PERFORMANCE:
Peptide Targets for Glioblastoma Against Novel Cytomegalovirus Antigens

Investigational Review Board Number Pro00034208
Food and Drug Administration Investigational New Drug Permit 15,846

Duke University Medical Center
Gary E. Archer, Ph.D.
Annick Desjardins, M.D.
Henry S. Friedman, M.D.
James E. Herndon, II, Ph.D.
Denise Jaggers, RN, B.S.N.
Margaret Johnson, M.D.
Roger E. McLendon, M.D.
David M. Ashley, MBBS, FRACP, PhD (Principal Investigator)
Sarah Woodring
Elizabeth S. Miller
Katy Peters, M.D., Ph.D.
Kent Weinhold, Ph.D.
Weihua Xie, M.S.
John H. Sampson, M.D., Ph.D.
Dina Randazzo, D.O.

Version Date	Sections Revised	Purpose	Institutional Dates	Federal Dates (1571 #)
20111018		Initial Protocol		
20120221	Study Synopsis, Sections 10.1, 16.1	Initial Protocol IRB Modifications Updated pregnancy testing language	20120227	20131126 (0)
20130219	Cover Page Study Synopsis, Sections 11.1, 11.4.4 Study Synopsis, Sections 7.1, 11.1, 11.4.4 Study Synopsis, Sections 7.2, 8.1, 8.2, 11.1, 11.4.4	New IND Submission and Updated Cover Page Clarified PEP-CMV dose Clarified and Corrected GM-CSF dose Clarified day of vaccine administration in relation to temozolomide cycles		20131211 via email
20130307	Study Synopsis, Section 11.1 Study Synopsis, Section 11.1 Table 3 Section 19.1 Section 15.3	Revised DLT definition per FDA recommendation Primary Safety Assessment changed to occur on day 28 of the 1st adjuvant cycle of TMZ Table provided for Schedule of Events Inserted standard language for prophylaxis treatment for Pneumomcystis Carinii Clarified Primary Endpoint for Immune Monitoring and comparison between the 3 arms		20131211 via email
20131220	Section 11.5.1	Clarified vaccine treatment will end if DLT occurs	20140724 (AMD0003)	20131223 via email and via

	Sections 11.6, 11.7, 11.8, 11.9, 11.10 Section 16.1	Added these sections to cover treatment period, end of treatment, end of study, follow up and withdrawal of subjects Corrected name of contact to receive pathology slides		FED EX (1)
20140801	Cover Page and Section 11.5.4	Changed the PI	20140808 (AMD0005)	
20160714	Study Synopsis, Study Schema Section 7.2, Sections 8, 11, 12, 13, 15, 16 & 19	Updated and clarified the hypotheses and objectives; removed the reference to following RTOG 0525 for eligibility and updated the inclusion/exclusion criteria to match BTC studies, removing supratentorial component, clarifying complete or partial resection, & increasing KPS to 70% and adding Curran Group Status; removed one of the 3 randomization arms; added Td booster as a requirement & Td pre-conditioning before vaccine #1; changed enrollment numbers & clarified time of enrollment; clarified primary objective; changed first 3 vaccines to bi-weekly; clarified difference between 5-day & 21-day TMZ and delay between cycles 1 and 2; added definition and information on pseudoprogression; removed radiographic response section & referred to BTC SOPs; changed from term “DLT” to “unacceptable toxicities” and updated definition; updated/clarified immune monitoring time points; updated randomization model; updated/clarified definitions of “evaluable subject”, “on study”, “off study”, and “end of study”; removed XRT/TMZ time restraint and referred to SOC as a guideline thus removing those sections and referring reader to Appendices; updated follow up requirements; updated Schedule of Events; changed from paper documentation to electronic medical record;	20160714 (AMD0014)	20160714 (4)

	Cover Page	clarified progressive disease requires removal from study; deleted data and specimen collection section & outdated recruitment section & enrollment tables; included an accrual pause (rather than halt) for safety evaluation & updated the statistics, updated name of contact for pathology slides; added DSMB/COI language; updated references and bibliography throughout. Added Dr. Fecci to cover page.		
20160929	Cover Page Study Synopsis, Table of Contents, Sections 7, 8, 9, 10, 11, 12, 13,15, 18, and Appendices	Removed Sharon Norman and added Denise Jaggers Updated study drug, limited total vaccines to 20, changed timing of consent to after XRT/TMZ, updated hypotheses/objectives, updated CDX-11- drug information, included information on ELEVATE trial, added in missing exclusion criterion, removed all sections on XRT/TMZ, clarified TMZ therapy information, clarified timing of evaluable subject assessment, clarified CBC and CMP draws and window of vaccine administration, included the description of the Td pre-conditioning mixture, removed withdrawal information related XRT, updated follow-up requirements, updated immune monitoring and removed immunologic testing not being done due to change in study drug components, changed study “coordinator” to study “team”, updated schedule of events for these changes and included the study suspension after first 6 subjects, updated the statistical section for change in PEP-CMV components, and included DCI SOC description. Finally, removed the reference to radiation and TMZ therapy in protocol as this is done prior to enrollment, as well as referred the reader to the TMZ PI uploaded in eIRB rather than in the		20160929 (5)

		appendices, – and with these removals, updated the Table of Contents and References.		
20170106	Study Synopsis, Sections 11 and 12	Clarified that screening consent will only be done if CMV status is not already known.	20170106 (AMD0027)	
20170110	Study Synopsis and Section 10.1	Added to inclusion criteria that the tumor must have a supratentorial component.	20170110 (AMD0028)	
20170221	Sections 10, 11.1, 11.2.2, 12, 13, 14.2.2	Removed one immune monitoring time point and clarified the other immune monitoring time point (Sections 11.1, 12, 13). Modified TMZ completion requirement. Modified criteria for adjuvant TMZ initiation (Section 11.1.) Updated inclusion/exclusion criteria (Section 10). Added dose reduction option (Section 11.2.2). Section 14.2.2 added to allow for the use of EMLA cream at the injection site.		
20170726	Study Synopsis, Sections 10.1, 11.1, 11.4.2, 11.4.3, 11.5.1, 11.5.2, 12.5, 13.6, 15.3, and Table 3	Curran Group status removed and specifications to steroid use added to the inclusion criteria (Sections 10.1 and 12.5), required as baseline measurement. A Study Drug Safety Arm was added to Sections 11.1, 11.4.3, and 15.3. Pre-medication with Zofran and extended follow-up procedure was added (Section 11.4.2). A post-vaccine monitoring plan was added (Section 11.5.1). Added Allergy Testing (Section 11.5.2). Removed MMSE from Table 3 and Section 13.6. Removed appendix including the safety oversight committee. Changed PI to David Ashley. Removed protocol footer.		
20180124	Sections 11.4.3, 15.3, Table 3, and Figure 13	Added immune monitoring blood draw volumes and modified tube top color (Table 3 and Section 11.4.3). Clarified the immune monitoring schedule for draws after vaccine #2 (Section 11.4.3). Updated Figure 13. Modified Table 3 footnote to clarify that pre- and post-XRT screening activities do		

