RESIST: Patients with IDH1 Positive Recurrent Grade II Glioma Enrolled in a Safety and Immunogenicity Study of Tumor-Specific Peptide Vaccine

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Principal Investigator
Katherine Peters, MD, PhD
[redacted]

Sub-Investigator(s)
Gary Archer, Ph.D.
[redacted]

Annick Desjardins, M.D.
[redacted]

Henry Friedman, M.D.
[redacted]

Darell Bigner, M.D.
[redacted]

John Sampson, M.D., Ph.D.
[redacted]

Dina Randazzo, D.O.
[redacted]

Elizabeth Reap, Ph.D.
[redacted]

Peter Fecci, M.D., Ph.D.
[redacted]

Hai Yan, M.D., Ph.D.
[redacted]

Brian Soher, Ph.D.
[redacted]

Statistician
James E. Herndon II, Ph.D.
[redacted]

Lead Study Coordinator
Sarah Woodring
[redacted]

Regulatory Coordinator
Rachel Hesler, Ph.D.
[redacted]

Therapeutic Material Manufacture Manager
Gary Archer, Ph.D.
[redacted]

Data Manager
Eric Lipp
[redacted]

Original version:	20140718	
Amended version 1:	20150831 for IND Submission	Updated information in Figure 4 and in paragraph describing Figure 4 and inserted reference (Section 7.2.4); Added

		NCT# (Section 7.2.6); Inserted references (Section 9.2); Updated the study drug manufacturing company (Section 10.3); Corrected and updated reconstitution information of the study drug (Section 10.4.2); Updated references (Section 17); removed Td manufacturer name (Sections 9 and 10).
Amended version2:	20151005 for FDA modifications 20151216 for IRB AMD002	Removed the saline injection to the contralateral leg (Section 10.4.1); Removed reference to femoral artery for administering vaccines (Sections 9.1 and 10.4.2); Added CBC and CMP blood draw time point in the table of scheduled assessments for leukapheresis screening with ionized calcium and pregnancy testing per Apheresis practice in footnote (Section 12); Clarified "continues to do well" by defining criteria for vaccine therapy if pseudoprogressioin is suspected and clarified definition of progression vs pseudoprogression (Section 12.7.3); More clearly defined the definition of unacceptable toxicity (Sections 8, 9.1.1, & 15.4.1); Updated the definition of SAE to match 21CFR312.23 (Section 13.2); Added the FDA for all adverse event reporting (Section 13.2.1); Corrected mis-numbering of inclusion criteria (Section 11.1); Added the exclusion of subjects unable to undergo MRI (Sections 11.2 & 12.8.2); Removed the word "transmural" from the 4th exclusion criterion (Section 11.2); Removed ACD description of red top tubes (Section 12); Added an additional clinic visit with blood work to assess for delayed toxicity 1 month following last vaccine (Section 12).
Amended version 3	20151216 for IRB AMD002	Removed exploratory objective comparison to historical controls (Section 8); Better described evaluable subjects (Sections 9.4 and 15.4.2); Updated location of paper documents (Section 0); Changed DLT to unacceptable toxicity (12.6.1); Removed statistical estimate (Section 15.4.2)
Amended version 4	20160201 for IRB AMD004	Added investigators Dr. Fecci & Dr. Yan to cover page. Typographical error corrected in Section 11 for inclusion criterion on KPS score from 60 to 70%. Changed wording from "recurrent" to "progressive" (Sections 8, 9, 11, 12, and 15). Removed the prior chemotherapy exclusion criterion (Section 11).
Amended version 5	20160517 for IRB AMD010	Updated eligibility criterion on pregnancy testing to within 72 hours prior to leukapheresis (Section 11).
Amended version 6	20160519 for IRB AMD011	Clarified difference between Td booster and pre-conditioning by adding route of administration as well as changed booster for ALL subjects (Sections 6, 9, 10, & 12); Allowed subjects that transition to a higher grade glioma to continue on vaccines monthly during SOC XRT/TMZ (Sections 6, 8, 9, 12, and 15); Updated immune monitoring blood work and

		biomarker testing and removed urine sampling (Sections 6, 8, 9 12, & 15); Added genomic analyses (Sections 8, 9, 12, and 15); Added MRS for 2HG measurements (Sections 6, 8, 9, 12 & 15); Changed wording from specifically study coordinator to study team (Section 9); Included standard language for leukapheresis preparation (Section 12); Increased the dexamethasone from 2 mg to 4 mg max (Section 12); clarified KPS and physical exam to occur during standard BTC visit with the Td pre-conditioning (Section 12); clarified tumor sampling for IHC analysis (Section 12); added language regarding antihistamine usage (Section 12); Included SOC language for XRT/TMZ and adjuvant TMZ in the appendices as a reference (Sections 18.4 & 18.5).
Amended version 7	20160808 for IRB AMD015	Adjusted timing of immune monitoring and genomic proteomic analysis in those who've transformed to a higher grade (Sections 6, 9.4, and 12); Clarified the description of study procedure following consent (Section 9); Clarified pregnancy testing and MRIs in table of events as well as pathology testing (Section 12); Corrected error in timing of vaccine #1 (Section 12); allowed for every 3 month MRI in low grade setting (Section 9 and 12); Updated screening examination to match current practice and updated location of paper CRFs (Section 12); and Corrected error that vaccine #3 is actually given on day 29 not day 30 (Sections 6, 9, and 12).
Amended version 8	20161128 for IRB AMD023	Formatted for FDA electronic submission; Changed the lead study coordinator and regulatory coordinator; Reformatted Table 2: Screening and On-Study Tests and Procedures (Section 12); Clarified that vaccine will be given on day 22 (+2), not day 21 (+/-2) of monthly cycles of TMZ because vaccine cannot be given on the same day as TMZ (Section 6, throughout); Clarified that Single-Voxel MR will be done before Td booster (Section 6, Section 9.5); Removed requirement that leukapheresis be performed within 2 weeks of consent (Section 9.1, Section 12); Corrected guidelines for dose delay, reduction, and discontinuation to fit 21-day TMZ, not standard 5-day TMZ (Section 18.5.2); Grade 3 subjects receiving RT/TMZ can delay TMZ more than a week without coming off study (Section 18.5.1); Corrected CTQA to the new name OARC (Section 4, Section 14.2); Immunization mixture will be given half in right groin and half in left groin for each vaccine (Section 9.1, Section 10.4.2, Table 2); Changed SOC abbreviation to standard of care, not Safety Oversight Committee (Section 13.3)
Amended version 9	20170106 for IRB AMD027	Added additional MRS time points (Sections 6, 9.1, 9.5, 12); Corrected MRS data acquisition time from 30 minutes to

		1-2 hours (Section 9.5); Baseline MRS is before PEP_{IDH1M} vaccine #1 (Section 9.5)
Amended version 10	20170127 for IRB AMD030	Removed recommendation that the needle be 25 gauge for injection of study vaccine because it is too narrow a bore for the vaccine (Section 10.4.2); Added that EMLA ® cream, or equivalent topical analgesic, can be offered to subjects who experience local pain at the injection site (Section 12.8.2); Corrected the number of patients to 24 in the Study Schema (Section 6); Testing of leukapheresis blood for HIV, hepatitis, and syphilis was re-moved (Section 12.8.2)
Amended version 11	20170215 for IRB AMD035	Added immune monitoring time point at vaccine #6 (Sections 6, 9.1, and 12)
Amended version 12	20170223 for IRB AMD037	Changed Principal Investigator (title page, Section 13.2.1)
Amended version 13	20171011 for IRB AMD054	Primary objective wording changed from "proportion" to "percentage" (Sections 8 and 15.4.1); Changed wording of the timing of the Td from "12-24 hours" before vaccine #1 to "one day" before (Sections 6, 9.1, 10.4.1, and 12); Clarified documentation of review of aggregate summary of AE information (Section 13.1.1); Added that providers will take pictures of the injection site at clinic visits at pre-op time point and at other time points at discretion of physician (Sections 9 and 12); Added collection of research blood at end of vaccine treatment (1 month after vaccine #15 or time the subject comes off study, whichever comes first) (Section 12): Changed total enrollment from 24 to 28, keeping the number of evaluable subjects that will receive at least 6 vaccines at 20 (Sections 6 and 15.4.2)

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4 LIST OF ABBREVIATIONS

2HG	R-2-Hydroxyglutarate
Ab	Antibody
ABC	Automated Blood Count
ACD	Acid Citrate Dextrose
ACLS	Advanced Cardiac Life Support
ACTH	Adrenocorticotropic Hormone
AE	Adverse Event
αKG	Alpha-ketoglutarate
AML	Acute Myeloid Leukemia
APAAP	Alkaline Phosphatase Antialkaline Phosphatase Complex
ALT	Autologous Lymphocyte Transfer
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
AT	Ambient Temperature
BBB	Blood Brain Barrier
β-HCG	Beta-Human Chorionic Gonadotropin
BMT	Bone Marrow Transplant
BTC	Brain Tumor Center
BUN	Blood Urea Nitrogen
Ca^{++}	Calcium
caBIG®	Cancer Biomedical Informatics Grid
CAMRD	Center for Advanced MR Development
CAR	Chimeric Antigen Receptor
CBC	Complete Blood Count
CCL3	C-C motif Chemokine Ligand 3
cDNA	Complimentary Deoxyribonucleic Acid
CFA	Complete Freund's Adjuvant
CFC	Cytokine Flow Cytometry
CLIA	Clinical Laboratory Improvement Act
CMP	Comprehensive Metabolic Panel
CNC	Clinical Neurologic Change
CNS	Central Nervous System
CPC	Cancer Protocol Committee
CIMP	CpG-Island Methylator Phenotype
CRF	Case Report Form
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T-Lymphocyte
DAR	Drug Accountability Record
DBTIP	Duke Brain Tumor Immunotherapy Program
DC	Dendritic Cell
DCCC	Duke Comprehensive Cancer Center
DCI	Duke Cancer Institute
DOB	Date of Birth
DSMB	Data and Safety Monitoring Board
DSMP	Data and Safety Monitoring Plan
DTH	Delayed-type Hypersensitivity
DUHS	Duke University Health System
EAE	Experimental Autoimmune Encephalomyelitis
EBRT	External Beam Radiation Therapy
eCRFs	Electronic Case Report Forms
ELISA	Enzyme-Linked ImmunoSorbent Assay

ELISPOT	Enzyme-linked Immunospot
EGFR	Epidermal Growth Factor Receptor
EGFRvIII	EGFR variant III
FACS	Fluorescence Activated Cell Sorting
FDA	Federal Drug Administration
FEV	Forced Expiratory Volume
GBM	Glioblastoma
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HMG	Hemoglobin
HMO	Health Maintenance Organization
HPLC	High Pressure Liquid Chromatography
IBA	Ion Beam Applications
IC	Intracerebral(ly)
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IDH1	Isocitrate Dehydrogenase 1
IEC	Independent Ethics Committee
IFA	Incomplete Freund's Adjuvant
IFN-γ	Interferon-gamma
IHC	Immunohistochemistry
IL-4	Interleukin-4
IL-12	Interleukin-12
IL-13	Interleukin-13
IRB	Institutional Review Board
KLH	Keyhole Limpet Hemocyanin
KPS	Karnofsky Performance Status
LAL	Limulus Amoebocyte Lysate
LGG	Low Grade Glioma
MAb	Monoclonal Antibody
MedDRA	Medical Dictionary for Regulatory Activities
MG	Malignant Glioma
MHC	Major Histocompatibility Complex
MRI	Magnetic Resonance Imaging
MRN	Medical Record Number
mRNA	Messenger Ribonucleic Acid
MRS	Magnetic Resonance Spectroscopy
MTD	Maximally Tolerated Dose
NA	Non-adherent
NCI CTC	National Cancer Institute Common Toxicity Criteria
NIH	National Institutes of Health
NK	Natural Killer
OARC	Office of Audit, Risk, and Compliance
OS	Overall Survival
PBL	Peripheral Blood Lymphocyte
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate-Buffered Saline
PET	Positron Emission Tomography
PD	Progressive Disease
PHA	Phytohemagglutinin

PFS	Progression Free Survival
PI	Principle Investigator
PRTBTC	Preston Robert Tisch Brain Tumor Center
PT	Prothrombin Time
PTT	Partial Thromboblastin Time
PTMs	Post-Translational Modifications
QC/QA	Quality Control/Quality Assurance
R-2HG	R-2-hydroxyglutarate
RANO	Revised Assessment in Neuro-Oncology
RDSPs	Research Data Security Plans
RECIST	Response Evaluation Criteria in Solid Tumors
reGBM	Recurrent Glioblastoma
reLGG	Recurrent Low Grade Glioma
RIO	Research Integrity Office
RNA	Ribonucleic Acid
RT	Radiation Therapy
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Severe Adverse Event
scFV	Single Chain Variable Antibody Fragment
SFC	Spot-Forming Cells
SOC	Standard of care
SOP	Standard Operating Procedure
SPECT	Single Photon Emission Computed Tomography
TCA	Trichloroacetic Acid
TCR	T cell Receptor
Td	Tetanus-diphtheria toxoid
TGF-β	Transforming Growth Factor-β
TMZ	Temozolomide
TNF-α	Tumor Necrosis Factor-α
T_{regs}	Regulatory T cells
TTP	Time to Progression
TTRNA	Total Tumor mRNA
VDLNs	Vaccine-site Draining Lymph Nodes
WHO	World Health Organization
WOCBP	Women Of Childbearing Potential
XRT	Radiation Therapy

5 PROTOCOL SYNOPSIS AND SUMMARY

Please see separate document (available upon request).

6 STUDY SCHEMA

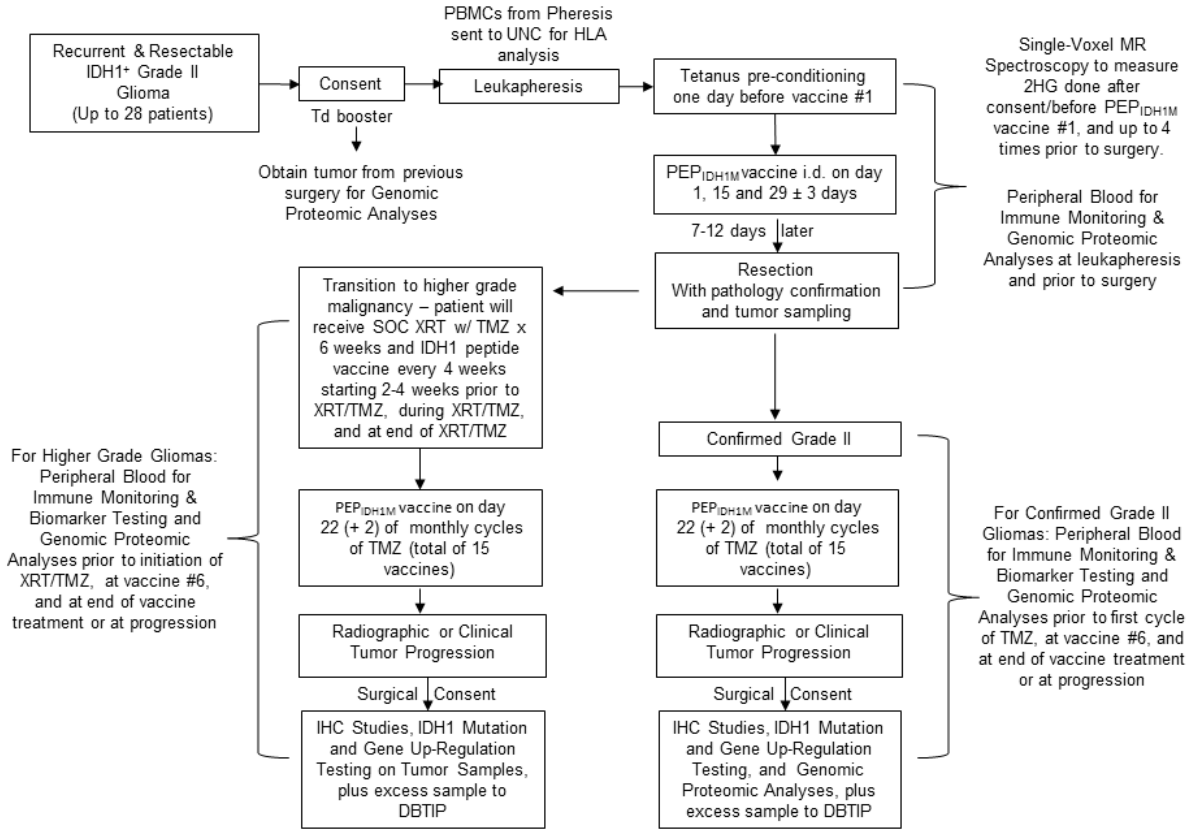

Figure 1. Study Schema

7 BACKGROUND AND SIGNIFICANCE

7.1 Study Disease

Malignant primary brain tumors are the most frequent cause of cancer death in children and young adults and account for more deaths than cancer of the kidney or melanoma.[3] Gliomas are the most common primary brain tumors with GBMs, astrocytomas and oligodendrogliomas comprising the large majority of subtypes.[4] Primary GBM, WHO grade IV, occurs *de novo* and is the most common and aggressive glioma with a median OS of <15 months.[5] Patients with lower grade II or III astrocytomas, oligodendrogliomas or mixed gliomas have a more favorable OS. However, lower grade astrocytomas can progress to secondary GBM and malignant progression is assured in almost all cases with patients succumbing to disease within a decade. Moreover, current therapy for malignant primary brain tumors is incapacitating[6] and limited by non-specific toxicity to systemic tissue or surrounding eloquent brain.

