AVeRT: Anti-PD-1 monoclonal antibody (nivolumab) in combination with DC Vaccines for the Treatment of Recurrent Grade III and Grade IV Brain Tumors

DUKE CANCER INSTITUTE

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Amended version 3: CPC Modifications and PI-initiated corrections	08/24/2015	.Added abbreviation (Section 4); Removed research summary (Section 5); Updated study schema (Section 6); Added more background on nivolumab (Section 7); Moved randomization (Sections 9, 12, 15); Moved surgery (Sections 9, 12); Removed specific Td manufacturer (Sections 9, 10, 12); Corrected dose of Td/Saline for pre-conditioning (Sections 9, 12); Corrected cell concentration error (Section 10); Corrected name of pharmacy dispensing Td (Section 10); Removed specific research nurse name (Section 10); Updated Tests & Procedures Table (Section 12); Corrected # of vaccines for primary analysis (Section 15).
Amended version 4: CPC Modifications Request	9/8/2015	Clarified rationale for surgery following nivolumab ± DC vaccine and subjects will be removed if surgery must be performed prior to plan (Sections 7, 9 and 12).
Amended version 5: FDA Modification Requests	9/16/2015	Clinical benefit in Section 12.2.1 is clarified; tolerating study drug in same section also clarified; Time point as listed in the PRTBTC Imaging SOP is clarified in Section 18.3.
Amended version 6: PI modification requests	1/20/2016	Included Apheresis blood work prior to each leukapheresis (Section 12); updated the blood work drawn prior to leukapheresis and each infusion of nivolumab (Section 12), corrected the total number of vaccines required for replacement of patients for primary endpoint evaluation (Section 15.7); and updated the documents in the appendices by removing them from protocol and uploading as separate attachments in eIRB (Section 18).
Amended version 7: PI modification requests	2/4/2016	Use of antihistamines before, during and following vaccine `administration recommendation (Section 12.7.3).
Amended version 8: PI modification requests	5/12/2016	Overall formatting change to meet FDA standards for electronic submission (entire document); Changed Primary Study Coordinator (Cover Page); Removed reference to “alone” from Group 2 description since giving nivolumab alone is done prior to randomization (Sections 7.3 & 9.1.6); Clarified blood draws (immune monitoring, and pre-nivo) timing, volume, and tubes, and removed CCL3 blood assessments (Sections 6, 8, 9.1, 12.1, 12.2, 12.7.5 & 15.6); Clarified subjects whose cells fail to qualify will not undergo repeat leukapheresis (Section 9.1); Described how Td booster will be administered on all enrolled subjects, removed saline injections during Td pre-conditioning (Sections 9.1,12.2); Changed timing of study treatments to allow more approximation and flexibility (Section 9.1, 9.1.4, & 15); Changed listing Dr. John Sampson to simply Duke Neurosurgeon (Sections 9.1 & 12.2); Updated AE and SAE collection to starting at time of consent through to 30 days after study ends, and includes leukapheresis (Sections 9.1.3, 9.1.4, 12.6.2, 13.1, & 13.2.1); Added language regarding a deviation log for non-reportable events for missed appointments (Section 9.1.4); Clarified that research team will document concomitant medications, not just study coordinator (Section 9.1.5); Added more language on nivolumab infusion and timing (Section 9.2); Updated location of leukapheresis (Section 10.1); Clarified eligibility criteria (Section 11); Increased dexamethasone from 2 mg/day to 4 mg/day (Section 11.2); Updated the Schedule of Events table(s) (Section 12); Removed WHO Performance Status (Sections 12.1 & 18.6); Simplified description of standard of care MRI (Sections 12.1 & 12.2.1); Removed references to a medical monitor (Sections 12.2.2 & 12.2.3); Changed nivolumab dose interruption timing from >6 weeks to >8 weeks (Section 12.2.2); Removed steroid usage as a

		criterion for early withdrawal and moved to own section (Sections 12.6.1 & 12.7.4); Added description of vital sign monitoring with nivolumab and vaccine administrations (Section 12.7.2); Changed the wording from "bi-weekly" to every other week (QOW) (Sections 4, 6, and 15); Clarified that TUMS are given three times a day instead of "with meals" (Section 12.2); Updated consenting by PI to include her designee (Section 16.3); and fixed minor edits and formatting errors (throughout).
Amended version 9: PI modification requests	8/5/2016	Use of bevacizumab in subjects that develop inflammation was added (Section 9.1.6); Use of Antihistamines (Section 9.1.4) and Use of Corticosteroids (Section 9.1.5) were moved from Study Assessments (Section 12.7) to Study Design (Section 9.1); added definition of WBRT to the List of Abbreviations; changed Primary Regulatory Coordinator to Rachel Hesler; clarification of the timing of MRIs and tests and procedures at Nivolumab infusion #1 (Section 12); Removed reference to BMS Guidelines in appendices in inclusion criteria (Section 11.1) and fixed minor formatting errors (throughout).
Amended version 10: PI modification requests	8/29/2016	Due to a change in the quality control timeline, the vaccine schedule for Group II subjects was changed to 3 vaccines prior to surgery and 5 vaccines after surgery, keeping the total number of vaccines at 8 (Section 6, Section 9.1, Section 12, Section 15); Changes were made to Group I vaccine timeline to keep consistent (Section 6, Section 9.1, Section 12); As a result of these changes to the vaccine timeline, the immune monitoring timeline was updated (Section 6, Section 9.1, Section 12) and the primary safety analyses will now focus on all subjects who complete at least 3, not 4, vaccinations or terminate early due to unacceptable toxicity (Section 9.1, Section 15); Clarified that Nivolumab will be infused over 30-60 minutes (Section 9.2); Clarified that the surgery is for de-bulking purposes (Section 7.3); fixed minor formatting errors (throughout).
Amended version 11: PI modification requests	02/13/2017	At leukapheresis #2, subjects (group 1 and group 2) do not need to have a physical exam, neuro exam, KPS, O2 saturation (Section 12). Subjects will continue to receive these exams at the Nivolumab infusion after leukapheresis #2 (1 day to 2 weeks later); Corrected Table 3 to indicate that patients who have completed at least 3 (not 4) vaccinations will be included in safety analysis (Section 15.7); For subjects whose first leukapheresis yields 8 or more vaccines, the duration of the second leukapheresis can be 2 hours instead of 4 hours (Section 12.2); Complications following resection added to definition of unacceptable toxicity (Section 9.1); Vital signs will be taken right after injection of DC vaccine, not 30-60 minutes post vaccine (Section 12.7.2, Section 12); In the Criteria for Early Withdrawal section (Section 12.6.1), correction of reference to section defining unacceptable toxicity (Section 9.1.1) instead of nivolumab discontinuation criteria (Section 12.2.2); added that a subject can continue on one study drug if the other study drug is discontinued due to unacceptable toxicity; Removed restatement of the definition of unacceptable toxicity and instead referred to definition in Section 9.1.1 (Section 15.4.1); Correction of reference #112 (Section 17)
Amended version 12	03/15/2017	PI changed (title page and Section 13.2.1)

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4 LIST OF ABBREVIATIONS

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
BMS	Bristol-Myers Squibb
BMT	Bone Marrow Transplant
BTSC	Brain Tumor Stem Cells
Ca^{++}	Calcium
CCL3	C-C motif Chemokine Ligand 3
cDNA	Complimentary Deoxyribonucleic Acid
CFA	Complete Freund's Adjuvant
CFC	Cytokine Flow Cytometry
CLIA	Clinical Laboratory Improvement Act
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
CRFs	Case Report Forms
CT	Computed Tomography
CTL	Cytotoxic T-Lymphocyte
CTQA	Clinical Trials Quality Assurance
DAR	Drug Accountability Record
DC	Dendritic Cell
DCI	Duke Cancer Institute
DILI	Drug Induced Liver Injury
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic Acid
DSMB	Data Safety and Monitoring Board
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
FFPE	Formalin-Fixed, Paraffin-Embedded

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	Intracellular Cytokine Staining
I.D.	Intradermal
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
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
MLE	Maximum Likelihood Estimator
MMSE	Mini-Mental Status Examination
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
ORR	Overall Response Rate
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
QOW	Every Other Week
qPCR	Real-time PCR
RANO	Response Assessment in Neuro-Oncology
RECIST	Response Evaluation Criteria in Solid Tumors
RIO	Research Integrity Office
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
TAA	Tumor-Associated Antigens
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
ULN	Upper Limit of Normal
VDLNs	Vaccine-site Draining Lymph Nodes
WBI	Whole Body Irradiation
WBRT	Whole Brain Radiation Therapy
XRT	External Radiation Therapy

5 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

Please see eIRB for separate document uploaded in Section 6.

6 STUDY SCHEMA

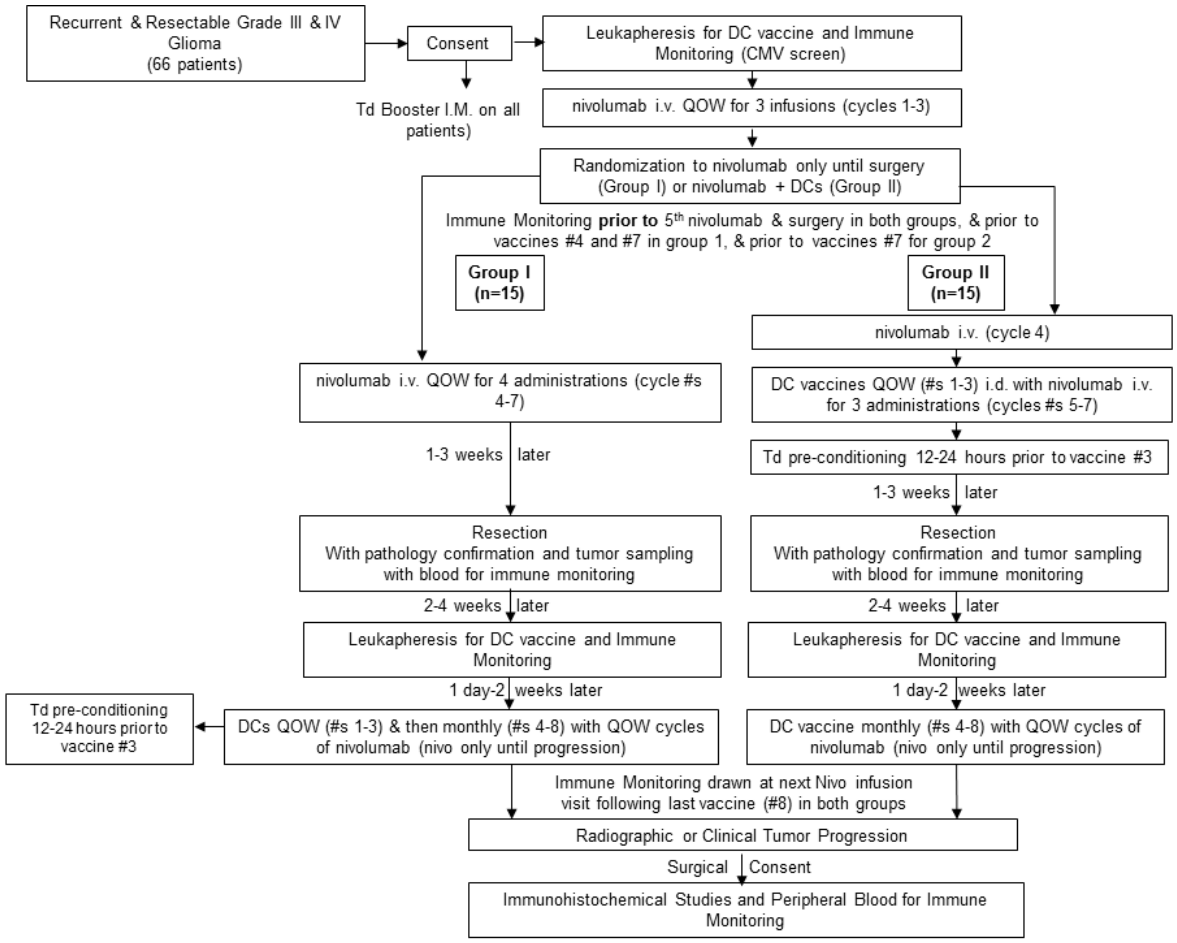

Figure 1. Study Schema

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, 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[1-6]. 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[7, 8]. Moreover, the non-specific nature of conventional therapy for brain tumors often results in incapacitating damage to surrounding normal brain and systemic tissues[9, 10]. 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

