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ELEVATE

Evaluation of overcoming Limited migration and Enhancing *Cytomegalovirus*-specific dendritic cell Vaccines with Adjuvant TEtanus pre-conditioning in patients with newly-diagnosed glioblastoma

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Amended version 1:	Date: 20140818 (CPC Mods)	
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Amended version 4 (AMD 001):	Date: 20150518 (PI-initiated)	Randomization moved up; Blinding of pre-conditioning; tissue testing; updated information on where to send samples; Hepatitis C and autoimmune disease exclusion criteria added; and clarification on RT/TMZ therapy requirements for participation.
Amended version 5 (AMD 005):	Date: 20150702 (PI-initiated)	Added a 3rd arm to provide basiliximab (Sections: 6, 7.2, 7.3, 8, 9.1, 9.4, 10, 12.2, 12.6, 12.7, 13.1, 15, and 18.7) Added Curran Group Status to eligibility criteria (Section 11 and 12.7.2) Updated language on source docs (Section 12.1) Updated withdrawal section to match TMZ treatment plan (Section 12.6) Updated emergency unblinding language (Section 13.3) Updated objectives with additional arm (Sections 8, 9, 12, and 15) Made all subjects receive Td booster regardless of prior immunization history to mirror previous DC migration study (Section 9)
Amended version 6 (AMD 007)	Date: 20150821 (PI-initiated)	To correct minor error on the cover page & update funding; to remove the company name of Td (Sections 9 & 10); correct the name of the pharmacy providing the Td (Sections 4, 9 & 10); correct cell concentration error (Section 10); correct wording on randomization (Section 9); and clarified treatment summary table by adding rows for leukapheresis and correcting blood draw errors (Section 12).
Amended version 7 (AMD 012)	Date: 20151016 (PI-initiated)	To include 1 red top tube prior to each leukapheresis for immune monitoring (Section 12); to increase the enrollment and chemoradiation timeframe (Section 9, 12, & 18); corrected reference errors (Section 15).
Amended version 8 (AMD 014)	Date: 20160210 (PI-initiated)	Clarified allowance of prevention of prior cancer treatment in exclusion criterion (Section 11); Use of antihistamines before, during and following vaccine administration recommendation (Section 12.7.3).
Amended version 9 (AMD 020)	Date: 20160518 (PI-initiated)	Included blood to evaluate CCL3 levels prior to the Td booster given IM (Sections 12.2 & 12.7.5); allowed repeated leukapheresis if less than 3 vaccines generated from initial pheresis and again as needed for continued vaccinations, clarified that Tums is taken 3 times a day, and that a central line may be needed for the leukapheresis (Sections 9.1, 10.1 & 12.2), reduced the total number of vaccines from 20 to 10 (Sections 6, 9.1, 12.2, and 12.3).
Amended version 10 (AMD 026)	Date: 20160727 (PI-initiated)	Removed LSQ lab test from study (Section 12.2) and included the already planned CMP with removal of LSQ (Section 18.4.2); added IHC assays to tumor samples already being obtained (Section 12.1).
Amended version 11 (AMD 027)	Date: 20160806 (PI-initiated)	To update the location of the cell processing suite with inclusion of associated abbreviations (Sections 4, 10.3, and 10.4).
Amended version 12 (AMD 029)	Date: 20160819 (PI-initiated)	To allow consenting and RT/TMZ to occur within 6weeks of OR (Section 12.1).
Amended version 13 (AMD 32)	Date: 20160915 (PI-initiated)	To remove reference to appendices with TMZ drug dosing information and removed section in appendices. Instead, referred the reader to the TMZ package insert uploaded in eIRB (Sections 9.1 and 18.4).
Amended version 14 (AMD 34)	Date: 20161017 (PI-initiated)	To include tumor analysis up front and at recurrence and add abbreviation for IDH (Sections 4 and 12).
Amended version 16 (AMD 45)	Date:20170223 (PI-initiated)	Changed the PI (title page, Sections 9.1.4, 12.1, 13.2.1, and 13.3); changed the primary study coordinator and primary regulatory coordinator (title page);
Amended version 15 (AMD 49)	Date: 20170324 (PI-initiated)	Randomization to Group III is closed due to results from a related study (Sections 9.1, 9.1.1, and 15); Due to the end to

		enrollment in Group III, adjusted the projected number of subjects in the study from 102 to 79. Up to 100 people may sign the consent to reach this goal (Sections 9.1, 9.1.1, and 15); Fixed inconsistencies in timing of Td Booster, which must occur before the DC vaccine #1 but can occur the same day as DC vaccine #1 (Sections 6, 9.1, and 12.7.5); Clarified the timing of blood draws for immune monitoring and subclinical autoimmunity at DC Vaccine #1 (Sections 12.2 and 12.7.5); Removed the blood draws for CCL3 levels pre-/ post-Td Booster, CCL3 is still measured from the IM blood taken at vaccine #1 and the SPECT/CT imaging visits (Sections 12.2 and 12.7.5); Clarified that after cycle 1, all remaining TMZ cycles will be 5 (±1) weeks (Section 9.1); Clarified the Follow-up Period for subjects (Sections 12.4 and 12.5); Clarified the recording of AEs (Section 13.1); Clarified MMSE and immune monitoring in Table 4 (Section 12.2); For baseline MRI, changed "standard 5mm slices with 2.5mm spacing" to "per standard of care" (Sections 12.1 and 12.2); Clarified inclusion criteria regarding MRI at time of consent (Section 11 and 12.1); Added "Patients should start RT within approximately 6 weeks of surgery" (Section 9.1)
Amended version 16 (AMD 58)	Date: 20170523 (PI-initiated)	Made inclusion criteria for pregnancy more clear by clarifying that the test will be performed within 48 hours of leukapheresis, the first study-related procedure (Section 11); Removed Curran as eligibility criteria but kept it as study assessment performed at time of consent (Sections 11 and 12.7.2); Clarified immune monitoring blood draws time points after completion of study vaccines (Sections 12, 12.4, and 12.7.5)
Amended version 17 (AMD 65)	Date: 20170914 (PI-initiated)	Clarified language in inclusion criterion #1 regarding definitive resection (Section 11); Corrected error in Section 9.1.4 regarding timing of recording AEs, which was missed in previous amendment (AMD 49); Clarified the information about subsequent therapies that needs to be recorded during follow-up period (Section 12.4); Clarified length of radiation is approximately 6 weeks to make Section 9 and Section 12.6.1 consistent; Updated description of person delivering vaccine from MPACT to clinic and added that each vaccine will have a separate cooler (Section 10.4): Clarified dose adjustment of TMZ (Section 9.1.2)
Amended version 18 (AMD 73)	Date: 20180406 (PI-initiated)	Study closed to enrollment (Section 9); Analysis plan updated (Section 15)
Amended version 19 (AMD 80)	Date: 20181120 (PI-initiated)	Added that patient's cells from the leukapheresis procedure can be transferred to the INTERCEPT trial (Pro00083828) if they become progressive and are eligible (Sections 9.1, 10.1, and 12.3).

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Ab	Antibody
ABC	Automated Blood Count
ACD	Acid Citrate Dextrose
ACLS	Advanced Cardiac Life Support
ACTH	Adrenocorticotropic Hormone
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
APAAP	Alkaline Phosphatase Antialkaline Phosphatase Complex
AST	Aspartate Aminotransferase
AT	Ambient Temperature
AUC	Area Under the Curve
β-HCG	Beta-Human Chorionic Gonadotropin
BMT	Bone Marrow Transplant
BTSC	Brain Tumor Stem Cells
Ca^{++}	Calcium
CCL3	Chemokine (C-C motif) ligand 3
cDNA	Complimentary Deoxyribonucleic Acid
CFA	Complete Freund's Adjuvant
CFC	Cytokine Flow Cytometry
cGMP	Current Good Manufacturing Practice
CLIA	Clinical Laboratory Improvement Act
CLN	Cervical lymph Nodes
Cmax	Maximum Concentration of Drug in Plasma or Serum
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CNC	Clinical Neurologic Change
CNS	Central Nervous System
Con-A	Concanavalin A
CPC	Cancer Protocol Committee
CT	Computed Tomography
CTL	Cytotoxic T-Lymphocyte
CTQA	Clinical Trials Quality Assurance
DAR	Drug Accountability Record
DC	Dendritic Cell
DCI	Duke Cancer Institute
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic Acid
DSMP	Data Safety and Monitoring Plan
DTH	Delayed-type Hypersensitivity
DUMC	Duke University Medical Center
EAE	Experimental Autoimmune Encephalomyelitis
EBRT	External Beam Radiation Therapy
ELISA	Enzyme-Linked ImmunoSorbent Assay
ELISPOT	Enzyme-linked Immunospot
EGFR	Epidermal Growth Factor Receptor
EGFRvIII	Epidermal Growth Factor Receptor variant type III
EGFRvIII-KLH	EGFRvIII conjugated to Keyhole Limpet Hemocyanin
FACS	Fluorescence Activated Cell Sorting
FDA	Federal Drug Administration

FEV	Forced Expiratory Volume
GBM	Glioblastoma
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HAMA	Human Anti-Murine Antibody
H&E	Hematoxylin and Eosin
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HMO	Health Maintenance Organization
I.C.	Intracerebral
ICS	Investigational Chemotherapy Services
I.D.	Intradermal
IDH	Isocitrate Dehydrogenase
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-12	Interleukin-12
IL-13	Interleukin-13
I.M.	Intramuscular
IRB	Institutional Review Board
ISH	In Situ Hybridization
I.V.	Intravenous
KLH	Keyhole Limpet Hemocyanin
KPS	Karnofsky Performance Status
LAMP	Lysosomal-associated Membrane Protein
Lf	Flocculation unit
MAb	Monoclonal Antibody
MG	Malignant Glioma
MGMT	Methylguanine Methyltransferase
MHC	Major Histocompatibility Complex
mL	MilliLiter
MMSE	Mini-Mental Status Examination
MPACT	Molecular Products and Cellular Therapies
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MTD	Maximally Tolerated Dose
NA	Non-adherent
NCI CTC	National Cancer Institute Common Toxicity Criteria
ng	NanoGram
NIH	National Institutes of Health
NK	Natural Killer
OS	Overall Survival
OVA	Ovalbumin
PBLs	Peripheral Blood Lymphocytes
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reactions
PD	Progressive Disease
PHA	Phytohemagglutinin
PFS	Progression Free Survival
PI	Principle Investigator

PO	By Mouth
PRTBTC	Preston Robert Tisch Brain Tumor Center
PT	Prothrombin Time
PTT	Partial Thromboblastin Time
RANO	Response Assessment in Neuro-Oncology
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic Acid
RT	Radiation Therapy
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Severe Adverse Event
S.C.	Subcutaneous
SFC	Spot-Forming Cells
SOC	Standard of Care; Safety Oversight Committee
TCR	T cell Receptor
TD	Tetanus-Diphtheria
TGF-β	Transforming Growth Factor-β
T_H2	T helper type 2
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
WBI	Whole Body Irradiation

5 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

Please see Research Summary section in eIRB.

6 STUDY SCHEMA

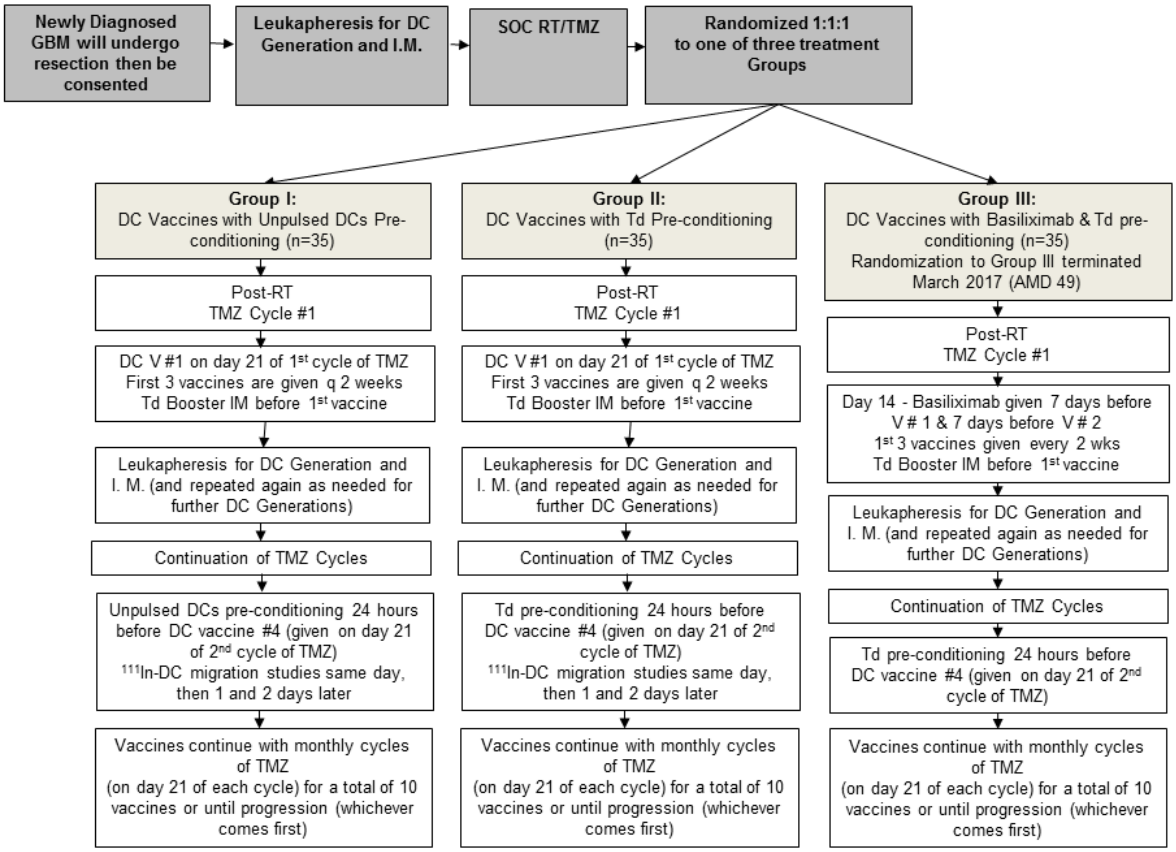

Figure 1. Study Schema

7 BACKGROUND AND SIGNIFICANCE

7.1 Study Disease

Malignant primary brain tumors are more common than Hodgkin's disease and account for more human deaths than melanoma or than cancer of the bladder or kidney. Despite aggressive, computer-guided tumor resection [3], high-dose external beam RT or brachytherapy, and multi-mechanistic chemotherapy delivered at toxic doses, most patients with malignant primary brain tumors live <15 months from the time of diagnosis, and patients with recurrent tumors usually survive <12 weeks [4-9]. The estimated cost of treatment for each patient with a malignant brain tumor is between $30,000 and several hundred thousand dollars annually. Thus, the annual treatment cost alone for these patients, not mentioning the lost earning potential of afflicted individuals, is greater than the entire annual budget of the National Institute of Neurological Diseases and Stroke. In fact, conventional therapy for patients with malignant brain tumor is the most expensive medical therapy per quality-adjusted life-year saved currently provided in the United States [10, 11]. Moreover, the non-specific nature of conventional therapy for brain tumors often results in incapacitating damage to surrounding normal brain and systemic tissues [12, 13]. Thus, in order to be more effective, therapeutic strategies will have to precisely target tumor cells while minimizing collateral damage to neighboring eloquent cerebral cortex. The rationale for employing the immune system to target brain tumors is based on the premise that the inherent biologic specificity of immunologic reactivity could meet the clear need for more specific and precise therapy**.**

7.2 Study Agent

Human *Cytomegalovirus* pp65-lysosomal-associated membrane protein (pp65-LAMP) mRNA-pulsed autologous dendritic cells

DCs are potent immunostimulatory cells that continuously sample the antigenic environment of the host and specifically activate CD4+ and CD8+ T cells and B-cells [14, 15]. They are at the crossroads of many of the elegant networks of the immune system, and DCs represent the most promising contemporary biologic entity for realizing the promise of immunotherapy. Potent immune responses and encouraging clinical results have been seen in Phase I and II human clinical trials in systemic cancers[16-32].