In contrast, immunotherapy promises an exquisitely precise approach and substantial evidence suggests that immunotherapeutic intervention can eradicate well-established tumors in mice and humans, even when tumors reside within the "immunologically privileged" brain. (See Figure 2, Figure 3, and Figure 4)[1,2,7,8] There is a clear and urgent need for the development of targeted therapeutics that can improve outcomes for patients with glioma without adding significant toxicity to current treatment regimens. Immunotherapy targeting tumor-associated antigens holds significant promise to meet this need.

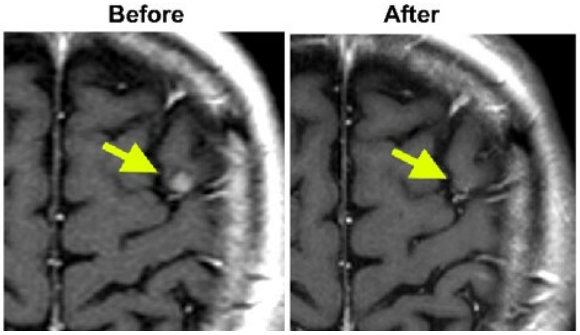

Figure 2. Highly reactive transferred cells traffic to brain and destroy tumor deposit in melanoma patients. Arrows represent location of melanoma metastases. (From Johnson *et al.*, Blood 2009)[1]

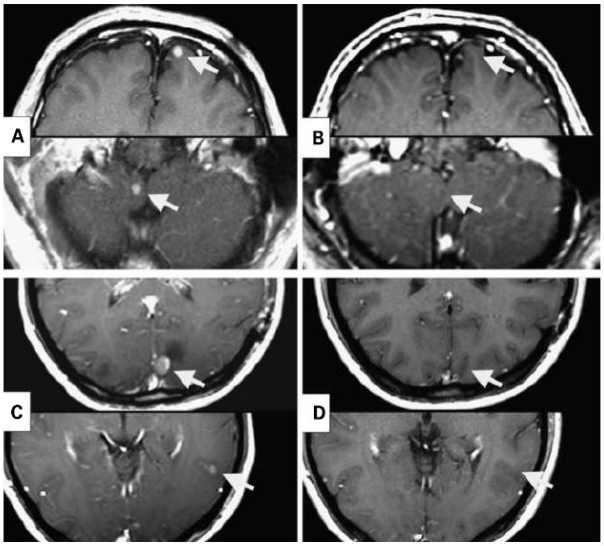

Figure 3. Host-derived antitumor T cells treat lesions in the brain. Arrows represent location of metastases. (A&C) Pretreatment (B) 14 months after cell transfer (D) 6 months after cell transfer. (From Hong et al., Clinical Cancer Research)[2]

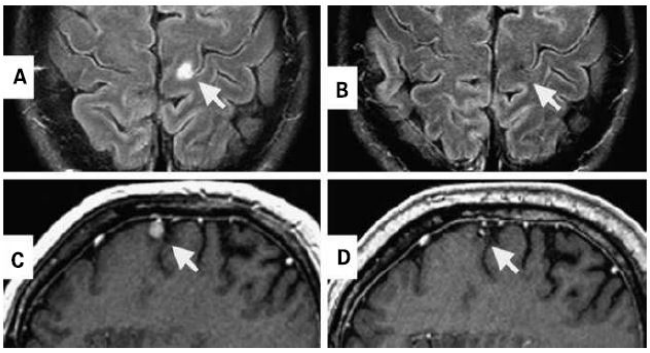

Figure 4. Genetically engineered antitumor T cells treat lesions in the brain Arrows represent location of metastases. (A&C) Pretreatment (B) 6 months after cell transfer (D) 30 months after cell transfer. (From Hong et al., Clinical Cancer Research)[2]

7.2 Study Agents

The study agent will consist of a 25 amino acid peptide (PEP_{IDH1M}) and the adjuvants GM-CSF and Montanide ISA-51.

7.2.1 PEP_{IDH1M}

PEP_{IDH1M} is a 25 amino acid peptide that spans the mutated region of IDHR132H.

7.2.2 GM-CSF

Recombinant GM-CSF, Leukine® (sargramostim), is a normal human cytokine that increases the number and function of granulocytes (neutrophils, eosinophils, and basophils) and monocytes. It has been FDA-approved for clinical use for more than a decade. It is usually used following induction chemotherapy in older adult patients with AML to shorten time to neutrophil recovery, but has also been used previously as a vaccine adjuvant.[9] It is contra-indicated in patients with excessive leukemic myeloid blasts (which we would not expect in the disease under study in this protocol), patients with known hypersensitivity to GM-CSF, yeast-derived products, or any component of Leukine. Serious allergic or anaphylactic reactions have been reported with Leukine.

7.2.3 Montanide ISA 51 (Incomplete Freund's Adjuvant)

Montanide ISA 51 or Incomplete Freund's Adjuvant (IFA) is a water-in-oil (w/o) emulsion with immunomoadjuvant activity. Montanide ISA 51 enhances the immune system's CTL response against antigen(s) in vaccines. Peptide vaccinations employing GM-CSF and Montanide ISA-51 as adjuvants have generally been well tolerated in human patients in numerous phase I-III trials.

7.2.4 Tetanus-diphtheria toxoid (Td)

The current use of Td toxoid is for active immunization in children and adults against infection with the bacteria Clostridium tetani and Corynebacterium diphtheria. Tetanus infection is manifested primarily by neuromuscular dysfunction caused by a potent exotoxin released by C. tetani. Diphtheria is an acute toxin-mediated infectious disease caused by toxigenic strains of C. diphtheriae. Protection against disease is due to the development of neutralizing antibodies to the diphtheria toxin. Td toxoids adsorbed are readily available as several approved administrations [i.e. Daptacel (DTaP), Infanrix (DTap), Tenivac (Td adult), Boostrix (Tdap)[10]. Protection against disease is due to the development of neutralizing antibodies to the tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assays, is considered the minimum protective level. A level ≥0.1 IU/mL by ELISA has been considered as protective[11]. A serum diphtheria antitoxin level of 0.01 IU/mL, measured by neutralization assays, is the lowest level giving some degree of protection; a level of 0.1 IU/mL by ELISA is regarded as protective. Diphtheria antitoxin levels ≥1.0 IU/mL by ELISA have been associated with long-term protection.

Following deep s.c./i.m. administration of the tetanus toxoid vaccine, toxoid molecules are taken up at the vaccination site by immature DCs, which are professional antigen-presenting cells. Within these cells, they are processed through the endosomal pathway (involving the phagolysosome) where they are bound to MHC type II molecules on the surface of DCs. The MHC II:toxoid complex then migrates to the cell surface. While this process is happening within the cell, the now activated mature DC at the vaccine site migrates along lymph channels to the draining lymph node where they encounter naive TH2 cells, each with their own unique TCR. Identifying and then binding of the MHC II:toxoid to the specific TH2 receptor then activates the naive T cell, causing it to proliferate. Simultaneously, toxoid molecules not taken up by DCs pass along lymph channels to the same draining lymph nodes where they come into contact with B cells, each with their own unique B-cell receptor (BCR). Binding to the B cell through the specific immunoglobulin receptor that recognizes tetanus toxoid results in the internalization of toxoid, processing through the endosomal pathway and presentation on the cell surface as an MHC II:toxoid complex, similarly to DCs undergoing the same process[12].

These two processes occur in the same part of the lymph node with the result that the B cell with the MHC II:toxoid complex on its surface now comes into contact with the activated TH2 whose receptors are specific for this complex. The process, termed linked recognition, results in the TH2 activating the B cell to become a plasma cell with the production initially of IgM, with a later switch to IgG antibodies produced. Additionally, a subset of these B cells becomes memory cells[12].

The novelty of using Td toxoid vaccination lies in the ability of this potent recall antigen to enhance antitumor responses as part of a cancer vaccination protocol. Td toxoid induces an inflammatory milieu within the intradermal vaccine site, thereby promoting the migration of injected tumor-specific DCs. Additionally, in the context of vaccinating the host with tumor-derived peptides, conditioning the vaccine site with Td toxoid has demonstrated enhanced immunogenicity with these peptides.

Our data from the ATTAC clinical trial (Duke IRB Protocol # Pro00003877) demonstrating the capacity to enhance DC migration to VDLNs via Td pre-conditioning of the vaccine site offer potential therapeutic interventions whereby we can enhance the immunologic responses to ultimately overcome the inherent challenges in faithfully eradicating established tumors. In a completed randomized clinical trial, we found that migration of injected DCs to VDLNs following vaccine site pre-conditioning with Td toxoid was significantly increased compared to controls and that the efficiency of DC migration was strongly associated with clinical outcomes of patients with newly-diagnosed GBM, the most fatal type of malignant brain tumors[13]. To address this observation, we took our Td pre-conditioning platform back into the preclinical setting using transgenic mouse models and were able to corroborate the effects of Td pre-conditioning on increasing the lymph node homing of intradermally administered DCs. Moreover, Td administration at a single vaccine site increases the migration of a bilateral DC vaccine to both inguinal lymph nodes. Regardless of the side of the Td intradermal skin prep, DC migration to bilateral inguinal VDLNs was equally increased, supporting a systemic response to recruit peripherally administered DCs.

Our Td pre-conditioning platform in the context of DC vaccination also elicited superior anti-tumor responses compared to controls receiving DC vaccines without Td pre-conditioning. In our clinical trial, patients with newly-diagnosed GBM who were administered the Td skin prep before DC vaccination revealed significantly longer progression-free and overall survival rates compared to the control cohort. In evaluating the relationship between DC migration and clinical responses, we observed a modest positive correlation between levels of DC migration and survival. In our preclinical model, Td pre-conditioning prior to vaccination with tumor antigen-specific DCs dramatically suppressed the growth of established and highly aggressive B16-F10/OVA tumors. The use of Td with a DC vaccine increased antitumor responses in an antigen-specific manner, as non-specific DC vaccines were not potentiated with Td pre-conditioning. Furthermore, in a challenge setting, where mice are administered the treatment platform prior to challenge with tumor inoculation, Td pre-conditioning at the vaccine site induced a significant survival benefit compared to controls.

For this clinical trial evaluating PEPIDH1M, we are also proposing to use Td as an adjuvant based on the pre- clinical data described below. Here, mice were immunized with 25mer mutant and wildtype peptides with two clinically relevant adjuvants: granulocyte-macrophage colony stimulating factor (GM-CSF), a

cytokine known to have a principle role in the activity of antigen presenting cells, and imiquimod, a topical TLR7 agonist administered at the vaccine site and tetanus-preconditioning. Immunogenicity of each adjuvant/vaccine combination was measured via IFNγ ELISpot; administration of imiquimod at the site of vaccination results in a more robust mutant-specific immune response compared to GM-CSF, Notably, immunogenicity of the mutant peptide in mice that received imiquimod emulsified in IFA/IFA/IFA is comparable to the immune response seen in mice that received CFA/IFA/IFA, a treatment course with widely known strong adjuvant activity (Figure 5).

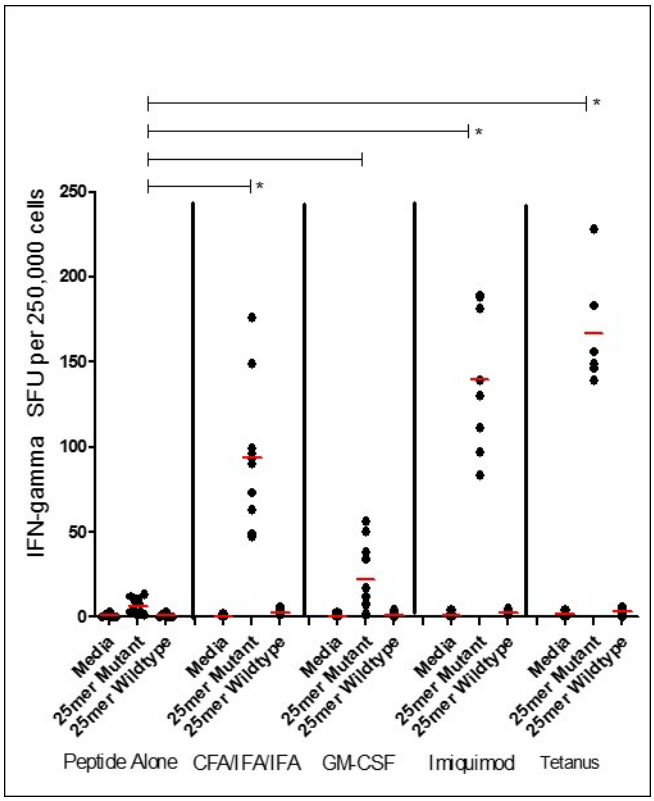

Figure 5. Tetanus pre conditioning, imiquimod and GM-CSF increases the number of IDH1 mutant specific T cells after vaccination. Mice were immunized with (three weekly 100 ug of peptide emulsified in IFA/IFA/IFA), and immunogenicity was measured via IFNγ ELISPOT against both the wild-type and mutant 25mer peptides.

7.2.5 Pre-clinical experience

We have developed a functional murine homologue (Figure 6) of the human IDH1^{R132H} mutant enzyme, cloned syngeneic murine gliomas that express this mutation, mapped peptide sequences specific to this mutation in either species (Figure 7), and demonstrated mutation-specific immunogenicity of these peptides (Figure 8 and Figure 9). Preliminary ELISpot data from our laboratory demonstrates that splenic lymphocytes isolated from mice immunized with peptides of various lengths containing the IDH1^{R132H} mutation (PEP_{IDH1M}) are activated by mutant but not wild type peptide, demonstrating both specificity and immunogenicity (Figure 10). Vaccination with PEP_{IDH1M} in 3 different strains of mice has produced no toxicity.

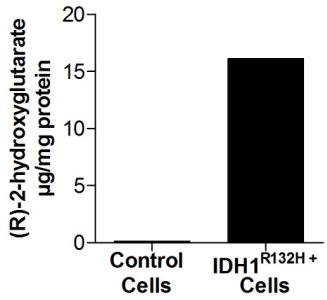

Figure 6. The murine homologue of the IDH1R132H mutation is functional. Murine cells were transduced with the IDH1R132H mutation or left untransduced as controls. Whole cell extracts were submitted for quantification of R-2-hydroxyglutarate by LC-negative electrospray ionization-MS/MS. Values were normalized to total protein levels in each sample.