7.2.1 Nivolumab

Programmed cell death 1 (PD-1) is a key immune receptor expressed by activated T cells that mediates immunosuppression. Blockade of PD-1 may help overcome immune resistance. Nivolumab is a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands promote immune responses and antigen-specific T cell responses to both foreign and self-antigens. PD-1 receptor blockade by nivolumab is a new approach for immunotherapy of tumors. Results from a Phase I/II study CA209003 indicate that nivolumab is active in multiple tumor types. Nivolumab 3 mg/kg monotherapy is currently being studied in phase 3 clinical trials in advanced melanoma, renal cell carcinoma (RCC) and non-small cell lung carcinoma (NSCLC).

GBM, the most common primary brain tumor in adults, has an aggressive clinical course and a median survival of 12–15 months post first-line therapy with maximal surgical resection, radiation, and temozolomide. Bevacizumab is approved in the United States for patients with progressive disease following therapy; no data has shown durable improvement of disease-related symptoms or OS. With limited efficacy of current therapy, more effective treatments to extend survival and preserve quality of life are needed. Ipilimumab, a cytotoxic T-lymphocyte antigen-4 receptor blocking antibody, has shown clinical activity in advanced melanoma patients with brain metastases; preclinical studies demonstrate a benefit from combining a PD-1 pathway inhibitor with radiation in a mouse glioma model. Duke has a phase IIb, randomized, open-label study to evaluate the efficacy and safety of nivolumab alone or with ipilimumab, versus bevacizumab in patients with recurrent GBM. Patients with KPS ≥70, grade IV malignant glioma treated with radiotherapy and temozolomide, and documented first GBM recurrence within 28 days of randomization are eligible. Patients with >1 recurrence of GBM, extracranial disease, autoimmune conditions, or previous VEGF inhibitor or anti-angiogenic treatment are ineligible. Safety cohort 1; nivolumab 3 mg/kg [n=10; every 2 weeks x 4] and nivolumab 1 mg/kg + ipilimumab 3 mg/kg [n=10; every 3 weeks x 4 followed by nivolumab 3 mg/kg every 2 weeks] will establish safety and tolerability in GBM patients. Upon successful completion of cohort 1, efficacy cohort 2 will enroll up to 240 pts with recurrent GBM, randomized 1:1:1 to receive nivolumab, nivolumab + ipilimumab (dosed as cohort 1), or bevacizumab (10 mg/kg every 2 weeks). The primary objectives are to evaluate safety in cohort 1 and OS in cohort 2, vs bevacizumab, with secondary objectives of PFS and ORR. Responses will be

assessed (Response Assessment Neuro-Oncology criteria) at the end of weeks 6 and 12, and every 8 weeks until progression or treatment discontinuation. Clinical trial information: NCT02017717.

7.2.2 DC Vaccine

DC vaccine: Human Cytomegalovirus pp65-lysosomal-associated membrane protein mRNA-pulsed autologous dendritic cells with granulocyte macrophage-colony stimulating factor.

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 [11, 12]. 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[13-29].

Human CMV is an endemic β-Herpesvirus that does not usually cause significant clinical disease[30]. 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[30]. Herpesviruses have also been implicated in a number of human malignancies including lymphoma, nasopharyngeal cancer, cervical cancer, and Kaposi's sarcoma[31, 32]. 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[33-35]. 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[35]. 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[35], 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, 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[36-39]. In addition, T-cell mediated immunotherapy has proven highly effective in the treatment of CMV-associated disease within the CNS and in the treatment of acute CMV infections[37, 40]. 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[41-46]. 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.

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[47, 48]. 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[49-51].

Vaccination specifically against CMV [52-56] has effectively reduced the risk of viral infection and transmission to fetuses in animal models [57-59] and in clinical trials[52, 56, 60-63]. Human clinical trials have also demonstrated some benefit of administering neutralizing antibodies in the treatment of human CMV infection[64-67], 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, and DCs pulsed *in vitro* with CMV antigens have been shown to be potent inducers of CMV-specific CTL responses in several studies[68-72], 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 [73-78]. In fact, there is accumulating evidence that RNA transfection represents a superior method for loading antigens onto DCs [75, 79]. 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 [80-82]. Furthermore, in direct comparisons, RNA-loaded DCs have been found to be better stimulators of antigen-specific T-cells than other approaches [79]. 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., have demonstrated that tumor mRNA-loaded DCs can elicit a specific CD8+ CTL response against autologous tumor cells in patients with MG.

7.2.3 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)] [83, 84]. 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 [85]. A serum diphtheria antitoxin level of 0.01 IU/mL, measured by neutralization assays, is the lowest level giving some degree of protection; a level of 0.1 IU/mL by ELISA is regarded as protective. Diphtheria antitoxin levels ≥1.0 IU/mL by ELISA have been associated with long-term protection.

Following deep s.c./i.m. administration of the tetanus toxoid vaccine, toxoid molecules are taken up at the vaccination site by immature DCs, which are professional antigen-presenting cells. Within these cells, they are processed through the endosomal pathway (involving the phagolysosome) where they are bound to MHC type II molecules on the surface of DCs. The MHC II:toxoid complex then migrates to the cell surface. While this process is happening within the cell, the now activated mature DC at the vaccine site migrates along lymph channels to the draining lymph node where they encounter naive 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.

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.

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[86]. 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.

7.2.4 Pre-Clinical Experience

7.2.4.1 Nivolumab

Preclinical animal models of tumors have shown that blockade by PD-1 by monoclonal antibodies (mAbs) can enhance the anti-tumor immune response and result in tumor rejection. Antitumor activity by PD-1 blockade functions in PD-L1-positive tumors as well as in tumors that are negative for the expression of PD-L1. This suggests that host mechanisms (ie, expression of PD-L1 in antigen-presenting cells) limit the antitumor response. Consequently, both PD-L1 positive and negative tumors may be targeted using this approach. In humans, constitutive PD-L1 expression is normally limited to macrophage-lineage cells, although expression of PD-L1 can be induced on other hematologic cells as well, including activated T cells. However, aberrant expression of PD-L1 by tumor cells has been reported in a number of human malignancies. PD-L1 expressed by tumor cells has been shown to enhance apoptosis of activated tumor-specific T cells in vitro. Moreover, the expression of PD-L1 may protect the tumor cells from the induction of apoptosis by effector T cells.

Preclinical data indicate that the combination of PD-1 and CTLA-4 receptor blockade may improve antitumor activity. In vitro combinations of nivolumab plus ipilimumab increase IFN-γ production 2- to 7-fold over either agent alone in a mixed lymphocyte reaction. Increased antitumor activity of the combination was also observed in 3 of 5 syngeneic murine cancer models. In a murine melanoma vaccine model, blockade with either CTLA-4 or PD-1 antibodies increased the proportion of CTLA-4 and PD-1-expressing CD4/CD8 tumor infiltrating T effector cells, and dual blockade increased tumor infiltration of T effector cells and decreased intratumoral T regulatory cells, as compared to either agent alone[87].

In a 4-week toxicity study of nivolumab in combination with ipilimumab conducted in cynomolgus monkeys demonstrated that the combination of nivolumab and ipilimumab resulted in dose-dependent gastrointestinal (GI) toxicity. Histologic findings included inflammatory changes in the large intestine, which increased in incidence and severity in a dose-dependent manner. GI toxicity/colitis was not observed in cynomolgus monkeys administered nivolumab alone, but was observed in monkeys receiving ipilimumab. Nivolumab in combination with ipilimumab was also associated with lymphoid hypocellularity of the cortex and/or medulla of the thymus and with acinar cell degranulation in the pancreas. Additional findings included interstitial mononuclear cell infiltrates in the kidneys, portal mononuclear cell infiltrates in the liver and myeloid hypercellularity in the bone marrow. Nivolumab in combination with ipilimumab at the high-dose level (ie, 50 mg/kg and 10 mg/kg, respectively) was associated with the death of 1 animal, attributed to acute gastric dilatation without histopathological evidence of colitis upon pathology evaluation of the GI tract. Please see nivolumab IB[88] for more information.

7.2.4.2 DC Vaccines

In our laboratories and those of others, systemic immunization using DCs co-cultured with uncharacterized tumor homogenate[89], whole tumor RNA[90], unidentified peptides eluted from tumor cells by gentle acid washing[91], or a distinct peptide encompassing the tumor-specific EGFRvIII mutation[92] 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, 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[93-97].

In another report from our laboratory[90], 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, 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.

7.2.5 Clinical Experience

7.2.5.1 Nivolumab

CA209003 is an ongoing Phase 1 open label, multiple dose escalation study in 306 subjects with select previously treated advanced solid tumors, including melanoma, RCC, NSCLC, colorectal cancer, and hormone-refractory prostate cancer. Subjects received nivolumab at doses of 0.1, 0.3, 1, 3, or 10 mg/kg intravenously every 2 weeks, up to a maximum of 2 years of total therapy. As of 18-Mar-2013, a total of 306 melanoma subjects were treated with nivolumab in the dose range of 0.1 - 10 mg/kg.