		not need to occur at the same visit. Updated Schedule B, Vaccine #2 corrective action (Section 15.3) to be consistent with wording in previous section, Section 11.4.3.		
20180622 (submitted to FDA only)	Sections 1, 9, 11.1, 11.4.2, 11.4.3, 11.5.1, 11.6, 12.1, 13, 15.1, and 15.3	With this amendment, we complete the enrollment to the study drug safety arm. This amendment is to change the vaccine administration procedure after completion of the Study Drug Safety Arm. In addition to an administrative review, the following changes have been made: Updated key personnel on cover page. Added pre-medication with Tylenol (Sections 11.1 and 11.4.2). Updated the number of enrolled patients (Sections 1, 11.1, 15.1). Updated vaccine administration (Sections 1 and 11.1). Updated immune monitoring blood draws (Sections 1, 11.1, 12.1, 13). Removed old TMZ requirements (Section 11.1). Clarified clinical endpoint evaluable patients (Section 11.1). Specified that the Study Drug Safety Arms procedures are “not applicable to patients enrolled on protocol version 6/12/18 or later” (Sections 11.1, 11.4.3, 15.3). Updated follow-up schedule (Section 11.5.1). Removed accrual suspension after 12 patients (Section 11.6). Toxicities will be formally monitored without accrual suspension according to Section 15.1. Additional administrative edits were made.		
20180719 (includes 20180622 mods + FDA mods)	Sections 1, 10.1, 11.1, 11.5.3, 14.2.4, and Table 3	This amendment is in response to FDA concerns from amendment protocol version 20180622. Specified physiologic doses of hydrocortisone therapy (Sections 1 and 10.1). Added potential risk of steroid-induced adrenal suppression, including justification for steroid supplementation with physiologic levels of hydrocortisone		

		(Section 14.2.4). Added cortisol levels to screening and prior to each vaccine (Table 3). Updated unacceptable toxicity language to include vaccine-related ≥ Grade 3 events, ≥ Grade 3 toxicity of any duration, and Grade 2 urticaria as a criteria for early withdrawal (Sections 1, 11.1, and 11.5.3). Minor administrative changes in the Appendix.		
20181218	Sections 1, 7.1, 8.1, 8.2, 11.1, 15.1, 11.4.2, 11.5.1, 11.5.4, 12.6, 13, 17.2, and Table 3	Updated the clinical research coordinator to Kristen Fisher. Removed antibody analysis, which is irrelevant without Component B (Sections 1, 7.1, 8.1, 8.2, removed previous Section 13.3). Created a Group 1 that follows the original treatment plan and then a Group 2 that does not have Component B and has modified TMZ schedules - removed all but the initial adjuvant TMZ cycles for unmethylated patients (Sections 11.1, 15.1, Table 3); removed Component B and changed Component A administration to be half in the right groin and half in the left groin for Group 2 (Sections 11.1 and 11.4.2). Made post-vaccine monitoring schedule consist with the rest of the protocol (Section 11.4.2). Removed FDA reporting of unrelated SAEs (Section 11.5.4). Due to the minimal toxicity seen in the 6 patient study drug safety cohort, the vital monitoring has been changed to every 30 (± 5) minutes (Sections 11.4.2, 11.5.1, 12.6, Table 3). Added MGMT methylation screening to Table 3. Added a 3-day interval to the 2nd and 3rd vaccine (Sections 1, 11.1, 11.4.2). Re-worded MRI schedule due to removal of TMZ cycles for unmethylated patients (Section 1). Updated immune monitoring schedule for Group 2 due to removal of Component B (Sections 1, 7.2, 13, Table 3). Clarified		

		Component A and Component B administration volumes (Sections 11.4.2 and 11.4.4).		
20190312	Sections 1, 7.1, 7.2, 10.2, 11.1, 11.4.1, 11.4.2, 13, 13.4, 15.1, 15.2, 15.3, 15.4.1, 15.4.2, 15.4.3, 15.4.4, and Table 3	Updated investigators. Removed Component B for all active and future patients (Sections 1, 7.1, 7.2, 11.1, 11.4.2, 15.1, 15.2, 15.3, 15.4.1, 15.4.2, 15.4.4). Delete exclusion criterion for known allergy to ingredients of Component B (Sections 1, 10.2). Change immune monitoring schedule due to removal of Component B (Sections 1, 13, 15.4.3). Change details regarding MGMT promoter methylation testing (Section 11.1, Table 3). Remove incorrect language related to randomization (Section 11.4.1). Fix and update footnote numbering in Table 3). Update Polyfunctional Analysis section for current practices (Section 13.4).		
20190730	Sections 11.1, 11.4.2, 11.5.1, 12.1, 12.6, and Table 2	Description of recent adverse events (Section 11.5.1). Revised/extended post-vaccine monitoring times after vaccines with associated updates to vital signs taken during the monitoring times (Sections 11.4.2, 12.1 [Table 2], and 12.6). Addition of optional oral prednisone for patients with post-vaccine reaction(s) (Sections 11.1 and 11.4.2).		
20200117	Sections 11.1, 11.4.2, 11.5.1, 11.5.3, 11.10.1, 12.6, 14.1, and 14.2.1	Add new supportive measures pre-vaccine and in the event of a post-vaccine reaction (Sections 11.1, 11.4.2, and 11.5.1). Qualify potential prednisone premedication as oral (Sections 11.5.1 and 12.6). Add history of vaccine reactions in study at interim analysis (Sections 11.5.1 and 14.2.1). Summarize results of recent immune analyses (Sections 11.5.1, 14.1, and 14.2.1). Add CRS Management Plan (Section 11.5.1). Revise unacceptable toxicity definition (Section 11.5.3). Reword criteria		

		for early withdrawal pertaining to corticosteroids (Sections 11.1 and 11.10.1). Clarify enrollment goals for consenting, randomizing, and evaluable (Sections 1, 11.1, 15.1, and 15.2).		
20200826	Sections 1, 5, 11.1, 11.4.2, 11.5.1, 11.5.2, 11.5.4, 11.5.5, and 16.4	Updated investigators. Definition of unacceptable toxicity revised per FDA request under IND (Sections 1, 11.5.4). New abbreviation added (Section 5). Language added regarding how indeterminate MGMT methylation will be handled (Section 11.1). Updates to safety precautions (Sections 11.1, 11.4.2). Revised CRS Management Plan per FDA request under IND (Section 11.5.1). New section added regarding use of reduced dose of bevacizumab for inflammatory response (Section 11.5.2). CTCAE version clarified as 4.03 (Sections 11.5.4, 11.5.5, 16.4)		