Figure 7. Human IDH1R132H protein sequence and human and mouse wild type sequences. The mutation is highlighted in yellow. Purple highlights nearest sequence difference between human (S) and mouse (T) protein which is not included in the 25mer IDH1R132H (PEPIDH1M) peptide. PEPIDH1M (red text) is identical between human and mouse.

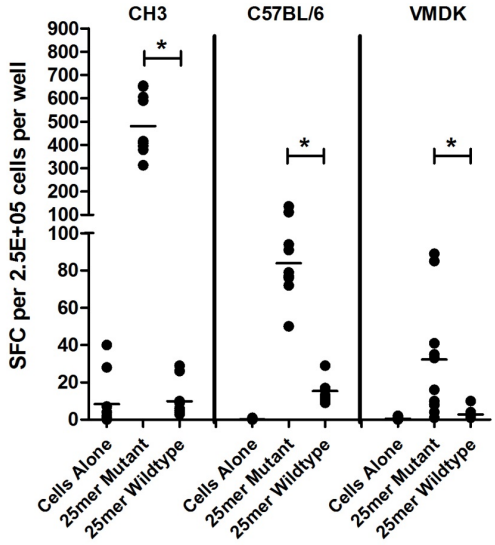

Figure 8. PEPIDH1M vaccination is immunogenic and specific in several mouse strains. Different mouse strains were immunized weekly for 3 weeks with PEPIDHM1 in CFA. After 7 days splenic lymphocytes were derived and stimulated with mutant or wildtype IDH1 peptide and IFNy secretion was assessed by ELISpot. *p<0.05 by t-test

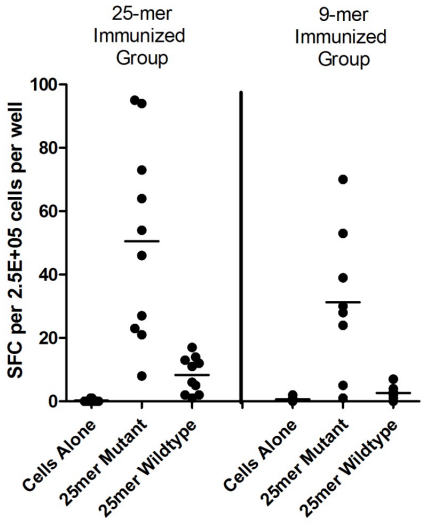

Figure 9. PEPIDH1M vaccination is immunogenic and specific in the context of human HLA. HLA-A2 Transgenic mice were immunized weekly for 3 weeks with 25mer or 9mer length peptides in CFA containing PEPIDHM1. 9mer peptides consisted of a peptide mix of 9 different peptides all spanning the IDH1R132H mutation. After 7 days, splenic lymphocytes were derived and stimulated with mutant or wild type IDH1 25mer peptide and IFNy secretion was assessed by ELISpot.

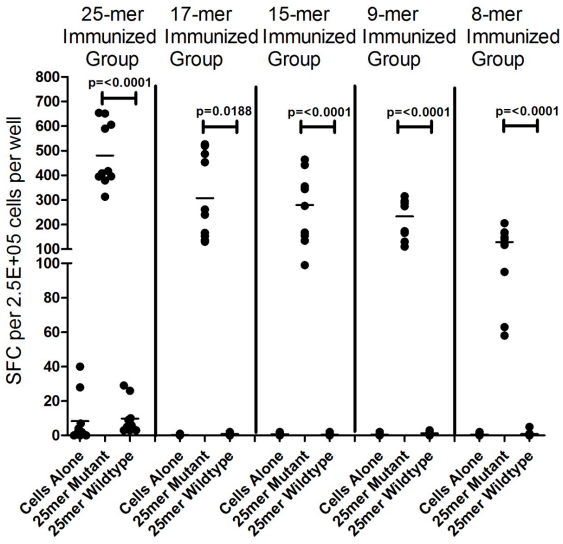

Figure 10. Splenocytes from mice immunized with different lengths of mutant IDH1 peptides. Mice were immunized weekly for 3 weeks with different length peptides in CFA containing the $IDH1^{R132H}$ mutation. After 7 days, splenic lymphocytes were derived and stimulated with the 25mer mutant peptide (PEP_{IDH1M}) or wildtype IDH1 peptide and IFNy secretion was assessed by ELISpot.

7.2.6 Clinical experience

This is a Phase I safety study. PEP_{IDH1M} has not been used in humans previously. Recombinant GM-CSF is FDA-approved for human use.

Our previous experience with a peptide vaccine corresponding to a similar tumor specific mutation, EGFRvIII, in clinical trials has shown that a dose of 500 µg of peptide with 150 µg of GM-CSF yields effective immunity against tumors in the brain without any toxicity. EGFRvIII is a tumor-specific mutation of the epidermal growth factor receptor that is expressed in primary GBMs and other common neoplasms.[14] Because the wild type sequences of human and mouse overlap adjacent to the EGFRvIII mutation, we

were previously able to create a murine homologue of this mutation to test its immunogenicity without concerns that the results would be influenced by xenogeneic responses.[15] Using this approach, we demonstrated that a peptide vaccine (PEPvIII) specific for the tumor-specific mutation EGFRvIII is capable of eliciting EGFRvIII-specific immune responses in mice with orthotopic gliomas.[16] This was possible despite these mice exhibiting profound, tumor-induced immunosuppressive deficits that mirror those seen in humans with malignant brain tumors.[9,16-18] Moreover, these immune responses were capable of eliminating EGFRvIII-expressing tumor cells in these tumors and extending survival of tumor-bearing mice.[16] When translated into humans with EGFRvIII-expressing GBM, similar results were seen.[9,16-18] Despite no evidence of EGFRvIII-specific immune responses prior to vaccination, EGFRvIII-specific immune responses were generated in almost all patients and EGFRvIII-expressing tumor cells were nearly universally eliminated without attendant toxicity.[16-18] Based on these data, this EGFRvIII-targeted vaccine is now being tested in an international Phase III trial (NCT01480479). Unfortunately, EGFRvIII is heterogeneously expressed and tumors recur as a result of outgrowth of the EGFRvIII negative tumor cells.[9] Montanide ISA 51 has proved to be a very efficient adjuvant, activating the cellular and the humoral immune response. It has been administered to more than 10,000 patients throughout the world.

Our overall goal is to utilize our vast experience in peptide-based immunotherapy to provide a PEP_{IDH1M} vaccine that is both safe and immunogenic in patients with IDH1 mutant-expressing WHO Grade II gliomas.

7.3 Study Purpose and Rationale

7.3.1 Temozolomide, Lymphopenia, and Homeostatic Proliferation

TMZ is an alkylating chemotherapeutic that has recently been shown to prolong survival in patients with GBM.[5] As a result, it has become part of the standard regimen used to treat these patients. TMZ induces a transient lymphopenia, and although counterintuitive, we and others have shown that following periods of lymphopenia, immune responses can be markedly enhanced.[19-23] Probably as a result of a surge in cytokines (IL-7, IL-15) in response to lymphopenia, lymphocytes undergoing homeostatic proliferation enjoy a reduced activation threshold,[24,25] differentiate directly into effector memory T-cells capable of rapid and intense response to antigen,[26] display increased expression of anti-apoptotic molecules, and are less sensitive to immunosuppressive NK cell-mediated lysis.[26] Still, lymphocytes must encounter their cognate antigen and compete for limiting amounts of these homeostatic cytokines to proliferate under these conditions.[25] Thus, B- or T-cells specific for antigens that predominate during this recovery period, like those provided in the form of a vaccine, have a competitive advantage and become disproportionately over-represented in the recovering lymphocyte population both in murine models[19,27] and in humans.[23] These skewed homeostatic responses have been shown to enhance antitumor immunity[19,27,28] but can also increase the risk of autoimmunity.

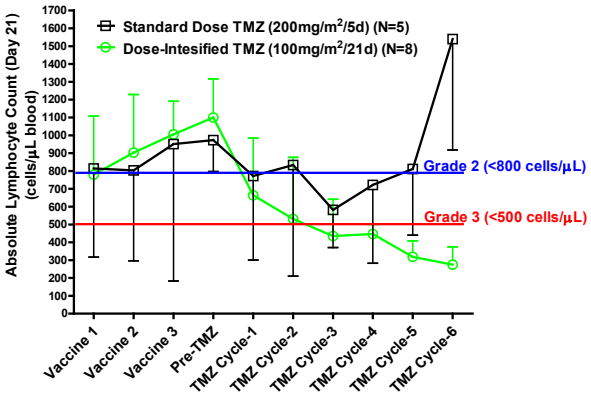

Figure 11. Lymphocyte counts of patients with newly-diagnosed GBM receiving standard dose adjuvant TMZ (200 mg/m²/5d) and dose-intensified TMZ (100 mg/m²/21d). Standard dose TMZ induces Grade 2 lymphopenia in 100% of patients (<800 cells/uL; blue line) and transient Grade 3 lymphopenia in 40% of patients. Sustained Grade 3 lymphopenia (<500 cells/uL; red line) was induced by dose-intensified TMZ cycle #3 in all patients.

Leveraging this principle, Rosenberg and colleagues have produced dramatic clinical responses[21,29-32], along with some autoimmune toxicity, in patients with advanced malignant melanoma.[20,22] After lymphodepletion, T-cells can expand dramatically and tumor-specific T-cells can constitute up to 90% of the host's T-cell repertoire and can be maintained for months,[20,33] and in these studies clinical regressions correlate with the frequency of tumor-specific T-cells achieved in the peripheral blood and persistence of these cells *in vivo*.[21,29-32,34-36]

TMZ produces a survival benefit in patients with GBM and has become a routine part of the therapy for these patients. Due to the known myelosuppressive and immunosuppressive effects of TMZ[37-40] and its potential negative implications for immunotherapy targeting GBMs, we evaluated the impact of TMZ treatment, in the context of our EGFRvIII peptide vaccine trial (now CDX-110, Pfizer, Inc.), on the lymphocyte compartment and immunologic responses of patients with newly-diagnosed GBM undergoing two different dose regimens of adjuvant TMZ. These regimens were evaluated in a randomized, Phase III trial (RTOG 0525[41]). After concurrent TMZ with EBRT, patients received TMZ for either 5 days (200 mg/m^2) or 21 days (100 mg/m^2) of each 28 day cycle. 100% of patients receiving the standard 5 day schedule exhibited Grade 2 lymphopenia (<800 cells/uL) **(**Figure 11**)** with nadirs occurring 14-21 days after the first dose (n=5). Grade 3 lymphopenia was observed in only 1 patient with this regimen. Sustained Grade 3 lymphopenia (<500 cells/uL) was induced in all patients receiving the 21 day regimen, however, by the fifth cycle of TMZ**.** Despite the profound lymphopenia, we have been able to induce and maintain potent EGFRvIII-specific immune responses **(**Figure 12 and Figure 13**)** in patients with GBM receiving serial cycles of TMZ.[42]

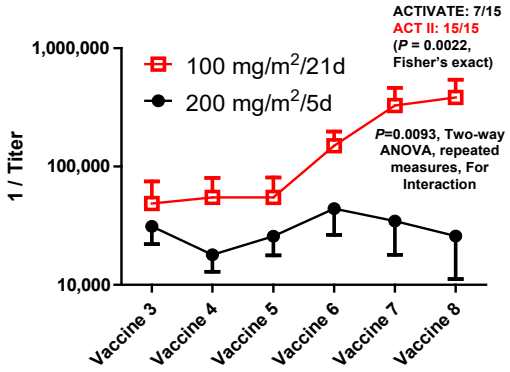

Figure 12. Antibody titers in patients during standard-dose TMZ (200 mg/m^2/5d) and dose-intensified TMZ (100 mg/m^2/21d). All patients receiving the 21 day dose regimen developed EGFRvIII-specific antibodies with titers detectable at greater than 1:100,000 dilution. Humoral and cellular immune responses have been dramatically better in patients receiving the higher TMZ dose.

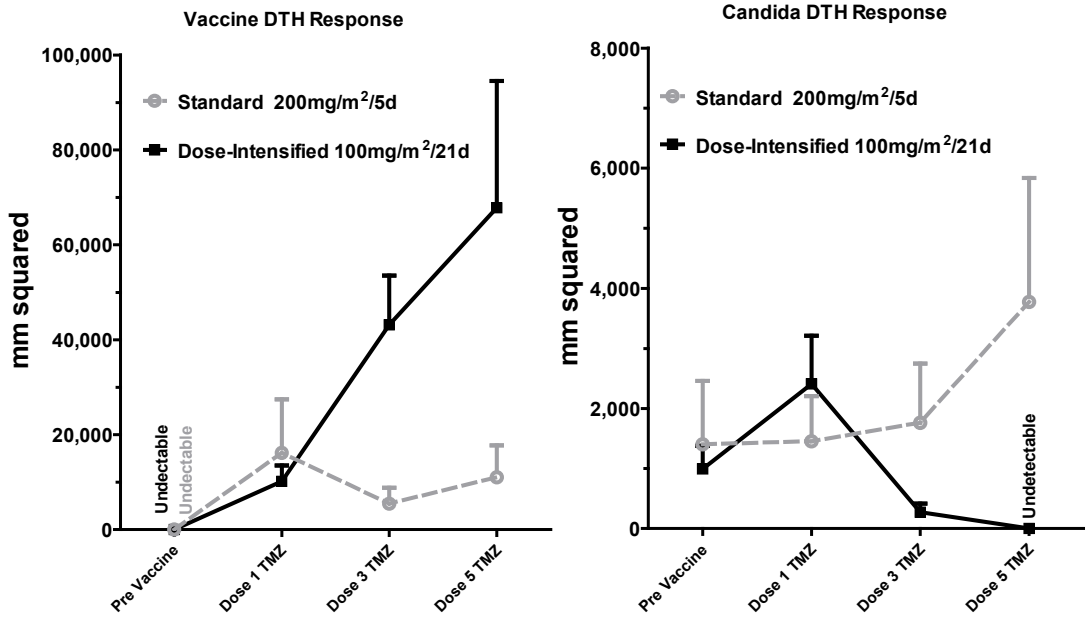

Figure 13. DTH responses to EGFRvIII vaccination. DTH responses to EGFRvIII vaccination (Left) in patients receiving lymphodepletive TMZ (100 mg/m^2/d x 21 days) cycles (black) were increased when compared with standard dosing (200 mg/m^2/d x 5 days) with continued vaccination. DTH responses to the recall antigen Candida (Right) in patients receiving lymphodepletive TMZ (100 mg/m^2/d x 21 days) cycles (black) were decreased when compared with standard dosing (200 mg/m^2/d x 5 days) with continued cycles of TMZ.

7.3.2 Peptide Vaccines for Cancer

Peptide vaccines encoding minimal CD8+ CTL epitopes have been demonstrated in many contexts to induce protective immunologic responses in experimental animals and mediate regressions of established tumors.[43,44] However, with few several notable exceptions,[36,45-48] vaccine-based cancer immunotherapy in humans is in need of significant improvement in immunogenicity and clinical outcomes.[21]

The addition of CD4+ T cell helper epitopes to peptide vaccine formulations is widely believed to be an advantageous strategy for enhancing anti-tumor immunity.[49] This presumption is supported by the observation that CD4+ T cell directed therapy can mediate effective anti-tumor immunity in humans and experimental animals.[50-52] CD4+ T helper cells deliver assistance for CD8+ effector cells by fully activating dendritic cells through the CD40-CD40L signaling pathway.[53] However, vaccination with T helper epitope encoding peptides has yielded mixed results in human clinical trials as a method to enhance anti-tumor cytotoxic T-cell responses *in vivo*[50,54-56] and thus warrants systematic evaluation.