Human *CMV* is an endemic β-Herpesvirus that does not usually cause significant clinical disease[33]. During primary maternal infection, however, human *CMV* can cause severe encephalitis in fetuses and lead to congenital brain defects. Human *CMV* disease is also a significant problem in immunocompromised adults such as organ transplant recipients or patients with AIDS[33]. Herpesviruses have also been implicated in a number of human malignancies including lymphoma, nasopharyngeal cancer, cervical cancer, and Kaposi's sarcoma[34, 35]. Recently, expression of proteins unique to human *CMV* has been reported within a large proportion of malignant tumors including colorectal carcinoma, prostate cancer, and malignant astrocytomas[36-38]. Universal detection of the human *CMV* immunodominant protein pp65, immediate early gene 1 protein (IE1), and several other early antigens was demonstrated using IHC in Grade II-IV astrocytomas[38]. Presence of the virus in these samples was confirmed with ISH, PCR for human *CMV*-specific glycoprotein B (UL55), electron microscopic detection of

intact virions[38], and direct detection of the virus from fresh operative samples in the shell vial assay (unpublished data). Human *CMV* antigens were not detected in surrounding normal brain samples, meningiomas, or brains affected by ischemia, Alzheimer's disease, paraneoplastic encephalitis, or *Cryptococcal* cerebritis.

The presence of highly-immunogenic human *CMV* antigens within MGs affords a unique opportunity to target these tumors immunologically. There is a vast amount of experience with both the safety and efficacy of immunotherapy targeting human *CMV*[33], and the presence of this virus within brain tumors may allow this experience to be leveraged toward the effective eradication of MG expressing human *CMV* antigens. Adoptive T cell therapy has been used to safely and successfully protect against *CMV* reactivation in myelodepleted BMT patients [39-42]. In addition, T cell mediated immunotherapy has proven highly effective in the treatment of *CMV*-associated disease within the CNS[43] and in the treatment of acute *CMV* infections[40, 44]. Tumors associated with other human *Herpesviruses*, such as Epstein-Barr virus-associated lymphoma, including tumors within the CNS, have also been effectively treated and even large tumors have been cured by immunotherapy[45-50]. More recently, a vaccine directed against the potent viral antigens of human papilloma virus has also been shown to reduce the incidence of human papilloma virus-related cervical intraepithelial neoplasia in a prospective, randomized, double-blind trial[51].

The potential for non-specific targeting of normal tissues is thought to be minimal in seropositive patients. After initial infection, *CMV* establishes lifelong latency in the infected individual, with cells of the myeloid lineage constituting a major reservoir for persistence of the virus. Virus can be detected within myeloid progenitors in the bone marrow, with a small portion of these cells demonstrating viral DNA replication without any detectable gene expression[52, 53]. Also a small proportion (typically 1 in 1,000 to 1 in 10,000) of peripheral blood monocytes can be found to contain *CMV* DNA, while detection of viral RNA (gene expression) is not detected[54-56]

Vaccination specifically against *CMV* [57-61] has effectively reduced the risk of viral infection and transmission to fetuses in animal models [62-64] and in clinical trials[57, 61, 65-68]. Human clinical trials have also demonstrated some benefit of administering neutralizing antibodies in the treatment of human *CMV* infection[69-72], highlighting the importance of the development of vaccination strategies that elicit both cellular and humoral immune responses. DCs strongly activate both T cell and B-cell responses *in vivo*[15], and DCs pulsed *in vitro* with *CMV* antigens have been shown to be potent inducers of *CMV*-specific CTL responses in several studies[73-77], in addition to our own work which is outlined below.

The use of RNA to encode tumor antigens for DCs was pioneered at Duke University in Dr. Gilboa's laboratory, but the ability of RNA-loaded DCs to stimulate potent antitumor immunity has been independently confirmed in murine and human systems [78-83]. In fact, there is accumulating evidence that RNA transfection represents a superior method for loading antigens onto DCs [80, 84]. This novel and innovative approach to DC antigen loading has multiple conceptual advantages over other forms of antigen delivery as well. RNA-based antigen loading does not require knowledge of major MHC restriction, and responses are not restricted to single MHC haplotypes or to a narrow B or T cell repertoire. This diversity increases the likelihood of

inducing effective and sustained antitumor immune responses by simultaneous activation of both CTLs and helper T cells [85-87]. Furthermore, in direct comparisons, RNA-loaded DCs have been found to be better stimulators of antigen-specific T cells than other approaches [84]. Finally, RNA also carries a significant safety advantage, not possessed by other nucleic acid or viral vectors, in that it cannot be integrated permanently into the host genome. In addition to the preliminary data we present below, Kobayashi *et al.* [88] have demonstrated that tumor mRNA-loaded DCs can elicit a specific CD8+ CTL response against autologous tumor cells in patients with MG.

Temozolomide

TMZ, a methylating agent with good blood-brain barrier penetration, has recently been shown to increase survival by a small, but statistically significant, 2.5 months in a subset of patients with newly-diagnosed GBM if given in conjunction with RT following initial resection of the tumor[2, 89, 90]. Leukopenia is essentially the only known human toxicity of TMZ. Although initially counter-intuitive, this TMZ-induced leukopenia may actually be advantageous in treating patients with immunotherapy due to the subsequent homeostatic proliferation it induces. In this protocol patients will receive standard of care doses of TMZ concurrent with RT following initial resection of the tumor. We believe that the myelosuppression induced by therapeutic TMZ treatment, if carefully timed before vaccination will actually enhance the proliferation and maintenance of these tumor-specific T cells through the natural forces that drive T cell homeostatic proliferative recovery. In addition, TMZ has also been shown to preferentially deplete T_{Regs}[91]. Thus, this combination strategy will uniquely exploit the toxicity of one effective therapy for GBMs, TMZ, to enhance another already promising therapy, immunotherapy. In preparation for this protocol, we have evaluated, in animal models, TMZ and sublethal WBI, as a positive control, as methods for induction of treatment-induced lymphopenia in order to determine the ability of TMZ-induced lymphodepletion to enhance active and adoptive immunotherapy. These studies were initially performed in a murine T cell receptor transgenic model in which the antigen-specific T cells can be followed *in vivo* in mice receiving adoptive transfer of lymphocytes. In this model system, we found that adoptive lymphocyte transfer coupled with DC vaccination (DC + ALT) is a potent mechanism for inducing antigen-specific T cell expansion after TMZ treatment. TMZ was found to be an effective agent for inducing homeostatic proliferation of transferred CD4+ and CD8+ T cells and for enhancing DC or peptide vaccinations with or without ALT (Figure 2).

Markedly elevated levels of antigen-specific T cells could be achieved and maintained in mice receiving ALT after therapeutic TMZ-induced lymphodepletion compared to normal hosts receiving ALT. The precursor frequency of OVA-specific CD8+ T cells in the peripheral blood of untreated mice receiving OVA peptide vaccine after transfer of OT-I transgenic T cells was 1.5%. Mice pretreated with TMZ and given OVA peptide vaccine had achieved a mean precursor

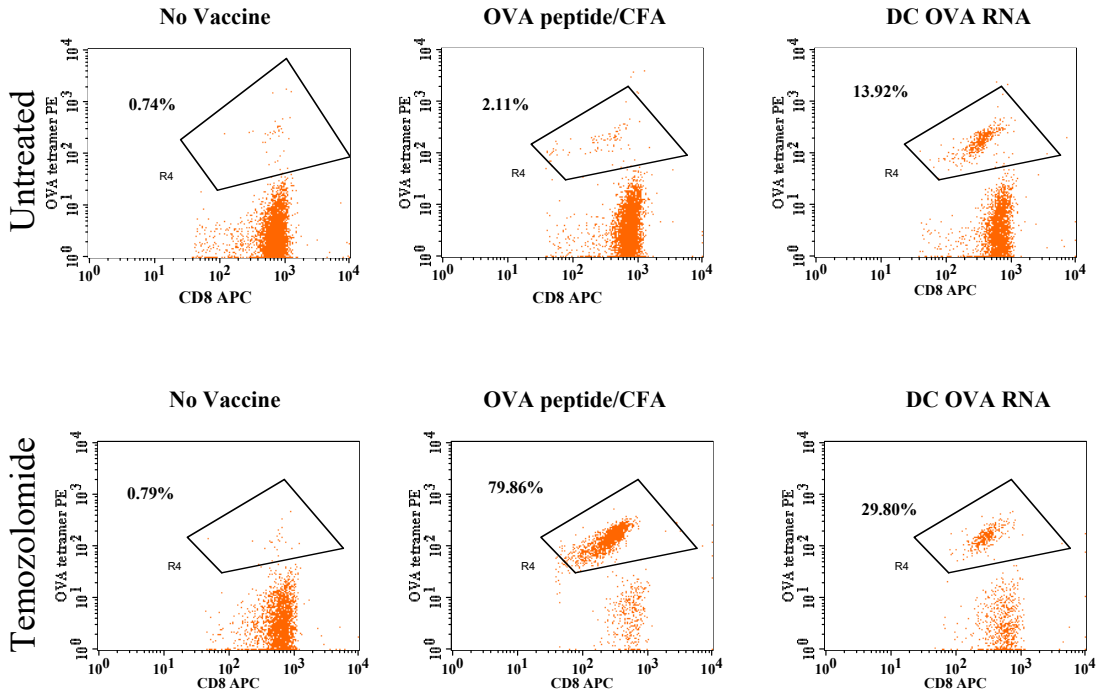

Figure 2. Homeostatic lymphodepletion induced by temozolomide enhances antigen-specific immune responses. Untreated mice or mice receiving non-myeloablative high-dose temozolomide treatment (5 days x 60 mg/kg) were administered a mixture of OVA-specific and normal lymphocytes intravenously and received either no vaccine, vaccination with DCs transfected with OVA RNA, or OVA peptide vaccination in complete Freund's adjuvant. The peripheral blood of treated animals was collected and analyzed 6 days after vaccination for the expansion of OVA-specific T cells in the circulation using αCD8 monoclonal antibodies and a specific tetramer. As shown above, mice treated with temozolomide prior to ALT demonstrated markedly increased T cell expansion after vaccination compared to untreated hosts receiving ALT.

frequency of 54%, exhibiting markedly increased levels of OVA-specific T cells in the blood (P<0.0001). Similar results were found in mice receiving DC vaccination, with untreated animals having an average precursor frequency of 3.6% and TMZ pretreated animals achieving an average of 11.9% OVA-specific CD8+ T cells after a single vaccination. These results demonstrate that active and adoptive immunotherapy administered during hematopoeitic recovery from TMZ treatment may be a very effective way to enhance the efficacy of immunotherapy.

Basiliximab (Simulect®)

Basiliximab (Simulect®) is a chimeric (murine/human) monoclonal antibody (IgG1K), produced by recombinant DNA technology, that functions as an immunosuppressive agent, specifically binding to and blocking the interleukin-2 receptor α-chain (IL-2Rα, also known as CD25 antigen) on the surface of activated T-lymphocytes.
Basiliximab has been most frequently used as an immunosuppressant in combination with other broadly active immunosuppressants, such as cyclosporine and corticosteroids that may target the redundancy pathways for effector T cell development[92]. As an immunosuppressant, it is administered within 2 hours prior to transplantation surgery (Day 0) and the second dose administered on Day 4 post-transplantation. The regimen of basiliximab was chosen to provide

30-45 days of IL-2Rα saturation during which time effector T cells may be expected to up-regulate IL-2Rα expression and be inhibited. Thus, we believe, and our preclinical animal studies support, that the activity of IL-2Rα blockade is entirely dependent on the context in which it's given. We believe that blocking IL-2Rα signaling during recovery from TMZ-induced lymphopenia will predominantly impact the recovery, function, and survival of T_{Regs} without having negative effects on the recovery, function and survival of tumor-specific T cells activated through vaccination or through cross-priming at the tumor site where the role of other cytokines will predominate. Please see package insert in the appendices (see Section 18.7).

^{111}Indium-labeling of Cells for in vivo Trafficking Studies

The *in vivo* distribution of DCs will be evaluated using ^{111}Indium (^{111}In)-labeled pp65-LAMP mRNA loaded mature DCs. ^{111}In-labeled DCs have been used extensively for evaluation of adoptively transferred tumor infiltrating lymphocytes, natural killer cells, granulocytes, dendritic cells, and whole blood leukocytes, for *in vivo* localization studies in humans[93-95]. DCs will be labeled at the Duke Radiopharmacy Lab according to standardized protocols. Briefly, 2 x 10^7 pp65-LAMP mRNA loaded mature DCs will be labeled with ^{111}In (50 µCi / 5 x 10^7 DCs) (Nycomed-Amersham, Chicago, IL) prior to injection. Intradermal DC injections sites will be carefully marked at 10 cm from the groin crease. Gamma camera (dual-headed) images will then be taken immediately after injections, and at 24 and 48 hours after injections to compare DC migration from the inguinal intradermal injection sites to the inguinal lymph nodes (SOP-DBTIP-078 "Administration of ^{111}In-labeled Dendritic Cells for Trafficking"). Regions of interest will be analyzed using decay corrected counts. These correlative studies are designed to determine the effects of skin site preparation have on DC migration.

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)] [96, 97]. 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 [98]. 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 [99].

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 T_H2 cells, each with their own unique TCR. Identifying and then binding of the MHC II:toxoid to the specific T_H2 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 [100].

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 T_H2 whose receptors are specific for this complex. The process, termed linked recognition, results in the T_H2 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 [100].

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. 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.

7.2.1 Pre-Clinical Experience

In our laboratories and those of others, systemic immunization using DCs co-cultured with uncharacterized tumor homogenate[101], whole tumor RNA[102], unidentified peptides eluted from tumor cells by gentle acid washing[103], or a distinct peptide encompassing the tumor-specific EGFRvIII mutation[104] have been shown to induce humoral and cell mediated systemic immune responses and to prolong the survival of rodents with brain tumors.

In our laboratory[101], inbred VM/Dk mice received three or four weekly intraperitoneal injections of autologous bone marrow-derived DCs transiently co-cultured with tumor homogenate. The homogenate was derived from a syngeneic murine astrocytoma cell line derived from a spontaneously occurring astrocytoma in the inbred VM/Dk mouse strain. Splenocytes from mice immunized in this way were able, in vitro, to lyse the astrocytoma cell line that was used to generate the tumor homogenate. They were also able to lyse other astrocytoma cell lines derived from the same inbred mouse strain, but they had no effect against syngeneic fibroblasts. Similarly, these immunized mice also demonstrated a significantly increased antibody titer against the astrocytoma cell line used to generate the homogenate. In addition, mice immunized with DCs transiently co-cultured with tumor homogenate that were subsequently challenged with a lethal dose of this astrocytoma cell line intracerebrally were found to have a median survival >160% longer than those immunized with DCs cultured without tumor homogenate (P=0.016). In addition, 50% of the mice treated with the tumor homogenate-supplemented DCs survived long-term without any evidence of tumor growth and also survived a rechallenge of tumor cells indicating that a sustained antitumor immune response had been established. These findings are especially significant in light of the fact that the astrocytoma cell line used is known to secrete the immunosuppressive agent TGF-β which is secreted by most human gliomas[105-109].