No maximal tolerated dose was identified in CA209003. The incidence, severity and relationship of AEs were generally similar across dose levels and tumor types. Nivolumab related AEs of any grade occurred in 75.2% of subjects. Of the 306 treated subjects, 303 (99.0%) subjects have at least 1 reported AE regardless of causality. The most frequently reported AEs were fatigue (54.9%), decreased appetite (35.0%), diarrhea (34.3%), nausea (30.1%), and cough (29.4%). Treatment-related AEs were reported in 230 (75.2%) of the 306 subjects. The most frequently reported treatment-related AEs were fatigue (28.1%), rash (14.7%), diarrhea (13.4%), and pruritus (10.5%). Most treatment-related AEs were low grade. Treatment-related high grade (Grade 3-4) AEs were reported in 52 (17.0%) of subjects. The most common treatment-related high grade AEs were fatigue (2.3%) and diarrhea (1%).

Drug-related SAEs occurred in 11.5% of subjects. Grade 3-4 drug-related SAEs reported in at least 2 subjects included: diarrhea (3 subjects, 1.0%), pneumonitis (3 subjects, 1.0%), pneumonia (2 subjects, 0.7%) and lipase increased (2 subjects, 0.7%).

Select AE categories (events with a potential inflammatory mechanism requiring more frequent monitoring and/or unique intervention such as immunosuppressants and/or endocrine replacement therapy) include: GI AEs, pulmonary AEs, renal AEs, hepatic AEs, skin AEs, and endocrinopathies. In addition, select AEs include a category for infusion reactions. Each category is composed of a discrete set of preferred terms, including those of greatest clinical relevance. These select AEs are considered events of interest based on the mechanism of action and were previously referred to as immune-related AEs or immune-mediated AEs.

The 10 mg/kg cohort had numerically greater frequency of high-grade select AEs including the subcategories of endocrinopathies, GI, pulmonary, and infusion reactions. Most high grade events resolved following the treatment guidelines for the treatment of pulmonary events, GI events, hepatic events, renal events, and endocrine events, respectively.

In CA209003 (MDX1106-03), the clinical activity of nivolumab was demonstrated in a variety of tumor types and across a range of doses (0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 10 mg/kg). As of the clinical cut-off date of 05-Mar-2013, a total of 306 subjects with melanoma, RCC, and NSCLC have been treated with nivolumab. All subjects initiated treatment at least one year prior to analysis. A response of either CR or PR, as determined by investigator assessed tumor evaluations based on modified RECIST 1.0, has been reported at all dose levels.

Among 107 patients with advanced melanoma who received nivolumab, the preliminary objective response rate was 33/107 (31%). Responses occurred at each dose level, with 6/17 (35%), 5/18 (28%), 11/35 (31%), 7/17 (41%), and 4/20 (20%) melanoma subjects responding at 0.1, 0.3, 1, 3, and 10 mg/kg, respectively. Duration of response ranged from 24.1 to 48.7^{+}, 18.4 to 66.3^{+}, 32.4 to 108.1^{+}, 40.1^{+} to

115.4+, and 73.9 to 117.0+ months in melanoma subjects treated at 0.1, 0.3, 1, 3, and 10 mg/kg, respectively. An additional 7% of melanoma subjects had stable disease for 24 weeks or longer. Across dose levels, melanoma subjects achieved a median overall survival of 16.8 months (95% CI: 12.5, 31.6), with a 2-year overall survival rate of 43%.

Here at Duke, we are currently enrolling in CA209143, "A Randomized Phase 3 Open Label Study of Nivolumab Versus Bevacizumab and a Safety Study of Nivolumab or Nivolumab in Combination With Ipilimumab in Adult Subjects With Recurrent GBM" where we evaluate the efficacy and safety of nivolumab alone or with ipilimumab, vs bevacizumab in subjects with recurrent GBM. Subjects with KPS ≥70, grade IV malignant glioma treated with radiotherapy and temozolomide, and documented first GBM recurrence within 28 days of randomization are eligible. Those with >1 recurrence of GBM, extracranial disease, autoimmune conditions, or previous VEGF inhibitor or anti-angiogenic treatment are ineligible. Safety cohort 1; nivolumab 3 mg/kg [n=10; Q2W x 4] and nivolumab 1 mg/kg + ipilimumab 3 mg/kg [n=10; Q3W x 4 followed by nivolumab 3 mg/kg Q2W]) will establish safety and tolerability in GBM pts. Upon successful completion of cohort 1, efficacy cohort 2 will enroll up to 240 pts with recurrent GBM, randomized 1:1:1 to receive nivolumab, nivolumab + ipilimumab (dosed as cohort 1), or bevacizumab (10 mg/kg Q2W). The primary objectives are to evaluate safety in cohort 1 and OS in cohort 2, vs bevacizumab, with secondary objectives of PFS and ORR. Responses will be assessed (RANO criteria) at the end of weeks 6 and 12, and every 8 weeks until progression or treatment discontinuation. Clinical trial information: NCT02017717. To date, nivolumab has been well tolerated in this patient population – 20 subjects have been treated on the nivolumab monotherapy arm.

Please see nivolumab IB[88] for more information.

7.2.5.2 DC Vaccines

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 immunosuppressed either from the use of steroids or due to the fact that malignant brain tumors secrete immunosuppressive agents like TGF-y. 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 NK cell lineage markers, CD19 and CD56, respectively, which is consistent with published DC phenotypes.

DC immunotherapy in patients with MGs has been evaluated in 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.[98] 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 radiation, have had a nearly complete response in our prior vaccination study which also included the use of chemotherapy agents such as TMZ. 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). 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)[99]; radiation and concurrent TMZ (14.6 months); or radiolabeled anti-tenascin monoclonal antibodies performed at Duke University (18.3 months)[100].

7.3 Study Purpose/Rationale

Immune checkpoint blockade is a rapidly advancing therapeutic approach in the field of immuno-oncology and treatment with investigational agents targeting this mechanism has induced regressions in several types of cancer. Cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and programmed death 1 (PD-1) receptor are two important cellular targets that play complementary roles in regulating adaptive immunity. Whereas PD-1 contributes to T cell exhaustion in peripheral tissues, CTLA-4 inhibits at earlier points in T cell activation. In preclinical models, combined blockade of PD-1 and CTLA-4 achieved more pronounced antitumor activity than blockade of either pathway alone[87].

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.

We have shown that immunosuppressive T_{Regs} are disproportionately represented in the blood and tumors of patients with malignant gliomas (MGs). This finding is accompanied by significant T cell dysfunction resulting in a profound immunosuppression[101, 102] that likely significantly limits endogenous antitumor immune responses. Programmed cell death 1 (PD-1) is a key immune receptor expressed by activated T cells that mediates immunosuppression. Blockade of PD-1 may help overcome immune resistance. Nivolumab is a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands promote immune responses and antigen-specific T cell responses to both foreign and self-antigens. PD-1 receptor blockade by nivolumab is a new approach for immunotherapy of tumors.

We believe the use of serial vaccination in conjunction with an anti-PD-1 monoclonal antibody, is a novel platform for enhancing the efficacy of tumor-specific vaccines. In addition, immune checkpoint receptors such as program death- ligand (PD-L)1/B7-H1/CD274, a transmembrane receptor ligand and negative regulator of T cell signaling, have been reported to be upregulated in gliomas. Studies have also suggested an association between malignancy grade of astrocytic tumors and tumor cell PD-L1 expression. Additionally, use of an inhibitor of PD-L1 in mouse glioma models suggests benefit in combination with radiation therapy.

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[89, 92, 103]. 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.

Surgery alone has not been established as the standard of care treatment in the recurrent brain tumor population, rather, it is used in combination with medical treatment. Therefore, it is our belief that providing nivolumab treatment ± DC vaccine prior to surgery will be safe and well tolerated. However, subjects will be closely monitored for any indication that surgical resection should be performed sooner than the planned schedule. Therefore, we would like to evaluate the safety of nivolumab in combination with DC vaccinations for the treatment of bevacizumab-naïve subjects with first or second recurrent, resectable WHO Grade III and IV MGs and to assess the immunologic changes from nivolumab therapy with and without DC. In order to do that, we will randomize subjects to two groups: Group I will receive nivolumab monotherapy until surgical resection, and Group II will receive nivolumab with DC vaccine therapy until surgical resection. During surgical resection blood and tumor samples will be assessed and compared. Following surgery, both groups will continue to receive DC vaccines (total of 8) and nivolumab therapy until confirmed progression. The purpose of the surgical resection is de-bulking of the tumor. The subject will continue on study treatment following surgery, at the discretion of the treating physician, until they have radiographic or clinical progression (as described in Section 12.2.1), regardless of the pathology results from surgery.

8 OBJECTIVES AND ENDPOINTS

Table 1: Objectives and Endpoints

	Objective	Endpoint	Analysis
Primary	To evaluate the safety of nivolumab in combination with DC vaccinations for the treatment of bevacizumab-naïve subjects with first or second recurrent, resectable WHO Grade III and IV MGs.	Proportion of patients with unacceptable toxicity.	See Section 15.4
Secondary	To describe overall survival and progression free survival.	Median overall and progression-free survival from initiation of protocol treatment.	See Section 15.5
Exploratory	To evaluate PBMCs for immunologic response to pp65 in the presence of nivolumab.	Mean or median change from baseline at each follow-up assessment in PBMC-based immune measures	See Section 15.6.1
Exploratory	To assess whether treatment modulates T cell costimulatory markers	Mean or median change from baseline in the expression of costimulatory markers.	See Section 15.6.2
Exploratory	To assess the effect of treatment on cytokines and other soluble factors	Mean or median change from baseline in the expression of cytokines and other soluble factors	See Section 15.6.3
Exploratory	To assess the impact of pre-surgical treatment on gene expression	Mean or median change in the level of gene expression in PBMCs, and mean or median level of gene expression in tumor	See Section 15.6.4
Exploratory	To determine if evidence exists for epitope spreading intratumorally or systemically after treatment.	Changes in TCR repertoire diversity TILs, and PBLs.	See Section 15.6.5

9 INVESTIGATIONAL PLAN

9.1 Study Design

This two-arm randomized trial (please see section 9.1.9 for details) will evaluate the safety of nivolumab in combination with DC vaccinations for the treatment of bevacizumab-naïve subjects with first or second recurrent, resectable WHO Grade III and IV MGs. Up to 66 patients will be enrolled and treated with the goal of accruing 30 patients (15 per arm) that will receive nivolumab and at least 3 vaccines. After enrollment, leukapheresis will be done for generation of DC vaccines and immunologic monitoring (please see section 12.7.5 for details). Following consent, **all** subjects will undergo standard of care tetanus

booster vaccination with 0.5 mL of Td (tetanus and diphtheria toxoids adsorbed) intramuscularly (I.M.) into the deltoid muscle to ensure adequate immunity to the tetanus antigen. All subjects will initially return every 2 weeks and receive approximately 3 infusions of nivolumab 3 mg/kg IV while the DC vaccines are being prepared from the initial leukapheresis, Nivolumab infusions will continue until the DCs are ready for vaccinations. All subjects will then be randomized 1:1 to one of 2 arms (Group I: nivolumab only pre-surgery; Group II: nivolumab with DC vaccines pre-surgery). Patients who are unable to tolerate nivolumab will be withdrawn from the study and replaced. Patients whose DCs or PBLs fail to meet release criteria will continue to receive nivolumab only and will not undergo repeat leukapheresis. For patients whose leukapheresis yields less than 4 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 will not cause a significant delay in treatment for the patient. For patients that need to come off study due to adverse events, they will be eligible for surgery and other treatment modalities. Patients will be monitored closely and those patients that have clinical indications for surgical resection prior to the timeframe indicated below will be withdrawn from the study and undergo surgical resection. Peripheral blood will be drawn for immune monitoring prior to treatment with the 5th cycle of nivolumab (second post-randomization infusion of nivolumab).