Recent studies have highlighted the use of longer peptides (15-30 amino acids in length) instead of minimal CTL epitopes (typically 8-10 amino acids) for induction of a more robust, long lived CD8+ cytotoxic T-cell responses in experimental animals.[36,57] The expanded length of the peptide requires it be processed by antigen-presenting cells. Minimal CTL epitopes do not require processing by antigen-presenting cells and thus are free to bind directly to T-cell receptors. This can induce tolerance due to a lack of co-stimulatory molecules that are presented to T-cells during antigen presentation by dendritic cells, however this area of peptide formulation requires further evaluation.[58]

Peptide vaccination in humans has exhibited an excellent safety profile with virtually no dose limiting toxicities.[36,56] Most toxicities occur when peptide vaccines are used in conjunction with immunostimulatory cytokines such as IL-2, which are expected results of IL-2 therapy.[59] This feature of peptide vaccinations allows it to be used in combinatorial immunotherapeutic strategies and/or as salvage therapies in resistant disease.[60,61]

7.3.3 Summary of Study Purpose/Rationale

Primary brain tumors are the most frequent cause of cancer death in children and young adults. GBM, the most malignant primary brain tumor, has a median OS of <15 months,[5] and patients with lower grade gliomas progress to this universally lethal tumor type within ten years. Moreover, current therapy for these tumors is incapacitating[6] and limited by non-specific toxicity. In contrast, immunotherapy promises an exquisitely precise therapeutic approach, and substantial evidence suggests that activated T-cells can eradicate large tumors, even within the "immunologically privileged" brain.[1]

EGFRvIII is a tumor-specific mutation that is expressed in primary GBMs.[14] We have previously demonstrated that EGFRvIII-specific immune responses could be generated in mice, and this predicted the ability to generate similar immune responses in humans.[9,16-18,62] These were sufficient to eliminate orthotopic gliomas expressing a murine homologue of EGFRvIII in mice and were sufficient to consistently eliminate all EGFRvIII-expressing tumors cells in mice and humans without toxicity.[9,17,63,64] Based on these observations, our EGFRvIII-targeted vaccine is now being tested in an international Phase III trial. Unfortunately, EGFRvIII is heterogeneously expressed and tumors recur as a result of outgrowth of the EGFRvIII negative tumor cells.[9]

Recently, unbiased, genome wide sequencing has been shown to be a powerful approach for identifying other recurring mutations like EGFRvIII. Using next-generation sequencing, we discovered tumor-specific mutations exclusively in codon 132 of the active site of IDH1[65,66] – an evolutionarily-conserved enzyme essential to cell function.[67] Further study revealed that IDH1 mutations occur in >70% of almost all glioma subtypes, except primary GBM. Greater than 90% of all IDH1 mutations occur from a substitution of histidine for arginine resulting in the highly conserved and tumor-specific mutation, $IDH1^{R132H}$.[68] Immunohistochemical analysis shows that $IDH1^{R132H}$ is homogeneously expressed in all tumor cells, including single infiltrating tumor cells[69] but is absent in normal cells.[66,70] The high frequency, specificity, and homogeneous expression of the IDH mutation make it an ideal target for therapeutic intervention.

Although IDH mutations can be seen in tumors with longer overall survival,[71] IDH status has been occasionally misunderstood to function as an inhibitor of tumor growth. Rather it denotes a genetically distinct subset of tumors. Within those tumors, however, IDH mutations are oncogenic and cause grossly aberrant methylation as well as impaired differentiation that promotes tumorigenesis.[72-78] While wildtype IDH1 converts isocitrate to αKG as part of cellular respiration, $IDH1^{R132H}$ mutations impair normal function resulting in the conversion of αKG into the onco-metabolite R-2HG.[76,79] R-2HG impairs αKG-dependent histone and DNA demethylases resulting in a global epigenetic CIMP, obstructed differentiation, and tumorigenesis.[72-78]

Mutant enzyme inhibition in gliomas using recently discovered small molecule inhibitors, while inhibiting tumor growth slightly, does not induce apoptosis, and tumor cells maintain logarithmic growth.[76] Thus enzyme inhibition may only be partially effective as a therapeutic approach.[72,76,80] In contrast, T cells targeted to these tumor-specific mutations are agnostic to the biologic function of the mutation and kill cells expressing the mutation indiscriminately. Our ability to eliminate EGFRvIII positive cells in GBM using a vaccine strategy similar to this proposal, also argues strongly for the potential of IDH-targeted immunotherapy. Moreover, the homogeneous expression of $IDH1^{R132H}$ and the apparent dependence on $IDH1^{R132H}$ for tumorigenicity may protect against the antigen escape seen with EGFRvIII-targeted vaccines.

Here we propose to leverage our experience targeting tumor-specific antigens to assess the potential of an IDH1 mutation-specific vaccine as a treatment for patients with tumors containing $IDH1^{R132H}$ mutations.

8 OBJECTIVES AND ENDPOINTS

Table 1. Objectives and Endpoints

	Objective	Endpoint	Analysis
Primary	To assess the safety of the PEP_{IDH1M} vaccine alone and in combination with adjuvant TMZ and/or XRT/TMZ in adult patients with progressive, resectable WHO grade II gliomas that are confirmed Grade II or transition to a higher grade glioma at the time of surgery.	The percentage of patients who experience an unacceptable toxicity defined as any Grade 3 toxicity at least possibly attributed to the vaccine (or vaccine + TMZ and/or RT) that does not resolve to baseline within 3 weeks, any Grade 3 hypersensitivity reactions requiring steroids, any Grade 4 toxicity, including neurologic events not due to progressive disease, or any life threatening-event not attributable to concomitant medication, co-morbid event, or disease progression.	See Section 15.4
Secondary	To assess the immunogenicity of the PEP_{IDH1M} vaccine with adjuvant TMZ using ELISpot.	Mean difference in the number of SFC per 10^6 lymphocytes for cells cultured with and without IDH1 peptide	See Section 15.5
Exploratory	To describe PFS and OS.	Proportion of patients alive without disease progression 6 months after initial vaccine treatment; median progression-free survival and median overall survival.	See Section 15.6
Exploratory	To determine if ELISpot results after vaccination predict PFS or OS.	Number of SFC per 10^6 lymphocytes above pre-vaccine baseline is a predictor of PFS or OS	See Section 15.6
Exploratory	To estimate radiographic response rate prior to surgical resection and PFS after resection using RANO criteria.	Proportion of patients with radiographic response (complete + partial response) observed prior to surgical resection. Proportion of patients alive without disease progression 6 months after resection.	See Section 15.6
Exploratory	To determine if tumors are IDH1 negative by immunohistochemical analysis and microarray analysis at the time of post-vaccine surgery.	Proportion of tumors that are IDH1 negative at the time of surgery	See Section 15.6
Exploratory	To characterize immunologic cell infiltrate in tumors at the time of post-vaccine surgery.	Median number of immunologic cell infiltrates at the time of surgery	See Section 15.6
Exploratory	To determine if tumor-specific circulating DNA containing the c.395G>A (R132H) mutation can be detected in plasma before, during, and after study drug treatment.	Detection of tumor-specific circulating DNA containing R132H mutation. Mean changes in R132H mutation from baseline.	See Section 15.6
Exploratory	To determine if 2-HG, a metabolite specifically produced by tumors with IDH R132H, can be detected in plasma by mass spectrometry before, during, and after study drug treatment.	Detection of 2-HG in plasma. Mean changes in 2-HG in plasma from baseline.	See Section 15.6
Exploratory	To explore the impact of PEP_{IDH1M} vaccination on genetic alternations, differential protein expressions or post-translational modifications (PTMs) using quantitative proteomic strategies in patient-derived samples.	Changes between baseline and surgery in genetic alternations, differential protein expressions and post-translational modifications (PTMs)	See Section 15.6

Exploratory	To demonstrate quantitative repeatable measures of metabolism change in IDH1+ gliomas via a standardized MRS protocol, patients will be scanned on the research MR scanner to establish same-day and longitudinal metabolite coefficient of variance and possible longitudinal metabolite changes.	Difference in levels of metabolism measured at same assessment. Changes from baseline in metabolism levels at each follow-up assessment.	See Section 15.6

9 INVESTIGATIONAL PLAN

9.1 Study Design

Subjects with radiographic and/or clinical progressive and resectable Grade II glioma with expression of $IDH1^{R132H}$ from the primary tumor (initial diagnosis) will be enrolled after signing informed consent. After consent has been signed, **all** subjects will undergo standard of care vaccination with 0.5 mL of Td (tetanus and diphtheria toxoids adsorbed) intramuscularly (I.M.) into the deltoid muscle to ensure adequate immunity to the tetanus antigen. The tetanus vaccine will be obtained through Duke ICS Pharmacy.

Eligible subjects will undergo a 1-2 hour leukapheresis for immune monitoring. Within 48 hours of leukapheresis, subjects will receive a vaccine site pre-conditioning as a single dose of Td toxoid (1 flocculation unit, Lf, in a total volume of 0.4 mLs saline). This will be administered one day prior to receiving PEP_{IDH1M} vaccine intradermally (i.d.) to the RIGHT groin area (as described below). The peptide vaccine is administered in the groin area approximately 10 cm below the inguinal ligament as vaccine # 1. Subsequent vaccines will be given on day 15 ±3 days (vaccine #2) and day 29 ±3 days (vaccine #3). These injections will occur without TMZ. Each injection of the peptide vaccine will be given half on the right groin and half on the left groin.

Seven to 12 days after the 3rd vaccine, subjects will undergo surgical resection of tumor, which is part of the SOC for these subjects. If possible, the provider will take a photo of the injection site at the pre-op appointment seven to twelve days after the 3rd vaccine to document the subject's reaction to the vaccine. Tumor samples from the surgical resection will be evaluated as outlined in the Exploratory Objectives.

Based on tissue obtained at surgical resection, subjects with stable histologic grade at recurrence will then be treated with vaccine and adjuvant TMZ at a targeted dose of 50-100mg/m^2/d for 21 days every 28 days for up to 12 cycles (please see Section 18.5 in the Appendices for TMZ). During monthly cycles of TMZ, subjects will receive the vaccine on day 22 (+ 2 days) for a maximum of 15 total vaccines (which includes the first 3 bi-weekly vaccines). Patients that have transitioned to a higher Grade brain tumor will receive TMZ and radiation therapy per standard of care and monthly vaccines.

All Adverse Events will be collected from time of consent until the end of the treatment phase of the study. The treatment phase of the study will end 1 month after the last vaccine (Section 12.3). Patients will be followed only for overall survival, progression-free survival, and subsequent therapies thereafter. For higher grade gliomas undergoing RT (please see Section 18.4 in the Appendices for SOC RT), vaccine #4 will be given during the SOC clinic visit prior to initiating RT, and vaccine # 5 will be given 4 weeks later (± 1 week) during RT. Vaccine #6 will be given at the end of RT during the SOC clinic visit to review MRI and begin post-RT TMZ, if MRI stable. Then, subjects in this treatment group will receive vaccines monthly on day 22 (+2 days) with post-RT cycles of TMZ to a maximum of 15 vaccines.

For immune monitoring and biomarker testing, subjects will have blood collected, as described in Section 12, during the following times: prior to vaccine therapy (at leukapheresis visit), prior to surgical resection (at the pre-op screening visit), prior to either RT/TMZ or first cycle of adjuvant TMZ (at SOC clinic visit) depending on tumor pathology, at vaccine # 6, and at End of Treatment (follow-up visit 1 month after vaccine #15; If a subject decides to stop participating in the study prior to vaccine #15, blood will be obtained at that time, if possible; If a subject progresses prior to vaccine #15, blood will be obtained at that time, if possible) .For genomic proteomic analyses, blood and tumor samples will be obtained as described below in Section 9.4.

Subjects will be imaged with contrast-enhanced MRI according to the SOC every 10 weeks (+/- 4 weeks) while on TMZ and afterward, per the treating neuro-oncologist's recommendation. RANO criteria will be used for assessment of pseudoprogression and tumor progression (please see Section 12.7.3 for further detail). Subjects demonstrating definitive progression will be removed from study.

In addition, to demonstrate quantitative repeatable measures of 2HG in IDH1+ gliomas, subjects will be imaged using magnetic resonance spectroscopy (MRS) at baseline (prior to vaccine #1 following consent), and up to 4 times prior to surgery (please see Section 9.5 below for further information).

As part of standard care for these subjects, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure, consent will be obtained separately. Subjects that have this procedure done within the Duke University Health System may be approached to participate in the Duke Brain Tumor Center Biorepository study (Pro00007434). Tissue obtained from subjects who consented to the Duke Brain Tumor Center Biorepository will be used to assess immunologic cell infiltration, antigen expression, and biomarkers for immunologic response.

9.1.1 Definition of Unacceptable Toxicity

Toxicities will be graded according to the NCI CTCAE version 4 criteria. An unacceptable toxicity is defined as any Grade 3 toxicity at least possibly attributed to the vaccine (or vaccine + TMZ +/- RT) that does not resolve to baseline within 3 weeks, any Grade 3 hypersensitivity reactions requiring steroids, any Grade 4 toxicity, including neurologic events not due to progressive disease, or any life–threatening event not attributable to concomitant medication, co-morbid event, or disease progression.

Please refer to the Potential Risks Section 12.8.2 of this protocol.

9.1.2 Concomitant Medications

Concomitant medications will be managed by the treating neuro-oncologist and recorded at each study visit by the study team.

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

The PEP_{IDH1M} vaccine dose that will be evaluated in this trial will be 500 µg with 150 µg of GM-CSF mixed 1:1 with Montanide ISA 51 administered intradermally as vaccine # 1. Subsequent vaccines will be given on day 15 ±3 days (vaccine #2) and on day 29 ±3 days (vaccine #3). The same dose will be administered on day 22 (+ 2 days) of each monthly cycle of adjuvant TMZ following surgical resection for those with confirmed Grade II. For those who transition to a higher grade glioma, they will be undergoing RT (please see Section 18.4 in the Appendices for SOC RT), thus, vaccine #4 will be given during SOC clinic visit prior to initiating RT, and vaccine # 5 will be given 4 weeks later (± 1 week) during RT. Vaccine #6 will be given at the end of RT during the SOC clinic visit to review MRI and begin post-RT TMZ, if MRI stable. Then, subjects in this treatment group will receive vaccines monthly on day 22 (+ 2 days) with post-RT cycles of TMZ to a maximum of 15 vaccines, as the confirmed Grade IIs.

The nearly universal expression of IDH1 in secondary GBMs and low-grade gliomas may make excellent anti-tumor immunotherapeutic targets. Vaccination and adoptive T-cell strategies in humans in other contexts have been safe and effective. Therapeutic TMZ induces a profound lymphopenia that may enhance anti-tumor vaccination responses when given during the homeostatic T-cell proliferation that occurs in response to lymphodepletion. Other peptide vaccines given to patients with high-grade gliomas during recovery from TMZ-induced lymphopenia have produced potent tumor-specific immune responses and have been well tolerated[9,17,81].

9.3 Rationale for Correlative Studies

While the primary goal of this study is to assess safety of the PEP_{IDH1M} vaccine, a secondary endpoint will be to determine whether this vaccine is capable of eliciting an immune response in patients with IDH1 mutation-expressing tumor. The primary measure of immunogenicity will be by ELISpot to the immunizing peptide. For the immune system to recognize the tumor specific IDH1 mutant, this mutant peptide must be presented by human leukocyte antigens (HLA) on the surface of a tumor cell for recognition by a T cell. There are a tremendous number of HLA alleles with variable expression from person to person. As certain peptides are able to bind with much greater affinity to certain HLA alleles, the ability of one individual to present the IDH1 mutant peptide will be dependent upon their unique set of HLA alleles and may differ greatly from another individual with differing HLA. Therefore, it is critical to know the HLA type of a patient receiving the peptide vaccine as this may reveal if certain HLA types are associated with enhanced vaccine induced immune responses. Such information may suggest certain HLA types present the mutant peptide in a superior fashion and may be valuable in stratifying patients for clinical trials using this vaccine. We

intend to identify HLA types from PBMCs taken at the leukapheresis time point. The purpose of collecting these data will be to determine whether there may be immune correlates that associate with clinical outcomes such as PFS and OS following vaccination with the PEP_{IDH1M} vaccine. Although this Phase I study is not designed to be definitive towards this end, these analyses will provide the initial data necessary to adequately power future studies. Any evidence of tumor response will be determined according to the Duke PRTBTC SOP (available upon request). RANO criteria[82] will be used for assessment of pseudoprogression. Tumor progression will need to be documented histologically, unless there are clinical contraindications, to exclude inflammatory responses presenting as radiographic or clinical changes, which could indicate potentially toxic or therapeutic responses and not tumor progression. Patients will be followed until death.