In another report from our laboratory[102], C57BL/6 mice received three weekly intraperitoneal injections of autologous bone-marrow derived DCs co-cultured with tumor homogenate or whole tumor RNA derived from the poorly immunogenic B16F10 melanoma cell line. Standard in vitro cytotoxicity assays again revealed that splenocytes harvested from mice immunized with DCs transiently co-cultured with either tumor-derived homogenate or whole tumor RNA were able to lyse B16F10 melanoma cells but not unrelated tumor cells from the same MHC) background. In this experiment, mice immunized with autologous bone-marrow derived DCs co-cultured with tumor homogenate or whole tumor RNA increased median survival by >233% (P=0.0006) and 48% (P=0.0001), respectively, relative to mice immunized with DCs co-cultured with tumor homogenate or whole tumor RNA derived from an unrelated tumor with the same MHC background. In addition, 8/13 (61.5%) in the specific homogenate group and 4/10 (40%) in

the specific RNA group survived beyond the endpoint of the study without evidence of tumor. Immunization of mice with pre-existing tumors with specific tumor homogenate also demonstrated the potency of this immunization approach by increasing survival by 62.5% relative to controls. In these mice an inflammatory infiltrate composed of mononuclear cells and polymorphonuclear leukocytes was identified only in mice treated with DCs co-cultured with tumor homogenate that matched the intracerebral tumor challenge.

In a recent report from another laboratory[103], the survival of tumor-bearing rats injected subcutaneously with autologous bone marrow-derived DCs co-cultured with peptides eluted from tumor cells with a gentle acid wash was significantly prolonged compared to tumor-bearing rats receiving equivalent numbers of DCs co-cultured with peptides acid-eluted from normal astrocytes ($P< 0.05$). Median survivals in these groups were 35 and 22 days respectively. In addition, three of the twelve rats (25%) treated with DCs co-cultured with acid-eluted tumor peptides remained alive at the end of the experiment. In addition, immunohistochemical analysis of five animals from each group in this experiment documented an increased peritumoral and intratumoral infiltration of CD8+T cells, and to a lesser extent CD4+ T cells and macrophages, in the group treated with DCs co-cultured with peptides acid-eluted from tumor cells when compared to controls.

Inhibition of T_{Reg} Function with Anti-CD25-specific Monoclonal Antibodies

We have reported that our murine model of glioma indeed recapitulates MG-induced changes to the human peripheral blood CD4 and T_{Reg} compartments. Specifically, tumor-bearing mice exhibit CD4 lymphopenia, while $CD4^+CD25^+Foxp3^+GITR^+$ T_{Regs} come to represent an increased fraction of the peripheral blood $CD4^+$ T cells that remain, despite themselves being reduced in number. Extending study to other sites, similar phenomena are observed in the spleens and CLN, while the reverse scenario emerges in bone marrow.

We employed this model to investigate the in vivo effects of T_{Reg} removal on anti-glioma immune responses. The current doctrine is that effective depletion of T_{Regs} may be achieved simply by systemic administration of anti-CD25 mAb[110, 111]. Likewise, in peripheral, non-CNS tumor models, the administration of anti-CD25 mAb has been employed in attempts to remove T_{Regs} and has effectively elicited prolonged survival to subcutaneous tumor challenge[112-114]. These studies, however, predated our ability to examine Foxp3 expression in T_{Regs} with antibody staining.

Following in vivo administration of anti-CD25 mAb (PC61), we discovered that $CD4^+Foxp3^+GITR^+$ cells failed to entirely disappear, despite the present thinking. Instead, they persisted at significant levels in all sites tested. When isolated based on CD4 and GITR expression, however, these cells demonstrated none of the typical suppressive capacities of $CD4^+CD25^+GITR^+$ T_{Regs} in vitro. Accordingly, systemic anti-CD25 mAb proved capable of enhancing T cell proliferation, IFN-γ production and glioma-specific CTL responses in treated mice. These effects translate to spontaneous tumor rejection in a murine model of established intracranial glioma (Figure 3).

Furthermore, when combined with a DC-based immunization strategy, anti-CD25 mAb elicited glioma rejection in 100% of challenged mice without attendant induction of EAE[115, 116]. Systemic anti-CD25 mAb administration therefore appears to counter the suppressive effects of

T_{Regs} without comprehensively eliminating the cells in vivo. This activity proves permissive for potent antitumor immunity in a murine glioma model that aptly recapitulates tumor-induced changes to the CD4 and T_{Reg} compartments.

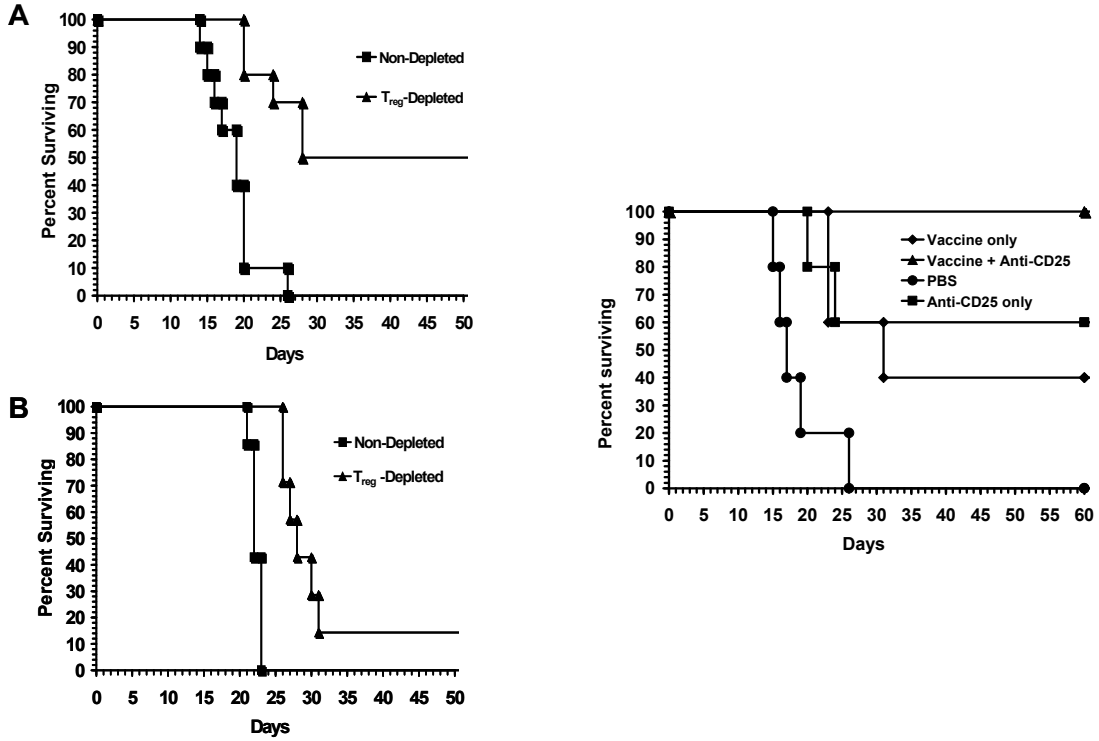

Figure 3. PC61 in vivo extends survival and permits tumor rejection in a murine model of intracranial glioma. A. VM/Dk mice (n=10 per group) received i.p. PC61 or isotype control on Day -4. On Day 0, all mice were challenged intracranially with 10,000 syngeneic SMA-560 astrocytoma cells. Anti-CD25 produced 50% long-term survivors with no evidence of tumor. Differences in survival curves are significant (p=0.0002). B. Effects of PC61 in a therapeutic model. Treated mice (n=7) received i.p. PC61 three days following tumor implantation, when tumor was established. PC61 significantly extended median survival (p=0.0003) and produced nearly 15% long-term survivors with no evidence of tumor. C. Combination of anti-CD25 and DC vaccine produce 100% survival following intracranial tumor challenge. VM/Dk mice (n=5 per group) were adminisitered 0.5 mg PC61 (anti-CD25) or isotype control antibody i.p. on Day -10. On Day -7, mice were vaccinated with 2.5x105 DC electroporated with total tumor RNA from the SMA-560 glioma cell line or injected with an equivalent volume of PBS. Injections were delivered s.c. at the base of each ear. On Day 0, all mice were challenged i.c. with 10,000 syngeneic SMA-560 cells. Kaplan-Meier survival data are presented as the percentage of mice surviving in each group. Differences in survival were determined by log rank test. Anti-CD25 (p=0.0198) and DC vaccine (p=0.0153) alone each produced significant survival benefits, but the combination of the two elicited 100% long-term survival (p=0.0018).

7.2.2 Clinical Experience

Prior Experience in Patients with Intracerebral Tumors

The occurrence of human DCs in the peripheral blood is low (0.15% of circulating mononuclear cells), and procedures to isolate circulating DCs are cumbersome, relying on negative selection techniques to deplete the mononuclear cell fraction of contaminating monocytes and lymphocytes. Furthermore, brain tumor patients are characteristically immnosuppressed either from the use of steroids or due to the fact that malignant brain tumors secrete immunosuppressive agents like TGF-β. We have been using a simple method described

previously[117], to generate human DCs by culturing PBMCs in media supplemented with GM-CSF and IL-4. We have compared the ability to generate DCs from patients with malignant brain tumors and patients undergoing craniotomy for non-tumor related procedures. The phenotype of DCs from both tumor and normal populations were identical and were characterized as being highly positive for HLA-ABC and HLA-DR, the co-stimulatory molecules CD80 and CD86, and the DC/monocyte marker CD11c, but negative for the monocyte marker CD14. The cells were negative for the B and natural killer (NK) cell lineage markers, CD19 and CD56, respectively, which is consistent with published DC phenotypes.

DC immunotherapy in patients with MGs has been evaluated only a few studies. In the published study by Yu *et al.* [118], patients received biweekly intradermal injections of peripheral blood derived DCs pulsed with uncharacterized peptides eluted from the surface of autologous glioma cells by gentle acid washings. All patients were required to complete a course of RT and were off steroids at the time of immunization. Toxicity was minimal and included only mild fever and lymphadenopathy. There was no clinical or radiographic evidence of autoimmune encephalomyelitis in any patient and no serious adverse events occurred. The immunization resulted in enhanced CTL activity in 4/7 patients and both cytotoxic and memory T cells were found to have infiltrated the patient's tumors whom underwent reoperation after immunization. Although this study was performed in a selected population of patients, the median survival of 455 days in the treated group compared very favorably with an institutional control group where median survival was only 257 days. Similarly, when immunized patients were compared to expected outcome per Curran's recursive partition analysis, which controls for known prognostic factors (Karnofsky Performance Status, histology, surgery, mental status, etc), the results still appeared quite favorable. Unfortunately, no clinical responses were seen and any antigen-specific immune response could not be characterized because the immunizing antigens were not characterized.

A study by Kikuchi *et al.* (2001) [119]used autologous DCs fused with autologous tumor cells as an immunogen in an 8 patient trial. The immunization schedule consisted of 3 to 7 vaccinations 3 weeks apart given intradermally. All vaccinations were well tolerated.

In another Phase I/II trial, tumor lysate pulsed DCs were given to ten patients who received immunizations every three weeks for a minimum of one and a maximum of 10 immunizations. There were only two minor clinical responses seen. Of five patients evaluated by ELISPOT before and after vaccination T cells reactive against tumor lysate-pulsed DCs were increased in two patients[120]. In a more recently published study, patients with GBM were treated with 1 x 10^6 to 1 x 10^7 DCs pulsed with acid eluted autologous tumor peptides[121]. There was no evidence of DLT or serious adverse events. One patient had an objective clinical response documented by magnetic resonance imaging, and six patients developed measurable systemic antitumor CTL responses.

In a more recent study, Kikuchi et al.[122] investigated the safety and clinical response to immunotherapy using fusions of DCs and glioma cells combined with recombinant human IL-12 for the treatment of MG. Fifteen patients with MG participated in this study. Cultured autologous glioma cells were established from surgical specimens in each case. Fusion cells were prepared from DCs and glioma cells using polyethylene glycol. All patients received fusion cells intradermally on day 1. IL-12 was injected subcutaneously at the same site on days 3 and 7. No

serious adverse effects were observed. In four patients, magnetic resonance imaging showed a greater than 50% reduction in tumor size. One patient had a mixed response. In our ongoing Phase I/II clinical trial (BB IND 9944) patients with newly-diagnosed MGs are vaccinated with mature DCs loaded with a peptide spanning the fusion junction of EGFRvIII conjugated to keyhole limpet hemocyanin (PEPvIII-KLH) (500 mcg/immunization), mixed with GM-CSF (approx. 150 mcg/immunization). EGFRvIII is a tumor specific antigen, which is expressed on approximately 47% of all MGs. The vaccination protocol consists of 3 vaccines 2 weeks apart of PEPvIII-KLH loaded, mature DCs beginning 2 weeks following completion of post-resection RT. To date, 19 patients have been enrolled with 16 completing vaccination with no adverse events. No patient showed a positive delayed-type hypersensitivity reaction to KLH or PEPvIII before vaccination and of the evaluable patients after vaccination, 14/15 (93.3%) patients reacted to KLH and 11/15 (73.3%) reacted to PEPvIII. *In vitro* proliferation in response to PEPvIII was seen in 11/12 (92%) and to KLH in 9/12 (75%) of patients tested. Two patients, one with anaplastic astrocytoma and one with GBM with residual radiographic disease after resection, and

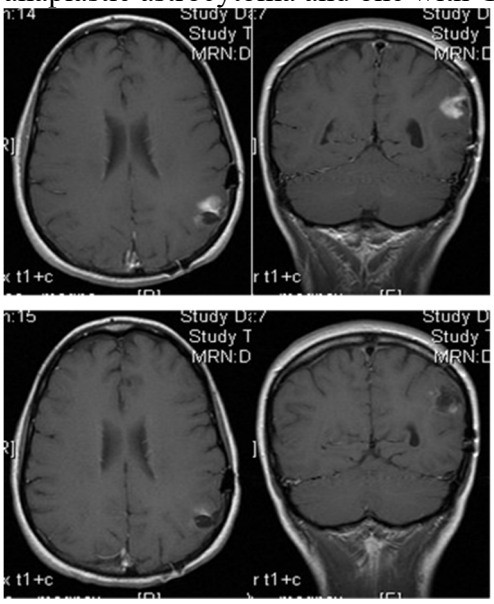

Figure 4. Patient from prior vaccination study with a nearly complete response. Progressive MG after radiation and temozolomide (top) with nearly complete response, 3 months after PEPvIII-pulsed DC vaccine (bottom).

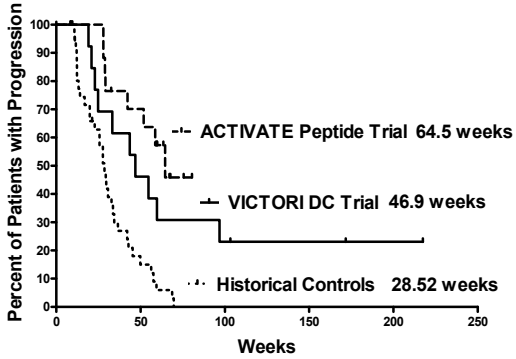

Figure 5. TTP comparison between VICTORI Trial, ACTIVATE Trial, and a historical control. TTP comparison in patients treated with PEPvIII-KLH loaded DCs (VICTORI Trial), patients treated with PEPvIII-KLH alone (ACTIVATE Trial), and a historical control group that had a 95% resection of an EGFRvIII-positive tumor. There

was no significant difference in the TTP between the patients in the VICTORI and ACTIVATE trials, but both were significantly better than historical controls (P<0.001).

radiation, have had a nearly complete response in our prior vaccination study which also included the use of chemotherapy agents such as TMZ (Figure 4). These patients have remained stable for 174.9 and 217.3 weeks. Of the 14 patients without radiographically evident disease, 4/14 (28.6%) have not progressed at 102.7, 171.3, 180.7, 430.7 weeks with a median overall time to progression of 10.4 months comparing favorably with a historical unvaccinated cohort (EGFRvIII positive and gross total resection) that had a median TTP of 7.1 months (n=39). (Figure 5) For patients with GBM, the median survival time was 20.0 months which compares favorably with recently published trials evaluating newly-diagnosed patients with GBM treated with GLIADEL®(13.9 months)[1]; radiation and concurrent TMZ (14.6 months)[2]; or radiolabeled anti-tenascin monoclonal antibodies performed at Duke University (18.3 months)[123] (Figure 7). This Phase I study has been completed. No toxicity has been seen (Figure 6). PBMC were isolated and analyzed by flow cytometry. $CD4^+$ is shown as percent of lymphocytes and T_{Regs} are shown as percent of CD4. T_{Regs} defined as $CD4^+CD25^+Foxp3^+$. Based on these results, the dose for this study will be 20 mg.