All vaccines will be administered intradermally near each groin, divided into 2-4 injections in each side as tolerated in order to inject the full amount without leakage. It is recommended that the injections be given approximately 10 cm below the inguinal ligament. Vaccine injections must be performed using an appropriate needle for intradermal administration. A 25-gauge needle is recommended.

Group I Treatment Plan (Nivolumab Only Pre-Surgery)
After randomization, patients in Group I will receive nivolumab 3 mg/kg IV every 2 weeks x approximately 8 weeks. The subject will then undergo surgical resection of tumor within approximately 1-3 weeks by a Duke neurosurgeon. Approximately 2–4 weeks later, leukapheresis is repeated for generation of DC vaccines and immunologic monitoring. Approximately 1 day to 2 weeks after leukapheresis, the subject will resume nivolumab 3mg/kg IV every two weeks with DC vaccine administration intradermally for a total of 3 vaccines. At the time of the third DC vaccine, patients will receive vaccine site pre-conditioning. A single dose of Td toxoid (1 flocculation unit, Lf, in 0.4 mLs of saline) will be administered to a **single** side of the groin i.d. (as described above for all vaccine administrations)12-24 hours prior to the third DC vaccine, which is always given bilaterally at the groin site. At the vaccine #3 visit, prior to vaccine # 3 administration, erythema and induration measurements will be taken of pre-conditioning site. Group I subjects will then receive monthly DC vaccine administrations intradermally for 5 months or until progression (whichever comes first). Total vaccines to be administered will be 8 post-surgery unless subject is removed. Nivolumab will continue until progression. At the clinic visit following the last vaccine (#8), subjects will have peripheral blood drawn for immune monitoring prior to infusion of nivolumab.

Group II Treatment Plan (Nivolumab with DC Vaccines Pre-Surgery)
After randomization, patients in Group II will receive the fourth cycle of nivolumab then receive nivolumab 3 mg/kg IV along with DC vaccines intradermally every 2 weeks x approximately 6 weeks for a total of 3 vaccines. At the time of the third DC vaccine, patients will receive vaccine site pre-conditioning. A single dose of Td toxoid (1 flocculation unit, Lf, in 0.4 mLs of saline) will be administered to a **single** side of the groin i.d. (as described above for all vaccine administrations)12-24 hours prior to the third DC vaccine, which is always given bilaterally at the groin site. At the vaccine #3 visit, prior to vaccine #3 administration, erythema and induration measurements will be taken of pre-conditioning site. The subject will then undergo surgical resection of tumor within approximately 1-3 weeks by a Duke neurosurgeon. Approximately 2-4 weeks later, leukapheresis is repeated for generation of DC vaccines and immunologic monitoring. Approximately 1 day to 2 weeks after leukapheresis, the subject will resume nivolumab 3mg/kg IV every two weeks. When DC vaccines have completed processing and are available for administration, DC vaccine administrations as described above will resume monthly for 5 months or until progression (whichever comes first). Total vaccines to be administered will be 8 (3 pre- and 5 post-surgery) unless subject is removed. Nivolumab will continue until progression. At the clinic visit following the last vaccine (#8), subjects will have peripheral blood drawn for immune monitoring prior to infusion of nivolumab.

9.1.1 Definition of Unacceptable Toxicity

Toxicities will be graded according to the NCI CTCAE version 4 criteria. An unacceptable toxicity is defined as any grade 3, 4, or 5 adverse event that is possibly, probably, or definitely related to either nivolumab or DC vaccination treatment during concurrent treatment, or any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment. In addition, any complication following resection (ex. excessive intracranial bleeding, delays in wound healing) that are prolonged longer than 4-6 weeks post-surgery will be considered an unacceptable toxicity.

9.1.2 Dose Modification

The DC vaccine and nivolumab dose will not be modified in this trial. Please see the nivolumab IB and Section 12.2.2 for discontinuation of nivolumab related to adverse events.

9.1.3 Safety Considerations

Management of Toxicities
If a Grade 3 NCI CTC or greater toxicity is seen that is not attributable to a concomitant medication (other than nivolumab or vaccine), co-morbid event, or disease progression that has been documented radiographically or clinically, the next scheduled study-specific treatment for that patient (nivolumab and/or DC vaccine) 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.

Long Term Survivors
For subjects still actively receiving study treatment ≥ 3 years from study initiation, 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 a toxicity attributable to vaccine or nivolumab treatment nor will have nivolumab held or delayed.

Adverse Event Reporting and Documentation
An “Adverse Event” will be defined as any adverse change from the subject’s pre-treatment baseline condition, including any clinical or laboratory test abnormality that occurs during the course of research after initiation of nivolumab. Adverse events will be categorized and graded in accordance with the NCI CTC (Version 4). AEs and SAEs will be collected starting at the time of consent.

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.
Please see section 11 for further information.

Collection and reporting of SAEs will be done through 30 days post end of study visit. Please see the BMS Guidelines and the management algorithms provided by BMS in the appendices of this protocol.

9.1.4 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.

9.1.5 Use of Corticosteroids

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.

9.1.6 Use of Bevacizumab

In the event that a patient demonstrates neurologic or radiographic signs suggestive of an inflammatory reaction that requires steroid management, patients will be initiated on bevacizumab at the discretion of the treating physician. Bevacizumab treatment will begin anytime four weeks or more post-resection. Bevacizumab will be administered at 5mg/kg IV QOW following nivolumab infusion and can be increased to 10mg/kg IV QOW if needed. Bevacizumab will not be provided by the study.

9.1.7 Missed Doses

To ensure that repetitive infusions of nivolumab and DC vaccines will be given to patients, both will be given within approximately ± 2 day window. A margin of 2 days surrounding each treatment will make scheduling more feasible. Nivolumab infusions will continue until progression, however, DC vaccinations will only be given for a maximum of 8. At the discretion of the study PI, patients who miss either the nivolumab infusion or the vaccine administration for whatever reason will have their appointment re-scheduled to administer treatment as soon as possible and this will be considered a non-reportable deviation will be and will be added to the deviation log. For continued non-compliance with the scheduled nivolumab or vaccine appointments, the subject may be removed from the trial at the discretion of the study PI. Please see the nivolumab IB[88] on management of missed doses of nivolumab.

9.1.8 Concomitant Medications

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

9.1.9 Randomization

Permuted block randomization will be used to assign patients to one of two treatment arms (Group I: nivolumab monotherapy until surgery and Group II: nivolumab with DC vaccine therapy until surgery). The randomization module for the Duke electronic database system (Title 21 CFR Part 11 Compliant) will be used for this purpose.

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

Nivolumab

Nivolumab will be infused at a dosage of 3 mg/kg IV every other week (+/- approximately 2 days) over approximately 30-60 minutes. Each infusion of nivolumab will be considered one cycle. On the days where patients receive both nivolumab and DC vaccine, the order of the administration does not matter (i.e., DC vaccine can be administered before or after nivolumab and does not need to be given in the same order at follow-up visits).

Single-dose pharmacokinetics (PK) of nivolumab was studied in 39 subjects with cancer. The single-dose PK of nivolumab was linear and dose-proportional in the range of 0.3 mg/kg to 10 mg/kg. The mean terminal T-HALF of nivolumab ranged between 17 and 25 days across the dose range of 0.3 mg/kg to 10 mg/kg. Geometric mean total clearance varied from 0.13 mL/h/kg to 0.19 mL/h/kg, while mean volume of distribution varied between 83 mL/kg and 113 mL/kg across doses. The clearance and half-life of nivolumab are consistent with that of IgG4.

The multiple-dose PK of nivolumab given every 2 weeks was determined from MDX1106-03 study as well as by opulation PK using data from 669 subjects across nivolumab studies. Multiple-dose PK of nivolumab following every 2 week dosing was linear with dose-proportional increase in Cmax and AUC (TAU) in the studied range of 0.1 mg/kg to 10 mg/kg. Nivolumab accumulation with every 2 week dosing

frequency was in the range of 2.9 to 3.3 based on AUC (TAU), 2.0 to 2.4 based on Cmax, and 3.1 to 4.8 based on Cmin.

The PK, clinical activity, and safety of nivolumab have been assessed in approximately 32 clinical studies sponsored by BMS or Ono Pharmaceutical Co., Ltd. (ONO): 11 Phase 1 studies, 13 Phase 2 studies, and 8 Phase 3 studies. Approximately 4,000 subjects have received nivolumab monotherapy in single- or multiple-dose Phase 1/2/3 studies or studies with nivolumab in combination with other therapeutics (ipilimumab, cytotoxic chemotherapy, anti-angiogenics, and targeted therapies). The description and status of all studies are outlined briefly in the nivolumab IB[88]. Results from the ongoing studies are preliminary and are subject to change.

DC Vaccine
Only 1 dose level of DCs (2×10^7) will be given.

Vaccination and adoptive T-cell strategies in humans in other contexts have been safe and effective. Therapeutic TMZ induces a profound lymphopenia that may enhance anti-tumor vaccination responses when given during the homeostatic T-cell proliferation that occurs in response to lymphodepletion. Other DC vaccines given to patients with high-grade gliomas during recovery from TMZ-induced lymphopenia have produced potent tumor-specific immune responses and have been well tolerated.

In one of our dendritic cell immunotherapy trials (Pro00003877 ATTAC), we evaluated the impact of vaccine site pre-conditioning with Td toxoid. 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[86].

9.3 Rationale for Correlative Studies

Peripheral blood and tumor specimens are collected in this study for use in assessing the impact of nivolumab and DC vaccination treatment on levels of immune function. Although this Phase I study is not designed to be definitive towards this end, these analyses will provide the initial data necessary to adequately power future studies.

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

Please refer to section 15 for detail concerning which patients will be included in which analyses.

Once the patient signs an ICF, that subject will be considered “on study” and the patient will be monitored for all AEs and SAEs. 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

Nivolumab
Nivolumab (also referred to as BMS-936558 or MDX1106) is a fully human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 is a negative regulatory molecule expressed by

activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligands 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T cell responses to both foreign antigens as well as self-antigens. Nivolumab is expressed in Chinese hamster ovary (CHO) cells and is produced using standard mammalian cell cultivation and chromatographic purification technologies. The clinical study product is a sterile solution for parenteral administration. Please see nivolumab IB[88] for more information.

DC Vaccine
Human *CMV* pp65-LAMP mRNA-pulsed autologous DCs is the name of the study drug given with every vaccine. The class of action for the DC vaccine is a biological.