1 STUDY SYNOPSIS

Title	PERFORMANCE Trial
Study Drug:	PEP-CMV is a vaccine originally comprised of two components (referred to as Component A and Component B). Component A is a pp65 synthetic long peptide (SLP). Component B consists of a neutralizing antibody epitope from human CMV glycoprotein B (gB) conjugated to KLH. Component B was removed from the vaccine as of 20190130 (change reflected in 20181218 version of the protocol). Component A is administered as a stable water:oil emulsion in Montanide ISA 51 (Incomplete Freund's Adjuvant). The previously included Component B was administered in aqueous solution with 150 µg of GM-CSF.
Rationale:	Radiation (RT) and temozolomide (TMZ) have efficacy in GBM, but are not curative. Adjuvant therapies are desperately needed. CMV antigens have been identified in GBM and may make excellent anti-tumor immunotherapeutic targets. Vaccination and adoptive T-cell strategies targeting CMV in humans in other contexts have been safe and effective. Therapeutic TMZ induces a profound lymphopenia that may enhance anti-tumor vaccination responses when given during the homeostatic T-cell proliferation that occurs in response to lymphodepletion. Other peptide vaccines given to patients with GBM during recovery from TMZ-induced lymphopenia have produced potent tumor-specific immune responses. Our Td pre-conditioning platform in the context of pp65 RNA-pulsed autologous dendritic cell (DC) vaccination also elicited superior anti-tumor responses compared to controls receiving DC vaccines without Td pre-conditioning. In our clinical trial (Pro00003877 ATTAC), patients with newly-diagnosed GBM who were administered the Td skin pre-conditioning before DC vaccination revealed significantly longer PFS and OS compared to the control cohort receiving unpulsed DCs. In evaluating the relationship between DC migration and clinical responses, we observed a modest positive association between levels of DC migration and survival.
Primary Objectives:	1. To assess the safety of PEP-CMV vaccination in combination with adjuvant TMZ. 2. To determine the TMZ regimen that produces the highest number of T cells that specifically secrete IFNγ by ELISPOT in response to component A of PEP-CMV.
Secondary Objective:	1. To determine if tumors are CMV antigen negative by immunohistochemical analysis and microarray analysis at the time of disease progression/recurrence.
Exploratory Objectives:	1. To quantitate the immune response to PEP-CMV vaccine by pp65 ELISPOT. 2. To determine the quality of the CMV pp65 immune response by polyfunctional flow cytometry 3. To evaluate if Treg levels remain the same or decrease after vaccination. 4. Identify if any HLA haplotypes respond better to the vaccine. 5. To characterize immunologic cell infiltrate in tumors at the time of disease progression/recurrence. 6. To describe PFS and OS within the two treatment groups. 7. To estimate radiographic response rate to PEP-CMV in the subset of patients with residual disease.
Inclusion Criteria:	1. Age ≥ 18 years. 2. Histopathologically proven newly-diagnosed primary glioblastoma with complete or partial surgical resection. Biopsy not acceptable. 3. Patients must be CMV seropositive.

	4. The tumor must be supratentorial. 5. Karnofsky performance status of ≥ 70. 6. Stable or decreasing steroid dose (≤ 4 mg/day) at time of post-XRT adjuvant TMZ initiation. If patients are decreasing steroid use, once they are at 2 mg/day, they may be supplemented with physiologic replacement hydrocortisone therapy (20-30 mg/day in divided doses), at the discretion of the treating oncologist. 7. Hematology • ANC ≥ 1500 cells/μL • Platelet count ≥ 100,000 cells/μL • Hemoglobin ≥ 9.0 g/dl 8. Chemistry • ALT/AST ≤ 3.0 times the upper limit of normal • Total bilirubin ≤ 1.5 x the upper limit of normal *(Exception: Patient has known Gilbert's Syndrome or patient has suspected Gilbert's Syndrome, for which additional lab testing of direct and/or indirect bilirubin supports this diagnosis. In these instances, a total bilirubin of ≤ 3.0 x ULN is acceptable.)*
Exclusion Criteria:	1. Radiographic or cytologic evidence of leptomeningeal or multifocal disease at any time prior to randomization. 2. Prior conventional antitumor therapy, other than steroids, RT or TMZ therapy given for glioblastoma. 3. Pregnant or need to breast feed during the study period. 4. Not adhering to pregnancy prevention recommendations. 5. Active infection requiring intravenous antibiotics or an unexplained febrile (> 101.5^{o} F) illness. 6. Immunosuppressive disease or human immunodeficiency virus infection. 7. Patients with unstable or severe intercurrent medical conditions such as severe heart or lung disease. 8. Allergic or unable to tolerate TMZ for any reason. Any patient that successfully completed at least 5 weeks of Temodar during standard of care XRT/TMZ and whose blood counts meet the eligibility requirements (inclusion #7) within 5 weeks post XRT/TMZ is eligible. 9. Patients with previous inguinal lymph node dissection, radiosurgery, brachytherapy, or radiolabeled monoclonal antibodies. 10. Prior allogeneic solid organ transplant. 11. Currently receiving or ever received immunosuppressive therapy for an autoimmune disorder or an organ transplant.
Study Design:	If their CMV serostatus is unknown, patients will be asked to consent to CMV screening either prior to or following XRT/TMZ. Patients are enrolled to the study following XRT/TMZ and prior to initiation of post-XRT cycles of adjuvant TMZ, provided they meet all eligibility criteria. After signing main consent, patients will undergo intramuscular (I.M.) Tetanus-diphtheria booster vaccination with 0.5 mL of Td (tetanus, diphtheria toxoid, adsorbed). After meeting all eligibility criteria, patients will be randomized to one of two arms: • Arm 1 will receive standard TMZ (150-200 mg/m^2/day on days 1-5 of each 28-day cycle) with vaccination on Day 23 (-1 day, +2 days) of each TMZ cycle • Arm 2 will receive dose-intensified TMZ (75-100 mg/m^2/day on days 1-21 of each 28-day cycle) with vaccination on day 23 (±1 day) of each TMZ cycle.

