will be maintained at the University of Florida.

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** and **SOP-UFBTIP-127**. Samples will be labeled with, at a minimum, the subject's name and study ID code. 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.

TRNA 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 and SOP-UFBTIP-125**. Products will be labeled with, at a minimum, the subject's name and study ID code.

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.

19.2 TTRNA isolation

Surgically resected reMB/PNET specimens are collected aseptically and transferred from the operating room to the research laboratory in accordance with **SOP-UFBTIP-116**. All specimens obtained at outside hospital facilities will be transferred aseptically under FDA guidelines (BB IND 13630 (Sampson), BB IND 14058 (Mitchell)) in accordance with **SOP-UFBTIP-120** or **SOP-UFBTIP-130**. The tumor is washed of excess blood and minced using sterile scissors. Minced tumor fragments are then snap frozen and stored in liquid nitrogen until RNA extraction. Because tissue thawing in the absence of ribonuclease inhibitors leads to RNA degradation, frozen tumor tissue is homogenized immediately prior to RNA extraction. Total cellular RNA is then extracted using the RNeasy kit (Qiagen, Valencia, CA) following the manufacturer's specifications. Extracted RNA is quantified with a NanoDrop at 260 nm and subsequently used in amplification reactions in accordance with **SOP-UFBTIP-102**.

19.3 Amplification of RNA

For larger samples the total RNA is isolated using RNeasy (Qiagen Cat# 74104). For small tumor samples SuperScript III CellDirect cDNA Synthesis System (Invitrogen) is used to isolate total RNA from the tumor cells. Invitrogen's CellDirect Resuspension/Lysis Enhancer Buffers (Invitrogen, Cat# 11739-010) are mixed 10:1 and added directly to the autologous tumor cells. The extracted RNA mixture is heated for 10 minutes at 75^{O}C. An aliquot of the CellDirect lysate or extracted RNA is used for 1st-strand cDNA synthesis with using the primers 5'-AAG CAG TGG TAT CAA CGC AGA GTA CTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTV N-3'; (CDS Primer IIIA), and 5'-AAG CAG TGG TAT CAA CGC AGA GT/G GCC ATA TTG GCC rGrGrG/3AmMC6-3'(SMART III) at 70^{O}C for 3 minutes, 42^{O}C for 20

minutes and 70^{O}C for 15 minutes. The 1st-strand cDNA libraries were amplified with ClonTech's Advantage 2 Polymerase PCR kit (ClonTech Cat# 639201) or ClonTech's polymerase mix (ClonTech Cat# 639201) by primers (CDS Primer IIIA; and T7 –Smart: 5'-CCA TCC TAA TAC GAC TCA CTA TAG GTA TCA ACG CAG AGT GGC CAT ATT G-3') at 95^{O}C for 2 minutes, 30-50 cycles of 95^{O}C for 25 seconds, 65^{O}C for 45 seconds, 68^{O}C for 6 minutes and 68^{O}C for 10 minutes. The integrity of the amplification is evaluated by Real-Time PCR on a 7900HT Fast Real-Time PCR System (Applied Biosystems) or equivalent using gene expression levels of human housekeeping genes (ACTB, EGFR, GAPDH or HPRT). PCR amplification conditions may be modified to optimize amplifications as assessed by housekeeping gene detection. The detection of at least one housekeeping gene and a cDNA smear extended to 1Kb or greater is required to declare the template suitable for RNA synthesis. The amplified cDNA PCR products are purified with QIAquick PCR Purification kit (QIAGEN Inc., Valencia, CA, Cat# 28104). *In vitro* mRNA transcription was done using the same products and procedures as those previously approved for INDs 12,839, 13,240 and 13,630. The amplified mRNA is synthesized using T7 mMessage mMachine™ Transcription Kit (Ambion Inc., Austin, TX, Cat# 1344) from PCR amplified TTRNA cDNA library templates, and cleaned with RNeasy™ kit (QIAGEN, Cat# 74104) and quantified with a NanoDrop at 260 nm. The integrity of the amplified mRNA is verified as defined in the RNA Release Criteria. The RNA amplification process will be conducted in accordance with **SOP-UFBTIP-103 through SOP-UFBTIP-115** and released in accordance with **SOP-UFBTIP-119** as outlined below.

RNA Release Criteria	
Nucleic acid/protein (260/280)	≥1.8
Size	RNA product range > 1kB by agarose gel electrophoresis.
Sterile	Negative aerobic and anaerobic bacterial cultures and fungal cultures by Clongen.

19.4 Procedure for TTRNA-DC and TTRNA-xALT Generation

Blood will be obtained by leukapheresis and transported to the UF cGMP facility in accordance with **SOP-UFBTIP-121** for cells collected at the University of Florida or **SOP-UFBTIP-127** for cells collected at external sites. PBMCs will be isolated in accordance with **BR-UFBTIP-001** and plated into flasks for *in vitro* generation of DCs. Non-adherent cells will be frozen in accordance with **BR-UFBTIP-004** and subsequently used for production of antigen-specific T cells. Immature DCs will be generated from the PBMCs *in vitro* by 7-day culture with GM-CSF and IL-4. At the end of the 7 day incubation, the immature DCs will be harvested and electroporated with TTRNA. The electroporated immature DCs are placed in a flask with AIM V media with HABS containing GM-CSF, IL-4, TNF-α + IL-6 + IL-1β at 37ºC, 5% CO_2 for 18-24 hours for maturation. The cells are washed with PBS and frozen in 80% human AB serum, 10% Cryoserv, and 10% Dextrose in accordance with **BR-UFBTIP-002**. A portion of the DCs are frozen and stored in liquid nitrogen until needed for DC vaccination. Another portion of the mature DCs is used for an *in vitro* 21 day stimulation of antigen-specific T cells in accordance with **BR-UFBTIP-003**. After freezing and prior to release, an aliquot of cryopreserved cells will be thawed, prepared, and sent to Clongen for QA/QC release testing in accordance with FDA IND BB-14058 and **SOP-UFBTIP-122 and SOP-UFBTIP-123**. Testing will include sterility (aerobic and anaerobic bacterial cultures, fungal cultures), endotoxin, and mycoplasma. A separate aliquot will be prepared for viability and phenotypic analysis for release criteria in accordance with **SOP-UFBTIP-100**.

For each vaccination, DCs will be rapidly thawed at 37ºC, washed with PBS and counted. The DC concentration will be adjusted to 1 x 10^7 cells per 400 µL of preservative free saline and placed into a sterile tuberculin syringe with a 25 gauge needle in accordance with **SOP-UFBTIP-124**. The final thawed and

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

Autologous ex vivo expanded TTRNA-specific lymphocytes (xALT)

Non-adherent cells will be obtained from the initial leukapheresis and frozen in accordance with **BR-UFBTIP-004** and will consist of the cells remaining after the plastic adherence step described above for DC generation in accordance with **BR-UFBTIP-002**. These non-adherent cells are stimulated *in vitro* for 21 days with mature DCs. $2x10^7$ non-adherent cells are placed into T-25 flasks along with $2x10^6$ matured TTRNA loaded DCs, in 10 mLs AIM V containing 5% HABS and 3,000IU/mL IL-2 and incubated at 37^OC for 5 days. After 5 days, the cells will be treated with 100IU/mL IL-2 every 3 days if needed based on observation of cell clusters and PH measurement of media. The cells are expanded into progressively larger flasks as needed. At the end of 21 days, the TTRNA stimulated T cells are harvested, washed in PBS, counted and viably frozen in 90% HABS + 10% Cryoserv in accordance with **BR-UFBTIP-003**. After freezing and prior to release, an aliquot of cells is thawed and sent for QA/QC release testing according to FDA IND BB-14058 and **SOP-UFBTIP-123**. This includes aerobic and anaerobic bacterial cultures ($1x10^6$ TTRNA-xALT), fungal cultures ($1x10^6$ TTRNA-xALT), and endotoxin testing. Viability (≥70%) and phenotypic analysis will also be conducted for release criteria in accordance with **SOP-UFBTIP-100**.

In preparation for TTRNA-xALT, cells are rapidly thawed at 37ºC, washed with saline + human albumin, and counted. The cell concentration will be adjusted to 3×10^7 cells/kg (or all cells available if less than target dose) in preservative free saline with 1% human serum albumin and placed into a syringe in accordance with **SOP-UFBTIP-125**. The final thawed and formulated TTRNA-xALT will be stored at 2-8°C for up to 6 hours. After this time the material will be disposed of if not given to the patient.

20. LABORATORY EVALUATIONS

20.1 Baseline and Eligibility

To be enrolled, patients must be scheduled for definitive resection, biopsy, and/or cytology collection of reMB/PNET or, in select cases, be at an outside institution and have had surgical resection and tumor tissue snap frozen and available for TTRNA. Clinical and laboratory evaluations will be obtained within 2 weeks of enrollment for the purposes of meeting Inclusion and Exclusion Criteria as outlined above. Final pathologic diagnosis of reMB/PNET will be made by the study pathologist, or by certified outside pathology report.