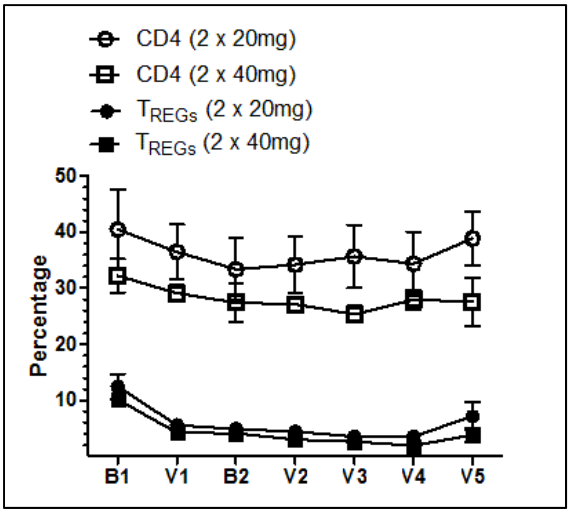

Figure 6. Basiliximab reduces TRegs in patients with GBM receiving DC vaccination. (B=Basiliximab, V=vaccine)

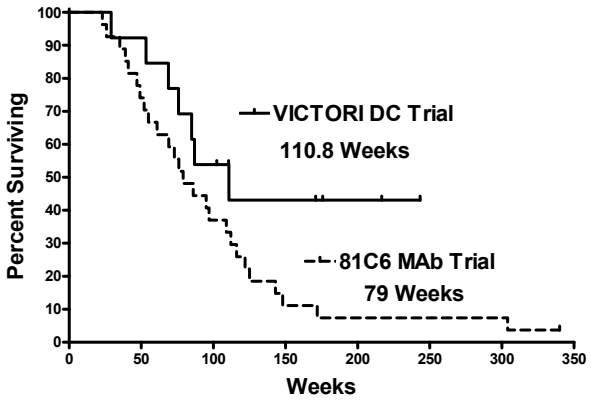

Figure 7. Survival comparison of patients with GBM treated with PEPvIII-KLH loaded DCs (VICTORI Trial) with patients treated at Duke University with 81C6 anti-tenascin ^{131}I labeled MAb. Both patient populations had similar eligibility criteria. Published median survival for such patients treated with Gliadel™ is 59.6 weeks [1] and with concurrent RT and temozolomide is 58.4 weeks [2]

7.3 Study Purpose/Rationale

We have demonstrated in murine models that DCs loaded with tumor-specific antigens in the form of mRNA can induce potent and specific humoral and cell-mediated immune responses that are effective against murine i.c. tumors, including a syngeneic murine astrocytoma, without inducing autoimmunity [101, 104, 124]. Our previous clinical experience has also shown that DC vaccines in combination with standard of care radiation therapy and chemotherapy are capable of generating potent, tumor-specific immune responses and clinical radiographic responses in patients with MGs. We and others have also shown that antigens derived from *CMV* are contained within MGs and may serve as potent and specific immunotherapy targets. Vaccination and adoptive T cell strategies targeting *CMV* in humans in other contexts, including the targeting of lesions within the CNS, have been safe and effective. We have also shown that DCs generated from patients with GBM and loaded with pp65-LAMP mRNA are capable of generating CD4+ and CD8+ T cells that produce IFN-γ and kill malignant astrocytes infected with *CMV* in an antigen-specific fashion. We have found that TILs isolated from these patients are significantly enriched for T cells that specifically recognize *CMV* antigens, suggesting that this response may be important in the biology of these tumors.

CMV antigens have been identified in GBM and may make excellent antitumor immunotherapeutic targets. Vaccination and adoptive T cell strategies targeting CMV in humans in other contexts have been safe and effective. DC vaccinations targeting other antigens in GBM have been safe and effective. Other peptide vaccines given to patients with GBM during recovery from TMZ-induced lymphopenia have produced potent tumor-specific immune responses. TMZ has recently shown modest efficacy, but is not curative, in some patients with newly-diagnosed GBM and is now frequently given to these patients during and after RT. Preclinical studies demonstrate that vaccine responses are enhanced during the recovery from TMZ-induced lymphopenia, but a preferential recovery of immunosuppressive T_{Regs} during this homeostatic event likely serves to limit tumor-antigen directed immune responses. Preclinical studies have shown that unarmed antibodies specific for the high affinity IL-2 receptor constitutively expressed on T_{Regs} abrogate their immunosuppressive phenotype and indirectly enhance effector T cell responsiveness. This is consistent with the notion that the only non-

redundant function of IL-2 at physiologic levels is the maintenance of T_{Regs}. This may be especially true within the context of recovery from lymphodepletion when high levels of homeostatic cytokines such as IL-7 and IL-15 may drive activated T cell division in the absence of IL-2. Basiliximab a chimeric (murine/human) monoclonal antibody (IgG_{1K}), produced by recombinant DNA technology, functions as an immunosuppressive agent, specifically binding to and blocking the interleukin-2 receptor α-chain (IL-2Rα, also known as CD25 antigen) on the surface of activated T-lymphocytes that may abrogate the functional recovery of T_{Regs} in patients with GBM recovering from TMZ-induced lymphopenia and enhance tumor-specific immune responses.

DC vaccines have shown considerable promise in the treatment of cancer. However, generally <5% of injected DCs actually reach the vaccine-site draining lymph nodes, limiting the induction of anti-tumor immune responses. We conducted a clinical trial with newly-diagnosed GBM patients who were randomized to pre-immunization with Td toxoid or unpulsed DCs before vaccination with *CMV* pp65 RNA-pulsed DCs (*ATTAC Trial*: FDA-IND-BB-12839). Strikingly, Td-treated patients had systemically enhanced DC migration and significantly improved PFS and OS compared to controls (PFS >36.6 vs 4.4 months, OS >36.6 vs 11.6 months). Our murine studies corroborated that Td pretreatment systemically enhanced DC migration and suppressed tumor growth. In the proposed clinical study, we aim to: 1) enhance the migration of *CMV* pp65 RNA-pulsed DCs with Td, and 2) to evaluate the effect of basiliximab and Td on survival outcomes when combined with DCs.

8 OBJECTIVES AND ENDPOINTS

Table 1. Objectives and Endpoints

	Objective	Endpoint	Analysis
Key Primary	To examine the impact of Td pre-conditioning with or without basiliximab on survival	Median survival from randomization	See Section 15.5.1
Other Primary	To determine whether pre-conditioning of the vaccine site with Td toxoid systemically increases the migration of *CMV* pp65 RNA-pulsed DCs to site-draining inguinal lymph nodes	The percentage of ^{111}In-labeled DCs reaching inguinal nodes will be calculated from the initial signal at the injection site in the groin to 48 hours after the 4th vaccination	See Section 15.5.2
Other Primary	To examine the impact to Td pre-conditioning on survival among patients who are CMV positive	Median survival from randomization	See Section 15.5.3
Other Primary	To assess the impact of Td pre-conditioning on survival among patients who are CMV negative	Median survival from randomization	See Section 15.5.4
Key Secondary	To examine the impact of Td pre-conditioning with and without basiliximab on progression-free survival	Median progression-free survival from randomization	See Section 15.6.1
Other Secondary	To examine the impact of Td pre-conditioning at the vaccine site on progression-free survival among patients who are CMV positive	Median progression-free survival from randomization	See Section 15.6.2
Other Secondary	To examine the impact of Td pre-conditioning on progression-free survival among patients who are CMV negative	Median progression-free survival from randomization	See Section 15.6.3
Exploratory	To describe changes from baseline in immune response as measured by IFNγ ELISpot	Median change from baseline in ELISpot at each follow-up assessment	See Section 15.7
Exploratory	To assess T cell polyfunctionality when ELISpot results are positive	Proportion of patients with 1, 2 and 3 functions at each assessment	See Section 15.7
Exploratory	To describe changes in T_{Regs} over time	Median change from baseline in T_{Reg} levels at each follow-up assessment	See Section 15.7
Exploratory	To measure levels of CCL3 and other inflammatory soluble factors	Median change from baseline in CCL3 and other inflammatory soluble factors.	See Section 15.7

9 INVESTIGATIONAL PLAN

9.1 Study Design

A maximum of 100 patients with resected, newly-diagnosed WHO Grade IV glioma will be enrolled in this study with the expectation that approximately 79 patients will be randomized to subsequent treatment after completion of radiation treatment with concurrent temozolomide. At the time that accrual to Groups I and II are complete an interim analysis will be conducted in order to make a decision concerning the accrual of additional patients to address efficacy objectives within patients subgroups defined by CMV status (see Section 15.8). The study was

closed to enrollment in April 2018 (Amendment 73). All patients previously enrolled will continue on study as planned.

All enrolled patients will undergo a leukapheresis after resection for harvest of PBLs for generation of DCs. Patients will then receive approximately 6 weeks of standard of care RT and concurrent TMZ at a standard targeted dose of 75 mg/m^2/d (please see Package Insert uploaded in eIRB for standard therapy). Patients should start RT within approximately 6 weeks of surgery. Patients who either experience progressive disease during radiation, are dependent on steroid supplements above physiologic levels at time of first vaccination, are unable to tolerate TMZ, or whose DCs or PBLs fail to meet release criteria will be withdrawn from the study and replaced and will not undergo repeat leukapheresis. For patients whose initial leukapheresis yields less than 3 vaccines, repeat leukapheresis may be obtained a minimum of 2 weeks from the previous leukapheresis (and may be repeated as needed) if pre-pheresis blood work is within the Apheresis Center's parameters and as long as this does not cause a significant delay in treatment for the patient. At the post-RT clinic visit where patient's MRI and blood work is evaluated prior to ordering the first post-RT cycle of TMZ, patients will be randomized to receive DC vaccination with one of three treating regimens: Group I: unpulsed DC pre-conditioning prior to vaccination #4; Group II: Td pre-conditioning prior to vaccination #4; or Group III: basiliximab infusions at vaccination #1 and #2 with Td pre-conditioning prior to vaccination #4. As of March 2017 (AMD 49), randomization of patients to Group III has been terminated. Please see Section 9.1.1 for a detailed outline of the randomization strategy for vaccine site pre-conditioning and Section 9.1.7 for a detailed description of the double blinding process. The study cycle of TMZ comprises a targeted dose of 150-200mg/m^2/d for 5 days every 5 (± 1) weeks. All patients will receive up to a total of 10 DC vaccines given bilaterally at the groin site unless progression occurs. DC vaccines will be given i.d. and divided equally to both inguinal regions. DC vaccines #1-3 will be given every two weeks, thus delaying the initiation of TMZ cycle 2 for all patients. All remaining TMZ cycles will continue every 5 (± 1) weeks. Before the first DC vaccination, patients will receive 0.5 mL of Td (tetanus and diphtheria toxoids adsorbed) intramuscularly into the deltoid muscle to ensure adequate immunity to the tetanus antigen. The Td vaccine will be obtained through Duke Investigational Chemotherapy Services (ICS) Pharmacy. All patients will undergo leukapheresis again for immunologic monitoring with specific assessment of baseline antigen-specific cellular and humoral immune responses and further DC generations 4 ± 2 weeks after vaccine #3.

If the patient is determined to have progressive disease, the patient may be considered for eligibility on the INTERCEPT study (INTracerebral EGFR-vIII Chimeric Antigen Receptor Gene-Modified T Cells for PaTients with Recurrent GBM, Pro00083828). If the patient is determined to be eligible for the INTERCEPT study (Pro00083828), the cells extracted from leukapheresis while they were on this study, ELEVATE, will be used to create the CAR T cells for administration in the INTERCEPT study.

Group I Treatment Plan (DC Vaccinations with pre-conditioning with Unpulsed DCs prior to the 4th DC vaccine) Patients in Group I will receive 1 x 10^6 autologous unpulsed DCs in saline administered to a single side of the groin, and 0.4 mLs of saline administered to the contralateral side one day prior to the fourth DC vaccine, which is always given bilaterally at the groin site. Patients will then receive ^{111}In-labeled DCs as the 4th vaccine to compare the effects of different

skin preparations on DC migration followed by SPECT/CT imaging immediately and at 1 and 2 days after injections.

Group II Treatment Plan (DC Vaccinations with pre-conditioning with Td prior to the 4th DC vaccine)
Patients in Group II will receive a single dose of Td toxoid (1 flocculation unit, Lf, in 0.4 mLs) administered to a single side of the groin, and 0.4 mLs of saline administered to the contralateral side one day prior to the fourth DC vaccine, which is always given bilaterally at the groin site. Patients will then receive ^{111}In-labeled DCs as the 4th vaccine to compare the effects of different skin preparations on DC migration followed by SPECT/CT imaging immediately and at 1 and 2 days after injection.

Group III Treatment Plan (DC Vaccinations with basiliximab infusions prior to 1st and 2nd DC vaccine with Td pre-conditioning prior to 4th DC vaccine)
Patients in Group III will receive the first 3 DC vaccines every 2 weeks same as Groups I and II only they will also receive basiliximab 20 mg I.V. 7 days before DC vaccine # 1 and 7 days before DC vaccine # 2. Prior to the 4th vaccine, patients will receive a single dose of Td toxoid (1 flocculation unit, Lf, in 0.4 mLs) administered to a single side of the groin, and 0.4 mLs of saline administered to the contralateral side one day prior to the fourth DC vaccine, which is always given bilaterally at the groin site. This group will not undergo migration studies.

Effective March 2017 (AMD 49), randomization of patients to Group III has been terminated due to results from the related REGULATE study (IRB # Pro00000581), a study that evaluated the safety, toxicity, and efficacy of T Reg inhibition with basiliximab. REGULATE was used as the model for Group III in ELEVATE. Recent analyses of REGULATE data have shown that although the regulatory T cells were depleted, the cells did not show pp65 reactivity and their activation status was reduced. Patients previously randomized to Group III will continue on study as planned.

With AMD49, approximately 79 patients will ultimately be randomized to subsequent treatment after completion of radiation treatment with concurrent temozolomide, including 9 patients previously randomized to Group III. As originally planned, an interim analysis will be conducted after approximately 35 patients have been randomized to Groups I and II each in order to make a decision concerning the accrual of additional patients to address efficacy objectives within patient subgroups defined by CMV status (see Section 15.8).

All Groups
All patients will then be vaccinated in conjunction with subsequent TMZ cycles every 5 ± 1 weeks for a total of 6 to 12 cycles after RT at the discretion of the treating oncologist. DCs will be given on day 21 ± 2 days of each TMZ cycle. DC vaccinations will continue during TMZ cycles up to a total of 10 vaccines unless progression occurs. Patients will be imaged bimonthly at the discretion of the treating oncologist without receiving any other prescribed anti-tumor therapy. Patients will undergo an additional leukapheresis for generation of DCs if needed to continue vaccinations. As part of standard care for these patients, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure consent will be obtained separately. However, if tissue is obtained, it will be used to confirm

tumor progression histologically and to assess immunologic cell infiltration and pp65 antigen escape at the tumor site.

9.1.1 Randomization of Vaccine Site Pre-Conditioning Strategies

A randomization will be performed to assign patients to one of the three treatment strategies after completion of radiation with concurrent temozolomide. A permuted block randomization scheme stratified by *CMV* serology will be used to assign patients to treatment groups: unpulsed DCs pre-conditioning prior to vaccination #4, Td pre-conditioning prior to vaccination #4, or basiliximab infusions at vaccination #1 and #2 and Td pre-conditioning prior to vaccination #4.

As previously noted, the randomization of subjects to Group III has been terminated as of AMD 49. With this amendment, the randomization scheme stratified by CMV serology has been modified to assign patients to one of two treatment groups.