	Beginning with protocol version 20181218, patients who are MGMT unmethylated will only receive one adjuvant cycle of the TMZ regimen according to their assigned randomized arm. Patients who are MGMT methylated or whose methylation status is inconclusive will continue with up to 12 cycles of TMZ. All patients enrolled prior to approval of protocol version 20181218 will receive 6-12 cycles of TMZ, independent of their MGMT methylation status. Patients who are dependent on steroid supplements above immune suppressive levels (4 mg) at time of vaccination or who are unable to tolerate TMZ will be withdrawn from the study and replaced before vaccination. Up to 70 patients may be consented on the main informed consent to meet the goal of 26 evaluable patients (13 per arm) for the primary outcomes. For both arms, the patients must be screened at Duke within 3 ± 1 weeks after the completion of standard of care radiation. For both arms, the initial cycle of adjuvant TMZ will begin as soon as possible following randomization. For Arm 1, the adjuvant TMZ cycle(s) will be given at a standard targeted dose of 150-200 mg/m^2/d for 5 days. For Arm 2, the adjuvant TMZ cycle(s) will be at the dose-intensified dose of 75-100 mg/m^2/d for 21 days. If a patient enrolls after approval of protocol version 20181218 and has an MGMT unmethylated tumor, they will discontinue TMZ after the 1st cycle. All patients will have blood drawn for immune monitoring and then receive a tetanus pre-conditioning injection intradermally (i.d.) in the RIGHT groin (as described below) on day 22 (+1 day) of cycle 1 of adjuvant TMZ. On the following day, the patients will receive study vaccine. The vaccine is administered as follows: 500 µg of PEP-CMV Component A mixed with Montanide ISA-51 administered i.d. with half on the right groin and half on the left groin. Vaccines #2 and #3 will be given at 2 week (+ 3 days) intervals; the MRI visit with immune monitoring will take place within 2 weeks (+3 days) of vaccine #3. Prior to the 20181218 amendment, vaccination consisted of two components: Component A and Component B. However, after that amendment, vaccination for all newly enrolled patients consisted of Component A only. For patients enrolled before approval of protocol version 20181218 and for patients enrolled after approval of protocol version 20181218 who have an MGMT methylated tumor or MGMT methylation status in inconclusive, TMZ cycle 2 will begin after that visit, but no sooner than 14 days post vaccine 3. This will result in at least ~35-day delay before starting TMZ cycle 2. MGMT unmethylated patients enrolled after approval of protocol version 20181218 will not receive subsequent cycles of TMZ, but will continue to receive vaccines approximately every 4 (+2) weeks. An unacceptable toxicity will be defined as any ≥ Grade 3 toxicity possibly, probably, or definitely related to the PEP-CMV vaccine with the following exceptions. A portion of the Grade 3 vaccine reactions noted in prior patient experiences with the PEP-CMV vaccine, described in Sections 11.5.1 and 14.2.1, will not be considered unacceptable if they are indicative of immune response. That is, toxicities such as Grade 3 flu-like symptoms, fever, and chills/rigors will not be considered unacceptable toxicities if the duration is less than 72 hours. Any ≥ Grade 3 organ toxicity (cardiac, renal, hepatic), including CRS-related toxicities such as hypotension and

	tachycardia, of any duration will be considered an unacceptable toxicity. Although not considered an unacceptable toxicity, any patient with ≥ Grade 2 urticaria will not receive further vaccines, will be withdrawn from the study, and will be replaced if less than 3 vaccines have been administered without unacceptable toxicity. The prevalence of unacceptable toxicities occurring during the initial 3 bi-weekly vaccinations or the vaccinations administered concurrently with temozolomide will be continuously monitored. If more than 25% of accrued patients experience unacceptable toxicities, then accrual will be suspended and reported toxicity will be carefully reviewed to determine if modifications to the protocol treatment should occur. Peptide vaccinations employing Montanide ISA-51 as adjuvants have generally been well-tolerated in human patients in numerous phase I-III trials. *Treatment Plan:* Patients will be vaccinated in conjunction with subsequent 28-day cycles for a total of 6 to 12 adjuvant TMZ cycles after RT if patient is continuing on TMZ (i.e., for patient's who's tumor is methylated or the status is inconclusive). Cycles are every 4 (+ 2) weeks, adjusting for slight delays on startup or scheduling of each 28-day cycle. For patients whose tumor is methylated or the status is inconclusive, the total number of TMZ cycles given will be at the discretion of the treating oncologist. During the 28-day cycles, vaccinations will occur on day 23 (-1 day, + 2 days) of each cycle for each arm: (Arm 1) standard TMZ (200 mg/m^2/day on days 1-5 of each 28-day cycle), or (Arm 2) dose-intensified TMZ (100 mg/m^2/day on days 1-21 of each 28-day cycle). All vaccines will be given i.d. approximately 10 cm below the inguinal ligament bilaterally. Component A will be administered half in the RIGHT groin and half in the LEFT groin at a maximum of 0.2 mL per intradermal injection. A target of six cycles with maximum of twelve cycles of TMZ may be given to patients enrolled before approval of protocol version 20181218 and to methylated patients enrolled after approval of protocol version 20181218, at the discretion of the treating neuro-oncologist. Unmethylated patients enrolled after approval of protocol version 20181218 will receive only one cycle of TMZ. After the completion of a patient's last TMZ cycle, vaccines will continue every 4-6 weeks for a maximum number of 20 vaccines (unless tumor progression occurs). Patients will be imaged with contrast-enhanced MRI within 2 weeks (+3 days) after vaccine 3 and then approximately every 8 weeks [every 2 cycles thereafter (i.e., end of cycles 3, 5, 7, 9, 11)]. RANO criteria will be used for assessment of pseudo-progression, and patients demonstrating definitive progression will be removed from study. Any patient removed prior to immune monitoring post vaccine 3 will be replaced for immunologic endpoints. Clinical endpoint comparisons will be made amongst patients successfully randomized to adjuvant TMZ treatment arms and receiving at least one vaccine. Blood for immune monitoring will be obtained: • Prior to Td pre-conditioning • During vaccines 1 and 2 (just prior to Component A, 1 and 2 hours after Component A • Prior to vaccine 3 • After vaccine 3 (within 2 weeks of vaccine 3 [+ 3 days]) • Prior to vaccines 4 and 6