Pathology slides consisting of 1 hematoxylin and eosin (H&E[44]) and 19 unstained slides for immunohistochemistry prepared on Fischer Plus glass or Histostix coated slides when available will be delivered to:

Duane Mitchell, M.D., Ph.D.
Department of Neurosurgery
1149 Newell Drive, Room L1-151
University of Florida
Gainesville, Florida 32611

Blood will also be sent for the following tests: CBC (Complete Blood Count) with manual differential. Initial clinical evaluations will also include a complete physical and neurologic examination.

A baseline brain and spine MRI and study eligibility MRI (with and without gadolinium enhancement taken post-surgical resection of recurrent disease) of the brain using standard 5 mm slices with 2.5 mm spacing for comparison to subsequent MRI images will also be obtained.

20.2 Data and Specimens to be Accessioned

Before undergoing surgical resection

- History and physical examination
- CBC, PT, PTT, Comprehensive metabolic panel & Type and Screen if clinically indicated
- Material in excess of that required for pathologic diagnosis will be transported to Dr. Mitchell's laboratory for RNA isolation in accordance with **SOP-UFBTIP-116** and autologous tumor cell targets for immunologic monitoring.

Prior to leukapheresis

- Tests as required by the Apheresis Center (total estimated blood volume required for these evaluations is 10-20 mLs based on patient weight per institutional guidelines)
  - β-HCG if female and greater than 12 years of age
  - Infectious disease testing to include, at a minimum, HIV, Hepatitis B, Hepatitis C and CMV
  - CBC with differential, Complete or Basic Metabolic panel (per institutional standard), T4/T8 ratio
  - To prevent the development of hypocalcemia from the citrate used for leukapheresis, all patients over age 3 will be given oral Tums, 2 tablets, b.i.d for 2 days prior to the leukapheresis procedure if standard of care. Patients who have lowered levels of Ca will be treated per Apheresis lab standard.

Prior to DC + xALT Therapy

- At the time of first vaccination, all patients will receive a standard 0.5 mlTd (2 Lf tetanus and 2 Lf diphtheria toxoids) booster intramuscularly. The rationale for this vaccination is to enhance DC trafficking to vaccine-site draining lymph nodes (VDLNs).
- Additionally, 24 hours prior to first immunization, blood will be drawn for the following purposes:
  - CBC with differential, T4/T8 ratio, and research blood. Total estimated blood volume required for these evaluations is 12 mL.

During and After Immunizations

- Clinical evaluations with each vaccine will include a general physical examination, complete neurologic examination, and KPS or LPS rating.
- Radiographic evaluations consisting of a contrast enhanced brain CT or MRI scan will be obtained every 8 $\pm$ 4 weeks.

Blood will be obtained for T4/T8 ratio, CBC with manual differential and for Immunologic Monitoring prior to non-mobilized leukapheresis, prior to immunotherapy, 1 day post Vaccine #1, 4 (+/- 1) days post Vaccine #1, weekly post Vaccine #1 for six weeks, and bimonthly, if feasible, until progression. Weekly blood draws should be drawn ***prior*** to subsequent vaccination. Blood will be processed in accordance with **SOP-UFBTIP-126**. Total estimated volume of blood required over the six week period for investigational purposes will be 80 mLs.

STUDY ROAD MAP:

Timepoint	Date	Study Requirements
Baseline		Inclusion & exclusion criteria
		Tissue consent
		Medical history, physical & neurological exam
		MRI brain & spine
		Performance Status: KPS or LPS
		CBC w/ differential, PT, PTT, comprehensive metabolic panel
		β-HCG pregnancy test
		Type & Screen if clinically indicated
Surgery		Resection, biopsy or cytology collection
		Tumor tissue for extraction of total tumor RNA
		Pathology slides for research: 1 H&E & 19 unstained slides
		MRI brain with & without gadolinium post-surgery
Non-Mobilized Leukapheresis		Treatment consent
		CBC w/ differential, comprehensive or basic metabolic panel (per institutional standard), T4/T8 ratio, research labs**
		β-HCG pregnancy test
		Infectious disease testing to include, at a minimum, HIV, Hepatitis B, Hepatitis C & CMV
		Oral tums if standard of care
Induction or Salvage Chemo Cycle 1 (28 days)		**Group A:** Cyclophosphamide, Mesna, GCSF **Group B:** Etoposide, Temozolomide, GCSF
Cycle 2 (28 days)		**Group A:** Cyclophosphamide, Mesna, GCSF **Group B:** Etoposide, Temozolomide, GCSF
Cycle 3 (28 days)		**Group A:** Etoposide, Temozolomide **Group B:** Etoposide , Temozolomide
Cycle 4 (28 days)		**Group A:** Etoposide ,Temozolomide **Group B:** Etoposide, Temozolomide
Mobilized Leukapheresis		CBC w/ differential, comprehensive or basic metabolic panel (per institutional standard), T4/T8 ratio
		β-HCG pregnancy test
		Infectious disease testing to include, at a minimum, HIV, Hepatitis B, Hepatitis C & CMV
		Oral tums if standard of care

Timepoint	Date	Study Requirements
Pre Consolidation or NMA Chemo		Medical history, physical exam
		MRI brain (spine if indicated)
		CBC w/ differential, comprehensive metabolic panel, GFR
		β-HCG pregnancy test
		ECHO or MUGA
		EKG (Group A only)
		DLCO, FEV1 & FVC or Crying Vital Capacity (Group A only)
		CXR & Pulse Oximetry (Group B & Group A subjects who cannot complete PFTs)
Consolidation or NMA Chemo		**Group A:** Carboplatin, Etoposide, Thiotepa, PBSCs , G-CSF **Group B:** Cyclophosphamide, Fludarabine , PBSCs, G-CSF
Prior to Immunotherapy (4 to 24 hours prior)		Td booster
		CBC w/ diff, T4/T8 ratio, research labs**
Immunotherapy Vaccine #1 **Group A:** Day +1 **Group B:** 96 hours post Fludarabine		Physical & neurological exam
		Performance Status: KPS or LPS
		Tylenol, Benadryl, Intravenous TTRNA-xALT
		EMLA, Intradermal TTRNA-DC
		1 day post: CBC w/ diff, T4/T8 ratio, research labs**
		4 (+/-1) days post: CBC w/ diff, T4/T8 ratio, research labs**
		Week 1 post: CBC w/ diff, T4/T8 ratio, research labs**
		Week 2 post: CBC w/ diff, T4/T8 ratio, research labs**
		Week 3 post: CBC w/ diff, T4/T8 ratio, research labs**
		Week 4 post: CBC w/ diff, T4/T8 ratio, research labs**
		Week 5 post: CBC w/ diff, T4/T8 ratio, research labs**
		Week 6 post: CBC w/ diff, T4/T8 ratio, research labs**
Vaccine #2 14 days post Vaccine #1		Physical & neurological exam
		Performance Status: KPS or LPS
		EMLA, Intradermal TTRNA-DC
Vaccine #3 14 days post Vaccine #2		Physical & neurological exam
		Performance Status: KPS or LPS
		EMLA, Intradermal TTRNA-DC
Bi-Monthly follow-up		Physical & neurological exam
		Contrast-enhanced Brain CT or MRI scan
		CBC w/ diff, T4/T8 ratio, research labs, if feasible**

****2 yellow (ACD-A) & 1 red (non-SST)

20.3 Laboratory and Clinical Evaluation Facilities

UF Health Clinical Laboratories

UF Health Clinical Laboratories, comply with federal regulations defined in the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) and the national accreditation standards defined by the College of American Pathologists (CAP).

UF Health Clinical Laboratories are accredited by the College of American Pathologist's Laboratory Accreditation Program.

UF Health Department of Radiology

The Department of Radiology at UF Health Shands Hospital consists of a large imaging facility for both inpatients and outpatients. The Neuroradiology Department will review all the radiological procedures in this proposal and be available to discuss the interpretation of any MRI findings that may be confusing in the setting of combinatorial therapy.

UF Health Brain Tumor Biorepository

This Biorepository or "tissue bank" has successfully obtained and stored brain tumor tissue and matched peripheral somatic cells from individual brain tumor patients. There are also peripheral somatic cells stored from non-brain tumor disease control patients and normal subjects.

Personnel from the Brain Tumor Biorepository will sterilely divide material from the operating room and preserve according to IRB approved biorepository protocol and SOPs. All patient information is entered into database at which time the program will generate a unique number for the specimen.

Outside Laboratories

Accredited outside laboratories used in patient assessment will be registered using Form FDA 1572 (21 CRF 312.53(c)).

21. DATA ANALYSIS

Samples collected from subjects during the phase 1 trial (FDA IND BB 14058; Duke IRB Pro00018020; PI: Duane A. Mitchell) will be analyzed as described below. Patients treated at the phase 2 established dose of T cells ($3x10^7$ cells/Kg) may be included in the statistical analysis summary. Patients treated below the targeted dose will be included in a descriptive analysis only.