9.4 Rationale for Genomic Proteomic Analyses

IDH1 mutation is a critical factor in glioma classification and a key determinant of clinical outcome. First, though the effect of mutant IDH1 in tumor cells have been well-established at the genetic and epigenetic levels (e.g., closely associated with several other genes, and has a profound impact in DNA methylome), the effect of the IDH1 mutant on the proteomic level is unclear, thus presenting a great opportunity for understanding glioma pathogenesis and developing new therapeutics. Second, in addition to IDH1 mutations, other genetic alterations and their associated mutant peptides may serve as multi variant factors underlying patient treatment outcome. It is critical to have a bird's eye view of and dissect all the genetic and peptide components that help stratify patients for clinical trials.

We intend to identify genetic alterations, differential protein expressions or post-translational modifications (PTMs) via our comprehensive 'inside-out' quantitative proteomic strategies in patient-derived tumor samples. Genetic alterations, differential protein expressions or PTMs that have been identified will be further tested in all patients enrolled in this clinical trial. We propose to obtain the following specimens, from patients both before and after treatment, for both genomic and proteomic analyses.

(1) Tumor tissues – 500 mg fresh frozen tumor tissue will be requested, as well as tissues from previous surgery. If frozen tissues from previous surgery are not available, fifteen 5 μm sections of FFPE samples are requested. If available, these samples will be used for genomic and proteomic analysis.

(2) PBCs – to determine proteomic profiling with a focus on immune system, and also for immune monitoring and tumor circulating DNA detection. Twenty mLs of peripheral blood (3 yellow top tubes) is requested (1) before vaccine with the leukapheresis visit, (2) at the pre-op screening visit before surgery, (3) At the SOC clinic visit prior to starting the 1st cycle of TMZ for those who are confirmed Grade II, or at the SOC clinic visit prior to starting RT/TMZ for those who have transitioned to a higher grade glioma, (4) Vaccine #6, and (5) End of Treatment (follow-up visit 1 month after vaccine #15). If a subject decides to stop participating in the study prior to vaccine #15, blood will be obtained at that time (if possible). If a subject progresses prior to vaccine #15, blood will be obtained at that time (if possible). PBMCs and plasma will be separated. Plasma will used for the detection of IDH1 mutation before surgery.

<u>These experiments will:</u>

- Identify genetic alterations and changes in protein before and after treatment with peptide vaccine in tumor cells.
- Identify proteins showing differential expressions or PTMs in immune cells, with and without IDH1 mutant, and before and after IDH1 vaccination, with a potential of revealing how immune cells response to IDH1 mutant, before and following vaccination.

We will perform proteomics, exome sequencing and RNA-sequencing on the resected tumors (after three IDH1 vaccines). We will use patient samples before IDH1 vaccination as controls. Radiographic and clinical tumor progress, as well as IDH1 mutation status in patient's plasma will be recorded.

9.5 Single-Voxel MR Spectroscopy

To demonstrate quantitative repeatable measures of metabolism change in IDH1+ gliomas, patients will be scanned on the research MR scanner using standardized MRS protocol to assess same-day repeatability and to assess longitudinal metabolite changes.

The IDH1 mutation causes extreme overexpression of the onco-metabolite R-2-hydroxyglutarate (2HG) in all tumor cells. This can be non-invasively measured using clinical MRS methods. 2HG is a specific marker for IDH1 mutated gliomas because its concentration in 'wild' IDH1 gliomas is below the level of measurement of MRS.

Duke Radiology has a standard protocol for 2HG measures using a proven 2HG MEGA-LASER pulse sequence for Siemens MR scanners and Dr. Soher's open source "Vespa" software for spectral simulation and fitting of the MEGA-LASER data. The protocol is available for immediate use on our research scanner as a part of the Duke Radiology's Center for Advanced MR Development (CAMRD).

We will assess subjects at up to 5 time points: baseline (after consent and before PEP_{IDH1M} vaccine #1), and up to 4 times prior to surgery. This will provide between 6-8 weeks of tumor/vaccine interaction. A typical 2HG protocol consists of 12-15 minutes of anatomical MRI and ~15-30 minutes of MRS data acquisition. An IDH1+ patient can usually be processed in a 1-2 hour time slot.

In order to establish same-day coefficients of variance and/or optimized acquisition parameters, patients may be scanned twice within a time point. Patients will be positioned and scanned in the MR scanner, then completely removed and put back into the scanner, and then positioned and scanned a second time. If necessary for patient comfort, this evolution can be done immediately or with a short rest period so long as scanning is finished within the booked period of time. Longitudinal acquisitions will also be analyzed for longitudinal metabolite coefficients of variance and possible longitudinal metabolite changes.

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

For the initial monitoring of adverse events (see Section 15.4.2), evaluable patients will include the first 6 accrued patients who received 3 vaccinations or experienced an unacceptable toxicity due to one of the first 3 vaccinations.

For the final analysis of the primary objective (See section 15.4.2), evaluable patients will include all patients who received at least 6 vaccinations or experienced an unacceptable adverse event that was related to vaccine (or vaccine + TMZ).

Once the patient signs ICF, that subject will be considered "on study." Rationale for taking patient off protocol treatment will be documented. End of study is defined as 2 months following last vaccine for purposes of toxicity monitoring.

9.7 Early Study Termination

This study can be terminated at any time for any reason by the PI-sponsor. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 12.5. Section 12.6 describes procedures and process for prematurely withdrawn patients.

10STUDY DRUG

10.1 Names, Classification, and Mechanism of Action

The name of the drug will be PEP_{IDH1M} vaccine. The class of action is a biological.

10.2 Packaging and Labeling

Name
MRN
DOB
Drug: PEP_{IDH1M}
Lot #: Lot 001
Caution New Drug Limited By Federal Law To Investigational Use

10.3 Supply, Receipt, and Storage

The PEP_{IDH1M} will be manufactured under GMP conditions by Bachem Americas, Inc., and stored in the investigational pharmacy. Montanide is supplied by Seppic and stored at 4^{O}C in the investigational pharmacy.

GM-CSF (LEUKINE®; sargramostim) will be obtained from commercial supply, as a sterile, white, preservative-free powder lyophilized powder in a vial containing 250 mcg to be reconstituted in 0.5 mL of sterile water for injection.

Store GM-CSF under refrigeration at 2-8°C (36-46°F). Vials must not be frozen.

Lyophilized GM-CSF vials contain no antibacterial preservative, and therefore solutions prepared with Sterile Water for Injection, USP should be administered as soon as possible, and within 6 hours following reconstitution. The vial should not be re-entered or reused. Do not save any unused portion for administration more than 6 hours following reconstitution. Bacteriostatic Water for Injection, USP (0.9% benzyl alcohol) should NOT be used.

Aseptic technique should be employed in the preparation of all GM-CSF solutions. To assure correct concentration following reconstitution, care should be exercised to eliminate any air bubbles from the needle hub of the syringe used to prepare the diluent. During reconstitution of lyophilized GM-CSF, the diluent should be directed at the side of the vial and the contents gently swirled to avoid foaming during dissolution.

PEP_{IDH1M} will be stored at -80^{O}C, thawed, and inspected visually for particulate matter and discoloration prior to administration. If particulate matter is present or the solution is discolored, the vial should not be used. Before use, the vial should be gently swirled to ensure uniform mixing of the contents. Avoid excessive or vigorous agitation; do not shake.

Td is indicated for active booster immunization against tetanus, diphtheria, and pertussis as a single dose; substitute 1-time dose of Tdap for Td booster, then standardly boost with Td every 10 years. Please refer to section 7.2 on the use of Td in this protocol. This study agent Td (Tetanus diphtheria toxoid adsorbed) is supplied by Duke Pharmacy and stored at 4^{o}C.

10.4 Dispensing, Preparation and Administration

10.4.1 Td

The Td booster vaccine will be administered at the initial consent visit only after the subject has signed consent. Administration will be done as per standard of care and according to package insert instructions (please see appendices in Section 18) with 0.5 mL of Td (tetanus and diphtheria toxoids adsorbed) I.M. into the deltoid muscle. In addition, vaccine site pre-conditioning will be performed as a single dose of Td toxoid (1 flocculation unit, Lf, in a total volume of 0.4 mLs saline) administered i.d. to the RIGHT side of the groin one day prior to the first vaccine.

10.4.2 PEP_{IDH1M} Vaccine with GM-CSF and Montanide ISA 51

The PEPIDH1M vaccine and GM-CSF will be mixed with Montanide ISA 51 (shortened to PEPIDH1M vaccine for the other sections of this protocol) in the investigational pharmacy and will be delivered directly to the bedside under the supervision of the research nurse. Vaccines will be administered according to

protocol. The patient's name, Study ID, DOB, and MRN will be double verified prior to vaccine administration.

PEPIDH1M will be co-administered with GM-CSF and mixed 1:1 with Montanide ISA 51.

Lyophilized GM-CSF should be reconstituted with Sterile Water for Injection, USP. Bacteriostatic Water for Injection, USP (0.9% benzyl alcohol) should NOT be used. During reconstitution, the diluent should be directed at the side of the vial and the contents mixed by gently swirling to avoid foaming during dissolution. Avoid excessive or vigorous agitation; do not shake.

Each vial of lyophilized GM-CSF (250 mcg/vial) should be reconstituted with 0.43 ml of Sterile Water for Injection, USP 0.35 ml of reconstituted GM-CSF will be withdrawn, under aseptic conditions, using a hypodermic needle and syringe.

Each vial of PEPIDH1M will be thawed at room temperature PEPIDH1M should fully thawed within 0.5 to 3 minutes, with no remaining visible frozen drug or particulate. The resultant solution of PEPIDH1M (0. 4 ml of 2.0 mg/ml PEPIDH1M) is a clear and colorless solution.

The syringe with the previously withdrawn GM-CSF (0.35 ml volume containing 203 mcg GM-CSF), will be used to withdraw 0.35 mL of peptide. The aqueous peptide/GM-CSF is mixed 1:1 with 0.7 mL of Montanide ISA 51 according to the manufacturer's instructions. One mL of the mixture will be administered by intradermal injection (in divided doses) in the patient's groin.

After mixing, the PEPIDH1M/GM-CSF/Montanide mixture will contain approximately 500 mcg PEPIDH1M and 150 mcg GM-CSF in 1 mL of mixture.

PEPIDH1M, GM-CSF and Montanide ISA 51 contain no antibacterial preservative, and therefore solutions prepared with Sterile Water for Injection, USP should be administered as soon as possible. The peptide mixture can be stored for not more than 6 hours at 2-8°C and for not more than 3 hours at room temperature.

Each PEP_{IDH1M}/GM-CSF/Montanide immunization should be administered i.d. near the groin, divided into 2-8 separate injections, as tolerated. It is recommended that the injections be given approximately 10 cm below the inguinal ligament. At each administration session, half of the immunization mixture will be given on the right groin and half on the left groin. Vaccine injections must be performed using an appropriate needle for intradermal administration.

Following administration of the PEP_{IDH1M} vaccine, the patient should be retained for observation for a minimum of 30 minutes.

10.5 Compliance and Accountability

Upon receipt from the manufacture the PEP_{IDH1M} and the GM-CSF/Montanide will be stored in the investigational pharmacy in a temperature controlled, locked access controlled storage unit. All drug transfers, receipts, and disposals are recorded in the Web-based drug accountability system. The vaccine will be signed out and distributed by the investigational pharmacist. The Duke investigational pharmacy personnel use safe medication practices to reduce the risk of medication errors and adverse events when setting up study drug procedures. They are clearly labeled with the identity of the study drug and other control numbers.

10.6 Disposal and Destruction

Unused drug will be autoclaved.

11 SUBJECT ELIGIBILITY

11.1 Inclusion Criteria

1. Age ≥ 18 years.
2. $IDH1^{R132H}$ expression in primary tumor
3. Clinical and/or radiographic, progressive and resectable Grade II glioma.
4. Signed informed consent.
5. For females of child-bearing potential, negative serum pregnancy test at screening (within 48 hours prior to leukapheresis).

6. Women of childbearing potential and male participants must agree to practice adequate contraception.
7. KPS of ≥ 70.
8. CBC/differential with adequate bone marrow function as defined below within 2 weeks of enrollment:
    a) Absolute neutrophil count, ≥ 1500 cells/mm^3.
    b) Platelet count, ≥ 100,000 cells/mm^3.
    c) Hemoglobin ≥ 10 g/dl. (Note: the use of transfusion or other intervention to achieve Hgb ≥ 10 g/dl is acceptable.)
9. Adequate renal function as defined below within 2 weeks of enrollment:
    a) BUN ≤ 25 mg/dl.
    b) Creatinine ≤ 1.7 mg/dl.
10. Adequate hepatic function as defined below within 2 weeks of enrollment:
    a) Bilirubin ≤ 2.0 mg/dl.
    b) ALT ≤ 3 x normal range.
    c) AST ≤ 3 x normal range.

11.2 Exclusion Criteria

1. Prior invasive malignancy (except for non-melanomatous skin cancer) unless disease free for ≥ 3 years. (For example, carcinoma in situ of the breast, oral cavity, and cervix are all permissible.)
2. Metastases detected below the tentorium or beyond the cranial vault.
3. Severe, active co-morbidity, defined as follows:
    a. Unstable angina and/or congestive heart failure requiring hospitalization.
    b. Myocardial infarction within the last 6 months.
    c. Acquired Immune Deficiency Syndrome (AIDS) based upon current CDC definition; note, however, that HIV testing is not required for entry into this protocol. The need to exclude patients with AIDS from this protocol is necessary because treatments involved in this protocol may be significantly immunosuppressive.
    d. Major medical illnesses or psychiatric impairments that in the investigator's opinion will prevent administration or completion of protocol therapy.
4. Pregnant or lactating women, due to possible adverse effects on the developing fetus or infant due to study drug.
5. Prior allergic reaction to temozolomide.
6. Patients treated on any other therapeutic clinical protocols within 30 days prior to study entry or during participation in the study.
7. Patients with known hypersensitivity to GM-CSF, yeast-derived products, or any component of Leukine®.
8. Allergy or hypersensitivity to tetanus vaccine or any component of the tetanus vaccine.
9. Unable to undergo MRI imaging.

12 SCREENING AND ON-STUDY TESTS AND PROCEDURES

Progressive patients will be offered treatment options during this PRTBTC clinic visit. CMP and CBC with differential, and physical and neurologic examination with KPS score will be performed within 6 weeks prior to consent and may be taken from the previous standard of care Duke PRTBTC clinic visit. All subject data is standard of care evaluations that occur for all patients being seen in the PRTBTC, regardless of study entry. Screening examination must include pathology report from initial diagnosis to confirm IDH1$^+$ WHO Grade II glioma diagnosis with MRI showing progressive lesion located in resectable areas. Once consent is obtained, subjects will receive Td booster immunization I.M. Study-mandated leukapheresis is scheduled to occur followed by Td i.d. pre-conditioning scheduled 48 hours or less. Vaccine #1 is then given one day after Td pre-conditioning. Within 72 hours prior to undergoing the leukapheresis procedure, patients will have blood samples taken for the following tests as required by the Duke Apheresis Center: CBC w/diff, CMP, ionized Calcium, and β-HCG (for females of child-bearing potential). Total estimated blood volume required for these evaluations is 12-15 mLs. For patients without sufficient venous access for leukapheresis, a temporary central intravenous catheter may be inserted. To prevent the development of hypocalcemia from the citrate used for leukapheresis, all patients will be instructed to take oral Tums, 2

tablets three times a day and at bedtime the day before and the day of the leukapheresis procedure. Patients who have lower levels of calcium will be treated per Apheresis lab standard protocols under the direction of apheresis attending physician. An aliquot of 10^7 PBMCs will be collected from this leukapheresis and sent to UNC Center for AIDS Research Virology, Immunology, & Microbiology Core Laboratory at 250 Bell Tower Drive, Genome Sciences Building, Room 2158, Chapel Hill, NC 27599-7291. This blood sample will be sent to UNC for HLA assessment and analysis (please see Section 9.3 for rationale).