	• At tumor progression (if feasible) As part of standard care for these patients, upon tumor progression, participants may undergo stereotactic biopsy or resection. As this is not a research procedure, consent will be obtained separately. Patients that have this procedure done here at Duke University Health System may be approached to participate in the Duke Brain Tumor Center Biorepository study (Pro00007434). Tissue obtained from patients who consented to the Duke Brain Tumor Center Biorepository will be used to assess immunologic cell infiltration, antigen expression, and biomarkers for immunologic response. *Study Drug Safety Arm:* Following the severe adverse event that occurred on 4/5/17, a small study drug safety arm of 6 patients was included in this study. These 6 patients will not contribute to the overall statistical evaluation of the study, but instead, will be used to determine how the study drug administration or the study drug itself should be modified to reduce the hypersensitivity reactions in patients. Protocol version 6/12/18 updates the protocol based on the information gathered from the completed Study Drug Safety Arm. All patients enrolled on the study after completion of the Study Drug Safety Arm toxicity follow-up (~6/6/18) will NOT follow the Study Drug Safety Arm modifications.
Statistical Analysis	Of primary interest in this study is a comparison of the 2 arms with respect to peak immune response (ELISPOT) in the immune monitoring blood that will be collected as described in the treatment plan. The ACT II study conducted at Duke provides antibody titer data that can be used to estimate an appropriate sample size for comparison of antibody response. ELISPOT data is not available. The ACT II dataset provides peak antibody titer to EGFRvIII data for 5 patients in Arm A (treated with 5 day TMZ) and 10 patients in Arm B (treated with 21 day TMZ). Given that the distribution of peak titers in the two arms is not normally distributed, resampling methods were used to estimate the power of a comparison using a one-tailed Wilcoxon rank sum test. Each of 10000 simulations for a fixed sample size per arm involved random sampling with replacement the peak antibody data separately within arm A and B, and then computing the Wilcoxon rank sum test. The percentage of tests that was statistically significant estimated the power of the statistical test. With 2 primary endpoints, power calculations assumed a type I error rate of 0.025. With 13 evaluable patients per arm having immune monitoring done prior to vaccine 4, there is 81% power to detect a difference similar to that observed in ACT II. With the goal of 26 evaluable patients (13 per arm) for the primary outcomes, a maximum of 70 patients may be consented on the main informed consent to meet this goal. With the 20190312 protocol amendment, the primary analysis dataset includes only patients who receive Component A alone (See Section 15.2). Hence, after this amendment, the study targets the accrual of 26 patients (13 per arm) who receive only Component A and are evaluable for the assessment of immunologic response. Following the safety events of vaccine reactions that were initially reported on 20170407, the study was amended to investigate, in a separate

	unrandomized drug safety cohort of 6 patients, the etiology of these events. Within this cohort, patients were consented to receive study vaccine with standard of care (SOC) 5-day TMZ at 150-200 mg/m^2/d for 28-day cycles with various sequences of vaccine components A and B. Details of this safety assessment plan are provided in Section 11.4.3. Upon completion of this unrandomized portion of the study, the randomized study was allowed to reinitiate with changes to vaccine administration procedures based on observations made in the safety cohort (protocol v. 20180719). Subsequently, Component B was removed from the vaccine for future patients due to problems with availability and supply (protocol v.20181218). On 20190130, Component B was removed from the vaccine for all patients (current and future) due to two new safety events observed and reported in January 2019.

2 TABLE OF CONTENTS

3 LIST OF FIGURES

4 LIST OF TABLES

5 LIST OF POSSIBLE ABBREVIATIONS

Ab	Antibody
ABC	Automated Blood Count
ACD	Acid Citrate Dextrose
ACLS	Advanced Cardiac Life Support
ACTH	Adrenocorticotropic Hormone
APAAP	Alkaline Phosphatase Antialkaline Phosphatase Complex
ALT	Autologous Lymphocyte Transfer
AST	Aspartate Aminotransferase
ASTCT	American Society for Transplantation and Cellular Therapy
AT	Ambient Temperature
β-HCG	Beta-Human Chorionic Gonadotropin
BTSC	Brain Tumor Stem Cells
Ca^{++}	Calcium
cDNA	Complimentary Deoxyribonucleic Acid
CFA	Complete Freund's Adjuvant
CFC	Cytokine Flow Cytometry
CLIA	Clinical Laboratory Improvement Act
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CNC	Clinical Neurologic Change
CNS	Central Nervous System
Con-A	Concanavalin A
CPC	Cancer Protocol Committee
CRS	Cytokine Release Syndrome
CT	Computed Tomography
CTL	Cytotoxic T-Lymphocyte
DC	Dendritic Cell
DI	Dose Intensified
DLT	Dose Limiting Toxicity
DTH	Delayed-type Hypersensitivity
EAE	Experimental Autoimmune Encephalomyelitis
EBRT	External Beam Radiation Therapy
ELISA	Enzyme-Linked ImmunoSorbent Assay
ELISPOT	Enzyme-linked Immunospot
EGFR	Epidermal Growth Factor Receptor
EGFRvIII-KLH	EGFRvIII conjugated to Keyhole Limpet Hemocyanin
FACS	Fluorescence Activated Cell Sorting
FDA	Federal Drug Administration
FEV	Forced Expiratory Volume
gB	Glycoprotein B
GBM	Glioblastoma
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
I.D.	Intradermal
IFN-γ	Interferon-gamma

IHC	Immunohistochemistry
IL-4	Interleukin-4
IL-12	Interleukin-12
IL-13	Interleukin-13
IRB	Institutional Review Board
KLH	Keyhole Limpet Hemocyanin
KPS	Karnofsky Performance Status
MAb	Monoclonal Antibody
MG	Malignant Glioma
MHC	Major Histocompatibility Complex
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MTD	Maximally Tolerated Dose
NA	Non-adherent
NCI CTC	National Cancer Institute Common Toxicity Criteria
NIH	National Institutes of Health
NK	Natural Killer
OS	Overall Survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PHA	Phytohemagglutinin
PFS	Progression Free Survival
PI	Principle Investigator
PT	Prothrombin Time
PTT	Partial Thromboblastin Time
RANO	Revised Assessment in Neuro-Oncology
RNA	Ribonucleic Acid
RT	Radiation Therapy
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Severe Adverse Event
SLPs	Synthetic Long Peptides
SOC	Standard of Care or Safety Oversight Committee
SPORE	Specialized Program of Research Excellence
TCR	T-cell Receptor
TGF-β	Transforming Growth Factor-β
TMZ	Temozolomide
TNF-α	Tumor Necrosis Factor-α
T_{Regs}	Regulatory T-cells
TTP	Time to Progression
TTRNA	Total Tumor mRNA
WHO	World Health Organization
XRT	External Beam Radiation Therapy
XRT/TMZ	Temozolomide During Concomitant Radiation Therapy

6 DEFINITION OF TERMS

Term	Definition
Date of diagnosis	Earliest confirmation by radiographic or histologic examination
Date of randomization	Date TMZ regimen is assigned.
Date of progression	Earliest date of surgical confirmation of disease progression. Where surgery is not feasible, earliest date of radiographic confirmation of progression.
Enrolled patient	Patient who signs the informed consent form and meets all eligibility criteria.