21.1 Progression Free Survival (PFS) and Overall Survival (OS)

The study statistician will be responsible for all statistical analysis. Estimating the potential efficacy of adoptive transfer with TTRNA-xALT plus TTRNA-DCs will be the primary goal of this Phase I/II evaluation.

Patients enrolled on this study who are confirmed to have a reMB/PNET will be followed for all parameters until death. The Kaplan-Meier estimator will be used to describe the progression-free survival and OS experience of vaccinated patients from the initiation of HDC as this is within 24hrs of the frame of reference for our historical control

comparison (PBSCT defined as Day 0 in historical control cohort). We will define the PFS and OS from the time of proximal tumor recurrence as a clinical reference point for further comparison to other treatment regimens within the published literature. We do not anticipate any appreciable data lost during this study due to premature patient drop out or lost to follow up.

21.2 Radiographic Response and Progression Criteria for Patients with Residual Disease

All radiographic responses will be based on MRI scans of the brain and spine 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) scan to document response. **However, 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.** 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)). For residual disease present at the time of the last MRI prior to initiating vaccination, radiographic response will be evaluated based on the NCI-endorsed, World Health Organization RECIST criteria and using a modified version of the MacDonald criteria[7,8] which is outlined below.

Complete Response

Complete disappearance of enhancing tumor on consecutive CT or MRI images at least 8 weeks apart, and neurologically stable or improved and at a stable or a lower steroid dose. If CSF cytology was previously positive, it should now be negative. To be assigned a status of "Complete Response" as a best overall response, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 8 weeks after the criteria for response is first met.

Partial Response

At least a 50% reduction in tumor size on consecutive MRI images at least 8 weeks apart, and neurologically stable or improved and at the same or a lower steroid dose. If CSF cytology was previously positive, it should now be negative. To be assigned a status of "Partial Response" as a best overall response, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 8 weeks after the criteria for response is first met.

Stable Disease

Insufficient change to qualify for Partial Response or Progressive disease. CSF cytology remains positive or becomes negative.

Progressive Disease

At least a 25% increase in the longest diameter on an axial image of any enhancing contiguous tumor on consecutive CT or MRI images. CSF cytology may be positive or negative.

Not Assessable

Progression has not been documented and one or more sites have not been assessed.

21.3 Radiographic Response and Progression Criteria for Patients with Complete Resection

A complete resection will be defined as contiguous contrast enhancement < 1 cm in 2 perpendicular measurements in each of 2 perpendicular planes (i.e., axial and coronal). Because neither the RECIST [8] nor MacDonald [7] criteria are completely appropriate for patients with minimal residual disease, a modified version of these criteria will be used as outlined below.

Complete Response

Complete disappearance of enhancing tumor on consecutive CT or MRI images at least 1 month apart, and neurologically stable or improved and at the same or a lower steroid dose. To be assigned a status of "Complete Response" as a best overall response, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response is first met.

Partial Response

At least a 30% reduction in longest diameter on an axial image of any contiguous enhancing tumor on consecutive CT or MRI images at least 1 month apart, and neurologically stable or improved and at the same or a lower steroid dose. To be assigned a status of "Partial Response" as a best overall response, changes in tumor

measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response is first met.

Stable Disease

Insufficient change to qualify for Partial Response or Progressive disease.

Progressive Disease

At least a 20% increase in the longest diameter on an axial image of any enhancing tumor on consecutive CT or MRI images with a minimum of ≥ 5 mm of contiguous contrast enhancement in 2 perpendicular measurements in each of 2 perpendicular planes (i.e., axial and coronal).

Not Assessable

Progression has not been documented and one or more sites have not been assessed.

21.4 Special Circumstances

- **If a scan is suspicious but not definitive for progressive disease (including indeterminate lesion size or enhancement that is not clearly disease progression) in the absence of clinical progression, an investigator may elect to confirm progression through alternate means, including biopsy, or repeat MRI at 4-8 weeks, symptomatic follow up or other modalities.**
  - Protocol treatment will continue until the investigator considers progression documented.
- **Disease that was measurable at baseline and appeared to be one solid mass splits to become 2 or more smaller lesions**
  - note that the lesion has split, to identify the separate lesions as unique and to record the measurements of each.
  - appearance of these individual split lesions will not automate a response assignment of PD; they will contribute to a determination of progression only if the sum of all lesions meets the criteria for PD.
- **2 or more lesions which were distinctly separate at baseline become confluent at subsequent visits**
  - note the occurrence and capture the required measurement(s) of the confluent mass.
- **A required region of a baseline scan may be unavailable or technically inadequate, and 1 or more lesions are detected on a subsequent scans.**
  - Such lesions are identified as additional evaluable lesions that do not contribute to determination of that visit's response.
  - If at a successive time point disease progression is noted, the overall response per time point will be PD.
  - For those areas inadequate at baseline, the first scan available will become the reference scan by which later scans are compared for evaluable progression.
- **1 or more lesions are detected in regions unavailable or inadequate at baseline that were also not required**
  - regarded as new lesions, resulting in an overall response of PD for that time point.
- **Regions are not available or adequate at post-baseline visit assessments that showed disease at baseline**
  - an overall response of NA will be assigned to that visit unless other available data shows progression.

Overall Response	Measurable Lesions	Evaluable Lesions	New Lesions	Overall neurological status	Steroid Use	Additional notes
PD	PD	PD	Yes	Unequivocal worsening	Stable or increased use	PD by any single component (not steroids by criteria) no sooner than 1 month after the first course of treatment
SD	***All other combinations, but please note the following particular combinations:***					
	CR, PR or SD	CR or SD	No	NA	Off steroids, stable dose	Note that a lack of steroid or neurological information could prevent CR or PR from being recorded
	CR, PR or SD	CR or SD	No	improved, unchanged, possible worsening	NA	Note that a lack of steroid or neurological information could prevent CR or PR from being recorded
	NA	NA	NA	improved, unchanged, possible worsening	Off steroids, stable use	
	CR, PR or SD	CR or SD	No	improved, unchanged, possible worsening	Off steroids, stable dose	A CR or PR overall response assignment is not possible with a neurological grading of -1.
PR	PR or CR	CR or SD	No	unchanged or improved	Off steroids, stable use	All conditions need to apply and CR criteria do not apply.
CR	CR	CR	No	unchanged or improved	Off steroids	All conditions need to apply.
NA	NA	NA	NA	NA	Off steroids, stable dose	NA applies when NA is true of both radiology and neurological status.

21.5 Survival

Survival is not an endpoint in this study but will be accessioned to derive hypotheses that may relate data obtained in this study with survival times. Survival will be calculated from the date diagnostic tissue confirming recurrence is first obtained and also from the time of Vaccine #1 for both Groups A and B.

Identification of Covariates

Descriptive statistics (e.g. frequencies and means/SD) will be used to summarize levels of polyfunctional T cells, cytotoxic T cell responses, tumor-specific antibodies, and serum cytokine levels in the peripheral blood measured bimonthly. Specifically, we will address the hypothesis that polyfunctional tumor-specific T cell responses may be important for mediating clinical efficacy of DC + xALT therapy. Hotelling's t-statistic will be used to determine whether changes in the polyfunctional T cells occurred during the 3 initial vaccinations. Cox proportional hazards model will be used to assess the effect of the pre-vaccination level of polyfunctional T cell on PFS-12 and OS with and without adjustment for known prognostic factors. The change observed in polyfunctional T cells during the first 3 vaccinations will be computed and its relationship with PFS-12 and PFS beginning at the time of the third vaccination with and without adjustment for known prognostic factors. Similar analyses may be conducted examining changes between pre-vaccination and other monthly follow-up assessments. The use of time-dependent variables within the Cox model for PFS-12 and OS beginning pre-vaccination will be explored. Logistic regression will be used to predict PFS-12 and 2yr-OS as a function of baseline polyfunctional T cell counts and previously defined changes. Given the large number of predictors that will be examined relative to the overall sample size, it is recognized that these analyses will be exploratory. These analyses are conducted to generate hypotheses only and will need to be validated in additional patients or studies. Statistically, joint effects will also be explored, but are unlikely to have sufficient power to identify such interactions. Assuming that half the sample size of 35 patients are positive for a particular measure (i.e. detection of tumor-specific antibodies at a particular post-vaccination time point) and that all have died, we will have 80% power for a log rank test conducted with α=0.05 to detect a survival hazard ratio of 2.6 comparing patients with a positive and negative measure.

21.6 Statistical Analysis of Longitudinal Immunologic Measures

All statistical analyses have been proposed and will be undertaken by the study statistician. Our laboratory have devoted considerable energy to co-developing and co-validating the high-throughput immunologic assays outlined above for monitoring TTRNA-specific CD8+ or CD4+ T cells in PBMCs from patients with GBM. The reproducibility of these assays in our hands ensures that they can be reliably used for monitoring of changes over time.