The administration of pre-conditioning agent (Td or unpulsed DCs) will be blinded for Groups I and II, however, the administration of the pre-conditioning agent (Td) and basiliximab for Group III will not be. Hence, patients and providers will know when a patient has been assigned to the third treatment group (please see randomization and blinding SOP 18.2 in appendices).

9.1.2 Definition of Unacceptable Toxicities

Toxicities will be graded according to the NCI CTCAE version 4 criteria. An unacceptable toxicity is defined as any Grade 3 toxicity that is possibly, probably, or definitely attributed to a study agent (basiliximab, pre-conditioning agent [Td or blinded Td/unpulsed DC], or pp65 DC vaccine) that does not resolve to baseline in 2-3 weeks, any Grade 3 hypersensitivity reactions or autoimmune toxicity requiring steroids or hormone replacement, 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. A Grade 3 or greater toxicity that is clearly related to TMZ (an expected and known side effect) that does not resolve and requires an interruption, dose adjustment to metronomic TMZ schedule, or stoppage will result in subject removal from this protocol.

9.1.3 Dose Modification

TMZ will be administered concomitant with standard external beam RT under the direction of the oncologist. Please see TMZ package insert uploaded in eIRB for standard of care treatment guidelines. The dose may be adjusted at the discretion of the treating oncologist.

The study drug (*CMV* pp65 RNA-pulsed DCs) dose will not be modified in this trial. Those subjects whose cells fail to meet release criteria will be removed and replaced.

9.1.4 Safety Considerations

Management of Toxicities

If a Grade 3 NCI CTC or greater toxicity is seen that is not attributable to a concomitant medication, co-morbid event, or disease progression that has been documented radiographically or clinically, the next immunization for that patient will be withheld for up to 2 months or until the NCI CTC toxicity improves to a Grade 2 or until the KPS score returns to within 10 points of baseline. However, planned procedures requiring hospitalization, or long-term clinical decline that is now seen in patients years from WBRT, which are clearly not related to study drug, but

are the natural development common in this patient population, will not be considered an unacceptable toxicity nor will have immunizations withheld.

Adverse Event Reporting and Documentation

An "Adverse Event" will be defined as any adverse change from the subject's pre-treatment baseline condition (which is assessed at the post-RT/TMZ clinic visit), including any clinical or laboratory test abnormality that occurs during the course of research after the time of randomization. Adverse events will be categorized and graded in accordance with the NCI CTC (Version 4).

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

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

All adverse events which are serious and unexpected should be reported immediately to Dr. Dina Randazzo (Pager: 919-970-9692) 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 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 Duke University Medical Center IRB using the appropriate SAE report form. At the time of the annual progress report to the Duke University Medical Center IRB, a summary of the overall toxicity experience will be provided.

9.1.5 Missed Doses

To ensure that repetitive DC vaccines will be given to patients, DCs will be given on day 21 ± 2 days of each TMZ cycle. Therefore, with a margin of 2 days surrounding each TMZ cycle Day 21, all patients will receive up to 10 DC vaccines or until progression, whichever comes first. At the discretion of the study PI, patients who miss vaccine administration for whatever reason will have their appointment re-scheduled to administer the vaccine as soon as possible and a deviation will be filed with the Duke IRB. For continued non-compliance with the scheduled vaccine appointments, the subject may be removed from the trial at the discretion of the study PI.

9.1.6 Concomitant Medications

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

9.1.7 Study Drug(s) Blinding

The Duke ICS Pharmacy will be responsible for the preparation of the Td and the saline used for the pre-conditioning done prior to the fourth vaccine. The unpulsed DCs used for pre-conditioning will be prepared in the DBTIP Laboratory and delivered to the ICS for the labeling and distribution of the blinded pre-conditioning drugs (i.e., Td, unpulsed DCs, and saline). Only those key personnel needed for the preparation and distribution of the pre-conditioning drugs will be unblinded (i.e., ICS pharmacy staff and DBTIP Lab study drug manufacturing personnel, as well as the statisticians). The patient and the remaining key personnel (including the PI and research nurses administering the pre-conditioning drugs) will be blinded to the pre-conditioning regimen. Emergency unblinding procedures are described in 13.3.

The administration of pre-conditioning agent (Td or unpulsed DCs) will be blinded for Groups I and II, however, the administration of the pre-conditioning agent (Td) and basiliximab for Group III will not be. Hence, patients and providers will know when a patient has been assigned to the third treatment group (please see randomization and blinding SOP 18.2 in appendices).

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

In our previous randomized clinical trial of 12 patients with newly-diagnosed GBM, we evaluated the impact of vaccine site pre-conditioning with Td toxoid[125]. Patients randomized to Td showed increased dendritic cell migration bilaterally and significantly improved progression-free and overall survival. Furthermore, we observed a modest association between effective DC migration to VDLNs and clinical outcomes. Based on the small sample size of the initial study, we would like to conduct a validation study with Td pre-conditioning that is powered sufficiently to evaluate the impact of DC migration on clinical outcomes. For that reason, we will conduct this validation trial with identical Td formulation, doses, and DC vaccine treatment schedules as in the original study. The 4^{th} vaccine is the first monthly vaccine and we believe that the influence of the skin preconditioning would be most isolated at that time. In the original study, Td was used based on our mouse studies which revealed a memory CD4 T cell response to tetanus and unpulsed DCs were used based on research[126] that showed injection of DCs 24 hours before DC vaccine increased migration. Therefore, 2 conditioning regimens were tested: Td versus unpulsed DCs with saline in the contralateral side as control.

9.3 Rationale for Correlative Studies

Please see Section 7.2 ^{111}Indium-labeling of Cells for in vivo Trafficking Studies.

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

Subjects evaluable for the analysis of the primary endpoints of DC migration will include all randomized patients in Groups I and II who receive the 4th DC vaccine. Survival analyses will include all randomized patients.

Once the patient signs an ICF, that subject will be considered "on study". Rationale for taking patient off protocol treatment will be documented.

9.5 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 12.7. Section 12.6 describes procedures and process for prematurely withdrawn patients.

10 STUDY DRUG

10.1 Names, Classification, and Mechanism of Action

Tetanus-diphtheria toxoid (Td toxoid adsorbed).

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.

DC vaccine

Human *CMV* pp65-LAMP mRNA-pulsed autologous DCs is the name of the study drug given with every vaccine. The name of the study drug at vaccine #4 will be 1 x 10^6 autologous unpulsed DCs or 1 Lf Td toxoid (depending on randomization). This vaccination will then consist of administration of 2 x 10^7 *CMV* pp65-LAMP mRNA loaded mature DCs as described above except that these DCs will be labeled with ^{111}In (50 μCi / 5 x 10^7 DCs) (Nycomed-Amersham, Chicago, IL) prior to injection. The class of action for all study drugs is a biological with the exception of the tetanus toxoid, which is an antitoxin.

Leukapheresis and dendritic cell vaccine generation

At least two leukaphereses will be performed on each patient enrolled on this protocol. The leukapheresis will be used for DC generation and immunologic monitoring. All leukaphereses will be approximately a 4-hour leukapheresis. It is estimated that 10-12 L of blood will be processed during this leukapheresis. An additional 4-hour leukapheresis will be requested from patients with positive immunological responses to the therapy for additional immunologic monitoring studies or for patients who need additional DCs generated to ensure a total of 10 vaccines.

DCs will be generated from the first leukapheresis *in vitro* by 7-day culture with GM-CSF and IL-4. PBMC for *in vitro* generation of DCs will be obtained by leukapheresis at the Duke Apheresis Unit and transported to the Cell Processing facility. For patients without sufficient venous access for leukapheresis a temporary intravenous catheter may be inserted.

At the end of the 7 day incubation for generation of DC a sample of the media is taken for mycoplasma testing, the cells are then harvested and electroporated with pp65-LAMP mRNA. The DCs are placed in a flask with AIM V media GM-CSF + IL-4 + TNF-α + IL-6 + IL-1β at 37^{O}C, 5% CO_2 for 18-20 hours for maturation. The cells are washed twice with PBS and frozen at 2-4 x 10^7cells/mL in 90% autologous human AB serum (Valley Biomedical, Winchester, VA 22602), 10% DMSO and 5% glucose in a controlled-rate freezer at a rate of 1ºC/minute.

The DCs are then stored until needed at –135ºC. After freezing, an aliquot of cells is thawed for QA/QC. This testing will look at viability, (>70%) endotoxin content, (<5 E.U. /Kg B.W.) mycoplasma contamination (negative) and sterility testing for aerobic and anaerobic bacterial cultures (1 x 10^6 DCs) and fungal cultures (1 x 10^6 DCs).

For each vaccination, cells that have passed QA/QC will be rapidly thawed at 37ºC, washed three times with PBS and counted. The cell concentration will be adjusted to 5 x 10^7 cells/mL and DCs will be resuspended in preservative free saline and placed into a sterile tuberculin syringe with a 27 gauge needle.

For <u>all</u> DC preparations, including unlabeled DCs for vaccination and ^{111}In-labeled DCs, from the final preparation a sample of cells will be sent for Gram stain and endotoxin testing prior to administration. DC vaccination will not be given until endotoxin testing has been passed (< 5.0 E.U/Kg) and the Gram stain has been found to be negative. An aliquot of cells will also be sent for aerobic and anaerobic bacterial cultures (1 x 10^6 DCs) and fungal cultures (1 x 10^6 DCs).

In the event of a positive sterility or mycoplasma test, the Principal Investigator or his or her designee will notify the treating physician and the patient. The FDA and IRB will be notified within 15 calendar days. The patient will be asked to be evaluated by a physician within 24 hours. If the patient has or develops a temperature >38.5 ºC or clinical evidence of infection at the injection site (drainage, erythema or edema) or systemically, the patient will have swabs taken from the injection sites (if possible), along with blood, urine and sputum (if possible) sent for bacterial, fungal, and mycoplasmal culture and sensitivity testing. The patient will be treated expectantly with antibiotics based on the sensitivities of the organisms identified from the immunization product, and an independent infectious disease consultation will be obtained to guide further therapy. Any remaining immunization samples will be sent for sent for bacterial, fungal, and mycoplasmal culture and sensitivity testing and endotoxin testing, and immunizations will proceed only if the patient fully recovers and subsequent samples are found to be sterile.

If the patient is determined to have progressive disease, the patient may be considered for eligibility on the INTERCEPT study (INTracerebral EGFR-vIII Chimeric Antigen Receptor Gene-Modified T Cells for PaTients with Recurrent GBM, Pro00083828). If the patient is determined to be eligible for the INTERCEPT study (Pro00083828), the cells extracted from

leukapheresis while they were on this study, ELEVATE, will be used to create the CAR T cells for administration in the INTERCEPT study.

Basiliximab

Because our Phase I study showed no difference between 20 and 40 mg of basiliximab in T_{Reg} depletion, patients will receive basiliximab 20 mg 7 days before vaccines # 1 and # 2 in the Infusion Center of the Duke Cancer Center.

A maximum tolerated dose of basiliximab has not been determined in brain cancer patients. During the course of clinical studies, basiliximab has been administered to adult renal transplantation patients in single doses of up to 60 mg, or in divided doses over 3-5 days of up to 120 mg, without any associated serious adverse events.
Basiliximab is contraindicated in patients with known hypersensitivity to basiliximab or any other component of the formulation.

Basiliximab functions as an IL-2 receptor antagonist by binding with high affinity (K_a = 1 x 10^{10} M^{-1}) to the alpha chain of the high affinity IL-2 receptor complex and inhibiting IL-2 binding. Basiliximab is specifically targeted against IL-2Rα, which is selectively expressed on the surface of activated T-lymphocytes. This specific high affinity binding of basiliximab to IL-2Rα competitively inhibits IL-2-mediated activation of lymphocytes, a critical pathway in the cellular immune response involved in allograft rejection. While in the circulation, basiliximab impairs the response of the immune system to antigenic challenges. Whether the ability to respond to repeated or ongoing challenges with those antigens returns to normal after basiliximab is cleared is unknown.

Single-dose and multiple-dose pharmacokinetic studies have been conducted in patients undergoing first kidney transplantation. Cumulative doses ranged from 15 mg up to 150 mg. Peak mean ± SD serum concentration following intravenous infusion of 20 mg over 30 minutes is 7.1 ± 5.1 mg/L. There is a dose-proportional increase in Cmax and AUC up to the highest tested single dose of 60 mg. The volume of distribution at steady state is 8.6 ± 4.1 L. The extent and degree of distribution to various body compartments have not been fully studied. The terminal half-life is 7.2 ± 3.2 days. Total body clearance is 41 ± 19 mL/h. No clinically relevant influence of body weight or gender on distribution volume or clearance has been observed in adult patients. Elimination half-life was not influenced by age (20-69 years), gender or race.

All patients will receive basiliximab mixed with 50-100 mLs of sterile 0.9% sodium chloride or dextrose 5% solution. Basiliximab solution will be administered in a peripheral or central vein over a 20-60-minute period. When mixing the solution, gently invert the bag in order to avoid foaming: do not shake. Infusion should be administered intravenously immediately after preparation. If it needs to be held longer, it should be refrigerated between 2° and 8°C (36° to 46°F) for up to 24 hours or at room temperature for 4 hours after which it should be discarded. Medications for the treatment of severe hypersensitivity reactions including anaphylaxis should be available for immediate use. Patients will be monitored for anaphylactic events for 1hour. Please see drug fact sheet in the Appendix – Section 18.7.

10.2 Packaging and Labeling of Study Agents

- For *CMV* pp65-LAMP mRNA-pulsed DCs:
  Name
  MRN
  DOB
  Drug: pp65DCs
  Lot #: Lot 001
  Caution New Drug Limited By Federal Law To Investigational Use
- For autologous unpulsed DCs:
  Name
  MRN
  DOB
  Drug: unpulsed DCs (blinded to personnel administering drug)
  Lot #: Lot 001
  Caution New Drug Limited By Federal Law To Investigational Use
- For tetanus diphtheria toxoid used in pre-conditioning
  Name
  MRN
  DOB
  Drug: Td (blinded to personnel administering drug)
  Lot #: Lot 001
- For *CMV* pp65-LAMP mRNA loaded mature DCs labeled with ^{111}In (50 μCi / 5 x 10^7 DCs)
  Name
  MRN
  DOB
  Drug: Radiolabeled pp65DCs
  Lot #: Lot 001
  Caution New Drug Limited By Federal Law To Investigational Use
- For basiliximab:
  Name
  MRN
  DOB
  Drug: basiliximab
  Lot #: Lot 001
- **Td™** (Tetanus diphtheria toxoid adsorbed); Stored at 4^{o}C used as Td booster vaccine
  Drug: Td
  Lot #
  Expiration Date

10.3 Supply, Receipt, and Storage

The DCs will be stored in a locked liquid nitrogen freezer in the Molecular Products and Cellular Therapies (MPACT) cGMP facility. The Nautilus LIMS (Laboratory Information Management System) database will track receipt and storage location.

Table 2. Storage of Study Agents

MPACT Facility	Duke ICS Pharmacy	Duke Radiopharmacy
CMV pp65-LAMP mRNA-pulsed DCs	Td used for booster and for pre-conditioning	*CMV* pp65-LAMP mRNA loaded mature DCs labeled with ^{111}In
Unpulsed DCs	Saline used for pre-conditioning	
	Basiliximab	

10.4 Dispensing and Preparation

The unblinded DC products (pp65 DC vaccines) will be delivered from the MPACT facility directly to the clinic under the supervision of the trained research staff. Each labeled patient vaccine will be transported to the clinic in a separate cooler. DCs will be administered according to protocol. The patient's name, Study ID, DOB, and Duke history number will be double verified prior to DC administration as is standard Duke transfusion procedure.

The radiolabeled DC products used with the migration studies within Groups I and II will be prepared from the pp65 DC vaccines sent from the MPACT facility to the Radiopharmacy and dispensed radiolabeled from the Duke Radiopharmacy.

Please see Section 9.1.7 for blinding, preparing and dispensing the pre-conditioning study drugs (i.e., saline, Td, and unpulsed DCs).