7 ABSTRACT AND STUDY SCHEMA

7.1 *Abstract*

Despite aggressive, computer-guided surgery, high-dose focused radiation, and toxic, multimechanistic chemotherapy, MGs remain almost universally fatal. Moreover, these inherently non-specific conventional treatments incapacitate patients as a result of damage to surrounding normal brain and systemic tissues[1]. The inherent biologic specificity of immunotherapy, however, offers the prospect of targeting neoplastic cells more precisely.

The recent discovery that GBMs, but not surrounding normal brain tissue, serve as a refuge for CMV reactivation provide an unparalleled opportunity to subvert, as tumor-specific antigens, the highly immunogenic viral proteins expressed by human CMV. The immunologic responses to CMV have been well-characterized and the immunodominant viral protein pp65 is highly-conserved. Finally, CMV-specific immunotherapy has been previously shown in humans to be safe and efficacious in combating CMV related disease within the CNS, and antitumor immunotherapy targeting viral proteins in human CNS tumors associated with Epstein-Barr virus (EBV), another *Herpesvirus*, have been curative[2-7]. Similarly, vaccinations directed against the highly immunogenic antigens of human papilloma virus have been shown to reduce the incidence of cervical intraepithelial neoplasia in a prospective, randomized, double-blind trial[8].

Chemotherapeutic agents frequently used in cancer therapy, however, often induce a profound lymphopenia that may inhibit even the most potent anti-tumor immune responses. TMZ, a methylating chemotherapeutic agent, has recently shown efficacy in some patients with newly diagnosed GBM who are MGMT methylated, and TMZ is now frequently given to these patients during and after RT. Therapeutic TMZ, however, also induces a profound lymphopenia. ALT after TMZ therapy may provide a source of T-cells that remain receptive to vaccination in this context while potentially protecting the patient from opportunistic infections as well. In addition, the homeostatic proliferation of T-cells that is triggered in response to lymphopenia may even potentiate anti-tumor vaccine or adoptive T-cell responses [9].

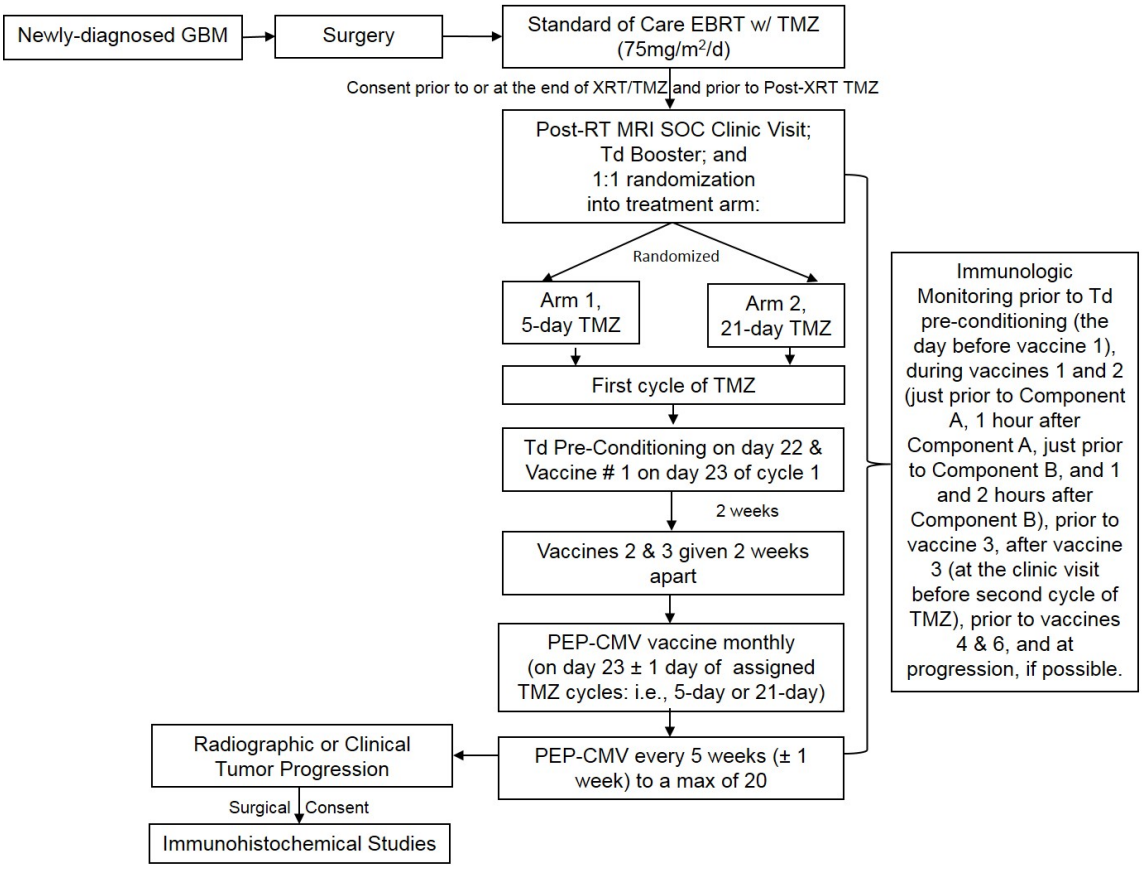
Our recent clinical trial with DC-based vaccination targeting the immunodominant CMV antigen pp65 has produced immunologic responses in patients with GBM undergoing standard of care TMZ along with encouraging PFS (15.4 months, CI_{95}: 10.0, 27.8). In order to advance vaccines targeting CMV in GBM, we have developed a CMV-specific peptide (PEP-CMV). PEP-CMV is a vaccine comprised of two components (referred to as Component A and Component B). Component A, pp65 synthetic long peptide (SLP) is a single peptide from human CMV matrix protein pp65SLP. Component B consists of a neutralizing antibody epitope from human CMV glycoprotein B conjugated to KLH (gB-KLH). Component A will be administered as a stable water:oil emulsion in Montanide ISA 51 (Incomplete Freund's Adjuvant). The gB-KLH conjugate (referred to as Component B) will be administered in aqueous solution with 150 μg of GM-CSF. Due to insufficient manufacturing of Component B followed by safety events in a subset of patients who received Component B, no patients will receive Component B as of 20190130.

Our preliminary data demonstrating the capacity to increase 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. 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 (Pro00003877 ATTAC), patients with newly-diagnosed GBM who were administered the

Td skin pre-conditioning before DC vaccination revealed significantly longer PFS and OS compared to the control cohort receiving unpulsed DCs. In evaluating the relationship between DC migration and clinical responses, we observed a positive association between levels of DC migration and survival. In addition, based on our preliminary data that Td vaccine site pre-conditioning enhances the immunogenicity of PEP-CMV in HLA-A2 transgenic mice, all patients will also receive a Td vaccine site pre-conditioning in the RIGHT groin and the following day receive the PEP-CMV vaccine.