Of interest in this study is 1) whether there exists a difference in the number of tumor specific T cells as measured by CFC (Cytokine Flow Chemistry) after TTRNA-xALT and TTRNA-DC vaccination and 2) whether there exists a change in a given immunologic measure over time with each patient serving as their own control. For (1) we will use simple two-sample t-tests for means derived from each group for each parameter. Assuming 35 patients will received DC + xALT therapy during the Phase II trial, we will have 80% power to detect a difference between groups equal to approximately 1.79 standard deviations of the immune response (i.e., coefficient of variation = 0.557) at the α=0.05 level (2-tailed).

For (2), we assumed for the power calculations, that measurements of immune response parameters will be normally distributed and correlated (r=0.5). All samples will be run in triplicate and the mean of the triplicate will be used for analysis. All statistical tests will be conducted at the 0.05 level of significance. In all assays proposed above, a paired t-test will be conducted unless assumptions required for conducting a t-test are not satisfied. In such cases, a non-parametric Wilcoxon signed rank test will be conducted. The difference detectable with a paired t-test from the mean at baseline in this study for a given patient is shown below:

Coefficient of Variation	Test	Percent change from baseline mean detectable (80% Power)	Percent change from baseline mean detectable (90% Power)
<0.10	Luminex, DTH, Serum Titers,	2.56%	6.14%
<0.15	CFC, Proliferation	3.67%	4.50%

Immunological response evaluations for baseline values will be conducted on a blood sample draw 24 hours before administration of HDC and prior to DC + xALT therapy and on a subsequent blood draw obtained two weeks after the third immunization. Immunologic response evaluations will also be performed from peripheral blood obtained prior to each vaccination. The total amount of blood required for this purpose will be 10 cc. A comparison of pre-therapy lymphocyte functions to those at intervals after each immunization will be made. These tests may provide evidence for the development of immune responses following DC + xALT therapy and will play an important role in the design of future immunotherapy clinical trials.

21.7 T cell Cytokine Production

CD4+ and CD8+ T cells derived from patients prior to and after DC + xALT therapy will be cultured in 96-well plates with 0, 5, or 10 μg of PHA, TTRNA pulsed DCs, and control (PBMC RNA) pulsed DCs. After 16 and 72 hours of culture, supernatants are harvested and processed in duplicate with a custom BioRad Bio-Plex 12-plex (IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p70), IL-15, IFN-γ, TNF-α, IP10) Cytokine Reagent Kit (BioRad) to differentiate responses into TH_1 and TH_2 types. Briefly, supernatants are incubated with anti-cytokine conjugated beads, followed by incubation with biotinylated detection antibody. Reaction mixture is detected with streptavidin-PE and analyzed on a Luminex 100 machine (Luminex Corporation, Austin, Texas). Unknown cytokine concentrations are calculated by BioPlex Manager software using standard curves derived from a recombinant cytokine standard.

A paired t-test will be used to compare cytokine differences between treatment groups as described above. A Bonferroni correction will be used to control for the multiple cytokines being evaluated such that $P<0.007$ will be required for statistical significance.

21.8 Cytokine Flow Cytometry

The CFC analysis will be done at the UF Brain Tumor Immunotherapy Program. This process involves the rapid early detection and analysis of the production of CD107a, γ-IFN, TNFα, and IL-2 prior to cellular secretion following antigen-specific stimulation *in vitro* as determined by CFC. The functional $CD4^+$ and $CD8^+$ immune response of the patients will be monitored using an 11 color CFC assay. Evidence for intra- and inter-molecular epitope spreading and evidence for the induction of autoimmunity will also be sought. For cellular immune responses a polyfunctional analysis [9-11] of T cell function will be performed as previously described [11]. Subsets of naïve ($CD45RO^-CD27^+$), effector ($CD45RO^+CD57^+$), effector memory ($CD45RO^+CD27^-$), central memory ($CD45RO^+CD27^+$), and terminal effector ($CD45RO^-CD57^+$) cells will be analyzed for production of IFNγ, TNFα, and IL-2 as well as induction of the cytolytic granule marker CD107.

Immediately preceding each vaccination, 90 mLs of peripheral blood will be drawn into vacutainer tubes containing ACD. PBMCs will be separated by density gradient centrifugation on ficoll-Hypaque. Cryopreserved PBMC samples are thawed and rested overnight at 37^0C/5% CO_2 in RPMI media containing 10% fetal calf serum. Cells are adjusted to $2x10^6$/well and incubated with 1 μg/mL of each of the co-stimulatory MAbs αCD28 and αCD49d with or without stimulation with autologous tumor, mRNA-pulsed DCs, and autologous normal brain mRNA (when available)(2 μg/mL) in the presence of Brefeldin A (5 μg/mL Sigma-Aldrich, St. Louis, MO) monensin (1μg/mL; Golgistop, BD Biosciences, San Diego, CA), and αCD107a PE-Cy5 for 5-6 hr at 37^0C and 5% CO_2. Following stimulation, cells will be treated with EDTA for 15 minutes at ambient temperature (AT, 18-22ºC). The cells will be washed and stained with MAbs specific for CD4 PerCP-Cy5.5-PE, CD8 APC-Cy7,

CD45RO PE-TR, CD27 APC, CD57 FITC, CD14/CD19 (PacBlue) and a vital-dye reagent (LIVE/DEAD Fixable Violet Dead Cell Stain Kit for Flow Cytometry; Invitrogen Corp., CA) for 20 minutes at RT. After two washes, 1xBD FACS Lysing solution (BD Biosciences, San Jose, CA) will be added and samples will be incubated for 10 minutes at RT. After one wash, 1xBD FACS Permeabilizing Solution 2 (BD Biosciences, San Jose, CA) will be added and samples incubated for 10 minutes at AT. After one wash, cells will be stained with αCD3 (AmCyan), αIFN-γ (PE-Cy7), αTNF-α (A700), and αIL2 (PE) for 30 minutes on ice, washed, and fixed in PBS containing 1% formaldehyde (Sigma-Aldrich, St. Louis, MO). In all experiments, a negative control (αCD28/49d), and a positive control (SEB, 10 μg/mL, Sigma-Aldrich) will be included. The samples are acquired on a custom LSRII polychromatic flow cytometer (BD Immunocytometry System, San Jose, CA) equipped for detection of 17 fluorescent parameters. We are planning to collect a minimum of 500,000 total lymphocytes from each sample, because we expect the frequency of responding cells to be between 0.05 and 1.0%. This number of events is required based on calculations performed by Dr. Holden Maecker (BD Bioscience, personal communication) to detect a statistically significant number of positive events that can be used for the analysis of the data and the characterization of the different populations.

Ideally, we would be able to select a validated surrogate immunologic response marker for clinical efficacy, but no such marker has been identified to date and studies to validate such a marker would need to be large and prospective and would be clearly beyond the scope of this protocol. It would be our intent, however, to incorporate any additional knowledge that becomes available at the time of data analysis to evaluate the relative biologic significance of the immune response markers that we have chosen.

Tetramer studies will not be routinely performed because the HLA restriction of all antigens present in the mRNA-pulsed DCs has not been determined. Tetramer analysis will be used, however, in patients with appropriate genetic haplotypes to quantitate changes in immunologic response to tumor-associated antigens differentially expressed in malignant brain tumors before and after vaccination as follows: IL-13R2$\alpha_{345\text{-}354}$, Her-2$_{369\text{-}377}$, survivin$_{96\text{-}104}$, gp100$_{209\text{-}217}$, and TRP-2$_{180\text{-}188}$.

21.9 Statistical Analysis for Polyfunctional Response

The analysis used here will be one that has been successful at distinguishing HIV progressors and non-progressors based on T cell phenotype [9-11]. All data will be background subtracted. For each measure, a lower threshold corresponding to two standard deviations above background is set to 0 based on a Poisson model essentially allowing T cells to be designated as positive or negative for a certain phenotypic marker. The number of positive phenotypic markers post-vaccination will be calculated for each patient. Additional exploratory analyses will be conducted using an exact binomial test that will compare treatment groups with respect to the proportion of patients with 5 positive markers, and 4 or more positive markers.

21.10 Serology

Antibody levels to specific antigens will be determined by FACS analysis of antigen immobilized on beads. Cellular lysate autologous tumor cells will be immobilized separately on Dynal M280 magnetic beads according to manufactures directions. Beads are reacted with patient serum for 30 minutes at AT. Washed beads are then developed with a fluorochrome conjugated goat anti-human polyclonal for an additional 30 minutes at AT. Beads are analyzed for fluorescent intensity on a BD FACSCalibur™. Differential levels of antibody binding from pre and post DC + xALT therapy samples to immobilized tumor lysate will be reported as humoral response to the tumor antigens. As a further test, pooled normal brain lysate (Zyagen, Inc.) will be used to demonstrate tumor-specificity or preabsorb normal brain binding antibodies. As a means of demonstrating specificity, serums will be pre-incubated with the same immobilized lysates prior to reacting with beads to absorb reactive antibodies.