Patients in Group III will receive basiliximab from the Duke ICS Pharmacy.

Table 3. Dispensing of Study Agents

MPACT Facility	Duke ICS Pharmacy	Duke Radiopharmacy
CMV pp65-LAMP mRNA-pulsed DCs	Td used for booster and for pre-conditioning (blinded for Groups I and II only)	*CMV* pp65-LAMP mRNA loaded mature DCs labeled with ^{111}In
	Saline used for pre-conditioning (blinded)	
	Unpulsed DCs for pre-conditioning (blinded)	
	Basiliximab	

10.5 Compliance and Accountability

All DC vaccines will be stored in the BTIP in a temperature controlled, locked access controlled storage unit. A drug log sheet will be used to track and document the drug. The products will be signed out and distributed by the BTIP laboratory manager. The Duke BTIP personnel use safe medication practices to reduce the risk of medication errors and adverse events when setting up study drug procedures. Investigational drugs are ordered, received, stored, and dispensed for BTIP protocols that are approved by the DUHS IRB. Investigational drugs are stored separately from other drugs in an area of limited access and in accordance with special storage requirements. They are clearly labeled with the identity of the study drug and other control numbers. All drug transfers, receipts, and disposal are recorded in the Duke Nautilus system.

10.6 Disposal and Destruction

Radioactively labeled drug will be disposed of according to standard Duke radiation safety practices. Un-labeled drug will be autoclaved.

11 SUBJECT ELIGIBILITY

Inclusion Criteria

- Age ≥18 years of age.
- WHO Grade IV Glioma with definitive resection prior to consent, with residual radiographic contrast enhancing disease on the post-operative CT or MRI of <1 cm in maximal diameter in any axial plane.
- MRI post RT does not show progressive disease at time of randomization
- Karnofsky Performance Status (KPS) of > 80%.
- Hemoglobin ≥ 9.0 g/dl, ANC ≥ 1,500 cells/µl, platelets ≥ 125,000 cells/µl.
- Serum creatinine ≤ 1.5 mg/dl, serum SGOT and bilirubin ≤ 1.5 times upper limit of normal.
- Signed informed consent approved by the Institutional Review Board.
- Female patients must not be pregnant or breast-feeding. Female patients of childbearing potential (defined as < 2 years after last menstruation or not surgically sterile) must use a highly effective contraceptive method (allowed methods of birth control, [i.e. with a failure rate of < 1% per year] are implants, injectables, combined oral contraceptives, intra-uterine device [IUD; only hormonal], sexual abstinence or vasectomized partner) during the trial

and for a period of > 6 months following the last administration of trial drug(s). Female patients with an intact uterus (unless amenorrhea for the last 24 months) must have a negative serum pregnancy test within 48 hours prior to first study procedure (leukapheresis).

- Fertile male patients must agree to use a highly effective contraceptive method (allowed methods of birth control [i.e. with a failure rate of < 1% per year] include a female partner using implants, injectables, combined oral contraceptives, IUDs [only hormonal], sexual abstinence or prior vasectomy) during the trial and for a period of > 6 months following the last administration of trial drugs.

Exclusion Criteria:

- Pregnant or breast-feeding.
- Women of childbearing potential and men who are sexually active and not willing/able to use medically acceptable forms of contraception.
- Patients with known potentially anaphylactic allergic reactions to gadolinium-DTPA.
- Patients who cannot undergo MRI or SPECT due to obesity or to having certain metal in their bodies (specifically pacemakers, infusion pumps, metal aneurysm clips, metal prostheses, joints, rods, or plates).
- Patients with evidence of tumor in the brainstem, cerebellum, or spinal cord, radiological evidence of multifocal disease, or leptomeningeal disease.
- Severe, active comorbidity, including any of the following:
    - Unstable angina and/or congestive heart failure requiring hospitalization;
    - Transmural myocardial infarction within the last 6 months;
    - Acute bacterial or fungal infection requiring intravenous antibiotics at the time of study initiation;
    - Chronic obstructive pulmonary disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy;
    - Known hepatic insufficiency resulting in clinical jaundice and/or coagulation defects;
    - Known HIV and Hepatitis C positive status;
    - Major medical illnesses or psychiatric impairments that, in the investigator's opinion, will prevent administration or completion of protocol therapy;
    - Active connective tissue disorders, such as lupus or scleroderma that, in the opinion of the treating physician, may put the patient at high risk for radiation toxicity.
- Co-medication that may interfere with study results; e.g. immuno-suppressive agents other than corticosteroids.
- Prior, unrelated malignancy requiring current active treatment with the exception of cervical carcinoma in situ and adequately treated basal cell or squamous cell carcinoma of the skin. (Treatment with tamoxifen or aromatase inhibitors or other hormonal therapy that may be indicated in prevention of prior cancer disease recurrence, are not considered current active treatment.)
- Patients are not permitted to have had any other conventional therapeutic intervention other than steroids prior to enrollment outside of standard of care chemotherapy and radiation therapy. Patients who receive previous inguinal lymph node dissection, radiosurgery, brachytherapy, or radiolabeled monoclonal antibodies will be excluded.

- Current, recent (within 4 weeks of the administration of this study agent), or planned participation in an experimental drug study.
- Known history of autoimmune disease (with the exceptions of medically-controlled hypothyroidism and Type I Diabetes Mellitus).

12 SCREENING AND ON-STUDY TESTS AND PROCEDURES

12.1 Screening Examination

The screening examination will take place at the Duke PRTBTC clinic visit. An informed consent must be signed by the patient before any screening procedure takes place. The baseline physical and neurologic examination with KPS score along with standard of care blood work will be performed and documented by the neuro-oncology team and verified by the study team during this PRTBTC clinic visit. All subject data is standard of care evaluation that occurs for all patients being seen in the PRTBTC. If the subject is considered a screen failure prior to vaccine treatment, the source documents for electronic data entry will be obtained from the Duke electronic medical record.

CMP and CBC with auto differential are drawn at clinic visits and prior to surgical procedures per standard of care. A *CMV* immune screen will also need to be drawn at one of these clinic visits following consent and prior to initiation of vaccine therapy.

Initial clinical evaluations will also include a baseline MMSE testing. A baseline and study eligibility MRI (with and without gadolinium enhancement) of the brain per standard of care for comparison to subsequent MRI images will also be obtained.

The patient must have had a definitive resection. Residual radiographic contrast enhancement on post-resection CT or MRI must not exceed 1 cm in diameter in any axial planes at time of consent. Patients with progressive disease after radiation will not be a candidate for the vaccine despite being previously consented and will be removed from the study and replaced (please see Section 12.7.4 for radiologic evaluations).

During collection of tumor sample at resection at Duke University, primary tissue will be banked and stored for IHC assays. Tissue will be stored in formalin-fixed blocks for subsequent partitioning of pathology slides. For initial resection at Duke, slides will be sent for MGMT promotor methylation status and IDH-1/2 mutation analysis as part of the routine molecular diagnostic assay performed at Duke. If the surgery is performed outside of Duke and if such institutions do not incorporate these initial molecular diagnostic assays, slides from surgical blocks will be ordered for subsequent MGMT promoter methylation status and IDH-1/2 mutation analysis, so as to ensure baseline molecular diagnostics of the tumor for each patient. At the discretion of the study investigator, for surgical blocks containing initial resection and biopsy at recurrence (if applicable) tissue, formalin-fixed paraffin-embedded slides will be requested for immunohistochemistry assays for the detection of CMV. These slides for CMV detection will not exceed 10% of the original specimen block, so as to preserve adequate tissue should patients prefer future diagnostic studies. Patient consent will be obtained for obtaining these slides in either scenario if a surgery/biopsy was performed at Duke University or at an external institution.

If resection is done outside of Duke University Hospital, formalin-fixed paraffin-embedded slides sectioned from outside blocks, not to exceed 10% of surgical specimen block tissue, prepared on Fischer Plus glass or Histostix coated slides when available will be mailed to:

Dina Randazzo, DO
Duke Brain Tumor Immunotherapy Program
Department of Neurosurgery
The Preston Robert Tisch Brain Tumor Center at Duke
Box 3050
220 Sands Building
Duke University Medical Center
Durham, North Carolina 27710, USA

After patients have been consented, they will be entered into the Velos eResearch system.

12.2 Treatment Period

All enrolled patients will then undergo a leukapheresis for generation of DCs and for baseline immunological monitoring. Within 48 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. 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. This first leukapheresis will be approximately a 4-hour leukapheresis, and it is estimated that 10-12 L of blood will be processed during this leukapheresis. One red top tube will be obtained prior to each leukapheresis for immune monitoring (approximately 4 mLs).

Table 4. Schedule of Events

Appointment	Time and Range	Tumor Pathology[1]	Physical and Neurologic Exam KPS, MMSE[2]	MRI[3]	CMV Screen [4]	CMP and CBC[5]	Blood for immunologic monitoring and Sub-clinical Autoimmunity[6]
Screening and Pre-enrollment	Prior to Consent	X	X	X		X	
Consent	Within 6 weeks of OR				X	X	
Leukapheresis[7]	After Consent					X	X
RT/TMZ	Per SOC (starts within 6 weeks of OR)					X	
Post-RT TMZ cycle 1	Within 4 (±2) weeks of RT			X		X	
Basiliximab Infusion (Group III only)	Day 14 (± 2 days)		X			X	X
Td Booster IM	Day 20 (± 2 days) so long as before V#1						
Vaccine #1	Day 21 (± 2 days)		X				X
Basiliximab Infusion (Group III only)	Day 28 (± 2 days)		X			X	X
Vaccine #2	Day 35 (± 2 days)		X				
Vaccine #3	Day 49 (± 2 days)		X				
Leukapheresis	4 (± 2) weeks after V#3					X	X

[1] WHO Grade IV Glioma with definitive resection prior to enrollment, with residual radiographic contrast enhancement on most recent CT or MRI of <1 cm in maximal diameter in any axial plane.

[2] Clinical evaluations with each vaccine will include a general physical examination, complete neurologic examination, and KPS rating. MMSE will be performed at baseline only.

[3] A baseline and study eligibility MRI (with and without gadolinium enhancement) of the brain per standard of care for comparison to subsequent MRI images will also be obtained. MRI or contrasted CT, every 8 ± 4 weeks per PRTBTC standard of care (may be adjusted by the treating neuro-oncologist).

[4] A *CMV* immune screen will also need to be drawn at one of these clinic visits following consent and prior to initiation of vaccine therapy.

[5] CMP and CBC (with auto differential) to confirm eligibility, and as per standard of care prior to and following surgery, and during SOC monthly and study cycles of TMZ and RT (if applicable).

[6] Blood work for immunologic monitoring will be drawn before each leukapheresis (1 red top only prior to phereses), basiliximab (Group III), on the day of vaccine #1 (9 yellow/2 red top only at this visit), and then prior to each vaccine after vaccine #3 (9 yellow/2 red top tubes) and/or at progression (whichever comes first). For subjects who complete all 10 study vaccines without progressing, an attempt will be made to obtain blood for immunologic monitoring 2-3 times a year at standard Duke Preston Robert Tisch Brain Tumor Center visits and at progression (whichever comes first). Peripheral blood for immunologic immune monitoring will be placed in 9 yellow tops and 2 red top tubes for a total of about 90 mLs and delivered to the BTIP lab for processing. Plasma from the yellow top tubes will be collected at each time point listed where immunologic monitoring is done. 2 yellow and 1 red top tubes will be obtained at the pre-conditioning visit (all Groups) and at the SPEC/CT imaging visits (Groups I and II only). Blood work for sub-clinical autoimmunity will be drawn before basiliximab (Group III), or vaccine #1 (all other groups), and repeated only if clinically indicated. Laboratory evaluations to detect subclinical disease will be obtained as follows: serum thyroglobulin Ab (Lab Code 1802105), rheumatoid factor (Lab Code 5560195), antinuclear Ab (Lab Code 5560214), Thyroid Profile (Lab Code 1850277), and CMP (Lab Code 1004261). Total volume for sub-clinical autoimmune testing is < 20 mLs.

[7] Initial leukapheresis will be scheduled following consent. Per standard Duke Apheresis Protocol, all subjects will have CBC, CMP, ionized Calcium, and βHCG within 48 hours prior to the procedure(s). Follow up leukapheresis will occur approximately 4 ± 2 weeks after third immunization to obtain PBMCs for Immunologic Monitoring and generation of additional DCs for continued vaccinations. Leukapheresis can be obtained up to every 2 weeks if needed according to the Duke Apheresis Center protocol, but these will likely be needed less than every 2 months throughout the study to generate enough DCs to continue vaccinations. Blood for immune monitoring will be drawn prior to each leukapheresis (1 red top tube – approximately 4 mLs).

Appointment	Time and Range	Tumor Pathology[1]	Physical and Neurologic Exam KPS, MMSE[2]	MRI[3]	CMV Screen [4]	CMP and CBC[5]	Blood for immunologic monitoring and Sub-clinical Autoimmunity[6]
TMZ Cycle 2 (slight delay start)	To start following V#3		X	X		X	
Pre-conditioning[8]	1 day before V#4		X			X	X
Vaccine # 4 [9] (^{111}In-labeled DCs) (Groups I & II only)	Day 21(± 2 days) of second cycle of TMZ						X
Migration studies[10]	1 day and 2 days after V#4						X
Vaccines with Cycles of TMZ[11]	On day 21 (± 2 days) of each cycle of TMZ		X	X		X	X
Monthly Vaccines After TMZ not to exceed 10	Every month (± 2 weeks)		X	X		X	X
Progression[12]		X		X			X

12.3 End of Treatment

Vaccines are given as described above for a total of 10 or until progression (whichever comes first). Once the vaccinations are complete, the treatment phase of the study will be over and the follow-up period will begin.

If the patient is determined to have progressive disease, the patient may be considered for eligibility on the INTERCEPT study (INTracerebral EGFR-vIII Chimeric Antigen Receptor Gene-Modified T Cells for PaTients with Recurrent GBM, Pro00083828). If the patient is determined to be eligible for the INTERCEPT study (Pro00083828), the cells extracted from leukapheresis while they were on this study, ELEVATE, will be used to create the CAR T cells for administration in the INTERCEPT study.

12.4 Follow-up Period

Patients will be followed for survival, progression, and subsequent therapies only, and this data will be recorded by the research team. For recording of subsequent therapies, the type of therapy(ies) that the subject receives will be recorded with approximate start and stop date, if

[8] On day before the fourth DC vaccine, patients will receive vaccine site pre-conditioning strategy (Group I-unpulsed DCs i.d.; Group II and III- Td i.d.). A single dose of Td toxoid (1 flocculation unit, Lf) or 1 x 10^6 autologous unpulsed DCs will be administered to a single side of the groin 12-24 hours prior to the fourth DC vaccine, which is always given bilaterally at the groin site. Standard of care blood for CBC and CMP will be drawn during the pre-conditioning visit. 2 yellow and 1 red top tubes for immune monitoring will be obtained during the pre-conditioning visit.

[9] At the day of the 4th vaccine (prior to administration), 9 yellow and 2 red top tubes obtained for immune monitoring. Patients in Groups I and II will then receive ^{111}In-labeled DCs to compare the effects of different skin preparations on DC migration followed by SPECT/CT imaging immediately.

[10] SPECT/CT imaging will be performed at 1 and 2 days after injection of Vaccine #4 for patients in Groups I and II. At the SPECT/CT imaging visits 1 and 2 days after Vaccine #4, 2 yellow and 1 red top tubes for immune monitoring will be obtained only on patients randomized to Groups I and II (not Group III).

[11] Monthly cycles of TMZ will be initiated at the discretion of the treating neuro-oncologist according to pathology.

[12] As part of standard care for these patients, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure consent will be obtained separately. However, if tissue is obtained, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and pp65 antigen escape at the tumor site.

possible. For subjects who complete all 10 study vaccines without progressing, an attempt will be made to obtain blood for immunologic monitoring 2-3 times a year at standard Duke Preston Robert Tisch Brain Tumor Center visits. If the subject progresses in the follow-up period, blood for immunological monitoring will be drawn at the time of progression, if possible.