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 5-10 years. Please refer to section 7.2 on the use of Td in this protocol. The class of action for the tetanus toxoid is an antitoxin.

Leukapheresis and dendritic cell vaccine generation
Two leukapheresis procedures will be performed in this protocol. The leukapheresis will be used for DC generation and immune monitoring. Leukaphereses will be approximately a 4-hour leukapheresis. It is estimated that 10-12 L of blood will be processed during this leukapheresis.

DCs will be generated from 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 either the Duke Apheresis Unit or Duke Bone Marrow Transplant 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 10^7 cells/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 1^{o}C/minute.

The DCs are then stored until needed at –135^{o}C. 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 37^{o}C, 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.

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^{o}C 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.

10.2 Packaging and Labeling

- For nivolumab: (Please see Appendices for BMS Guidelines for packaging and labeling)
  Name
  MRN
  DOB
  Drug: nivolumab
  Lot #: Lot XXX
  Caution New Drug Limited By Federal Law To Investigational Use

- For CMV pp65-LAMP mRNA-pulsed DCs (DC vaccine):
  Name
  MRN
  DOB
  Drug: pp65DCs
  Lot #: Lot 001
  Caution New Drug Limited By Federal Law To Investigational Use

- For tetanus diphtheria toxoid
  Name
  MRN
  DOB
  Drug: Td
  Lot #: Lot 001

10.3 Supply, Receipt, and Storage

The DCs will be stored in a locked liquid nitrogen freezer in the FDA-approved Clinical Processing Suite in the DBTIP Laboratory. The Nautilus LIMS (Laboratory Information Management System) database will track receipt and storage location.

These study agents are supplied and stored by the Duke Investigational Chemotherapy Services (ICS): nivolumab is supplied in a single use glass vial, Bristol-Myers Squibb, NJ; and Tetanus Diphtheria Toxoid adsorbed, stored at 4^{o}C.

10.4 Dispensing and Preparation

The DC products will be delivered from the DBTIP Laboratory directly to the bedside under the supervision of the research nurse, or his/her designee. 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 BMT transfusion procedure. The nivolumab will be dispensed from the Duke Investigational Chemotherapy Service to the Duke Cancer Center's Oncology Treatment Room where infusion will take place per standard operating procedures for that clinic. The tetanus will be dispensed from the Duke Investigational Chemotherapy Services (ICS) Pharmacy.

10.5 Compliance and Accountability

The 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 accountability, transfers, receipts, and disposal are recorded in the Duke Nautilus system.

Tetanus compliance and accountability will be managed by the Duke ICS Pharmacy.

10.6 Disposal and Destruction

Unused study drugs will be destroyed per Duke Policy and institutional guidelines.

11 SUBJECT ELIGIBILITY

11.1 Inclusion Criteria

- Age 18-80 years
- First or second recurrence of MG (WHO Grade III or IV glioma or astrocytoma) in surgically accessible areas with prior histologic diagnosis of MG
- Bevacizumab-naïve – no prior exposure to Bevacizumab
- KPS of ≥ 70%
- RT with ≥ 45 Gy tumor dose, completed ≥ 8 weeks prior to study entry
- Laboratory values must meet the following criteria (using CTCAE v4):
    1) WBC ≥ 2000/uL
    2) Neutrophils ≥ 1500/uL
    3) Platelets ≥ $100x10^3$/uL
    4) Hemoglobin ≥ 9.0 g/dL
    5) Serum creatinine ≤ 1.5x ULN or creatinine clearance (CrCl)≥ 40 mL/min (using the Cockcroft-Gault formula)
    a. Female CrCl = (140 - age in years) x weight in kg x 0.85 /72 x serum creatinine in mg/dL
    b. Male CrCl = (140 - age in years) x weight in kg x 1.00/72 x serum creatinine in mg/dL
    6) AST ≤ 3x ULN
    7) ALT ≤ 3x ULN
    8) Bilirubin≤ 1.5x ULN (except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL)
    9) Subjects must have resting baseline O2 saturation by pulse oximetry of ≥ 92% at rest.
- Patients of child bearing potential or with partners of child-bearing potential must agree to practice recommended contraceptive methods to prevent pregnancy during treatment and for 5 months after the last dose of nivolumab for women, 7 months after the last dose of nivolumab for men, and 6 months after the last dose of bevacizumab for subjects receiving bevacizumab as stated in the informed consent.

11.2 Exclusion Criteria

- Contrast-enhancing tumor component crossing the midline, multi-focal tumor, or tumor dissemination (subependymal or leptomeningeal)
- Clinically significant increased intracranial pressure (e.g., impending herniation), uncontrolled seizures, or requirement for immediate palliative treatment
- Pregnant or need to breast feed during the study period (Negative β-HCG test required), or unable to maintain use of contraception while on study and for 31 weeks after the last dose of nivolumab
- Active infection requiring treatment or an unexplained febrile (> 101.5o F) illness
- Known immunosuppressive disease, autoimmune disease or human immunodeficiency virus infection, Hepatitis B or Hepatitis C
- Known allergy or hypersensitivity to tetanus, or any other tetanus or diphtheria toxoid-containing vaccine, or any component of this vaccine (i.e., aluminum phosphate, formaldehyde)
- Known severe (Grade 3 or 4) infusion-related allergy or hypersensitivity to any monoclonal antibody
- Previous radiation therapy with anything other than standard radiation therapy (such as previous stereotactic radiosurgery) or previous treatment with an immune checkpoint inhibitor (i.e., nivolumab, pembrolizumab, ipilimumab)
- Unstable or severe intercurrent medical conditions such as severe heart (New York Association Class 3 or 4) or lung (FEV1 < 50%) disease, uncontrolled diabetes mellitus.
- Corticosteroid use > 4mg/day at time of consent
- Prior inguinal lymph node dissection.

12 SCREENING AND ON-STUDY TESTS AND PROCEDURES

Table 2. Screening and On-Study Tests and Procedures

Prior to Randomization:

	Screening	Leukapheresis #1	Nivolumab IV Cycle 1	Nivolumab IV Cycle 2	Nivolumab IV Cycle 3	Progression
Beta HCG	X[1]	X[2]	X[1,13]			
CBC w/diff	X	X[2]	X[13]	X	X	
CMP	X	X[2]	X[13]	X	X	
Ionized Calcium		X[2]				
LDH	X		X[13]	X	X	
Mg	X		X[13]	X	X	
Amylase/Lipase	X		X[13]	X	X	
Thyroid Profile and Free T3	X[3]					
CMV		X[4]				
Autoimmunity Testing	X[5]	X[5]	X[13]	X[5]	X[5]	X[5]
Blood for Immunologic Monitoring	X[4]	X[6]				X[7]
MRI	X[8]					X
Physical Exam, Neuro Exam, KPS, O2 Saturation[9]	X		X[13]	X	X	
Con Meds	X		X	X	X	
Baseline symptoms and medical history	X					
AE/SAE monitoring[10]	X	X	X	X	X	X
Tumor Pathology	X[11]					X[11]
MMSE	X					
Nivolumab			X	X	X	
Randomization					X[12]	

[1]Serum pregnancy testing is required at screening, prior to each leukapheresis, and prior to the first nivolumab infusion. Additional testing can be performed as the treating physician feels is necessary and a urine pregnancy test is allowed at any additional time points.
[2]Labs for leukapheresis should be drawn within 48 hours prior to leukapheresis.
[3]Thyroid profile and Free T3 should be drawn at screening. These tests will be repeated prior to cycle 4 and may be drawn sooner at the discretion of the treating physician.
[4]CMV screen will be drawn prior to 1st leukapheresis. One red top tube will be drawn and taken to the DBTIP Lab for processing. The CMV screen only needs to be drawn once and may be batch processed.
[5]Autoimmunity testing is not required and will be drawn at the discretion of the treating physician based on clinical symptoms at any time point deemed necessary by the treating physician.
[6]Immune monitoring prior to leukapheresis will consist of one red top tube. These tubes will be sent to the DBTIP lab for processing.
[7]Immune monitoring will be drawn at the time of progression, if possible.
[8]Baseline MRI can be performed prior to consent since this is a standard of care test. The baseline MRI is not required to be performed within a certain period of time before screening. A new baseline MRI can be performed prior to leukapheresis at the discretion of the treating physician. Additional MRIs can be performed at the discretion of the treating physician. If a patient is removed from the study due to clinical progression, a MRI is not required.
[9]O2 saturation only required at screening. O2 saturation will be rechecked at the discretion of the treating physician based on clinical symptoms.
[10]AEs and SAEs to be captured starting at the time of consent.
[11]Tumor pathology must be confirmed by at least outside pathology report prior to enrollment. It is preferable that pathology be confirmed by Duke Pathology Department prior to enrollment, but this is not required. Tissue may be obtained at the time of progression based on the discretion of the treating physician.
[12]Randomization will occur after cycle 3 of nivolumab and prior to cycle 4 of nivolumab in order for the DBTIP lab to prepare for the first DC vaccination.
[13]Labs and clinic visit (physical exam, neuro exam, KPS) do not need to be repeated prior to Nivolumab #1 if previously performed for screening with the same week.

Post-Randomization, Group 1:

	Nivolumab QOW (Cycles 4-7)	Surgery	Leukapheresis #2	Nivolumab QOW (Cycles 8-11) with DC Vaccine QOW (x 3)	Nivolumab QOW (Cycles 12-20) with DC Vaccine Monthly (x 5)	Nivolumab QOW (Cycles 21+)	Progression
Beta HCG			X[2]				
CBC w/diff	X	X	X[2]	X	X	X	
CMP	X	X	X[2]	X	X	X	
Ionized Calcium			X[2]				
LDH	X			X	X	X	
Mg	X			X	X	X	
Amylase/Lipase	X			X	X	X	
Thyroid Profile and Free T3	X[3]			X[3]	X[3]	X[3]	
Autoimmunity Testing	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]
Blood for Immunologic Monitoring	X[6]	X[6]	X[5]	X[6]	X[6]		X[6]
MRI	X[7]	X[7]		X[7]	X[7]	X[7]	X[7]
Physical Exam, Neuro Exam, KPS, O2 Saturation[8]	X			X	X	X	
Con Meds	X		X	X	X	X	
AE/SAE monitoring	X	X	X	X	X	X	X
Tumor Pathology		X[9]					X[9]
Nivolumab	X			X	X	X	
Td Pre-conditioning				X[6,10]			
DC Vaccine with vitals				X[11]	X[12]		