21.11 Statistical Analysis

All data will be background subtracted and compared using **paired t-tests** as described above.

21.12 Toll-Like Receptor Agonist Activity

Release of endotoxin, a TLR4 agonist, from gut microbial release into the bloodstream during myeloablative therapy has been shown to enhance the activation and expansion of adoptively transferred tumor-specific lymphocytes in murine models[12]. Endotoxin levels (LPS) in patients receiving HDC + PBCT and NMA salvage chemotherapy will be measured in the serum before induction of chemotherapy and serially afterwards during immune monitoring blood draws. The quantitative chromogenic Limulus amebocyte lysaste assay (QCL-1000, Whittaker M.A. Bioproducts, Walkersville, MD) will be used to measure serum LPS levels. Blood will be drawn aseptically into LPS-free tubes. Samples are subsequently diluted with pyrogen-free water and heat inactivated for 100 C for 10 minutes. *Escherichia coli* 055:B5 reference endotoxin will be used for standard curve analysis (Whittakeer M.A. Bioproducts) and LPS levels at varying time points of therapy will be determined compared to baseline with a paired t-test.

Upregulation of TLR expression and TLR signaling pathway genes in serially collected PBMCs will be analyzed by Oligo GEArray® Human Toll-Like Receptor Signaling Pathway Microarray allowing the profiling of 113 genes related to TLR-mediated signal transduction including all identified members of the TLR family[13-15].

21.13 Statistical Analysis

Data analysis will be conducted using Genespring (Silicon Genetics) and intensity dependent normalization will be performed on entire dataset. Triplicate samples of RNA extracted from various portions of tumor tissue (where sufficient material available) will be used to control for sampling variability and a threshold of a 2-fold change in expression relative to baseline, and a *p*-value cutoff of 0.05 will be performed to determine changes in TLR-specific gene expression. Data will be confirmed by real-time RT-PCR for a subset of TLR signaling pathway genes that show statistically significant changes after therapy. The primary analysis will focus on pre and post HDC + PBSCT and salvage chemotherapy time points.

21.14 Tumor Associated Antigen (TAA) Profile in Recurrent Tumors

The identification and validation of antigens in MB/PNETs that can serve as potent tumor rejection antigens may be important for the development of potent and specific immunotherapy against these tumors. RNA amplified from tumors used in vaccine preparation and from 2nd recurrences (where biopsy or resection is clinically indicated for confirmatory diagnosis and sufficient tissue obtained) will be analyzed for a panel of common TAAs to determine the frequency of expression of these antigens in reMB/PNETs and whether immunotherapy directed against total tumor RNA encoded antigens modulates the expression of these genes after treatment. The top 40 uniformly overexpressed transcripts in common malignancies that were not expressed in complementary normal tissues were determined using database mining of publically available SAGE and microarray analyses[16]. An analysis of MB/PNETs was not included in this data set but we will investigate the frequency of expression of these 40 TAAs (TAA-40) using a custom microarray chip containing the published Tag sequences for identity of each of these proteins (Agilent Made-to-Order Arrays, Affymetrix, Inc., Santa Clara, CA).

RNA (directly isolated where sufficient material or amplified) from first and second recurrent tumors will be analyzed by the TAA-40 expression chip. Tumors will be considered positive for a TAA-40 is expressed at $\geq$ 33 copies per cell and at least twofold higher than reference normal brain RNA from three donors (Zyagen, Inc.).

21.15 Statistical Analysis

Data analysis will be conducted using Genespring (Silicon Genetics) and intensity dependent normalization will be performed on entire dataset. Triplicate samples of RNA extracted from various portions of tumor tissue (where sufficient material available) will be used to control for sampling variability and a threshold of a 2-fold change in expression relative to control (normal brain), and a 2-way ANOVA with a *p*-value cutoff of 0.05 will be performed to determine changes in TAA-40 specific gene expression. Data will be confirmed by real-time RT-PCR for a subset of TAA-40 genes.

22. DATA MANAGEMENT

22.1 Data Management and Quality Control

The Department of Neurosurgery will be responsible for the management of clinical data.

22.2 Laboratory Data Transmission and Accountability

In addition to the observational clinical data, other material is collected, such as blood, tissue, and images, which must be transmitted, tracked, and accounted for by linking it to the appropriate patient and follow-up sequence. Also, expectations for the generation of these elements and the resultant data must be set and monitored closely to ensure their timely collection and movement through the system.

In the case of sample management, tissue and tissue-related data is managed by the Brain Tissue Bank.

23. DATA AND SAFETY MONITORING PLAN

23.1 Data Safety Monitoring Board

The University of Florida Health Cancer Center (UFHCC) is committed to the safety of patients participating in clinical trials at its institution. The UFHCC is also committed to data accuracy and protocol compliance. The UFHCC has established an institutional plan to assure data safety and monitoring for all clinical trials at the University of Florida Health Cancer Center. This plan is designed to comply with policies and guidelines regarding data and safety monitoring from the National Institutes of Health and the National Cancer Institute.

Data and safety monitoring takes place within the established process of protocol review and monitoring by the UFHCC Protocol Review and Monitoring Committee (PRMC), the Institutional Review Board (IRB), and the UFHCC's Data and Safety Monitoring Board (DSMB). The DSMB is the primary agent for assuring data and safety monitoring. The DSMB includes two co-chairs and multidisciplinary representation from clinical researchers, physicians and biostatisticians. The DSMB includes both voting and advisory members from the UF Health Cancer Center, UF Health Cancer Center-Orlando Health, and the UF Proton Therapy Institute – the three facilities it serves.

The UFHCC DSMB meets bi-annually. If an emergent situation arises the committee will convene a meeting. All studies identified by the PRMC as requiring review by the DSMB will be monitored. The DSMB will report final recommendations to the PRMC who reviews and then forwards these recommendations, along with any additional recommendations, to the Principal Investigator. The Principal Investigator is responsible for forwarding PRMC's recommendation to the Institutional Review Board. Final responsibility and authority for closing or amending of such trials will rest with the PRMC and Institutional Review Board.

Requirements for periodic data and safety monitoring are commensurate to the clinical trial's risk and complexity. The IRB, PRMC, and/or DSMB may require more frequent and/or additional monitoring to assure patient safety and trial integrity. Data safety and monitoring activities continue until all patients have completed their treatment and until all patients have been followed beyond the time point at which study-related adverse events would likely be encountered.

Circumstances that would prompt communication with the DSMB include:

- Any unexpected death
- Any circumstance felt by the medical monitor to warrant DSMB notification and/or input
- A frequency of DLTs that require suspension of enrollment

23.2 Independent Medical Monitor

For research involving more than minimal risk (as defined in 32 CFR 219.102(i)) to subjects, an independent medical monitor shall be appointed by name. The medical monitor will oversee the progress of this research protocol, especially issues of individual subject management and safety. The medical monitor shall be independent of the investigative team and shall possess sufficient educational and professional experience to serve as the subject advocate. The medical monitor shall promptly report discrepancies or problems to the PI. They

shall have the authority to stop a research study in progress, remove individual subjects from a study, and take whatever steps are necessary to protect the safety and well-being of research subjects until the PI can assess the medical monitor's report.

The Medical Monitor will review the general progress of the study in regard to the safety for the subjects in a written report discussed with the PI biannually.

23.3 Independent External Monitor

An independent external monitor will conduct data and safety monitoring for this study. The monitor will compare source documents with completed case report forms to ensure accuracy and tabulation of adverse events. The monitor will report all findings to the sponsor-investigator, who will notify the IRB when significant safety concerns are identified.

The independent external monitor will conduct monitoring visits to ensure subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, standard operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements. The monitor also provides ongoing education and resources to investigators and study teams to enhance data quality. The frequency of monitoring will be based on accrual and additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of an investigator or the IRB.

23.4 Principal Investigator

This clinical research study will also be monitored internally by the PI. In terms of internal review, the PI will continuously monitor and tabulate adverse events. Appropriate reporting to the UF IRB will be made. If an unexpected frequency of grade III or IV events occur, depending on their nature, action appropriate to the nature and frequency of these adverse events will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Stopping rules for toxicity and/or response are met;
- Risk/benefit ratio is not altered to the detriment of the subjects;
- Appropriate internal monitoring of adverse events and outcomes is done;
- Over-accrual does not occur;
- Under-accrual is addressed with appropriate amendments or actions;
- Data are being appropriately collected in a reasonably timely manner.

24. RISKS/BENEFITS ASSESSMENT

24.1 Potential Benefits

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

24.2 Potential Risks

24.2.1 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. Patients 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.