Subsequent vaccines will be given on day 15 ±3 days (vaccine #2) and day 29 ±3 days (vaccine #3). Seven to 12 days after the 3rd vaccine, subjects will undergo surgical resection which is part of the SOC for these subjects. Patients with re-confirmed Grade II will be treated with adjuvant TMZ and vaccine. Patients that have transitioned to a higher Grade brain tumor will receive TMZ and radiation therapy per standard of care and monthly vaccines. For higher grade gliomas undergoing RT, vaccine #4 will be given during SOC clinic visit prior to initiating RT, and vaccine # 5 will be given 4 weeks later (± 1 week) during RT. Vaccine #6 will be given at the end of RT during the SOC clinic visit to review MRI and begin post-RT TMZ, if MRI stable. Then, subjects in this treatment group will receive vaccines monthly on day 22 (+2 days) with post-RT cycles of TMZ to a maximum of 15 vaccines.

If possible, the provider will take a photo of the injection site at the pre-op appointment seven to twelve days after vaccine #3 to document the subject's reaction to the vaccine. The provider may take additional pictures of the injection site at other clinic visits at the discretion of the treating physician or Principal Investigator. There will be no identifiers on the photographs. The photographs will be taken by the subject's provider and may be uploaded to their electronic medical record or kept in a separate electronic or paper research file. The photographs will be uploaded to the electronic medical record by using electronic applications called Haiku for iPhones or Cantu for iPads. These applications allow for the photograph to be uploaded directly to the electronic medical record without the photographs being stored on the device taking the picture. A ruler will be included in the photo to allow measurement of the size of the injection site reaction.

To demonstrate quantitative repeatable measures of 2HG change in IDH1+ gliomas, patients will be scanned on the CAMRD MR scanner using a an MRS protocol. We will acquire MRS data at up to 5 time points: baseline (after consent and before PEP_{IDH1M} vaccine #1) and up to 4 times prior to surgery (please refer to Section 9.5 for more detail).

IDH1 R132H biomarker component of the protocol and immune monitoring:

a) Study of tumor-specific circulating nucleic acid from plasma of patients with gliomas with IDH1 R132H:

We plan to isolate circulating nucleic acids from the plasma of patients affected by glioma and attempt to detect tumor-specific circulating DNA containing the c.395G>A (R132H) mutation. We will follow the protocol as described by Boisselier, et al., Neurology 2012[83] for plasma collection. Twenty mLs of blood will be the appropriate amount for our analyses (should yield ~10mL of plasma). At the same time, we will collect peripheral blood for immune monitoring. Ideally, we would like to have these blood samples collected:

- (1) Prior to initiating vaccine therapy at the leukapheresis visit,
- (2) Prior to surgical resection at the pre-op screening visit,
- (3) Prior to first cycle of TMZ at the SOC clinic visit (confirmed Grade IIs), or prior to RT/TMZ at SOC clinic visit (higher Grade Gliomas),
- (4) Vaccine #6
- (5) End of Treatment (follow-up visit 1 month after vaccine #15). If a subject decides to stop participating in the study prior to vaccine #15, blood will be obtained at that time (if possible). If a subject progresses prior to vaccine #15, blood will be obtained at that time (if possible).

b) Study of 2-Hydroxyglutarate levels in patients with gliomas with IDH1 R132H:

The second approach to be used will be a mass spectrometry-based approach for detection of 2-hydroxyglutarate (2-HG), a metabolite specifically produced by tumors with the IDH1 c.395G>A (R132H) mutation. This metabolite can be sensitively detected by mass spectrometry and has been found to be elevated in the tissue of IDH1 R132H-containing gliomas as well as in the plasma of other solid tumors containing the same mutation (cholangiocarcinoma, subsets of breast carcinoma). A small portion of the

previously mentioned isolated plasma will be used for a LC-MS/MS based approach for sensitive detection of circulating 2-HG levels.

Tumor tissue – we are planning to obtain a snap-frozen tumor tissue sample after the surgical resection for possible presence of IDH1 mutation and gene up-regulation.

Tumor Sampling Analyses:

At the time of surgical resection, excess tumor tissue will be obtained through the Duke Biorepository, and IHC analyses will be performed to assess immunologic infiltrate which will be identified by cell type and reactivity state.

Table 2. Screening and On-Study Tests and Procedures

	Screening	Leukapheresis[2]	One day prior to vaccine #1	Vaccine #1	Vaccine #2	Vaccine #3	Pre-Surgery	Surgery[7]	Post-Surgery Pre TMZ visit[9]	Radiation Therapy and TMZ[8]	TMZ Cycles 1-12 [9,10]	End of Treatment (1 month after Vaccine #15)	Progression *followed for survival
ICF	X												
Medical History/ Baseline symptoms	X		X										
Tumor pathology	X							X					X *If possible*
Blood for Immune monitoring/ Biomarker testing[5]		X *3 red*					X *17 yellow, 1 red*		X *17 yellow, 1 red*	X *17 yellow, 1 red* At vaccine #6	X *17 yellow, 1 red* At vaccine #6	X *17 yellow, 1 red*	
CBC/diff and CMP[3]	X	X					X		X	X[8]	X	X	
Beta HCG *(for WOCBP)*		X					X		X				
Ionized Ca		X											
TD Booster[4]	X												
MRI[1]	X						X	X			X *every 2-3 months*		
MRS 2HG[6]		X		X	X	X	X						
Pre-conditioning TD[11]			X										
TMZ 50-100 mg/m^2 21 of 28 days x 12 cycles											X		
Radiation Therapy[8]										X[8]			
TMZ 75mg/m^2/day for 42 days[8]										X[8]			
Vaccine[10] Total of 15 vaccines				X	X	X				X[10]	X[10]		
Physical Exam, Neuro exam, KPS	X		X		X	X			X	X[8]	X	X	
Con Meds	X	X	X	X	X	X	X	X	X	X[8]	X	X	
AE/SAE collection	X	X	X	X	X	X	X	X	X	X[8]	X	X	
Photo of injection site (Optional to subject)[12]							X *If possible*						

[1] Initial standard of care MRI report to confirm eligibility must be performed within 10 weeks (+/- 4 weeks) of signing consent. MRI or contrasted CT, every 10 weeks (+/- 4 weeks) per PRTBTC standard of care (may be adjusted by the treating neuro-oncologist). MRI will be done pre- and post-surgery per surgeon discretion and standard of care.

[2] 1-2 hour leukapheresis within 2 weeks of enrollment. Within 72 hours of leukapheresis, patients will have blood samples taken for the following tests as required by the Duke Apheresis Center: CBC, CMP, ionized Calcium, and β-HCG (for females of child-bearing potential). Total estimated blood volume required for these evaluations is 12-15 mLs. An aliquot of PBMCs from this pheresis will be sent to UNC for HLA analysis.

[3] CMP and CBC (with differential) to confirm eligibility, and as per standard of care prior to and following surgery, and during monthly cycles of TMZ. CBC and CMP will be repeated along with a physical examination 1 month following last vaccine to assess for delayed toxicity to vaccine therapy.

[4] Td booster will be given I.M. into the deltoid muscle after consent on all subjects.

[5] Immune monitoring and Biomarker testing will be drawn before initiation of vaccine therapy (at leukapheresis visit), prior to surgical resection, prior to RT/TMZ and vaccine #4 or cycles of TMZ (depending on tumor pathology), at vaccine # 6, and at end of treatment (at the follow-up visit 1 month after vaccine #15, at the time come off study treatment, or at progression, whichever comes first). Blood for immune monitoring and Biomarker testing will be placed in 14 yellow containing ACD and 1 red vacutainer tube for a total of 90 mLs delivered to the BTIP lab for processing (3 red tops will be drawn with leukapheresis instead). Genomic proteomic analyses will include tumor sample at surgery and 20 mLs of blood (3 yellow ACD tubes) drawn at leukapheresis visit, at the pre-op screening visit prior to surgery, and depending on tumor pathology at the SOC clinic visit prior to starting either RT/TMZ (and receiving the 4th vaccine) or the 1st cycle of TMZ, at vaccine #6, and at end of treatment (at the follow-up visit 1 month after vaccine #15, at the time come off study treatment, or at progression, whichever comes first).

[6] To demonstrate quantitative repeatable measures of metabolism change in IDH1+ gliomas via a standardized MRS protocol, patients will be scanned on the research MR scanner at up to 5 time points per subject: baseline (after consent and before PEP_{IDH1M} vaccine #1), and up to 4 times prior to surgery. Up to 5 MRS scans can be scheduled between consent and surgery, but they are not required to be scheduled on the same day as PEP_{IDH1M} vaccines #1-3.

[7] Tissue obtained will be used to reconfirm the Grade and assess immunologic cell infiltration, antigen expression, and biomarkers for immunologic response, specifically IDH1 T cell response.
[8] Only for those whose pathology reveals transition from Grade II to Grade III at time of surgery.
[9] Monthly cycles of TMZ will be initiated and adjusted at the discretion of the treating neuro-oncologist.
[10] For higher grade gliomas undergoing RT, vaccine #4 will be given during SOC clinic visit prior to initiating RT, and vaccine # 5 will be given 4 weeks later (± 1 week) during RT. Vaccine #6 will be given at the end of RT during the SOC clinic visit to review MRI and begin post-RT TMZ, if MRI stable. Then, subjects in this treatment group will receive vaccines monthly on day 22 (+2 days) with post-RT cycles of TMZ to a maximum of 15 vaccines. Subjects with a stable grade glioma at time of surgery will receive vaccines #4-15 monthly on day 22 (+2 days) of TMZ cycles.
[11]Tetanus pre-conditioning will be done i.d. into the right groin one day before vaccine #1, as described in protocol.
[12] Photo may be repeated at additional clinic visits at discretion of the treating physician or Principal Investigator

12.1 Screening

Screening will take place at the Duke PRTBTC clinic visit. An informed consent must be signed by the patient before any study-specific procedure takes place. CMP and CBC with differential, and physical and neurologic examination with KPS score will be performed within 6 weeks prior to consent and may be taken from the previous standard of care Duke PRTBTC clinic visit. All subject data is standard of care evaluations that occur for all patients being seen in the PRTBTC. If the subject is considered a screen failure prior to initial protocol treatment, the source documents for electronic data entry will be stored in EPIC Electronic Medical Record or, if in paper form, a locked cabinet in a locked room in the PRTBTC.

12.2 Treatment Period

Patients that have consented onto this study will receive 3 vaccines as indicated above and then undergo surgical resection. Unless a patient is determined at the time of surgery to have transitioned to a higher grade brain tumor, vaccines will then continue monthly along with adjuvant cycles of TMZ on day 22 (+ 2 days) for a maximum of 15 vaccines, unless the patient has tumor progression prior to completing 12 cycles of TMZ. Subjects that have transitioned to a higher grade glioma at the time of surgery will continue on study treatment, however, they will need to undergo standard of care chemoradiation therapy. Therefore, the vaccines will be given before, during and following RT as described above. Subjects will return one month following the last vaccine for blood work (CBC and CMP) and clinical assessment to monitor for delayed toxicity.

12.3 End of Treatment

One month after final vaccination or once all the blood for immune monitoring has been collected following progression (whichever comes first), the treatment phase of the study will be completed.

12.4 Follow-up Period

Once the treatment phase has been completed, patients will be followed for OS, PFS, and subsequent therapies and data recorded by the study team. For recording of subsequent therapies, the type, agent, and duration of therapy(ies) that the subject receives will be recorded.

12.5 End of Study

End of study will be declared after the death of the last subject on the study. However, one month after the last enrolled subject receives the final vaccine, the investigational vaccine treatment will be considered as complete and collected data will be analyzed for safety and toxicity.

Subjects will be followed for OS, PFS, and subsequent therapies and data will be updated. Furthermore, in case of disease progression, every attempt will be made to obtain tissue for immune monitoring (if biopsy or resection performed).

12.6 Early Withdrawal of Subject(s)

12.6.1 Criteria for Early Withdrawal

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

- Progressive disease as documented by MRI or physical examination at any time after the initiation of post-op immunotherapy or post-RT immunotherapy (for those who've transitioned to a higher grade at time of surgery)

- Development of unacceptable toxicity, as defined in section 9.1.1
- Pregnancy
- Need for corticosteroids > 4mg/day at time of 1st vaccine
- Upon request of the subject
- If, in the investigator's medical judgment, further participation would be injurious to the subject's health or wellbeing
- Development of intolerable symptoms
- Protocol deviation
- Administrative reasons, such as a major violation of the clinical trial protocol
- Non-compliance of the subject
- Clinical decline or inability to be weaned off steroids following surgery
- Failure to undergo surgery following the 3rd vaccine
- Failure to tolerate TMZ therapy.

12.6.2 Follow-up Requirements for Early Withdrawal

Subjects treated on this study that are withdrawn by the PI for any of the aforementioned reasons will continue to be followed for survival by the study coordinator until death or are lost to follow up. Subjects that voluntarily withdraw will be asked permission to follow for survival. Subjects that are withdrawn prior to vaccine treatment for any of the aforementioned reasons will be considered eligibility failures and thus will not be followed for survival.

12.6.3 Replacement of Early Withdrawal(s)

Subjects who voluntarily withdraw prematurely (i.e. before receiving the first 3 vaccinations) or who are withdrawn by the PI prior to treatment will be replaced unless the subject experienced an unacceptable toxicity as defined in section 9.1.1.

12.7 Study Assessments

12.7.1 Medical History

Medical history will be obtained from the Duke electronic medical records system and from the subject and/or family at the screening visit and reviewed at each study visit. This data may include the following:

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

12.7.2 Physical Exam

Vital signs and physical and neurologic examinations will be assessed and recorded along with a KPS score prior to enrollment, at the Td pre-conditioning i.d. visit, and at each vaccine visit (with the exception of vaccine #1 where only vital signs will be assessed).

12.7.3 Use of Antihistamines

Subjects will be advised to avoid antihistamine use 48 hours prior to each vaccine administration, the day of vaccine administration, and for 48 hours following each vaccine administration. If the subject has a pre-existing condition that requires antihistamine usage, the PI and the treating oncologist will decide if it is safe and appropriate for the subject's antihistamines to be held before and following vaccine administrations.