[1]Serum pregnancy testing can be performed as the PI feels is necessary and a urine pregnancy test is allowed. Serum pregnancy test is required prior to leukapheresis.
[2]Labs for leukapheresis should be drawn within 48 hours prior to leukapheresis.
[3]Thyroid profile and free T3 should be drawn prior to cycle 4 and every 4 cycles thereafter. These tests can be repeated as necessary per the discretion of the treating physician.
[4]Autoimmunity testing to be done at the discretion of the treating physician based on clinical symptoms at any time point deemed necessary by the treating physician.
[5]Immune monitoring will be done prior to both leukapheresis procedures and will consist of one red top tube. This tube will be sent to the DBTIP lab for processing.
[6]Immune monitoring will consist of 10 yellow top ACD tubes and 1 red top tube. Immune monitoring will be drawn at Nivolumab cycles 5, 12, 18 and 21 (or the first cycle of Nivolumab two weeks after vaccine #8 is administered), and prior to surgery Immune monitoring will be drawn at the time of progression, if possible. After a year, an attempt will be made to obtain blood for immunologic monitoring 2-3 times a year at standard Duke neuro-oncology visits (10 yellow, 1 red).
[7]MRI to be done prior to cycle 4, before and after surgery per the discretion of the neurosurgeon, and prior to every 4th cycle of nivolumab for one year starting with cycle 12 (i.e., prior to cycles 12, 16, 20, etc.). Additional MRIs can be performed at the discretion of the treating physician. If a patient is removed from the study due to clinical progression, a MRI is not required.
[8]O2 saturation only required at screening. O2 saturation will be rechecked at the discretion of the treating physician based on clinical symptoms.
[9]Tissue will be collected at the time of surgery. Tissue may also be obtained at the time of progression at the discretion of the treating physician.
[10]Td preconditioning to be administered 12-24 hours prior to DC vaccine #3.
[11]DC Vaccines to be administered QOW x 3 (Nivo cycles 8-10). Vitals will be checked on the day of the DC vaccine administration at any time prior to administration as well as after DC vaccine administration.
[12]DC Vaccines to be administered monthly x 5 (Nivo cycles 12, 14, 16, 18 20). Vitals will be checked on the day of the DC vaccine administration at any time prior to administration as well as after DC vaccine administration.

Post-Randomization, Group 2:

	Nivolumab IV (Cycle 4)	Nivolumab QOW (Cycles 5-7) with DC Vaccine QOW	Surgery	Leukapheresis #2	Nivolumab QOW (Cycles 8-15) with DC Vaccine Monthly (x 5)	Nivolumab QOW (Cycles 16+)	Progression
Beta HCG	X[1]	X[1]		X[2]	X[1]	X[1]	
CBC w/diff	X	X	X	X[2]	X	X	
CMP	X	X	X	X[2]	X	X	
Ionized Calcium				X[2]			
LDH	X	X			X	X	
Mg	X	X			X	X	
Amylase/Lipase	X	X			X	X	
Thyroid Profile and Free T3	X[3]	X[3]			X[3]	X[3]	
Autoimmunity Testing	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]	X[4]
Blood for Immunologic Monitoring		X[6]	X[6]	X[5]	X[6]	X[6]	X[6]
MRI	X[7]		X[7]		X[7]	X[7]	X[7]
Physical Exam, Neuro Exam, KPS, O2 Saturation[8]	X	X			X	X	
Con Meds	X	X		X	X	X	
AE/SAE monitoring	X	X	X	X	X	X	X
Tumor Pathology			X[9]				X[9]
Nivolumab	X	X			X	X	
Td Pre-conditioning		X[6,10]					
DC Vaccine with vitals		X[11]			X[12]		

[1]Serum pregnancy testing can be performed as the PI feels is necessary and a urine pregnancy test is allowed. Serum pregnancy test is required prior to leukapheresis.
[2]Labs for leukapheresis should be drawn within 48 hours prior to leukapheresis.
[3]Thyroid profile and Free T3 should be drawn prior to cycle 4 and every 4 cycles thereafter. These tests can be repeated as necessary per the discretion of the treating physician.
[4]Autoimmunity testing to be done at the discretion of the treating physician based on clinical symptoms at any time point deemed necessary by the treating physician.
[5]Immune monitoring will be done prior to both leukapheresis procedures and will consist of one red top tube. This tube will be sent to the DBTIP lab for processing.
[6]Immune monitoring will consist of 10 yellow top ACD tubes and 1 red top tube. Immune monitoring will be drawn prior to surgery, prior to vaccines # 1, and 7, and prior to Nivolumab cycle 17 (or the first cycle of Nivolumab two weeks after vaccine #8 is administered). Immune monitoring will be drawn at the time of progression, if possible. After a year, an attempt will be made to obtain blood for immunologic monitoring 2-3 times a year at standard Duke neuro-oncology visits (10 yellow, 1 red).
[7]MRI to be done prior to cycle 4, before and after surgery per the discretion of the neurosurgeon, and prior to every 4th cycle of nivolumab for one year (i.e., prior to cycles 8, 12, 16, 20, etc.). Additional MRIs can be performed at the discretion of the treating physician. If a patient is removed from the study due to clinical progression, a MRI is not required.
[8]O2 saturation only required at screening. O2 saturation will be rechecked at the discretion of the treating physician based on clinical symptoms.
[9]Tissue will be collected at the time of surgery. Tissue may also be obtained at the time of progression at the discretion of the treating physician.
[10]Td preconditioning to be administered 12-24 hours prior to DC vaccine #3.
[11]DC Vaccines to be administered QOW x 3 (Nivo cycles 5-7). Vitals will be checked on the day of the DC vaccine administration at any time prior to administration as well as after DC vaccination administration.
[12]DC Vaccines to be administered monthly x 5 (Nivo cycles 8, 10, 12, 14, 16). Vitals will be checked on the day of the DC vaccine administration at any time prior to administration as well as after DC vaccination administration.

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 study-specific treatment is initiated. 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 stored in a locked cabinet in a locked room in the PRTBTC Offices.

CBC w/diff, complete metabolic panel, magnesium, LDH, amylase, lipase, thyroid profile, free T3, and beta HCG (if necessary) will be drawn at screening. The serum CMV screen (1 red top tube) will be drawn prior to the 1st leukapheresis during that visit. The CMV screen will be sent back to the DBTIP Lab where the test will be performed. These tests may be batched tested and results delayed.

Initial clinical evaluations will also include a baseline MMSE.

A baseline MRI of the brain (with and without gadolinium enhancement) will be obtained as per standard of care.

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

12.2 Treatment Period

After enrollment, leukapheresis will be done for generation of DC vaccines and immunologic monitoring. Within 48 hours of leukapheresis, patients will have blood samples taken for the following tests as required by the Duke Apheresis Center: CBC w/diff, CMP, ionized Calcium, and β-HCG (for females of child-bearing potential). Total estimated blood volume required for these evaluations is 12-15 mLs. For patients without sufficient venous access for leukapheresis, a temporary central intravenous catheter may be inserted. To prevent the development of hypocalcemia from the citrate used for leukapheresis, all patients will be instructed to take oral Tums, 2 tablets three times a day and at bedtime the day before and the day of the leukapheresis procedure. Patients who have lower levels of calcium will be treated per Apheresis lab standard protocols under the direction of apheresis attending physician. 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.

Following consent, all subjects will undergo standard of care vaccination with 0.5 mL of Td (tetanus and diphtheria toxoids adsorbed) I.M. into the deltoid muscle to ensure adequate immunity to the tetanus antigen. Each subject will initially return every 2 weeks and receive nivolumab 3 mg/kg IV while the DC vaccines are being prepared from the initial leukapheresis and then randomized to two treatment arms (Group I and Group II). Peripheral blood will be drawn for immune monitoring prior to treatment with the 5th cycle of nivolumab (second post-randomization infusion of nivolumab). Group I will receive nivolumab alone I.V. every 2 weeks x approximately 8 weeks and Group II will receive the 4th cycle of nivolumab then receive nivolumab 3 mg/kg IV every 2 weeks with DC vaccines administered intradermally and divided equally to both inguinal regions for a total of 3vaccines x approximately 6 weeks. At the time of the third DC vaccine, patients from both groups will receive vaccine site pre-conditioning. A single dose of Td toxoid (1 flocculation unit, Lf, in 0.4 mLs) will be administered to a single side of the groin 12-24 hours prior to the third DC vaccine, which is always given bilaterally at the groin site. At the vaccine #3 visit, prior to vaccine # 3 administration, erythema and induration measurements will be taken of pre-conditioning site. The subject will then undergo surgical resection of tumor within approximately 1-3 weeks by a Duke neurosurgeon. Approximately 2-4 weeks after surgery, leukapheresis is repeated for generation of DC vaccines and immunologic monitoring. For subjects whose first leukapheresis yields 8 or more vaccines, the duration of the second leukapheresis can be 2 hours instead of 4 hours. Approximately 1 day to 2 weeks after leukapheresis, the subject will resume nivolumab 3mg/kg IV every

two weeks along with DC vaccine administrations as described in section 7.1 (Group I and Group II differ) for a total of 8 vaccines or until progression (whichever comes first). Nivolumab will continue until progression. See section 12.7.5 for additional immunologic assessments.

If clinically indicated, patients will be assessed before initial infusion of nivolumab and as needed at the discretion of the treating oncologist, by a panel of clinical laboratory analyses to screen for the development of autoimmunity. The most common manifestations of autoimmunity seen in related trials[104-108] have included enterocolitis, dermatitis, uveitis, hepatitis, and hypophysitis.

12.2.1 Rationale for Continued Treatment in Cases of Suspected Progressive Disease

Accumulating clinical evidence indicates some subjects treated with immune system stimulating agents may develop progression of disease (by conventional response criteria) before demonstrating clinical objective responses and/or stable disease. This phenomenon was observed in approximately 10% of subjects in the Phase 1 study of nivolumab[109]. Two hypotheses have been put forth to explain this phenomenon. First, enhanced inflammation within tumors could lead to an increase in tumor size which would appear as enlarged index lesions and as newly visible small non-index lesions. Over time, both the malignant and inflammatory portions of the mass may then decrease leading to overt signs of clinical improvement. Alternatively, in some individuals, the kinetics of tumor growth may initially outpace anti-tumor immune activity. With sufficient time, the anti-tumor activity will dominate and become clinically apparent. Therefore, subjects initially meeting radiologic criteria for disease progression (see Section 12.1) will be allowed to continue study therapy until a second radiologic confirmation of progression performed approximately 8 weeks later as long as the following criteria are met: 1) the subject experiences investigator-assessed clinical benefit (i.e., no new clinical symptoms contributed to disease progression) and 2) the subject is tolerating the study treatment (i.e., no expected toxicities higher than grade 3 [with the exception of Grade 2 uveitis – see Section 12.2.2 below] or unexpected toxicities).

Any evidence of tumor response will be determined according to the Duke PRTBTC SOP (see APPENDICES). RANO criteria[110] will be used for assessment of pseudoprogression. Tumor progression will need to be documented histologically, unless there are clinical contraindications, to exclude inflammatory responses presenting as radiographic or clinical changes, which could indicate potentially toxic or therapeutic responses and not tumor progression. Patients will be followed until death.