24.2.2 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. Symptoms may include, but are not limited to, severe headache, confusion, lethargy, unresponsiveness, coma, or focal neurological deficits. Patients will be monitored throughout the course of the study and those patients with any signs or symptoms of cerebral edema may need their steroid doses increased, treatment with an osmotic diuretic, or surgical decompression. Edema that fails to respond to aggressive therapy may lead to permanent neurological impairment. The probability of this risk can be predicted to some degree based upon tumor size, location, pre-operative neurological impairment, and post-operative course prior to DC injections. Patients will be monitored throughout the course of the study.

24.2.3 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 patients, DCs must pass sterility tests in thiglycolate broth, tryptic soy blood agar, and inhibitory Sabouraud agar. Following injections, patients will be monitored throughout the course of the study for any signs and symptoms of infection. There have been no infections to date in the recent VICTORI (IRB #3108-05-9R4) or ACTIVATE (IRB #5421-05-1R1) clinical trials testing similar approaches in a similar patient population. If an active infection is suspected, patients will be cultured and treated with appropriate antibiotics.

24.2.4 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.

24.2.5 PHLEBOTOMY

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

24.2.6 LEUKAPHERESIS

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. Patients will be carefully monitored throughout the procedure by trained nursing and medical staff. Calcium gluconate (2 mg PO) will be given to minimize the risks of hypocalcemia, fluid supplementation will be given to minimize hypotension, and blood will be routinely screened for *HIV*, hepatitis, and syphilis to minimize the risk of transmitting infection.

24.2.7 MRI

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

24.3 Allergic Reactions to Contrast Agents

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

24.4 GM-CSF and G-CSF 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

Risks and side effects related to <u>Sargramostim (GM-CSF)</u> 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

24.5 Unknown Risks

The overall risk classification of this research is unknown. Clinical trials using DC-based immunizations on brain tumor patients only recently published in the literature. From our experience with 40 patients in ongoing and previous trials we have not seen any toxicities or serious unexpected AEs.

24.6 Confidentiality

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

24.7 Treatment Alternatives & Financial Reimbursement

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

25. KEY STUDY PERSONNEL

Duane A. Mitchell, M.D., Ph.D. (Principal Investigator) **is Associate Professor of Neurosurgery, Neuroscience, Pathology, and Immunology, Co-Director of the Preston A. Wells, Jr. Center for Brain Tumor Therapy, and Director of the University of Florida Brain Tumor Immunotherapy Program.** Dr. Mitchell has considerable translational research experience involving the immunologic treatment of cancer. Dr. Mitchell is a graduate of the prestigious NIH-sponsored Medical Scientist Training Program (MD/PhD Program) and received his Ph.D. under the guidance of Dr. Eli Gilboa, a world-renowned tumor immunologist and pioneer of the RNA transfected DC platform that will serve as the antigen presentation platform in this investigation. He has devoted his research efforts on the development of immunotherapeutic approaches to the treatment of malignant brain tumors, and has served as PI on seven FDA and IRB approved Phase I/II clinical trials investigating immunotherapy in patients with malignant brain tumors. He is the Chair of the Immunotherapy Working Group for the NCI Pediatric Brain Tumor Consortium and a panel member of the Immunotherapy Working Group for the NCI Brain Malignancy Steering Committee. The study outlined in this proposal will be conducted under FDA IND-BB-14058 covering the use of adoptive T cell therapy and amplified total tumor RNA-pulsed DCs in pediatric patients with recurrent MB/PNETs. He will be responsible for supervising all aspects of the study; including writing and disseminating all study results and reports. He will also be ultimately responsible for ensuring the regulatory compliant execution of this trial and coordinating the multidisciplinary interactions of the investigators and administrators involved with this study. Overall coordination of the multi-site phase II trial will be assisted by Dr. Gerald Grant, Stanford University (Neurosurgery Chair) and Sri Gururangan, University of Florida (Neuro-Oncology Chair). A Leadership Plan for this collaboration with Dr. Mitchell functioning as lead PI and Immunotherapy Chair has been developed and is outlined below (Section 21.1 Leadership Plan).

William Slayton, M.D. is Professor, Division Chief, and Program Director of Pediatric Hematology/Oncology at the University of Florida with over 20 years of experience in the care of pediatric patients. Dr. Slayton has an active clinical protocol involving the use of high-dose chemotherapy followed by autologous stem cell rescue in children with CNS tumors and extensive experience in treating children with CNS tumors using intensive chemotherapy that will be utilized as the backbone for the clinical trial design investigating the safety and immunologic effects of adoptive lymphocyte transfer and DC vaccination during hematopoietic

recovery from high-dose chemotherapy. http://hemonc.pediatrics.med.ufl.edu/faculty-and-staff/attending-physicians/dr-william-slayton/

John R. Wingard, M.D. is the Price Eminent Scholar and Professor of Medicine, Deputy Director for Research of the UF Health Cancer Center, and Director of the Bone Marrow Transplant Program for the University of Florida. Dr. Wingard is a renowned expert in bone and stem cell transplantation as well as active clinical researcher. He also oversees the pediatric and adult stem cell transplantation programs and in such capacity coordinates the care of patients receiving high-dose chemotherapy and stem cell transplantation at the University of Florida including children with CNS tumors. Dr. Wingard has extensive experience in the collection and dissemination of autologous and allogeneic cellular products to patients at multiple FACT-accredited institutions and will assist Dr. Mitchell in the multi-site coordination of collection of leukapheresis specimens, peripheral blood stem cell products, bar-coded storage and dissemination of cellular products, and batch records for the cellular products generated on this protocol. The UF stem and bone marrow transplant program maintains state-of-the-art facilities and technologies for autologous cell product delivery to pediatric and adult patients. http://hemonc.medicine.ufl.edu/about-us/meet-the-team/john-r-wingard-md/

David R. Nelson, M.D. is Professor of Medicine, Molecular Genetics and Microbiology, Director, UF Clinical and Translational Science Institute, and Associate Dean for Clinical Research. He has met with Dr. Mitchell several times to outline the many facets of this protocol that can leverage the extensive clinical research infrastructure supported by the NIH-funded CTSI at the University of Florida. Dr. Nelson will oversee the coordination of the multi-site collection and registration of clinical data into a clinical database, coordinate the availability of the clinical research hospital and infusion center for this protocol, as well as coordinate the interaction between clinical research sites. Additionally, the CTSI maintains extensive biorepository and tissue distribution capabilities as one of only 12 CAP-certified biorepositories in the United States. The CTSI-supported biorepository and distribution capabilities will be leveraged to coordinate the collection of tumor tissues and leukapheresis specimens from participating centers and disseminate autologous vaccine products to subjects enrolled at sites outside of the UF health network.
https://www.ctsi.ufl.edu/
http://biorepository.pathology.ufl.edu/
http://gastroliver.medicine.ufl.edu/hepatology/faculty-staff/david-r-nelson-m-d/

Scott Rivkees, M.D. (Other Significant Contributor) **is Professor and Chairman of Pediatrics and Chair of the Child Health Research Institute at the University of Florida**. Dr. Rivkees has met several times with Dr. Mitchell to coordinate the Re-MATCH protocol within the extensive UF pediatric health system. Dr. Rivkees currently oversees the active partnership between the UF and Orlando Regional Medical Center (ORMC) including Arnold Palmer Children's Hospital. ORMC extends the patient network of the UF health system by over 2 million subjects, and Dr. Rivkees is attending physician at both UF/Shands medical center and Arnold Palmer Children's with active on-site rotating work between both centers. He will facilitate the interactions between Dr. Mitchell and his clinical research team with the pediatric oncology and clinical research teams both at UF and ORMC to enhance recruitment and enrollment of subjects served by the Florida health system. He will also ensure that all physical and intellectual resources available within the Department of Pediatrics at the University of Florida are available to Dr. Mitchell in support of this protocol.
http://research.pediatrics.med.ufl.edu/researchers/research-faculty/scott-rivkees/

Brian D. Cleaver, Ph.D. is Director of the Powell Gene Therapy Center (PGTC) and Human Applications Laboratory at the University of Florida. Dr. Cleaver has extensive experience (>10 years) in the FDA-regulated cGMP manufacturing of cellular and gene vector products for use in clinical trials. He has directed

GMP manufacturing of products for use at single-site, multi-site, and even international clinical studies and is an expert in the regulatory compliant manufacturing of GMP materials. Dr. Cleaver and his staff within the PGTC will be responsible for the generation of cellular products used in this protocol and the regulatory oversight, record keeping, and release of qualified cellular products for this study.
http://www.gtc.ufl.edu/core/gtc-apps.htm
http://urology.ufl.edu/about-us/faculty-staff-directory/brian-d-cleaver-phd/