12.5 End of Study

Rationale for taking a patient off study will be documented (see section 12.6.1). In the Follow-up Period, patients will be followed for survival, progression, and subsequent therapies only, and this data will be recorded by the research team.

12.6 Early Withdrawal of Subject(s)

12.6.1 Criteria for Early Withdrawal

Subjects may voluntarily withdraw from the study at any time. Subjects may be withdrawn by the PI and considered screen failures prior to vaccine therapy and 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:

- Inability to complete approximately 6 weeks of RT with TMZ as per standard of care.
- Inability to tolerate TMZ (either requiring a delay in cycles of TMZ, dose adjustment, or discontinuation).
- Patients with an active infection requiring treatment or having an unexplained febrile illness (Tmax > 99.5° F).
- Patients requiring an increase in corticosteroids, with the exception of nasal or inhaled steroid, such that at the time of first vaccination they require a dose above physiologic levels, will be removed from the study and replaced. For the purposes of this study, physiologic dose will be defined as <2 mg of dexamethasone / day. Once vaccinations have been initiated, if patients subsequently require increased steroids, they will still be permitted to remain on the study, but every effort will be made to minimize steroid requirements.
- Adverse events
- Abnormal laboratory values
- Abnormal test procedure results (DCs fail to meet release criteria; inability to tolerate leukapheresis)
- Protocol deviation
- Administrative issues
- Disease progression
- Pregnancy

12.6.2 Follow-up Requirements for Early Withdrawal

Consented subjects that are withdrawn prior to randomization for any of the aforementioned reasons will be considered eligibility failures and thus will not be followed for survival. All other subjects will be followed by the study coordinator until death or are lost to follow-up.

Subjects that received **any** basiliximab (Group III only) or vaccine therapy will be assessed and followed or adverse event monitoring/safety analysis for at least 1 month following the last vaccine (or basiliximab infusion if in Group III).

12.6.3 Replacement of Early Withdrawal(s)

Subjects who voluntarily withdraw prematurely or who are withdrawn by the PI prior to randomization will be replaced.

12.7 Study Assessments

12.7.1 Medical History

Medical history will be obtained from the Duke electronic 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 and at each visit. Curran Group Status will be performed at time of consent. Please see appendices – Section 18.2 for Curran Group Status Form.

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 newly-diagnosed GBM 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 (see Appendices – Section 18.2). RANO criteria[127] will be used for overall assessment of tumor response and pseudoprogression. Pseudoprogression by the RANO response criteria is defined as follows: 1) $\geq$ 25% increase in the sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response; 2) significant increase in T2/FLAIR non-enhancing lesion compared with baseline scan or best response after initiation of therapy - not caused by comorbid events (eg, radiation therapy, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects); 3) any new lesion; clear clinical deterioration not attributable to other causes apart from the tumor (eg, seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, and so on); 4) failure to return for evaluation as a result of death or deteriorating condition. 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. If the subject continues to do well beyond the 3 months, they will continue on study as planned. Subjects demonstrating definitive progression will be removed from study. 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. If tissue is obtained through the Duke Brain Tumor Biorepository, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and antigen escape using IHC. Upon progression, patients may be treated on other therapies as directed by the treating Oncologist.

12.7.5 Immunologic Assessments

Immunological response evaluations for baseline values will be conducted on the leukapheresis sample used to generate the DCs and on a second leukapheresis sample obtained 4 ± 2 weeks after the third immunization. Blood work for immunologic monitoring (9 yellow and 2 red top tubes) will be drawn before basiliximab (Group III only), vaccine #1, and then as described above after vaccine #3 and/or at progression (whichever comes first). For subjects who complete all 10 study vaccines without progressing, an attempt will be made to obtain blood for immunologic monitoring 2-3 times a year at standard Duke neuro-oncology visits and at progression (whichever comes first). The total amount of blood required for this purpose will be about 90 mLs. The immune monitoring blood drawn at vaccine #1 will be analyzed for baseline CCL3 level. Plasma from the yellow top tubes will be collected at each time point listed where immunologic monitoring is done. Blood work for sub-clinical autoimmunity will be drawn before basiliximab (Group III only), or vaccine #1 (all other groups), and repeated only if clinically indicated. Laboratory evaluations to detect subclinical disease will be obtained as follows: serum thyroglobulin Ab (Lab Code 1802105), rheumatoid factor (Lab Code 5560195), antinuclear Ab (Lab Code 5560214), Thyroid Profile (Lab Code 1850277), and CMP (Lab Code 1004261). A comparison of pre-therapy lymphocyte functions to those at intervals after each immunization will be made. These tests may provide evidence for the development of immune responses following DCs immunization and will play an important role in the design of future DC-based clinical trials. Cellular immune responses to pp65 will be measured in a tiered approach. First, PBMCs will be assessed at pre-treatment (leukapheresis) and during treatment for responses to pp65 using a qualified IFN-y ELISpot. If positive in the ELISPOT assay, pp65 antigen-specific T cell response will be measured by polyfunctional T cell assays using 8-9 color panels previously optimized in our laboratory which includes detection of CCL3 on T cells. The effect of treatment on cytokines and other soluble factors will be assayed in the serum/plasma. Serum/plasma will be measured to determine levels of CCL3 from the blood drawn at the vaccine #1 visit (in ALL Groups), and repeated at the SPECT/CT imaging visits (2 yellow and 1 red top tubes, in Groups I and II only). T_{Reg} kinetics will be measured by using a constellation of markers specific for T_{Regs} such as foxp3, CD25 and CD4.

In addition, blood and tumor samples will be stored for possible future research in the Duke Brain Tumor Immunotherapy Program (DBTIP) Laboratory where all other samples from this study are stored. The samples being stored are ONLY for this study. Our mouse studies being done in the DBTIP Lab are identifying markers that may influence migration, therefore, as other markers are identified, we would like to investigate whether these same markers are identified in human subjects enrolled on this trial.

Gamma interferon-enzyme-linked immunospot (IFN-γ ELISpot) assay

Using a tiered approach as described above, 90 mLs of peripheral blood will be drawn into (9 yellow and 2 reds) vaccutainer tubes containing ACD. PBMC will be separated by density gradient centrifugation using Leukosep tubes. Antigen specific T cell responses to pp65 will be measured ex vivo by direct IFN-γ ELISpot assay. PBMCs at $2.5x10^5$ per well will be stimulated overnight with a pool of synthetic peptide (15-mers overlapping by 11 amino acids, JPT Peptide Technologies, Germany).

Single-use aliquots of the lyophilized pp65 peptide pool will be resuspended to a final concentration of 1 μg/mL. Each assay will include test PBMC cultured with no peptide, pp65 peptide pool, PHA (2.5 μg/mL), and positive and negative control PBMC wells for pp65 antigen. PBMCs from different visits for each patient will be batch analyzed on one plate. PBMC added to duplicate wells of ELISpot assay plates coated with mouse IgG1 anti-human IFN-γ mAb will be incubated overnight at 37°C, 5% CO_2, washed with PBS/Tween-20, incubated with biotinylated mouse IgG1 anti-human IFN-γ for 1 hour at room temperature, washed with PBS, incubated with avidin-peroxidase complex for 1 hour at room temperature, washed, incubated with substrate (3-amino-9-ethylcarbazole) for 4 minutes at room temperature and spot development will be stopped by distilled water rinse. Spot enumeration by automated analysis with a Zeiss KS ELISpot system will be performed. Results will be expressed as the mean SFC/10^6 PBMC after subtraction of counts from cells cultured with no peptide.

Polyfunctional Flow cytometry

Polyfunctional T cell analysis will be done in Dr. Reap's immunology laboratory on samples positive for secretion of IFN–γ by ELISpot after stimulation with pp65. Dr. Reap's immunology laboratory conducts all the clinical immune monitoring for the Duke Brain Tumor Center. Polyfunctional T cell panels have been developed in our laboratory and optimized for detection of CCL3 in T cells. This process involves the rapid early detection and analysis of the production of TNF-*a* CCL3, IFN-γ and IL-2 prior to cellular secretion following antigen-specific stimulation *in vitro*. The functional CD4 and CD8 immune response of the patients will be monitored using an 8 color assay.

Isolated PBMC will be stimulated for 6 hours with the pool of 138 peptides spanning the entire pp65 gene. The cells will be stained for the surface markers CD3 (to identify it as a T cell), CD4 (to define as specific helper cell), CD8 (to define antigen specific cytotoxic T cells), CD27, CD28, CD45RO (to identify maturation and activation state) and the intracellular cytokines IL-2, TNF-*a* and IFN-γ. This procedure allows for the highly sensitive detection of IFN-γ, TNF-*a* and IL-2-producing cells which are assumed to be specifically responding to the stimulating antigen. This will also allow us to quantitate the change in antigen specific effector, central memory, and effector memory cells following each vaccination and monitor polyfunctional T cell responses.

Serology/Multiplex Platform

CCL3 and levels of other inflammatory soluble factors may be assayed by multiplex platform. Antibody levels to the immunizing antigen may be measured.

T_{Reg} Kinetics

T_{Regs} will be analyzed by flow cytometry using a constellation of markers specific for T_{Regs} included CD4, CD25 and foxp3.

13 SAFETY MONITORING AND REPORTING

13.1 Adverse Events

An AE is any untoward medical occurrence in a subject receiving any of the study drugs (basiliximab, Td or unpulsed DCs for pre-conditioning, ^{111}In-labeled DCs, and pp65 DC vaccines) 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 DCs, whether or not related to use of the DCs. 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 prior therapy are considered adverse events.

From the time the subject is randomized through 1 month after the subject's last vaccine, all AEs must be recorded in the subject's medical record and 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 drugs) will be kept which will categorize the event by organ system, relationship to which treatment, its grade of severity, and resolution. Periodic review by the PI and monthly review at the PRTBTC Adverse Event meeting of the collective adverse events will occur with the intention of identifying any trends or patterns in toxicity. If any such trends are identified, depending on their severity and frequency, a protocol amendment will be considered.

13.2 Serious Adverse Events

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

- Fatal
- Life-threatening
- Constitutes a congenital anomaly or birth defect
- A medically significant condition (defined as an event that compromises subject safety or may require
- medical or surgical intervention to prevent one of the three outcomes above).
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption to conduct normal life functions.

13.2.1 Reporting of SAEs

All SAEs should be reported immediately to Dr. Dina Randazzo (Pager: 919-970-9692) 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 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 Duke University Medical Center IRB and the FDA using the appropriate SAE reporting process. At the time of the annual progress report to the Duke University Medical Center IRB and the FDA, a summary of the overall toxicity experience will be provided.

13.3 Emergency Unblinding of Investigational Treatment

In the case of an allergic reaction following the pre-conditioning part of the study, the research nurse administering the study drug(s) will alert the PI (or her designate) for PI notification and approval to initiate unblinding and the ICS will be contacted to obtain information on the pre-conditioning regimen the subject received. Dr. Randazzo (study PI) can be reached at 919-684-5301 during regular business hours and at 919-970-9692 (or 919-684-8111 for page office if designate) after hours and on weekends and holidays. The ICS Pharmacy can be reached at 919-668-0657.

13.4 Safety Oversight Committee (SOC)

The DCI SOC 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 SOC 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 SOC 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.

13.5 External Data and Safety Monitoring Board (DSMB)

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.

Due to potential for COI in relation to proprietary interest in the pp65CMV DC vaccine, a Data Safety and Monitoring Board (DSMBplus) has been established. Please see Appendix – Section 18.6 for detail on the Duke PRTBTC DSMBplus Charter.

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 SOC, 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 CTQA office may conduct audits to evaluate compliance with the protocol and the principles of GCP. The PI agrees to allow the CTQA auditor(s) direct access to

all relevant documents and to allocate his/her time and the time of the study team to the CTQA auditor(s) in order to discuss findings and any relevant issues.

CTQA audits are designed to protect the rights and well-being of human research subjects. CTQA 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.

CTQA 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.[13] 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 Procedures 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

All medical terms will be coded using CTCAE (version 4).

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

15 STATISTICAL METHODS AND DATA ANALYSIS

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

[13] 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) uses the term "source data/documents." For the purpose of this guidance, these terms describe the same information and have been used interchangeably.

15.1 Study Design Overview

This randomized phase II study will assess the impact of tetanus pre-conditioning on migration among newly diagnosed GBM patients who have undergone definitive resection and completed standard temozolomide and radiation treatment, as well as the impact of tetanus pre-conditioning and basiliximab together on survival. After completing standard of care radiotherapy with concurrent temozolomide, patients will be randomized to 1 of 3 treatment arms:

- Group I will receive DC vaccinations with ***unpulsed DC pre-conditioning*** prior to the 4th vaccination;
- Group II will receive DC vaccinations with ***Td toxoid pre-conditioning*** prior to the 4th vaccination; and
- Group III will receive ***basiliximab infusions*** prior to the 1st and 2nd DC vaccinations along with Td toxoid pre-conditioning prior to the 4th vaccination.

A permuted block randomization algorithm using a 1:1:1 allocation ratio will be used to assign patients to a treatment arm. Randomization will be stratified by CMV status (positive, negative), with the assignment to arms I and II being double-blinded (See Sections 9.1.1 and 9.1.7 for further details).

The study was initially activated with Groups I and II. However, approximately 2 months the study's initial activation, the study has been amended to include Group III.

Once accrual to Groups I and II are complete, a decision will be made concerning the accrual of additional patients to the Groups I and II in order to assess the impact of Td toxoid pre-conditioning within subgroups defined by CMV status. Options include continued accrual to both strata, accrual to only one stratum, or termination of accrual. Details are provided in Section 15.8.

Per Section 9.1, the study has been amended to terminate randomization to Group III. Rationale is provided in Section 9.1. Though randomization to Group III has been terminated, the analyses proposed for Group III will still be done even though the power of planned analyses will be greatly reduced due to a sample size smaller than anticipated within Group III.

The study was closed to enrollment in April 2018 (Amendment 73). At the time the study was closed to new enrollment, a sufficient number of subjects have been enrolled to power the migration endpoint described in Section 15.9.3. An interim analysis of survival was initially planned after 35 patients were randomized to each Arm A and Arm B (Section 15.8). Survival analyses will still be conducted, but they may not be powered for the comparisons of survival described in Section 15.9.2. Exploratory immune analyses described in Section 15.7 will be conducted as planned.

15.2 Analysis Sets

Subject to the availability of data, all randomized patients will be included in all analyses of correlative and efficacy endpoints.

15.3 Patient Demographics and Other Baseline Characteristics

Summaries of clinical and socio-demographic characteristics will be generated for all enrolled patients, as well as a summary stratified by assigned treatment group for patients randomized. Categorical descriptors will be summarized using frequency distributions; whereas, interval variables will be summarized using percentiles, as well as means and standard deviations.

15.4 Treatments

A frequency distribution will be generated for the number of vaccines received among patients within each treatment arm.

15.5 Primary Objectives

This study has one key primary objective and three other primary objectives (See Section 8). The key primary objective is to assess the impact of Td pre-conditioning with or without basiliximab on survival.

Other primary objectives include an examination of the effect of pre-conditioning of the vaccine site with Td toxoid as opposed to unpulsed DCs at the time of the 4th vaccination on the migration of *CMV* pp65 RNA-pulsed DCs to site draining inguinal lymph nodes, as well as an assessment of the impact of Td pre-conditioning within subgroups of patients defined by CMV status (negative/positive).

15.5.1 Key Primary Objective: Overall Survival

The Kaplan-Meier estimator will be used to describe the survival within each of the 3 treatment arms. Median survival will be estimated within each arm, with 95% confidence intervals. OS will be defined as the time between randomization and death, or last follow-up if alive. Two pairwise one-tailed log-rank tests will be conducted to assess the impact of Td and/or basiliximab. One test will compare the survival experience of patients in the TD toxoid arm (Group II) and the unpulsed DC arm (Group I), and the other test will compare the survival experience within the basiliximab arm (Group III) and the unpulsed DC arm (Group I). An intent-to-treat philosophy will be followed in these analyses.