Standard treatment for glioblastomas (including radiation therapy and temozolomide) may result in a transient increase in tumor enhancement (pseudoprogression) in a subset of subjects that eventually subsides without any change in therapy. Pseudoprogression may be difficult to differentiate from true tumor progression and may have important implications for patient management. Accumulating evidence also indicates that some subjects treated with immune system stimulating agents may also develop apparent progression of disease (by conventional response criteria) before demonstrating clinical objective responses and/or stable disease[109]. This phenomenon was observed in approximately 10% of subjects in the Phase 1 study of nivolumab and has also been reported for ipilimumab monotherapy[111].

In order to minimize premature discontinuation of study medication and distinguish pseudoprogression from progressive disease, subjects initially meeting radiologic criteria for disease progression may continue receiving study medication until confirmation of progression with an MRI performed approximately 8 weeks later.
In order to continue study treatment after assessment of initial radiological progression, the following criteria must be met:

1. The subject is believed to demonstrate clinical benefit as determined by the investigator.
2. The subject is tolerating study medication.

Subjects with confirmed progression (approximately 8 weeks after initially assessed progression) will discontinue study medication and enter the follow up/survival phase of the study. If progression is confirmed, then the date of disease progression will be the first date the subject met the criteria for progression.

12.2.2 Discontinuation Criteria for Nivolumab

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment.
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities: uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic toxicity, hypersensitivity reactions, and infusion reactions:
  - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic toxicity, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation.
  - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
    - ♦ Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation.
    - ♦ Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
      - AST or ALT > 8 x ULN
      - Total bilirubin > 5 x ULN
      - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN.
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis. It is recommended to consult with the PI or treating physician for Grade 4 amylase or lipase abnormalities.
  - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset.
- Any dosing interruption lasting > 8 weeks from the last dose with the following exceptions:
  - Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 8 weeks, the PI or treating physician must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted.
  - Dosing interruptions > 8 weeks from the last dose that occur for non-drug-related reasons may be allowed if approved by the PI or treating physician. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 8 weeks, the PI or treating physician must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted.
- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab and DC dosing.

12.2.3 Treatment of Nivolumab-Related Infusion Reactions

Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritus, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms. All Grade 3 or 4 infusion reactions should be reported within 24 hours to the PI and reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE (version 4) guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated)

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional nivolumab administrations.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g., antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for ≤ 24 hours).

Stop the nivolumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor subject until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further nivolumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the case report form (CRF). The following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) should be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms: (Severe reaction, Grade 3: prolonged [i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g., renal impairment, pulmonary infiltrates]). Grade 4: (life-threatening; pressor or ventilatory support indicated).

Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

12.3 End of Treatment

Nivolumab and DC vaccines are given as described above for a total of 8 vaccines and nivolumab until progression. Once all nivolumab doses and DC vaccinations have been administered, the treatment phase of the study will be over and the follow-up period will begin. Please see Section 12.2.1 above for details in treating subjects beyond suspected progression.

12.4 Follow-up Period

All patients will be followed for survival and data recorded by the study team.

12.5 End of Study

Rationale for taking a patient off study will be documented (see section 12.2.2). All patients will be followed for survival and recorded by the study 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. 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:

- Patients with an active infection requiring treatment or having an unexplained febrile illness (Tmax > 99.5 F).
- Adverse events
- Abnormal laboratory values
- Unacceptable toxicity (see section 9.1.1). If a subject discontinues from one study drug (nivolumab or DC vaccine) due to unacceptable toxicity attributed to that study drug, the subject can continue on the other study drug at the discretion of the investigator. If in the investigator's opinion the toxicity is related to both study drugs, the investigator can hold both study drugs.
- Protocol deviation
- Administrative issues
- Disease progression
- Pregnancy
- Clinical indications for surgical resection prior to treatment plan.

12.6.2 Follow-up Requirements for Early Withdrawal

Subjects that received **any** nivolumab and/or vaccine therapy will be assessed and followed for serious adverse event monitoring/safety analysis for 30 days following the last infusion and/or vaccine (please see section 15.1 for safety analyses of the lead-in phase).

12.6.3 Replacement of Early Withdrawal(s)

Patients who terminate protocol treatment without experiencing an unacceptable adverse event or completing 3 vaccinations 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. Vital signs will also be assessed prior to nivolumab and DC vaccines. Lastly, vital signs will also be assessed after DC vaccine administration.

12.7.3 Immunologic Assessments

Blood for serum CMV screen (1 red top tube) will be drawn prior to the 1st leukapheresis. Immunological response evaluations for baseline values will be conducted on the leukapheresis sample used to generate the DCs and on the second leukapheresis sample as well as from 1 red top tube done prior to each leukapheresis. Peripheral blood drawn for immunologic monitoring (10 yellow and 1 red top tubes) will be drawn before surgery, before the fifth nivolumab infusion, and at progression (if feasible) in BOTH groups. Group I will have blood drawn prior to the 12th and 18th infusions of nivolumab and Group 2 will have the blood drawn prior to the 7th vaccine. The total amount of blood required for 10 yellow/1 red top tubes will be about 100 mLs. Plasma from the yellow top tubes will be collected at each time point listed where immunologic monitoring is done. Lastly, 10 yellow/1 red top tubes for immune monitoring will be drawn **following** the last vaccine (#8) at the next clinic visit prior to infusion of nivolumab monotherapy in both groups. After a year, an attempt will be made to obtain blood for immunologic monitoring 2-3 times a year at standard Duke neuro-oncology visits (10 yellow and 1 red top tubes).

12.7.4 Correlative Assessments

Testing will be performed by 'optimal batch testing' in which samples will be thawed, rested overnight, and tested according to established/validated SOPs on file at Duke. All samples from each study participant will be analyzed together. The antigen to be tested will be pp65 with the CEF peptide and mitogen controls.

The proportion of specific lymphocyte subsets and expression levels of T cell co-stimulatory markers in PBMC preparations and TILS will be quantified by flow cytometry. Analyses may include, but not necessarily be limited to, the proportion of T, B, and NK cells, granulocytes, the proportion of memory and effector T cell subsets, and expression levels of PD-1, PD-L1, other B7 family members, ICOS, and Ki67.

Fresh tumor tissue will be collected and frozen for gene expression and T cell receptor sequencing. The expression level of genes in TILs and whole blood samples that are associated with response to nivolumab will be assessed by quantitative molecular methods such as microarray and/or quantitative RT-PCR analysis. Analysis may include, but will not necessarily be limited to, genes associated with immune-related pathways, such as T cell activation, antigen processing and presentation, and apoptosis.

As samples permit but not limited to soluble factors, such as cytokines, chemokines, soluble receptors, and antibodies to tumor antigens, will be characterized and quantified by immunoassays in serum. Analyses may include, but will not necessarily be limited to, soluble CD25, soluble PD-1, soluble LAG-3, CCL3, and CXCL-9. Collected serum samples will also be used for the assessment of tumor antigen-specific responses elicited following treatment with and the combination therapy to explore which antitumor antibodies are most associated with clinical response.

13 SAFETY MONITORING AND REPORTING

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

13.1 Adverse Events

An AE is any untoward medical occurrence in a subject receiving study drug (for purposes of this study, this is defined as nivolumab or DC vaccine) 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 nivolumab and/or DC vaccines, whether or not related. All abnormal laboratory findings that can be graded by the CTCAE version 4.0 will be collected as adverse events.

From the time the subject signs consent through the End of Study visit (as defined in Section 12.6), all AEs must be recorded in the subject medical record and adverse events case report form. All SAEs will be recorded from the time consent is signed until 30 days from the end of study visit.

AEs will be assessed according to the CTCAE version 4. 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 following the initiation of study intervention (nivolumab and DC vaccine):

- 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 that are thought to be possibly, probably, or definitely related to study treatment (including leukapheresis) should be reported immediately to Dr. Katherine Peters (Pager: 919-970-7591) or her designee (919-684-8111) and to the FDA. Fatal or life-threatening, unexpected adverse events will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the 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 Serious Adverse Events must be reported to BMS Worldwide Safety. The sponsor/investigator will be required to reconcile SAEs reported in the clinical database with SAE cases transmitted to BMS Global Pharmacovigilance (GPV&E); worldwide.safety@bms. BMS requests this is initiated by the sponsor

investigator up to quarterly and prior to the database lock or final data summary. The process will be further defined. During reconciliation, any events found to not be reported previously to BMS must be sent to Worldwide.Safety@BMS.com.

Potential drug induced liver injury (DILI) is also considered an important medical event. Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs. Potential drug induced liver injury is defined as:

1. ALT or AST elevation > 3 times upper limit of normal (ULN)

AND

2. Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)

AND

No other immediately apparent possible causes of AST/ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

Any overdose or pregnancy is considered a SAE regardless of CTCAE grade level and will be documented and reported.

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 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, including SAEs that are felt to be unlikely or unrelated to study treatment.

13.3 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 include 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.

13.4 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 Case Report Forms (CRFs)

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

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

14.3.3 Data Management 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. An audit trail will be kept of all subsequent changes to the data.

14.3.4 Coding

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

14.3.5 Study Closure

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

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.

Patients will be randomized to one of two treatment arms – Group I and Group II. In Group I, patients will receive nivolumab every 2 weeks for approximately 8 weeks followed by surgery. Following resection, nivolumab and DC vaccine will be administered every 2 weeks followed by QOW treatment with nivolumab and monthly DC vaccinations. In Group II, patients will initially receive the 4th cycle of nivolumab followed by nivolumab and DC vaccine every 2 weeks for a total of 3 vaccines for approximately 6 weeks, and then resection. Subsequent to resection, the patient will resume QOW treatment with nivolumab and monthly DC vaccinations. In both arms, a total of 8 DC vaccinations will be administered after which the administration of nivolumab will be continued QOW.

15.1 Analysis Sets

The primary safety analyses will focus on all patients, regardless of treatment arm, who complete at least 3 vaccinations in combination with nivolumab treatment, or terminate combination protocol treatment earlier after having experienced an unacceptable toxicity. Additional secondary safety analyses will include any patient who receives any DC vaccination.

Efficacy analyses will be conducted among patients who receive any nivolumab treatment and are able to tolerate nivolumab.

Correlative analyses will primarily focus on patients who undergo resection and have DCs or PBLs that meet release criteria. A patient within Group I who withdraws from protocol treatment after surgery will be included in the correlative analyses if the DC vaccine met the release criteria whether or not it was administered.

15.2 Patient Demographics and Other Baseline Characteristics

The socio-demographic and clinical characteristics of subjects randomized to each of the two treatment arms will be summarized using descriptive statistics (e.g. means/standard deviations/percentiles or frequencies).

15.3 Treatments

For each treatment arm, the number of doses of nivolumab and the number of patient vaccinations administered will be summarized with a frequency distribution.

15.4 Primary Objective

The primary objective of this study is to assess the safety of administering DC vaccines with nivolumab for the treatment of bevacizumab-naïve subjects with first or second recurrent, resectable WHO Grade III and IV malignant gliomas.