Anthony T. Yachnis, M.D. is Professor of Pathology and Medical Director of Anatomical Pathology at the University of Florida. He is a board-certified neuropathologist with over twenty years of clinical experience. In such capacity, he reviews and performs confirmatory diagnosis on all brain tumor specimens collected at University of Florida (>600 annually) and will ensure the pathologic eligibility criteria for patients enrolled in this study are met. He will also evaluate the histochemical analysis of lymphocytic infiltrates in tumor specimens, and perform all neurotoxicity evaluations. Dr. Yachnis will interact with the study team in the coordination of collection of tumor materials collected at the UF site and will perform the confirmatory diagnostic evaluation of specimens collected from external sites.
http://pathlabs.ufl.edu/team/pathologists/yachnis-a

William A. Friedman, M.D. (Other Significant Contributor) **is Professor and Chairman of Neurosurgery at the University of Florida and Co-Director of the Preston A. Wells, Jr. Center for Brain Tumor Therapy.** Dr. Friedman is a highly experienced neurosurgeon specializing in the care of patients with malignant brain tumors. As Co-Director of the Preston Wells Center with Dr. Mitchell, his major interests are in the advancement of novel therapeutics for pediatric and adult patients with malignant brain tumors. Dr. Friedman's role will be to facilitate the utilization of the vast financial, physical, and environmental resources within the Department of Neurosurgery and Preston Wells Brain Tumor Center in support of this protocol. He will meet regularly with Dr. Mitchell in the coordination of this study and ensure that all institutional resources are leveraged appropriately. Dr. Friedman is also actively overseeing the partnership with the Orlando Regional Medical Center's neurosurgical practices and thus will be able to assist in the recruitment and enrollment of subjects from the ORMC network. Additionally, Dr. Friedman will ensure that the clinical research and regulatory staff of the department are available in support of this protocol including requisite CRC, regulatory, administrative, and nursing support. Dr. Mitchell and Friedman are coordinately responsible for staffing any additional specialized needs in support of the research mission of the Preston A. Wells, Jr. Center for Brain Tumor Therapy. In such capacity, up to $20M in unrestricted funds are available to Drs. Mitchell and Friedman to support clinical research activities. The capacity to support unforeseen budget shortfalls from the clinical trial award supporting this protocol is an additional administrative role that Dr. Friedman would facilitate in collaboration with Dr. Mitchell.
http://neurosurgery.ufl.edu/faculty-staff/our-faculty/william-a-friedman-md/
http://neurosurgery.ufl.edu/faculty-staff/clinical-research-staff/
http://news.medinfo.ufl.edu/articles/lead-story/10-million-wells-foundation-gift-will-enable-uf-to-speed-brain-tumor-remedies/

Elias Sayour, M.D. is a pediatric oncologist who conducted his research fellowship on the immunotherapy of malignant brain tumors. Dr. Sayour will function as an integrative co-investigator at the University of Florida. He will assist in the enrollment, evaluation, and medical management of patients enrolled, and in the evaluation of clinical data and laboratory analysis on acquired tissue and blood specimens.

Jeffrey Drake is a research associate with considerable experience in molecular biology techniques and cell culture. He will be responsible for working with the cGMP facility and staff in the generation of clinical-grade RNA and the generation of autologous dendritic cells and tumor-specific lymphocytes.

Marcia B. Hodik, RN, BSHS, CCRC is a registered nurse with over 20 years of pediatric nursing experience. She has functioned as a study coordinator for Gastroenterology and Hepatology. She currently holds certification as a Certified Clinical Research Coordinator and is the president of the local ACRP Chapter. She will be responsible for screening and enrolling patients, ensuring protocol compliance and collecting and recording study data.

Paul Kubilis, MS is a biostatistician dedicated full-time to research efforts within the Department of Neurosurgery. He is committed to delivering understandable and useful results that help investigators achieve their research goals. He has extensive knowledge in study design, data analysis and presentation of data for publication. He will collaborate with Dr. Mitchell on data analyses and manuscript preparation.

25.1 Key Study Personnel External Sites

CHILDREN'S NATIONAL MEDICAL CENTER, WASHINGTON, D.C.
111 MICHIGAN AVE., N.W.
WASHINGTON, D.C. 20010
http://www.childrensnational.org/

Roger J. Packer, M.D. is Professor of Neurology & Pediatrics, Senior Vice President of the Center for Neuroscience, and Director of the Brain Tumor Institute at Children's National Medical Center in Washington, D.C. Dr. Packer is a renowned pediatric neuro-oncologist that leads the brain tumor program at CNMC, one of the nation's leading pediatric medical centers. He has been a colleague and associate of Dr. Mitchell's for several years through interactions within the NCI-funded Pediatric Brain Tumor Consortium (PBTC) and has a strong interest in the implementation of novel therapeutics for children with malignant brain tumors. He will supervise the implementation of the Re-MATCH protocol within CNMC and ensure that the vast expertise, infrastructure, and resources that the center maintains in support of the treatment of pediatric patients is appropriated in support of this protocol. Dr. Packer will be available to assist Dr. Eugene Hwang in the routine care of patients enrolled on this protocol as well. http://www.childrensnational.org/research/faculty/bios/cnr/packer_r.aspx

Anthony Sandler, M.D. is the Diane and Norman Bernstein Professor of Pediatric Surgery, Senior Vice President and Surgeon-in-Chief of the Joseph E. Robert Center for Surgical Care, and Principal Investigator of the Immunology Initiative in the Sheik Zayed Institute at Children's National Medical Center. Dr. Sandler's primary interest is in the immunologic treatment of pediatric brain tumors. The Shiek Zayad Institute at CNMC was established with a $150 million philanthropic gift to advance novel therapeutics for pediatric patients. Dr. Sandler has hosted Dr. Mitchell as a visitor to the Shiek Zayad Institute to tour the outstanding basic and clinical research facilities established at CNMC. In addition to his strong scientific and clinical interests in immunotherapy, Dr. Sandler is an experienced pediatric neurosurgeon. He will oversee the surgical obtainment and transfer of tumor specimens of suitable quality for vaccine preparation. He will also be responsible for the routine pre- and post-operative care of subjects enrolled on the Re-MATCH protocol.
http://innovationinstitute.childrensnational.org/about-us/team/1001

Eugene I. Hwang, M.D. is a pediatric neuro-oncologist and Director of the Pediatric Neuro-Oncology Fellowship Program at Children's National Medical Center. Dr. Hwang received his pediatric neuro-oncology fellowship training at Duke University and worked with Dr. Mitchell during the development of the Re-MATCH protocol. He has a strong interest in pediatric immunotherapy and is collaborating with Dr. Mitchell on

novel initiatives within the Pediatric Brain Tumor Consortium. Dr. Hwang will play an active role in the care of patients enrolled on this protocol and in the integration of the Re-MATCH protocol with the outstanding clinical research team at CNMC. He will also oversee the collection and transport of leukapheresis products to the GMP facility at University of Florida for vaccine and T cell preparation and be directly responsible for vaccine administration for subjects enrolled at CNMC.
http://www.childrensnational.org/findadoctor/profiles/eugene-hwang-4385.aspx

CHILDREN'S HOSPITAL LOS ANGELES
4650 SUNSET BLVD
LOS ANGELES, CA 90027
http://www.chla.org

Girish Dhall, M.D. is Clinical Director of Neuro-Oncology at Children's Hospital Los Angeles. He is an experienced pediatric hematology/oncologist who will oversee the management of subjects enrolled on the protocol at CHLA. Dr. Dhall will also direct the collection and dissemination of leukapheresis products for vaccine and T cell preparation to the University of Florida and the administration of vaccine products to subjects enrolled at CHLA.
http://www.chla.org/site/apps/kb/cs/contactdisplay.asp?c=ipINKTOAJsG&b=3832751&sid=ewJXIjPWJfLYKdP1KzG&r=1

Mark Krieger, M.D. is Division Chief of Pediatric Neurosurgery and Director of the Neurosurgery Fellowship Program at CHLA. Dr. Krieger is a highly-experienced pediatric neurosurgeon at one of the top ranked pediatric hospitals in the nation. He will oversee the protocol compliant collection and dissemination of tumor materials suitable for vaccine preparation at the CHLA site and in his duties as Division Chief will ensure the proper pre- and post operative management of subjects enrolled on this protocol. Dr. Krieger will also ensure coordination of information with the Neurosurgical Chair of the protocol, Dr. Gerald Grant at Stanford University.
http://www.chla.org/site/apps/kb/cs/contactdisplay.asp?c=ipINKTOAJsG&b=3832751&sid=dvKTKhPVIiI5IfMQJrF&r=1

25.2 Investigator Leadership Plan

Drs. Mitchell, Grant, and Gururangan have been collaborators on several initiatives in experimental therapeutics for pediatric brain tumors for more than a year now, and are co-investigators on a grant award from the Pediatric Brain Tumor Foundation of the United States that has provided support during the preclinical phases of this investigation and clinical trial planning period. These individuals have jointly developed the experimental strategy proposed in this application. This existing infrastructure and high-level expertise will be leveraged to integrate the novel immunotherapeutic platform that is the focus of this investigation. Drs. Grant and Gururangan have in the past and will continue to jointly oversee the surgical and post-surgical clinical care of the patients enrolled on this protocol.