15.5.2 Other Primary Objective: Migration

Within Groups 1 and 2, the percentage of ^{111}In-labeled DCs reaching inguinal nodes at vaccination #4 is calculated from the initial signal at the injection site in the groin to 48 hours post-vaccination. A Wilcoxon rank-sum test will be used to compare assigned treatment groups (Groups I and II) with respect to migration. This analysis will be based upon the first 12 patients within each arm (Groups I and II) that receive vaccination #4.

15.5.3 Other Primary Objective: Survival Among CMV Positive Patients

To assess the impact of Td pre-conditioning on survival among patients who are CMV positive, an analysis similar to those described for the key primary objective will be generated within the subgroup of patients who are CMV positive. Specifically, the Kaplan-Meier estimator will be used to describe the survival among CMV positive patients within Groups I and II. Median survival will be estimated within each of these 2 treatment arms, with 95% confidence intervals.

OS will be defined as the time between randomization and death, or last follow-up if alive. A log-rank test will be conducted to assess the impact of pre-conditioning. An intent-to-treat philosophy will be followed in these analyses.

Subgroup analyses will be underpowered and descriptive unless a decision is made to continue accrual and treatment of CMV positive patients after the completion of accrual to Group III (See Section 15.8).

15.5.4 Other Primary Objective: Survival Among CMV Negative Patients

Analyses similar to those described in section 15.5.3 for patients who are CMV positive will be conducted among patients who are CMV negative.

15.6 Secondary Objectives

Secondary objectives and endpoints that are presented in section 8 include an examination of the impact of Td pre-conditioning with and without basiliximab on progression-free survival, as well as the impact of Td pre-conditioning on progress-free survival within patient subgroups defined by CMV status.

15.6.1 Key Secondary Objective: PFS

The Kaplan-Meier estimator will be used to describe progression-free survival (PFS) within each arm. Median PFS will be estimated within each of the 3 treatment arms, with 95% confidence intervals. PFS will be defined as the time between randomization and initial failure (disease progression or death). If the patient remains alive without disease progression, PFS will be censored at the time of last follow-up. Two pairwise log-rank tests will be conducted to assess the impact of Td and/or basiliximab. One test will compare the PFS experience of patients in the TD toxoid arm (Group II) and the unpulsed DC arm (Group I), and the other test will compare the survival experience within the basiliximab arm (Group III) and the unpulsed DC arm (Group I). An intent-to-treat philosophy will be followed in these analyses.

15.6.2 Other Secondary Objective: PFS Among CMV Positive Patients

To assess the impact of Td pre-conditioning on PFS among patients who are CMV positive, analysis similar to those described for the key primary objective will be generated within the subgroup of patients who are CMV positive. Methods similar to those described in Section 15.5.3 will be used.

15.6.3 Other Secondary Objective: PFS Among CMV Negative Patients

Analyses similar to those described in section 15.6.2 for patients who are CMV positive will be conducted among patients who are CMV negative.

15.7 Exploratory Objectives

Changes from baseline in immune response as measured by ELISpot will be explored at each follow-up assessment and within each treatment group. Though analyses are exploratory, a Kruskal-Wallis will be used to compare treatment groups relative to maximum changes in ELISpot observed. A similar analysis will be conducted for levels of T_{Reg}, CCL3, and other

inflammatory soluble factors. Parametric analyses, such as an analysis of variance, may be used in lieu of a Wilcoxon test if underlying assumptions are satisfied.

When ELISpot results are positive, the extent of T cell functionality will be assessed. For each patient, the proportion of patients with 1, 2, and 3 functions will be determined. A negative ELISpot will be assumed to have 0 functions. Within each treatment group, changes from baseline in the percentage of patients with 0, 1, 2 and 3 functions will be explored Hotelling's T-test or a nonparametric equivalent. Additional statistical analyses may be conducted to changes in polyfunctionality, as well as to address other exploratory goals

15.8 Interim Analysis

Once accrual to Groups I and II are complete, analyses will be conducted to help determine whether accrual of patients to Groups I and II should continue in order to assess the impact of Td toxoid pre-conditioning within subgroups defined by CMV status. Based upon these analyses and other factors, one of 3 decisions will be made: 1) Continue accrual to both CMV positive and CMV negative strata within Groups I and II; 2) Continue accrual to only one CMV stratum within Groups I and II; or 3) Terminate accrual to all arms. It should be noted that there are no options to continue accrual to Group III once the initial sample size goals are attained.

At the time that this interim analysis is conducted, the number of deaths required per Section 15.9.2 for the overall comparison of survival within Groups I and II to have the desired power will not have yet been attained. With the assumptions described in Section 15.9.2, we anticipate that a total of 31 deaths will have occurred in Groups I and II combined by the time of these interim analyses if the alternative hypothesis is true.

Based upon data available at the time of the interim analysis, the conditional probability of observing a statistically significant result within each CMV subgroup will be generated. That calculation will account for the accrual rate observed to date within each subgroup, which reflects both overall accrual and the prevalence of CMV positivity. The sample size requirements provided in Sections 15.9.4 and 15.9.5 will also be adjusted to reflect subgroup accrual rates observed to date.

Factors that will be considered in the decision to extend accrual to address subgroup efficacy questions include the conditional probability referenced about, as well as the feasibility of completing extended subgroup accrual in light of accrual rate and competing priorities.

15.9 Sample Size Calculation

The following sections concerning sample size and power reflect the study as it was originally designed. Per Section 9.1, the randomization of patients to Group III has been terminated with AMD 49. Otherwise, the overall design of the rest of the study remains.

Our goal remains to randomize a total of 35 patients to Groups I and II each. As described in Section 15.9.2, a 1-tailed logrank test will be conducted at the 0.15 level of significance to compare survival in Group I and II. For this comparison to have 90% power to detect a hazard ratio of 0.5, 45 deaths need to be observed within the two arms being compared.

At this time, approximately 35 patients have been randomized to each of Groups I and II, the interim analysis described in section 15.8 will be conducted to determine whether accrual of patients to CMV subgroups should continue.

Included in the following sample size and power calculations are estimates of the time required to complete accrual and observe the required number of events. Though these estimates are no longer valid, re-estimates will not be generated due to the rate of randomizing patients to Groups I and II having varied over time due to the number of randomizing arms as well as early problems with vaccine contamination.

15.9.1 Overview of Sample Size Requirements

A maximum of 100 patients will be initially enrolled in this study over a period of 29 months with the expectation that approximately 79 patients will be randomized after completion of radiation with concurrent temozolomide. Justification for the required number of randomized patients for the key primary outcome variables are provided below. The sample size calculations described in Section 15.9.2 for the key primary objective and the primary subgroups objectives reflect the fact that accrual to Group III was initiated a few months after accrual was initiated on Groups I and II.

At the time that accrual to Groups I and II are completed, a decision will be made concerning the accrual of additional patients to address efficacy questions within CMV subgroups (see Section 15.8). A priori sample size requirements for subgroups are described in Sections 15.9.4 and 15.9.5; however, these calculations will be updated at the time of the interim analysis to reflect observed subgroup accrual rates. At the time of writing this protocol, we anticipate that a maximum of 64 additional patients will be accrued during the extended accrual period so that 58 additional patients will be randomized to Groups I or II.

Hence, a maximum total of 180 patients will be accrued to this study so approximately 160 patients will be randomized.

Sample size requirements for the migration studies are provided in Section 15.9.3. As described in that section, the first 12 patients in Groups I and II will undergo migration studies are be used in those analyses.

15.9.2 Key Primary Objective: Survival

The key primary goal of this study is to determine whether the addition of either Td pre-conditioning with vaccination #4 or basiliximab administration with vaccination #1 and #2 that is administered in conjunction with DC vaccination and temozolomide is worthy of investigation in a large phase III study. Two pairwise comparisons of survival are of primary interest: 1) Td Toxoid arm (Group II) versus Unpulsed DC arm (Group I), and; 2) Basiliximab arm (Group III) versus Unpulsed DC arm (Group I).

Though this phase II study is comparative, a false-positive rate of 0.15 will be used to test each hypothesis while maintaining reasonably high power[128-130]. This rate of false-positivity is used in lieu of a false positive rate of 0.2 as suggested in the literature in an attempt to partially adjust for there being multiple comparisons.

Within the ATTAC study, the median survival in the DC vaccine arm without pulsed DC pre-conditioning was approximately 12 months; whereas, within the Td pre-conditioning arm median survival was estimated to be approximately 36 months. Study results suggest a hazard ratio of approximately 0.33. Due to the small sample sizes in the ATTAC study, estimates are all associated with wide confidence intervals. Given these imprecise estimates and the potential for "regression to the mean" in this study replicating the ATTAC trial, we will target a smaller hazard ratio of 0.5 for a comparison of unpulsed DC (Group I) and Td (Group II). With pilot data not being available for the comparison of basiliximab (Group III) and unpulsed DC (Group I), we will also target a hazard ratio of 0.5 for that comparison.

For each pairwise comparison, a 1-tailed logrank test will be conducted at the 0.15 level of significance. In order that each comparison will have 90% power to detect a hazard ratio of 0.5, 45 deaths need to be observed within the two arms being compared[131].

We anticipate that patients will be accrued at the rate of 4 patients per month, with a randomization rate of 3.5 patients per month. As described earlier, the protocol was initially activated with only Groups I and II. However, with a later amendment, patients will be randomized to one of 3 arms. In the following calculations, we will assume that there is a period of 2 months during which patients are randomized to one of 2 arms after which patients will be randomized to one of 3 arms. Tabulated below is the anticipated accrual and randomization rate within each arm during each period of accrual.

Table 5. Anticipated Accrual and Randomization Rates

# of Arms Accruing	Accrual Rate (Patients / Month / Arm)	Randomization Rate (Patients / Month / Arm)
2 arms	2	1.75
3 arms	1.33	1.17

Approximately 32 patients will be randomized to Group III before accrual will be terminated to Group III and the interim analysis described in section 15.8 conducted. Per Section 9.1, the randomization of patients to Group III has been terminated with AMD 49. At the time approximately 35 patients have been randomized to each of Groups I and II, the interim analysis described in section 15.8 will be conducted to determine whether accrual of patients to CMV subgroups should continue. At the time of this interim analysis, we anticipate that approximately 35 patients will have been accrued to Groups I and II each and that a total of 31 patients within Groups I and II will be dead under the alternative hypothesis where the median survival in Group I is 12 months and the median survival in Group II is 24 months. Approximately 12 months are needed after the last patient randomization to observe 45 events[132-134].

In addition to the comparison of Groups I and II, we plan to compare the survival of patients enrolled on Arms I and III. Under the null hypothesis, approximately 13 month of follow-up after the last patient randomization is required to observe 45 events.

15.9.3 Other Primary Objective: Migration

One goal of this study is to validate observations made in the recently completed ATTAC trial concerning Td toxoid pre-conditioning. The design of this study (Groups I and II) is identical to

the ATTAC trial in that patients with newly diagnosed GBM underwent surgery followed first by SOC treatment (temozolomide + XRT) and then by 3 vaccinations. Prior to the 4th vaccination, patients were randomized to unpulsed DCs or Td tetanus pre-conditioning. The percentage of ^{111}In-labeled DCs reaching inguinal nodes was calculated from the initial signal at the injection site in the groin to 48 hours after the 4th vaccination.

In this study, the first 12 patients in Groups I and II will have their DCs labeled will undergo migrations studies to assess the migration objective.

A bootstrap approach was used to determine the sample size required for a comparison of migration rates using the Wilcoxon rank-sum test (also the Mann-Whitney U test) to have 95% power to detect a difference similar to that observed in the ATTAC study. Re-sampling with replacement from the pilot ATTAC study data was used in the 10,000 bootstrapping simulations.

With 12 patients in the Td toxoid arm (Group I) and 12 patients in the unpulsed DC arm (Group II) who have undergone migration studies at vaccination #4, simulations show that there is 95% power to detect the difference observed in the ATTAC trial assuming α=0.05.

15.9.4 Other Primary Objective: Survival Among CMV Positive Patients

One of the other primary goals of this study is to determine whether Td pre-conditioning at vaccination #4 in conjunction with DC vaccination and temozolomide is worthy of investigation in a large phase III study among patients who are CMV positive. That assessment involves a comparison of survival in Groups I and II among patients who are CMV positive.

The sample size requirements needed to address this subgroup question are similar to those described for the key primary objective (Section 15.9.2). A 1-tailed logrank test will be conducted at the 0.15 level of significance. To have 90% power to detect a hazard ratio of 0.5, 45 deaths need to be observed within the two arms being compared[131].

As described in section 15.9.2, we anticipate that patients will be accrued to the study at the rate of 4 patients per month, with a randomization rate of 3.5 patients per month. We also anticipate that approximately 50% of patients will be CMV positive.

As described in Section 15.9.2, the study will randomize patients to one of two arms, and after amendment, it will randomize patients to one of 3 arms. If a decision is made to continue patient accrual (See Section 15.8), the study will randomize patients to either Group I or II. Tabulated below is the anticipated accrual and randomization rate for CMV positive patients within each arm during each period of accrual.

Table 6. Anticipated Accrual and Randomization Rate for CMV Positive Patients

# of Arms Accruing	Accrual Rate (Patients / Month / Arm)	Randomization Rate (Patients / Month / Arm)
2 arms	1	0.88
3 arms	0.67	0.58

At the time of the interim analysis, we anticipate that approximately 36 CMV positive patients will have been accrued, with half being assigned to Group I and half to Group II. If accrual to

the CMV positive patient subgroup is continued for an additional 16 months, we expect to accrue 32 additional patients to that subgroup of which 28 will be randomized to either Group I or II. After completion of extended accrual, an additional 12 months of follow-up are anticipated to observe approximately 45 events assuming the treatment-arm median survivals are 12 and 24 months.

If all the assumptions made in this sample size calculation remain valid during the conduct of the study and accrual is extended for CMV positive patients, approximately 57 months would be required to address this subgroup objective. The 57 months assumes 2 months of 2-arm randomization, 27 months of 3-arm randomization, 16 months of expanded subgroup accrual, and 12 months of follow-up.

15.9.5 Other Primary Objective: Survival Among CMV Negative Patients

The final other primary goals of this study is to determine whether Td pre-conditioning at vaccination #4 in conjunction with DC vaccination and temozolomide is worthy of investigation in a large phase III study among patients who are CMV negative. That assessment involves a comparison of survival in Groups I and II among patients who are CMV negative. The sample size requirements needed to address this subgroup question are identical to those described in section 15.9.4 given that the prevalence of CMV negative is anticipated to be 50%.

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.

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 Oracle Clinical database, which is housed by the DCI. Access to electronic databases will be limited to the Principal Investigator, key personnel, statisticians, the Radiolabeled Pharmacy personnel, and the PRTBTC data manager. 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. For a more detailed description of the DSMP for this protocol, refer to attached plan in Appendices (Section 18.5).

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 Registration Procedure

After patients have been enrolled, subject registration will be entered into the Duke Velos eResearch system and the subject's visits associated in the Duke Epic Maestro Care system with this protocol which is entered after Duke IRB approval.

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18 APPENDICES

18.1 Research Summary

See eIRB.

18.2 SOPs and FORMS

Separate upload in the protocol section in eIRB.

18.3 Standard Radiation Therapy

Radiotherapy typically begins within ≤ 5 weeks of surgery. One treatment of 1.8-2.0 Gy/fraction will 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.

18.4 Standard Temozolomide Therapy

Please refer to the package insert uploaded in the drug section in eIRB.

18.5 DCI Data and Safety Monitoring Plan

Separate upload in the protocol section in eIRB.

18.6 Duke PRTBTC DSMBplus Charter

Separate upload in the protocol section in eIRB.

18.7 Basiliximab Package Insert

Separate upload in the protocol section in eIRB.