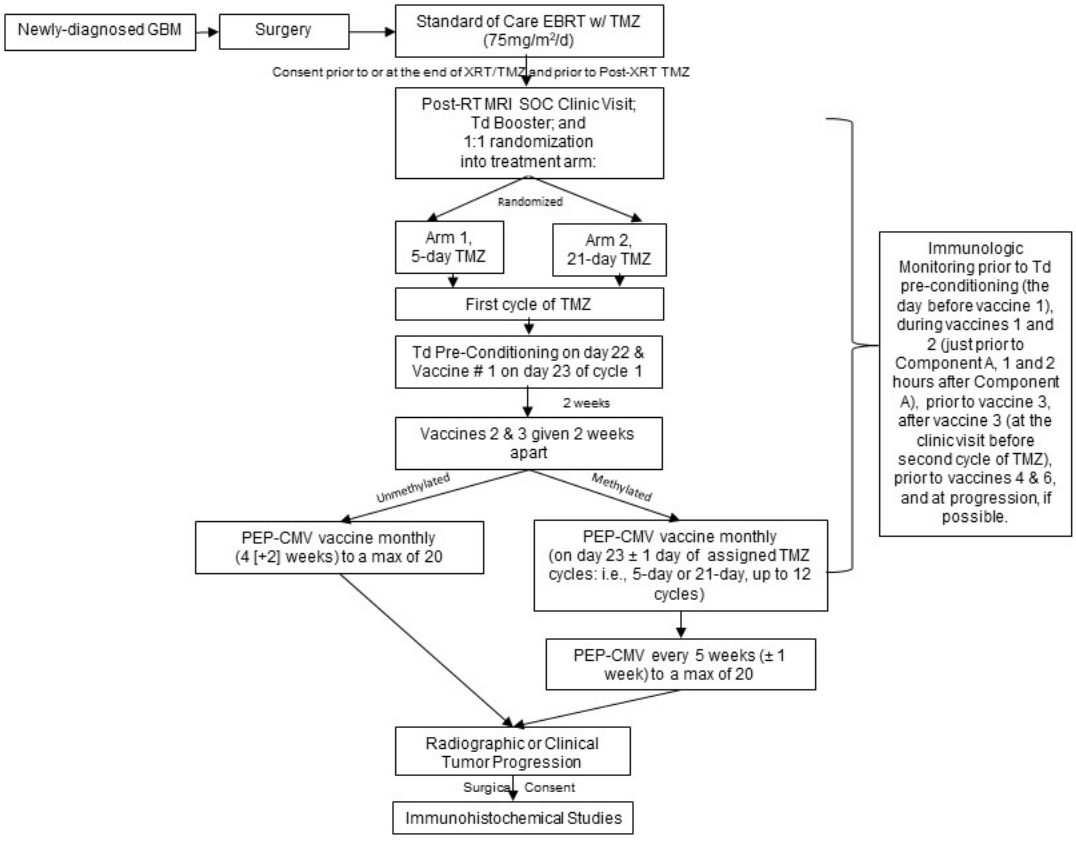
In this protocol, the goal is to determine the safety of PEP-CMV vaccine with adjuvant TMZ, and what TMZ regimen produces the highest number of T cells that specifically secrete IFNγ by ELISPOT in response to PEP-CMV component A.

7.2 *Study Schema*



[1]Figure 1. Study Schema for Patients Enrolled Prior to Approval of Protocol v. 20181217

[1] Originally, patients received both Component A and Component B and methylation status did not inform TMZ schedule. *Please note that a decision was made to eliminate Component B for all patients on study for safety reasons (January 2019) and this immediate change was approved via a protocol deviation.*



[2]Figure 2. Study Schema for Patients Enrolled After Approval of Protocol v. 20181217

[2] With approval of Protocol v.20181217, newly enrolled patients receive Component A alone and methylation status informs subsequent TMZ schedule. *Please note that Component B was initially eliminated in protocol v.20181217 for future patients due to issues with supply. The decision mentioned above in Footnote 1 and the aforementioned protocol deviation eliminated Component B for all current patients, in addition to future patients.*

8 HYPOTHESES AND OBJECTIVES

8.1 *Hypotheses*

- Our primary hypothesis is that:
    - Treatment of adult patients with newly diagnosed GBM using PEP-CMV during recovery from TMZ-induced lymphopenia in patients that are seropositive for CMV will be safe.
    - Treatment of adult patients with newly diagnosed GBM using PEP-CMV during recovery from TMZ-induced lymphopenia in patients that are seropositive for CMV will induce cellular immune responses through IFNγ secretion of total Component A specific T cells using ELISPOT.
- Secondary hypotheses include:
    - Tumors are CMV antigen negative by immunohistochemical analysis and microarray analysis at the time of disease progression/recurrence.
- Exploratory hypotheses include:
    - The immune response to the PEP-CMV vaccine is detectable by pp65 ELISPOT.
    - The quality of immune responses determined by polyfunctional flow cytometry is multifunctional or monofunctional using the pp65 peptide in PEP-CMV.
    - Tregs will decrease after vaccination with PEP-CMV in combination with TMZ.
    - Different HLA haplotypes respond better to PEP-CMV vaccine in combination with TMZ.
    - At the time of disease recurrence, immunologic tumor cell infiltrate will be evident.
    - That PFS and OS within the 2 arms will provide information on the effects between the 2 TMZ regimens.

Treatment of adult patients with newly diagnosed GBM using PEP-CMV during recovery from TMZ-induced lymphopenia in patients that are seropositive for CMV will induce objective radiographic responses in this patient population in the subset of patients with residual disease.

8.2 *Objectives*

8.2.1 Primary Objective

- To assess the safety of PEP-CMV vaccination in combination with adjuvant TMZ.
- To determine the TMZ regimen that produces the highest number of T cells that specifically secrete IFNγ by ELISPOT in response to PEP-CMV component A.

8.2.2 Secondary Objectives

- To determine if tumors are CMV antigen negative by immunohistochemical analysis and microarray analysis at the time of disease progression/recurrence.

8.2.3 Exploratory Objectives

- To quantitate the immune response to the PEP-CMV vaccine by pp65 ELISPOT.
- To determine the quality of the immune response by polyfunctional flow cytometry using the pp65 peptide in PEP-CMV.
- To evaluate if Treg levels remain the same or decrease after vaccination.
- Identify if any HLA haplotypes respond better to the vaccine.
- To characterize immunologic cell infiltrate in tumors at the time of disease progression/recurrence.
- To describe PFS and OS within the 2 arms.
- To estimate radiographic response rate to PEP-CMV in the subset of patients with residual disease.