This protocol will be carried out under an existing FDA-approved IND for use of amplified total tumor RNA loaded DCs in patients with malignant brain tumors (FDA IND-BB-14058) using standardized SOPs developed within Dr. Mitchell's laboratory that are centralized within the UF BTIP.

Dr. Mitchell and the multi-site key personnel will be responsible for informed patient consent enrollment of subjects for this investigation. Dr. Gururangan will ensure the informed consent of procedures involving induction chemotherapy and HDC + PBCT, and Dr. Mitchell will ensure the informed consent of experimental immunotherapy consisting of DC + xALT therapy. Dr. Mitchell and his group will be responsible for all experimental procedures involving generation, quality assurance and quality control assessment, and administration of DC + xALT therapy in patients with reMB/PNETs. Drs. Grant and Gururangan will be

responsible for the provision of neurosurgical and adjuvant chemotherapy respectively according to current practice guidelines. **No deviation from standard of care provision of therapy other than the addition of immunotherapy is mandated by enrollment on this protocol**. During admission to the BMT unit, HDC + PBSCT will be provided by the local primary team with Dr. Mitchell coordinating the interaction between these treating physicians and the research study team. Dr. Mitchell will be responsible for ensuring that all study data is accurately documented within patient CRFs by the research nurse and study coordinator. All AEs and serious adverse events (SAE) will be reviewed jointly by Drs. Mitchell, and Gururangan to assess whether deemed vaccine related and reporting to the DSMB when deemed necessary.

Drs. Mitchell, Gururangan, and external site key personnel will have a standing monthly conference call where this protocol and other collaborative projects are thoroughly reviewed. All relevant protocol issues including amendments, accrual, and any treatment related events are discussed during this meeting.

The detailed budget for this proposal has been allocated to cover the supply costs and personnel efforts required to meet the study objects. Dr. Mitchell, as the PI, will function as the coordinating investigator for supply expenditures related to this study and will be responsible for timely submission of required study progress reports.

New intellectual property relative to this project is not foreseen, and therefore it is unlikely that additional disclosures will result from this project. Publication decisions and authorship issues will be decided according to the relative contributions of the PIs.

26. WITHDRAWAL FROM THE PROTOCOL

In this protocol, children being treated for grave and often fatal illness will be treated. Reasons for withdrawal from the study are expected to fall into three general categories. First, being treated on this protocol will put the family of the treated child under social and financial hardship, since many of the children treated at UF are referred from a distance. This often means an interruption of income for one or both of the parents and difficulties in providing care for siblings, if there are any. Since the protocol spans over 1.5 years (beginning with surgical resection) it may become too difficult for some families to complete planned treatment. Second, a significant AE may convince the child's family and the child not to continue with the study. Third, a failure to cooperate with the treatment team or treatment schedules may lead to an investigator's need to remove a child from the study.

Any data collected from cases where the subject is withdrawn will be retained and the collected information regarding this case will be included in the reports of the study along with a brief reason for withdrawal from the study.

27. MODIFICATIONS TO THE PROTOCOL

The investigator/sponsor shall submit a protocol amendment describing any change in the Phase II trial that affects the safety of subjects, the scope of the investigation, or the scientific quality of the study.

Changes in the protocol will be distributed to all investigators and persons responsible for the conduct of the study in advance of the announced date of change. Where necessary, training will be provided and records of the training kept if the change is significant enough to require it.

28. CONTINUING REVIEW AND FINAL REPORT

A. The protocol will undergo continuing review and approval, at least annually, with the UFIRB. Once approved by the UFIRB, notification will be submitted to the USAMRMC Office of Research Protections (ORP) Human Research Protections Office (HRPO) for approval. Following completion of the study, a final report will be submitted to the UF IRB and HRPO as soon as these documents become available. All investigators will remain current with the Collaborative Institutional Training Initiative (CITI).

B. Case report forms will be used to collect data for each subject and a copy of these forms has been submitted as Section 28. (See below). All case report forms have been coded to protect confidentiality of the subject. Study records will be kept confidential as required by law. The subject will not be identified by name,

social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of UF. For records disclosed outside of UF, a unique code number will be assigned.

C. Study team (PI, Co-PIs, co investigators, study coordinator) will adhere to the following requirements and responsibilities issued by the USAMRMC ORP HRPO:

1) The protocol will be conducted in accordance with the protocol submitted and approved by the USAMRMC ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the USAMRMC ORP HRPO.
2) Accurate and complete study records will be maintained and made available to representatives of the US Army Medical Research and Material Command as part of their responsibility to protect human subjects in research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.
3) The knowledge of any pending compliance inspection/visit by the FDA, PHRP, or other government agency concerning clinical investigation research, the issuance of Inspection Reports, FDA From 483, warning letters, or actions taken by any Regulatory Agencies, including legal or medical actions, and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO.

29. REFERENCES

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30. APPENDICES

30.1 Referenced Documents

UFBTIP IDENTIFIER	DOCUMENT TITLE
BR-UFBTIP-001	Isolation of Peripheral Blood Mononuclear Cells (PBMC) Following Leukapheresis
BR-UFBTIP-002	Production of Total Tumor RNA Transfected Dendritic Cell Product
BR-UFBTIP-003	Procedure for the Production of Total Tumor RNA Specific T Cell Product
BR-UFBTIP-004	Non-Adherent Cell Freezing Procedure
SDW-UFBTIP-002	DC Vaccine Administration
SDW-UFBTIP-003	ALT Vaccine Administration
SDW-UFBTIP-004	Performance Status Assessment
SOP-UFBTIP-100	Assay for Dendritic Cell and T cell Phenotype
SOP-UFBTIP-102	RNA Extraction from total tumor sample in BSC
SOP-UFBTIP-103	Small Scale cDNA Production Using Total Tumor RNA From Patient Using Reverse Transcriptase
SOP-UFBTIP-104	Real Time PCR Assay to Determine the Presence of Housekeeping Genes in the cDNA From Total Tumor RNA
SOP-UFBTIP-105	Large Scale cDNA Production Using Total Tumor RNA From Patient Using Reverse Transcriptase
SOP-UFBTIP-106	Check of Large-Scale Reverse Transcription Reaction by PCR
SOP-UFBTIP-107	Large Scale PCR Amplification of cDNA from Total Tumor RNA
SOP-UFBTIP-108	Small Scale Amplified DNA Clean Up Using Qiaquick Purification Kit
SOP-UFBTIP-109	Small Scale IVT Reaction From Small Scale DNA Clean Up of the Large scale PCR Amplification using T7 mMessage mMachine Kit
SOP-UFBTIP-110	Clean Up of Small Scale IVT RNA with RNeasy kit from Qiagen
SOP-UFBTIP-111	cDNA Synthesis From Small Scale IVT RNA
SOP-UFBTIP-112	Real Time PCR Assay to Determine the Presence of Housekeeping Genes in the Small Scale IVT RNA
SOP-UFBTIP-113	Large Scale Clean Up of Large Scale PCR Amplification using Qiagen PCR Purification Kit
SOP-UFBTIP-114	Small Scale IVT Reaction From Small Scale DNA Clean Up of the Large scale PCR Amplification using T7 mMessage mMachine Kit
SOP-UFBTIP-115	Clean Up of Small Scale IVT RNA with RNeasy kit from Qiagen
SOP-UFBTIP-116	Procurement and Transport of the Tissue Sample From the Surgical Suite to the Laboratory
SOP-UFBTIP-119	RNA Release Criteria
SOP-UFBTIP-120	External Tissue Collection, Snap Frozen
SOP-UFBTIP-121	Leukapheresis Product Transport and Receipt Procedures
SOP-UFBTIP-122	Preparation of RNA Transfected DC Product for QC Testing
SOP-UFBTIP-123	Preparation of RNA Stimulated T Cell Product for QC Testing
SOP-UFBTIP-124	Procedure for DC Vaccine Preparation
SOP-UFBTIP-125	Procedure for Preparation of TTRNA-ALT for Adoptive Transfer

UFBTIP IDENTIFIER	DOCUMENT TITLE
SOP-UFBTIP-126	Isolation and Cryopreservation of Peripheral Blood Mononuclear Cells (PBMC) and Plasma From Whole Blood for Immune Monitoring
SOP-UFBTIP-127	Leukapheresis Transport
SOP-UFBTIP-128	Administration of DC Vaccine
SOP-UFBTIP-129	Vaccine Transport
SOP-UFBTIP-130	External Tissue Collection, fresh tissue
SOP-UFBTIP-131	Leukapheresis for Peripheral Blood Mononuclear Cells (PBMCs)
SOP-UFBTIP-132	Administration of ALT (adoptive lymphocyte transfer)

31. ADDENDUM A

31.1 Single Patient-Specific Protocol (UF Site Only)