15.4.1 Variable

To assess the safety of the combination of DC vaccination and nivolumab, the percentage of patients who experience unacceptable toxicity during combination treatment (i.e. DC vaccination + nivolumab) will be tabulated.

Unacceptable toxicity is defined in Section 9.1.1.

15.4.2 Statistical Hypothesis, Model, and Method of Analysis

15.4.2.1 Statistical Hypothesis, Model, and Method of Analysis

As described in section 15.8, the goal of the study is to determine whether the addition of the DC vaccine to nivolumab treatment increases the prevalence of unacceptable toxicity. Topalian et al.[112] reports that 14% (approximate 95% confidence interval: 10%, 18%) of patients treated with nivolumab alone experienced a grade 3 or 4 adverse event. If the true unacceptable toxicity rate with the combination treatment is approximately 10%, then the treatment is considered safe. However, if the true unacceptable toxicity rate is 35% or greater, the treatment combination as prescribed in this protocol is not safe. A one-sample binomial test will be used to differentiate between an unacceptable toxicity rate of 10% and 35%. Further details are provided in section 15.7. In addition, a 90% exact binomial confidence interval for the unacceptable toxicity rate will be generated.

15.4.2.2 Related Statistical Analyses

Adverse events experienced by protocol subjects during combination treatment (nivolumab + DC vaccine) will also be summarized in several other forms to satisfy scientific and monitoring needs, as well as various regulatory reporting needs (e.g. FDA, DCI SOC, and ClinicalTrials.gov):

- Among all patients who receive any combination protocol treatment, the frequency of adverse events that are possibly, probably, or definitely related to protocol treatment will be tabulated by the maximum grade for each type of adverse event.

- Among all patients who receive any combination protocol treatment, the frequency of adverse events regardless of attribution will be tabulated by the maximum grade for each type of adverse event.

- Among patients who terminate combination treatment due to an unacceptable toxicity or receive at least 3 vaccinations, the frequency of adverse events that are possibly, probably, or definitely related to protocol treatment during the 8 vaccinations will be tabulated by the maximum grade for each type of adverse event.

- Among patients who terminate combination treatment due to an unacceptable toxicity or receive at least 3 vaccinations, the frequency of adverse events regardless of attribution to protocol treatment during the 8 vaccinations will be tabulated by the maximum grade for each type of adverse event.

Given that nivolumab remains an investigational agent too, there will be a need to summarize adverse events experienced during the period of time when nivolumab is administered alone. Tabulations that summarize all adverse events as well as tabulations that summarize those that are possibly, probably, or definitely related to nivolumab treatment will be generated.

15.4.3 Handling of Missing Values, Censoring, and Discontinuations

If a patient terminates protocol treatment without experiencing an unacceptable toxicity and without receiving 3 vaccinations, the patient will be replaced for the primary analyses.

15.5 Secondary Objective

The secondary objective of this study is to describe the survival and PFS of subjects treated with any protocol treatment (i.e. nivolumab or DC vaccines). Survival is defined as the time between first initiation of nivolumab treatment and death, or last follow-up if the patient remains alive. PFS is defined as the time between treatment initiation and initial progression or death, or date of last follow-up if the patient remains alive without disease progression. The Kaplan-Meier estimator will be used to describe the OS and PFS experience of all patients. Median OS and PFS will also be calculated. Patients who are not able to tolerate nivolumab and are removed from the study will not be included in these analyses. Patients for whom DC vaccines cannot be manufactured will not be included in the survival analyses.

Though the primary analysis for this secondary objective will include all patients, additional exploratory analyses may be conducted. OS and PFS will be examined within each assigned treatment group using an intent-to-treat approach. Given the small sample size, any comparison of the treatment arms will have limited power to detect clinically meaningful differences. Additional descriptive analyses may also be conducted, including an examination of OS/PFS from resection.

15.6 Exploratory Objectives

Given the rapidly advancing field of check-point block inhibitors, additional exploratory statistical analyses related to immunologic function may be conducted in conjunction with the analyses described below.

Many of these analyses will primarily focus on patients who undergo resection and have DCs or PBLs that meet release criteria.

15.6.1 Exploratory Objective #1: Immunologic Response to pp65

Within each group, changes between baseline and a pre-resection assessment for various measures of immune response derived from PBMC plasma/serum will be described. When appropriate, these descriptive statistics may include mean and standard deviation, or medians and quantiles.

Within each group, a paired t-test will be conducted to assess the impact of nivolumab (Group I) or nivolomab with DC vaccination (Group II) on these changes in immune function. Assuming normality is not appropriate, a Wilcoxon signed-rank test will be conducted.

Analysis of covariance will be used to compare treatment groups with respect to change, with the baseline measure serving as a covariate. If normality is not an appropriate assumption, a Wilcoxon rank sum test will compare treatment groups with respect to the change in the various measures of immune response that are of interest.

15.6.2 Exploratory Objectives #2: T-cell Costimulatory Markers

Analyses similar to those described in section 15.6.1 will be conducted to assess the impact of nivolumab and DC vaccination on T-cell costimulatory markers.

15.6.3 Exploratory Objectives #3: Cytokines and Other Soluble Factors

Analyses similar to those described in section 15.6.1 will be conducted to assess the impact of nivolumab and DC vaccination on cytokines and other soluble factors.

15.6.4 Exploratory Objectives #4: Gene Expression

Gene expression will be measured in PBMC samples collected serially from patients, as well as from resected tumor. Analyses similar to those described in section 15.6.1 will be used to assess the impact of treatment on changes in gene expression as measured from PBMC samples. A two-sample t-test or Wilcoxon rank sum test will compare groups with respect to gene expression as measured within tumors.

15.6.5 Exploratory Objectives #5: Epitope Spreading

The goal of this objective is to assess whether evidence exists for epitope spreading intratumorally or systemically after vaccine and anti-PD-1 treatment. Among all patients with pre- and post-treatment collection of PBMCs, TCR repertoires of PBLs (pre- and post-treatment) will be assessed. The post-treatment collection occurs before resection. For each sample, we will compute the total number of unique clones. Within each group, a comparison of TCR repertoires pre- and post-treatment is of interest. To test whether the paired samples have different variances with respect to the number of unique TCR clones, the Fligner-Killeen median test for homogeneity of variance will be used. This test is robust against departures from normality. To assess the distribution of T cell clonal frequency, Shannon's entropy normalized by log2 (# of TCR unique clones) will be computed. Wilcoxon signed-rank test will compare the paired measures of entropy. The proportion of TCR overlap between matched specimens

will be calculated as the ratio of the number of TCR sequencing reads from clones found in both samples and the number of total TCR sequencing reads from both samples. Wilcoxon rank sum test will be used to compare groups with respect to post-treatment measures of entropy for PBLs.

The TCR repertoire of TILs obtained from the resection tissue will be assessed, and Shannon's entropy will be computed as described above. Wilcoxon rank sum test will be used to compare groups with respect to post-treatment measures of entropy for TILs.

15.7 Interim Safety Analysis

Rigorous monitoring of toxicities in a manner similar to that found in many Phase I dose-escalation studies would require accrual suspension between stages of patient accrual while relevant data matures. Accrual will not be suspended to formally assess the toxicity profile unless the following guidelines are satisfied. Rather, the study will be monitored continuously for the occurrence of unacceptable adverse events, with the primary focus being on adverse events occurring during combination treatment (i.e. DC vaccination + nivolumab). Included in these analyses are patients who have completed at least 3 vaccinations in combination with nivolumab or terminated combination treatment earlier after having experienced an unacceptable adverse event.

Aggregate summaries of adverse experiences will be generated and reviewed by the clinical team every 3-6 months for the first year, and then every 6-12 months thereafter.

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

Table 3. Conditions for Temporary Accrual Suspension

Number of patients who have received 3 DC vaccinations or experienced an unacceptable adverse event during combination treatment	Number of patients with unacceptable toxicity requiring accrual suspension
6-7	≥3
8-10	≥4
11-14	≥5
15-20	≥6
21-24	≥7
≥25	≥8

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

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

Underlying unacceptable toxicity rate	Probability of accrual suspension	Expected # of Patients
0.01	<0.0001	30

0.05	0.004	29.9
0.1	0.039	29.1
0.15	0.15	27.4
0.2	0.35	24.2
0.25	0.58	20.6
0.3	0.79	16.5
0.35	0.91	13.1
0.4	0.97	10.7
0.45	0.99	9.0
0.5	>0.99	7.9

Patients who terminate combination protocol treatment without experiencing an unacceptable toxicity during combination treatment and without receiving 3 vaccinations will be replaced for the evaluation of the primary endpoint.

15.8 Sample Size Calculation

Topalian, et al.,[112] reports that 14% (approximate 95% confidence interval: 10%, 18%) of patients treated with nivolumab alone experienced a grade 3 or 4 adverse event. If the true unacceptable toxicity rate with the combination treatment is approximately 10%, then the treatment is considered safe. However, if the true unacceptable toxicity rate is 35% or greater, the treatment combination is not safe as prescribed in this protocol. Based upon simulation studies with 30 subjects using the decision rules described in section 15.7, the probability of terminating accrual assuming a true unacceptable toxicity rate of 10% and 35% is 0.039 and 0.91, respectively.

With the 6-month progression-free survival rate of GBM patients standardly treated after one recurrence being 42.6% (Friedman, JCO, 2009), we anticipate that approximately 50% of patients will drop out between enrollment and the 3rd vaccination. In order to attain the goal of accruing 30 patients that will be included in the primary analyses, approximately 66 patients will be enrolled onto the study.

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 International Conference on Harmonization 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 or her designee must obtain written informed consent from the subject or a legally acceptable representative. 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 Study Documentation

Study documentation includes but is not limited to source documents, CRFs, 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.

A CRF will be the primary data collection document for the study. The CRFs will be updated within two weeks of acquisition of new source data. Only the Principal Investigator and study coordinator, research nurses, and investigators, are permitted to make entries, changes, or corrections in the CRF. For paper CRFs, errors will be crossed out with a single line, and this line will not obscure the original entry. Changes or corrections will be dated, initialed, and explained (if necessary). The Principal Investigator or authorized key personnel will maintain a record of the changes and corrections. For electronic CRFs, an audit trail will be maintained by the electronic CRF management system utilized at Duke.

16.5 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 a Title 21 CFR Part 11 Compliant 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.

16.6 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 Section 13.4 and Section 14.

16.7 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.

16.8 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 (if an IND is involved)
- at least two years after formal withdrawal of the IND associated with this protocol (if an IND is involved)
- at least six years after study completion (Duke policy).

16.9 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 Research Integrity Office (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.

16.10 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 Nivolumab Investigator Brochure(s)

Please see separate upload in eIRB.

18.2 I-O Safety Algorithms

Please see separate upload in eIRB.

18.3 PRTBTC Imaging SOP

Please see separate upload in eIRB.

18.4 CTRAF

Please see separate upload in eIRB.

18.5 MMSE

Please see separate upload in eIRB.

18.6 PRTBTC DSMBplus Charter

Please see separate upload in eIRB.