12.7.4 Radiologic Evaluations

Patients with progressive Grade II LGGs will be imaged by MRI as per standard of care for eligibility and baseline measurements, and to assess progression prior to vaccine therapy. Although the purpose of this study is not to detect tumor responses, any evidence of tumor response will be determined according to the Duke PRTBTC SOP (available upon request). RANO criteria[82] will be used for overall assessment of tumor response and pseudoprogression versus progression. Progression by RANO response criteria is defined as follows: 1) New contrast-enhancing lesion outside of radiation field on decreasing, stable, or increasing doses of corticosteroids; 2) ≥ 25% increase in the sum of the products of perpendicular diameters between the first post radiotherapy scan, or a subsequent scan with smaller tumor size, and the scan at 12 weeks or later on stable or increasing doses of corticosteroids; 3) Clinical deterioration not attributable to concurrent medication or comorbid conditions is sufficient to declare progression on current treatment but not for entry onto a clinical trial for recurrence; 4) For patients receiving antiangiogenic therapy, significant increase in T2/FLAIR non-enhancing lesion may also be considered progressive disease. The increased T2/FLAIR must have occurred with the patient on stable or increasing doses of corticosteroids compared with baseline scan or best response after initiation of therapy and not be a result of comorbid events (e.g., effects of radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects). Since some of these subjects will not be undergoing radiation therapy, and the possibility to exhibit pseudoprogression is likely due to inflammation from immune response at the site of the tumor, RANO criteria will be used to monitor for pseudoprogression versus progression. If pseudoprogression is suspected, the subject will continue with vaccine therapy for a minimum of 2-3 subsequent months so long as subject remains clinically and radiographically stable compared to the MRI showing suspected pseudoprogression as long as the following criteria are met: 1) the subject experiences investigator-assessed clinical benefit (i.e., no new clinical symptoms contributed to disease progression) and 2) the subject is tolerating the study treatment (i.e., no expected toxicities higher than grade 3 or unexpected toxicities). If the subject continues to do well beyond the 3 months, they will continue on study as planned.

12.8 Risk/Benefit Assessment

12.8.1 Potential Benefits

The potential benefits may include reduction 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.

12.8.2 Potential Risks

Td

Injection of Td toxoid may produce inflammation, edema, induration, erythema, or pruritus at the injection site. Administration may cause occasional pain and discomfort up to three days after the vaccine is given.

Allergic Reactions to PEP_{IDH1M}

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.

Injection Site

EMLA ® cream, or equivalent topical analgesic, can be offered to subjects who experience local pain at the injection site.

Cerebral Edema

Cerebral edema may be secondary to the disease process itself, the surgical procedure, radiation therapy, 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 and after PEP_{IDH1M} injections. Patients will be monitored throughout the course of the study.

Infection

The PEP_{IDH1M} vaccine injections may include the risk of infection due to potential contamination of the peptides 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 peptides will be strictly tested for sterility prior to each injection. The risk of infection due to potential contamination of the peptides 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, peptides 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.

Delayed Autoimmune Diseases

It is possible that delayed autoimmune disease(s) may develop as a result of injection with PEP_{IDH1M} vaccine. 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 peptide vaccination in glioma patients has not demonstrated evidence of autoimmunity in treated patients. Furthermore, the doses of TMZ used in this study for induction of lymphopenia are standard doses administered to patients with gliomas. It therefore, is unknown what the risk of delayed autoimmune disease is for this study.

Phlebotomy

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

Leukapheresis

As with any donation of blood, a variety of minor reactions may occur with leukapharesis, 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 gm) will be given to minimize the risks of hypocalcemia, fluid supplementation will be given to minimize hypotension.

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 if they are unable to undergo MRI imaging. Patients will also be checked to make sure that they do not bring any metal objects into the MRI facility. Dental fillings are less affected by the magnetic fields generated and are therefore permitted. It will be asked that patients let the physicians conducting this study know of any metal in their bodies other than dental fillings.

Allergic Reactions to Contrast Agents

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

Radiation Therapy

Early side effects of radiation include hair loss, scalp redness, inflammation of the ear canals, and fatigue. In a few patients, radiation contributes to headaches or nausea. Rarely, tumors may swell during radiation causing neurological symptoms such as weakness on one side, visual loss, or changes in mental function. There is a small chance of long-term effects from radiation, occurring months or years after completion. These may include worsening of mental function, hearing, vision, strength and coordination. Rarely, radiation may cause the development of benign or malignant tumors around the brain or skull. It is unknown whether temozolomide increases the risk of any of the early or late side effects of radiation.

Temozolomide

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

GM-CSF

Injection of GM-CSF may increase the risk of infection, lower platelets, or cause fluid retention. GM-CSF also may result in an allergic reaction, which could include redness and swelling at the injection site, itching, hives, flushing, syncope, 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.

Montanide ISA 51

Montanide ISA 51 adjuvant is well tolerated. Local reactions may include granuloma, local pain, tenderness and erythema. Montanide ISA 51 may also cause flu like symptoms, nausea and vomiting.

Unknown Risks

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

13 SAFETY MONITORING AND REPORTING

The PI is responsible for the identification and documentation of adverse events and serious adverse events, as defined below. At each study visit, the PI or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred.

13.1 Adverse Events

An AE is any untoward medical occurrence in a subject receiving study drug and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the PEP_{IDH1M} and GM-CSF, whether or not related to use of the vaccine. Abnormal laboratory findings without clinical significance (based on the PI's judgment) should not be

recorded as AEs. But laboratory value changes that require therapy or adjustment in therapy are considered adverse events. All lab values that are not deemed AEs by the PI will be noted to be Non-Clinically Significant in the subject record and source documents.

From the time the subject signs the informed consent form through the End of Treatment visit (as defined in Sections 12.3, 12.5 and 12.6), all AEs must be recorded in the subject medical record and electronic adverse events case report form.

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

Attribution of AEs will be indicated as follows:

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

13.1.1 Reporting of AEs

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

13.2 Serious Adverse Events

An AE is considered “serious” if in the opinion of the investigator it is one of the following outcomes:

- Death,
- A life-threatening adverse event,
- A congenital anomaly or birth defect,
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption in the ability to conduct normal life functions.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgement, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Furthermore, an important medical event that is not otherwise an SAE may be considered serious if the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

13.2.1 Reporting of SAEs

All SAEs should be reported immediately to Dr. Katherine Peters (Pager: 919-970-7591) or her designee (919-684-8111) and to the FDA. Fatal or life-threatening, unexpected adverse events will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the PI-sponsor followed by as complete a report as possible within 8 additional calendar days. Serious, unexpected adverse events that are not fatal or life-threatening will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the sponsor.

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

13.3 Safety Oversight Committee

The Duke Cancer Institute Safety Oversight Committee is responsible for annual data and safety monitoring of DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent DSMB. The primary focus of the Safety Oversight Committee is review of safety data,

toxicities and new information that may affect subject safety or efficacy. Annual safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The Safety Oversight Committee in concert with the DCI Monitoring Team (see Section 14.1 for Monitoring Team description) oversees the conduct of DUHS cancer-related, sponsor-investigator therapeutic intervention and prevention intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, SOPs, GCP, and applicable regulatory requirements.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring

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

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of DUHS and DCI leadership, the DCI Cancer Protocol Committee, the Safety Oversight Committee, the sponsor, the Principal Investigator, or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, including but not limited to the National Institute of Health, National Cancer Institute, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

14.2 Audits

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

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

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

14.3 Data Management and Processing

14.3.1 Study Documentation

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

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

14.3.2 Data Management

The subject's medical records will be the primary source document for the study. Source documents include all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical investigation used for reconstructing and evaluating the investigation[1]. Source documentations may also include paper eligibility checklists, data flowsheets, patient reported outcomes and other paper documents. The PI, study coordinator, study research nurse, data management team and all associated study key personnel, are permitted to make entries, changes, or corrections in the source documents or database per the study delegation of authority log.

Errors on the source documents will be crossed out with a single line, and this line will not obscure the original entry. Changes or corrections will be dated, signed, initialed, and explained (if necessary). Database changes will be tracked via electronic trail automatically.

14.3.3 Data Management and Data Verification

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

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

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries.

14.3.4 Coding of Adverse Events

All adverse events will be coded using CTCAE (version 4.0).

14.3.5 Study Closure

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

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

[1] In 21 CFR 312.62(b), reference is made to records that are part of case histories as "supporting data"; the ICH guidance for industry *E6 Good Clinical Practice: Consolidated Guidance* (the ICH E6 guidance) (available at http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm or http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm) uses the term "source data/documents." For the purpose of this guidance, these terms describe the same information and have been used interchangeably.

15 STATISTICAL METHODS AND DATA ANALYSIS

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

15.1 Analysis Sets

Subject to the availability of data, all patients who receive any protocol treatment will be included in analyses addressing secondary objectives. Statistical analyses for the primary objective will exclude patients who terminate protocol treatment prematurely (i.e. less than 6 vaccinations) without an unacceptable toxicity.

15.2 Patient Demographics and Other Baseline Characteristics

Summaries of clinical and socio-demographic characteristics will be generated for all patients who receive vaccine treatment. Categorical descriptors will be summarized using frequency distributions; whereas, interval variables will be summarized using percentiles, as well as means and standard deviations.

15.3 Treatments

A frequency distribution will be generated for the number of vaccines received by patients.

15.4 Primary Objective

To assess the safety of the PEP_{IDH1M} vaccine alone and in combination with adjuvant TMZ and/or RT in adult patients with progressive, resectable WHO Grade II gliomas that are confirmed Grade II or transition to a higher grade glioma at the time of surgery.

15.4.1 Variable

The percentage of patients with an unacceptable toxicity will be estimated, where unacceptable toxicity is defined as any Grade 3 toxicity at least possibly attributed to the vaccine (or vaccine + TMZ and/or RT) that does not resolve to baseline within 3 weeks, any Grade 3 hypersensitivity reactions requiring steroids, any Grade 4 toxicity, including neurologic events not due to progressive disease, or any life threatening-event not attributable to concomitant medication, co-morbid event, or disease progression.

15.4.2 Statistical Hypothesis, Model, and Method of Analysis

Up to 28 patients will be enrolled and treated with the goal of accruing 20 patients that will receive at least 6 vaccines (3 pre-surgery and 3 post-surgery). Less than 20% of patients are expected to drop out between enrollment and the 6^{th} vaccination.

Initial Study Monitoring For Adverse Events: Initially 6 subjects will be accrued to the study after which accrual will be suspended until the last patient enrolled undergoes surgical resection to review the toxicity experienced by these subjects during the first 3 vaccinations. During this period of time, some of the 6 patients may have initiated post-surgery treatment with TMZ and IDH1 vaccine. If 2 or less of these subjects experience an unacceptable toxicity, accrual will continue. Otherwise, modifications of the protocol will be considered before accruing additional subjects. If a patient who has not experienced an unacceptable toxicity does not complete 3 vaccinations, that patient will be replaced for initial study monitoring.

Overall Adverse Event Monitoring: Rigorous monitoring of long- and short-term toxicities in a manner similar to that found in many phase I dose-escalation studies would require accrual suspension between stages of patient accrual while relevant data matures. After the initial 6 patients are accrued and safely receive vaccine treatment, accrual will not be suspended to formally assess the toxicity profile unless the following guidelines are satisfied. Rather, the study will be monitored continuously for the occurrence of unacceptable adverse events.

Once accrual is resumed after the initial monitoring of the first 6 subjects, aggregate summaries of adverse experiences will be generated and reviewed by the clinical team every 6-12 months. The following are considered adequate documentation of review:

- PI review of the aggregate summary of adverse events that is generated every 6 months for the DSMBplus meeting.
- PI signature of the collective AE listing after review with the clinical research team. As described in Section 13.1.1, the PI will periodically review the collective adverse event listing with the clinical research team with the intention of identifying any trends or patterns in toxicity on a monthly basis.

Tabulated below are the conditions under which accrual will be temporarily suspended and data carefully reviewed to determine the appropriate action, including permanent study termination, continuation with patient accrual after appropriate amendment, or continuation with patient accrual with no modification of the protocol (Table 3). Accrual will also be suspended whenever a death occurs that is possibly, probably, or definitely related to treatment with vaccine alone or vaccine and TMZ.

Table 3. Conditions under Which Accrual will be Suspended

Number of patients accrued	Number of patients with unacceptable toxicity requiring accrual suspension
6-7	≥3
8-10	≥4
11-14	≥5
>14	≥5

These guidelines have not been adjusted for differential length of follow-up of accrued patients. The probability of accrual suspension as a function of the true unacceptable toxicity rate is tabulated below based upon simulation studies (Table 4). These statistics were generated assuming toxicity outcome was known at the time of accrual, and ignored issues such as time to toxicity, accrual rate, and length of follow-up.

Table 4. Probability of Accrual Suspension as a Function of the True Unacceptable Toxicity Rate

Underlying unacceptable toxicity rate	Probability of accrual suspension	Expected # of Patients
0.01	0.0002	20
0.05	0.005	19.9
0.1	0.034	19.6
0.15	0.12	18.8
0.2	0.27	17.5
0.25	0.46	15.6
0.3	0.65	13.6
0.35	0.80	11.7
0.4	0.90	10.0
0.45	0.96	8.6
0.5	0.98	7.8

Though few or no unacceptable toxicities are expected during pre-surgery vaccine treatment, unacceptable toxicities possibly attributable to either TMZ or vaccine post-surgery are expected. Yung[84] reports that 18% of patients treated with TMZ alone experienced a grade 3 or 4 adverse event. Assuming vaccine does not increase the chances of unacceptable toxicity and an unacceptable toxicity rate of 18% with TMZ, the probability of study suspension due to excessive unacceptable toxicity is 0.21. If the chances of unacceptable toxicity increase the overall true unacceptable toxicity rate, then the chances of study suspension due to excessive unacceptable toxicity increases to 0.80 with an underlying unacceptable toxicity rate of 35%.

Any patient who discontinues vaccination prior to the receipt of 6 vaccines will not be evaluable for the primary endpoint, unless the patient experienced an unacceptable toxicity. Per Section 12.6.3, these patients will be replaced. Patients who are determined at the time of surgery to have transformed from

Grade II to a higher grade will receive TMZ and radiation therapy per standard of care and monthly vaccines and will be included in the final analysis of the primary objective.

15.4.3 Handling of missing values, censoring, and discontinuations

If a patient does not receive any vaccine treatment, they will be excluded from all analyses.

15.5 Secondary Objective

An important secondary goal of this study is to assess the immunogenicity of the PEP_{IDH1M} vaccine alone and in combination with adjuvant TMZ using ELISpot. Levels of immune response will be examined after 3 vaccinations (i.e., pre-surgery) as well as after 3 additional post-surgery vaccinations. In previous research, the mean (± SD) number of SFC per 10^6 lymphocytes for cells cultured with no peptide in previous qualified assays has been 4.5 ± 3.6. The mean (± SD) response for cells cultured with an irrelevant peptide (i.e., actin) has been determined to be 4.2 ± 6.3. Values for cells cultured with IDH1 peptide, after subtraction of counts from cells cultured with no peptide, will be determined. A response will be considered positive if this value is greater than 20 SFC per 10^6 lymphocytes after determining the level of detection in the IDH ELISpot. With 20 patients, there is 80% power to detect a difference of 4.17 SFC per 10^6 lymphocytes between response for cells cultured in IDH1 peptide and those cultured without any peptide. This power calculation conservatively assumes the response to IDH1 peptide and no peptide to be independent, and did not adjust for multiplicity.

15.6 Exploratory Objectives

Exploratory objectives include: (1) estimation of overall survival and progression-free survival, (2) examination of the effect of ELISpot results on subsequent OS or PFS, (3) estimation of the radiographic response rate, (4) determination of whether tumors are IDH1 negative at surgery, (5) characterization of immunologic cell infiltrate, (6) determination of whether IDH R132H can be detected in plasma and whether the study treatment will affect the levels detected, (7) determination of whether IDH 2HG can be detected in plasma and whether the study treatment will affect levels detected, (8) genomic and proteomic analyses, and (9) an assessment of metabolic imaging.

1. The Kaplan-Meier estimator will describe the survival, PFS from vaccine #1, and PFS post-resection of patients treated with PEP_{IDH1M} vaccine. Survival is defined as the time between first vaccine and death, or last follow-up if the patient remains alive. PFS from vaccine #1 is defined as the time between vaccine #1 and initial progression or death, or date of last follow-up if the patient remains alive without disease progression. PFS post-resection is similarly defined but starting at resection.
   The proportion of patients alive without progression 6 months after resection will be estimated from the Kaplan-Meier estimator.
2. Cox proportional hazards model will be used to explore the association between ELISpot outcome and subsequent OS or PFS. The radiographic response rate (complete response + partial response) will be estimated with a 95% confidence interval.
3. The proportion of tumors that are IDH1 negative at the time of surgery will be estimated, with a 95% confidence interval.
4. Means and standard deviations, as well as quantiles, will be used to describe the distribution of immunologic cell infiltrates found in tumors at the time of surgery.
5. Descriptive statistics will be used to describe the changes over time in the level of IDH R132H as measured from tumor-specific circulating DNA.
6. In a similar manner, changes over time in levels of the 2-HG metabolite as measured by mass spectrometry will be described.
7. Genomic and proteomic analyses of the pre-treatment pathologic specimen and the post-vaccination surgical sample will be conducted. Changes in genetic alterations, differential protein expressions and PTMs will be described. If statistical tests are conducted, appropriate adjustment for multiplicity will be considered.
8. Measures of metabolism in IDH1+ gliomas will be assessed at several time points. Paired comparison (e.g. paired t-tests) will be used to assess whether same-day measures on a patient are the same. Assuming repeatability, changes over time in levels of metabolism will be described.

Additional exploratory analyses may be conducted. Among these additional exploratory analyses will be an examination of the relationship among changes in metabolism, genomic expression, 2-HG metabolite, and pathologic expression of IDH.

15.7 Interim Analysis

Efficacy: No interim efficacy analysis is planned.
Safety: See section 15.4.2.

15.8 Sample Size Calculation

See section 15.4.2.

16 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

16.1 Regulatory and Ethical Compliance

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

16.2 DUHS Institutional Review Board and DCI Cancer Protocol Committee

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

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form. The CPC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e., amendments affecting subject population, inclusion/exclusion criteria, agent administration, statistical analysis, etc.).

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

16.3 Informed Consent

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

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

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

The prospective participant will have as much time as he/she may need to make an informed decision about the study and all treatment related questions will be answered. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his/her family, if so desired by the patient. Before, during, and after the consent is signed, the research team and investigators will be available in person and by phone to answer any questions the participants may have. Any and all other available treatment options are offered to the patient in order to avoid undue influence. Participants are not offered compensation for this study in order to avoid any monetary coercion/influence.

For those who cannot read or are blind, the consenter will read the consent form verbatim to those who are illiterate or to those who are blind in the presence of a witness. If the subject decides to participate, they will sign the consent form, or if they are unable to sign, make another kind of mark (like an X) to indicate consent. The person obtaining consent will document at the bottom of the consent form that the consent form was read out loud to the patient by (name of person obtaining consent). If an X or mark is used instead of a signature, the person obtaining consent will note on the consent form that the subject wrote a mark or X instead of a signature. The witness will sign the consent form. Subjects who do not read/understand English may be potentially enrolled following DUHS HRPP policy, including obtaining IRB approval of either a short form or long form consent translation.

All of the potential subjects are patients of the PI and the PRTBTC. Patients will be recruited for this study as follows: Upon determination that a patient's tumor histology and radiographic findings are compatible with the eligibility criteria of this protocol, the clinical study will be briefly explained to the patient by the principal investigator (PI) or colleague. If the patient indicates interest in study participation, patient education sheets and possibly the protocol consent form will be provided to the patient as these provide the most comprehensive explanation of the study in lay terms. If the patient shows continued interest, the PI or designee will thoroughly explain the required elements of informed consent and all aspects of the study to the subject including inclusion/exclusion criteria, risks, benefits and alternatives to study participation.

16.4 Privacy, Confidentiality, and Data Storage

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

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

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

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

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

16.5 Data and Safety Monitoring

Data and Safety Monitoring will be performed in accordance with the DCI Data and Safety Monitoring Plan.

The Principal Investigator, CPC and DUHS IRB assess the adequacy of proposed Data and Safety Monitoring Plans during initial and ongoing review and ensure inclusion of adverse event reporting guidelines in each protocol. The sponsor-investigator is responsible for reporting all applicable events to the appropriate regulatory agencies as described in the protocol.

Protocols sponsored by the NCI Cooperative Group Program (e.g., ECOG, GOG, NSABP, ADOSOG, SWOG, RTOG, and CTSU) are monitored by established centralized data and safety monitoring programs within each cooperative group.

Phase III and IV protocols (e.g., NIH-supported, large-scale, multi-site phase III therapeutic intervention protocols which involve significant risk) are outside the scope of this monitoring and safety review system. Independent DSMBs for such studies should be established by the sponsor-investigator. NIH-supported phase III clinical protocols which involve only low risk (e.g. behavioral and nutritional interventions) will be reviewed by CPC on a case-by- case basis, as their sample size may be too large to be practically monitored by this system.

It is recognized that protocols with corporate sponsors and protocols sponsored by NCI cooperative groups are continually audited for compliance and assessed for scientific progress. DUHS sponsor-investigator protocols without outside sponsorship however, are not, and are the focus of this monitoring plan.

Safety Oversight Committee Protocol Review

The Safety Oversight Committee conducts annual data and safety monitoring for DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent DSMB. Annual safety reviews include review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. Studies are rated satisfactory when adequate accrual with lack of excessive toxicity is present.

The sponsor-investigator submits a Safety Oversight Committee Safety Report to the Safety Oversight Committee annually, upon request, or the data may be submitted simultaneously with required reporting, e.g., FDA annual report. Attachments (e.g., accrual tables, toxicities and reference literature) are also acceptable for Safety Oversight Committee review. The sponsor-investigator is notified in writing if additional information is needed and if protocol or operational changes are required. The Safety Oversight Committee will notify the sponsor-investigator and DUHS IRB when significant safety concerns are identified. The Safety Oversight Committee Chair has the authority to temporarily suspend accrual to the study pending acceptable changes. Any recommendation for temporary or permanent suspension of an NIH-funded clinical protocol will be reported by the sponsor-investigator via written communication to the responsible NCI grant program director.

DCI Monitoring Team

The DCI Monitoring Team is comprised of individuals who do not have a direct working relationship with the sponsor-investigator or research team. This independence of DCI Monitoring Team composition is intended to prevent conflicts of interest and breaches of confidentiality during monitoring visits.

The DCI Monitoring Team conducts monitoring visits as defined by CPC, on DUHS sponsor-investigator therapeutic intervention and prevention intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, SOPs, GCP, and applicable regulatory requirements. The Monitoring Team also provides ongoing education and resources to investigators and study teams to enhance the quality.

Standard monitoring for therapeutic intervention and prevention intervention studies is routine monitoring after the third subject is enrolled, followed by annual monitoring of 1-3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk. At the time of CPC review, the CPC may assign monitoring requirements in addition to the standard monitoring plan, as summarized in Table 1 on the next page. Also, additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of DUHS and DCI Leadership, CPC, Safety Oversight Committee, a sponsor, an investigator or the IRB.

SOC members serve as an expert resource and back-up for the DCI Monitoring Team. The DCI Monitoring Team reviews the adequacy of informed consent, enrollment of appropriate patients, implementation of protocol-specified procedures and treatment, adequacy of data collection, and appropriateness of adverse event monitoring and reporting.

16.6 Protocol Amendments

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

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

16.7 Records Retention

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

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

16.8 Conflict of Interest

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

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

16.9 Costs to the Subject

The costs of the study drug, Td, blood for immune monitoring, and the leukapheresis associated with this study are provided free of charge. The patient or third party payer (HMO or insurance company) will be responsible for all other medical costs including the standard of care chemotherapy, radiation therapy (if applicable), blood work, and MRI scans. Some insurance companies and HMOs may not reimburse patients for costs arising from investigational studies. No compensation for participation in this study will be given.

16.10 Registration Procedure

After patients have been enrolled, protocol-specific information and subject registration will be entered into the Velos e-Research software system. This system is managed by the School of Medicine. Patients will also be entered under this protocol study in the Duke Epic Maestro Care system.

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18 APPENDICES

18.1 Protocol Synopsis and Summary

Please see separate documents (available upon request).

18.2 Special or Representative SOPs and FORMs

Please see separate documents (available upon request).

18.3 DSMBplus Monitoring Plan

Please see separate document (available upon request).

18.4 Standard Radiation Therapy

Radiotherapy typically begins within ≤ 5 weeks of surgery. One treatment of 1.8-2.0 Gy/fraction should be given daily 5 days per week for a total of 59.4-60.0 Gy over <7 weeks. 3D conformal and intensity-modulated RT is permitted. All portals should be treated during each treatment session. Doses are specified as the target dose that shall be to the center of the target volume.

The gross target volume (GTV) for both the initial volume (GTV1) and the conedown volume (GTV2) should be based on the postoperative CT/MRI (and preferably the MRI; the preoperative scans may be used if postoperative scans are not available). This initial target volume (GTV1) should include the contrast-enhancing lesion (and should include the surgical resection cavity) and surrounding edema (if it exists) demonstrated on CT/MRI plus a 2.0-cm margin (this 2.0-cm margin-extended volume will be considered the initial planning target volume, or PTV1). The initial target volume should be treated to 46 Gy at 2Gy/fraction or 45-50.4 Gy at 1.8Gy/fraction. If no surrounding edema is present, the initial planning target volume (PTV1) should include the contrast-enhancing lesion (and should include the surgical resection cavity) plus a 2.5-cm margin. Please note that clinical judgment may be used to modify PTV1 to exclude sensitive structures such as the optic chiasm, non-cranial contents, or anatomic regions in the brain where natural barriers would likely preclude microscopic tumor extension, such as the cerebellum, the contralateral hemisphere, directly across from the tentorium cerebri, the ventricles, etc. After 46 Gy, the tumor volume (GTV2) for the conedown treatment should include the contrast-enhancing lesion (without edema) on the pre-surgery CT/MRI scan plus a 1.5-2-cm margin (PTV2). Treat to 14 Gy at 2Gy/fraction or 14.4-9.0 Gy at 1.8Gy/fraction to a total of 60.0 or 59.4Gy, respectively.

Dose is prescribed to the isodose line such that at least 95% of the target volume receives he prescribed dose.

The optic apparatus should be limited to a maximum of 54Gy and no more than 5% of the volume of the brainstem should receive >54Gy.

Radiation should be delayed or interrupted if the platelet count is < 20,000. Radiation should not begin or resume until the platelet count is ≥ 20,000. Hematologic toxicities should be rated on a scale of 0-5 as defined in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. If radiotherapy has to be temporarily interrupted for technical or medical reasons unrelated to the temozolomide administration, then treatment with daily temozolomide should continue. If radiotherapy has to be permanently interrupted then treatment with daily temozolomide should stop. The following should be recorded at entry into this study: daily treatment record, all isodose distributions (in color), dose volume histograms including the cumulative dose to the target volumes, optic chiasm, optic nerves and brain stem, and the radiotherapy summary.

18.5 Temozolomide Therapy

18.5.1 Concurrent TMZ and RT

For subjects who progress to Grade 3 at the time of surgery, TMZ should be administered concomitant with standard external beam RT under the direction of the study Neuro-Oncologists (listed on title page), or their designees, respectively, at Duke University or another institution at the standard dose per discretion of the treating neuro-oncologist. During standard RT, subjects will receive 75mg/m^2/day TMZ for 42 days (6 weeks).

Per the new NCI Temozolomide (NSC 362856) Action Letter:

- Liver function tests (or CMP) should be performed:
  - prior to treatment initiation. If abnormal, the decision to initiate temozolomide treatment should carefully consider the benefits and risks for the individual patient;
  - after each treatment cycle.
- For patients on a 42 day treatment cycle, liver function tests should be repeated midway during this cycle;
- For patients with significant liver function abnormalities the benefits and risks of continuing treatment should be carefully considered.

If any interruption occurs or dose reduction is required, TMZ may be resumed when re-treatment criteria are met as outlined in Table 5 and Table 6.

Dose reduction or alteration during this period due to toxicity is standardly performed as outlined in Table 5.

Table 5: Temozolomide Dosing Interruption or Discontinuation during Concomitant Radiotherapy

Toxicity	TMZ Interruption[a]	TMZ Discontinuation
Absolute Neutrophil Count	≥0.5 and <1.0 x 10^9/L	<0.5 x 10^9/L
Platelet Count	≥10 and <100 x 10^9/L	<10 x 10^9/L
Common toxicity criteria (CTC[2]) Non-hematological Toxicity (except for alopecia, nausea, vomiting)	CTC Grade 2	CTC Grade 3 or 4
a: Treatment with concomitant TMZ can be continued when all of the following conditions are met: absolute neutrophil count ≥1.0 x 10^9/L; platelet count ≥100 x 10^9/L; CTC non- hematological toxicity ≤Grade 1 (except for alopecia, nausea, vomiting).		

The dose may be modified as outlined in Table 6 at the discretion of the treating oncologist.

Table 6. Dose Modifications of Temozolomide during Radiation Therapy

Dose at Toxicity	Modified Dose
75 mg/m2 (during XRT)	60 mg/m2
60 mg/m2 (during XRT)	50 mg/m2

18.5.2 Guidelines for 21-day TMZ Therapy

Subjects with stable histologic grade at time of surgery (Grade 2) will be treated with adjuvant TMZ at a targeted dose of 50-100mg/m^2/day for 21 days every 28 days for up to 12 cycles. Subjects that progress to Grade 3 at the time of surgery will receive SOC RT with TMZ x 6 weeks following surgery. Once RT is complete, subjects that progressed to Grade 3 at the time of surgery will have a 3 ± 1 week break before

[2] CTC, common toxicity criteria (NCI)

resuming TMZ (Table 7). Then, subjects should receive TMZ at a targeted dose of 50-100 mg/m^2/day for 21 days every 28 days for up to 12 cycles. A CBC with auto differential (Lab Code 2010300) should be obtained at the end of each cycle for all subjects receiving 21-day TMZ.

Table 7. Criteria to Resume Temozolomide after Radiation

Absolute Neutrophil Count ≥ 1.0 x 10^9/L
Platelet Count ≥ 100 x 10^9/L
Resolution of CTC Non-hematologic toxicities to Grade 2 or less.

Because of the risk of opportunistic infections in patients receiving TMZ, patients may receive antibiotic prophylaxis at the discretion of the primary Neuro-Oncologist, consisting of inhaled pentamidine or oral levofloxacin. Antiemetic prophylaxis with metoclopramide or a 5-hydroxytryptamine3 antagonist will also be recommended before the initial doses of concomitant TMZ and may be used during the adjuvant 21-day course of TMZ.

All subjects will start 21-day TMZ cycle 1 at a dose of 50mg/m^2/day, and the dose may be increased up to 100mg/m^2/day in subsequent cycles at the discretion of the treating physician. During 21-day TMZ treatment (28 day cycles), dose adjustments to TMZ, if needed, may be conducted as outlined below:

Table 8. Temozolomide Dose Delay, Reduction, or Discontinuation During 21-day Treatment

Toxicity	Delay TMZ Dose [a]	Reduce TMZ by 25% (down to minimum of 50 mg/m^2) [b]	Discontinue TMZ
Absolute Neutrophil Count	≥0.5 and <1.0 x 10^9/L	≥0.5 and <1.0 x 10^9/L	<0.5 x 10^9/L
Platelet Count	≥10 and <100 x 10^9/L	≥10 and <100 x 10^9/L	<10 x 10^9/L
CTC Non-hematological Toxicity (except for alopecia, nausea, vomiting)	CTC Grade 3	CTC Grade 3	CTC Grade 4 [c]
	a: If dose is delayed, treatment with TMZ can resume when the following conditions are met: absolute neutrophil count ≥1.0 x 10^9/L; platelet count ≥100 x 10^9/L; CTC non-hematological toxicity resolved to baseline (except for alopecia, nausea, vomiting). b: For subjects whose dose was escalated above 50 mg/m^2 at the discretion of treating physician c: TMZ is to be discontinued if dose reduction to <50 mg/m^2 is required or if the same Grade 3 non-hematological toxicity (except for alopecia, nausea, vomiting) recurs after dose reduction or delay.		