9 BACKGROUND AND RATIONALE

9.1 *Disease and Current Therapy*

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 [10], 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[11-16]. 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. Moreover, the non-specific nature of conventional therapy for brain tumors often results in incapacitating damage to surrounding normal brain and systemic tissues[1, 17]. 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.

9.2 *Rationale for Immunotherapy*

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 precise antitumor therapy. Recently, TMZ, a myelosuppressive alkylating chemotherapy, has shown a benefit in patients with GBM who are MGMT methylated, but median survival for GBM is still less than 15 months[14]. Moreover, these conventional therapies lack specificity and result in incapacitating damage to surrounding normal brain and systemic tissues[1]. Immunotherapy may provide an opportunity to eliminate altered neoplastic cells without adding additional toxicity to multi-modality therapy, but the lymphopenia induced by cycle(s) of adjuvant TMZ, now the standard-of-care in patients with GBM, would be predicted to curtail the induction of productive antitumor immune responses. However, our vaccines targeting EGFRvIII, when given to patients with GBM during recovery from TMZ-induced lymphopenia, have produced strong immunologic responses that eliminate tumor cells expressing the targeted antigen and are accompanied by complete radiographic responses in all patients with residual disease and patient survival that significantly exceeds that of historical controls[18-21]. We believe, and our preliminary data in mice and humans strongly support, that an environment uniquely susceptible to the induction of potent immune responses is created within the host during recovery from TMZ-induced lymphopenia. This hypothesis is supported by basic investigations into lymphocyte homeostasis, which have shown that, during periods of lymphopenia, recovering lymphocytes have a significantly lowered threshold for activation, but they remain dependent on limiting amounts of homeostatic cytokines and antigen-specific stimulation for survival and proliferation. As a result, lymphocytes that encounter their cognate antigen during homeostatic proliferation, perhaps in the form of a vaccine, are given a competitive advantage. Although we have been able to successfully target

EGFRvIII in this context, only 25-30% of patients with GBM express this mutation and the outgrowth of recurrent tumors that no longer express EGFRvIII highlights the need to effectively target more ubiquitously expressed antigens in GBMs.

9.3 *CMV in GBM*

The recent discovery and confirmation by five independent laboratories[20, 22-26], including our own, that cytomegalovirus (CMV) propagates within a high proportion of GBMs (>90% of examined GBMs) without infecting surrounding normal brain provides an unparalleled opportunity to subvert the highly immunogenic antigens from CMV as tumor-specific targets. Cobbs *et al.,* first reported the associated of CMV with malignant gliomas[22]. We recently reported the first confirmatory report of the expression of CMV antigens in greater than 90% of GBM specimens without expression in normal brain[23]. To date, five independent laboratories from major academic medical centers (University of Alabama, Duke University, UCLA, MD Anderson Cancer Center, and the Karolinska Institute in Stockholm, Sweden) have confirmed this association. We and others have demonstrated the presence of CMV early and late antigens in GBM tumors by immunohistochemisty, *in situ* hybridization, real-time PCR, viral DNA sequencing, and electron microscopy in published materials[22, 23, 25, 26]. In additional data, we have confirmed that the detection of CMV proteins by monoclonal antibodies in IHC is specific for CMV antigens using Western blot analysis of freshly resected GBM specimens (Figure 3).

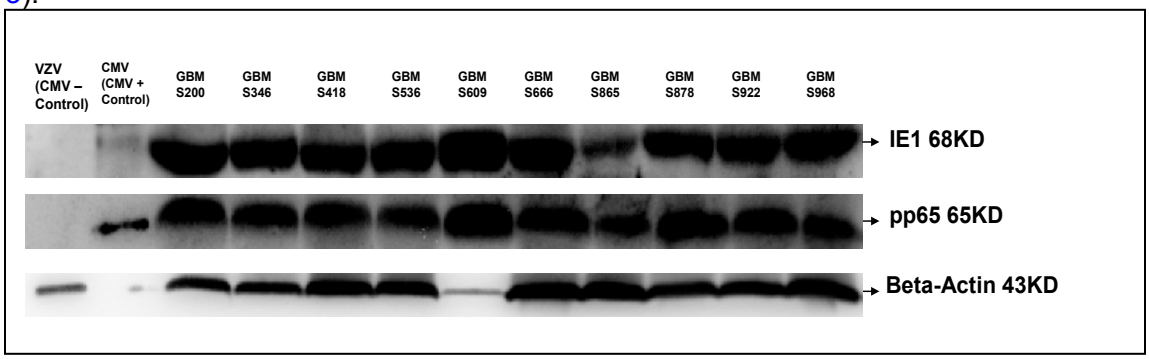


Figure 3: Western Blot Detection of CMV IE1, pp65, and gB Proteins in Freshly Resected GBM Samples. Single cell digest were prepared from freshly resected GBM specimens and washed with 2X in PBS to remove red cells and debri. Tumor cells were harvested and lysates prepared in the presence of proteinase inhibitors and Western blot analysis performed using IE1-specific MAb (810, Chemicon) and pp65 MAb (sc 71229, Santa Cruz). CMV- and VSV-infected fibroblast lysates were used as positive and negative control samples respectively. Due to the abundance of CMV proteins in laboratory strain infected lysates 1/40th the amount of lysate relative to GBM sample was loaded onto gels for Western blot detection. Detection of IE1 has been observed in 14/22 (63.6%) analyzed GBM samples and pp65 detection was positive in 19/22 (86.3%) analyzed tumors. gB envelope protein was detected in 16/16 (100%) tumors chosen based on positivity for pp65. Normal brain lysates derived from autopsy specimens were negative in 5/5 cases (median age 64) not shown (0%), $p<0.0001$.

CMV is an endemic β-*Herpesvirus* that does not usually cause significant clinical disease in adults. *Herpesviruses* have previously been implicated in a number of human malignancies including lymphoma, nasopharyngeal cancer, cervical cancer, and Kaposi's sarcoma[20, 27, 28]. Expression of proteins unique to CMV has been reported in a large proportion of GBMs[22]. Near universal detection of the CMV immunodominant proteins pp65, glycoprotein B (*UL55*), and immediate early gene 1 protein (IE1) in GBM has been

confirmed by us and others by immunohistochemistry (IHC), *in situ* hybridization (ISH), and polymerase chain reactions (PCR)[22-25] (Figure 4).

Presence of the virus in these samples was confirmed by electron microscopic detection of intact virions[22]. CMV antigens were not detected in surrounding normal

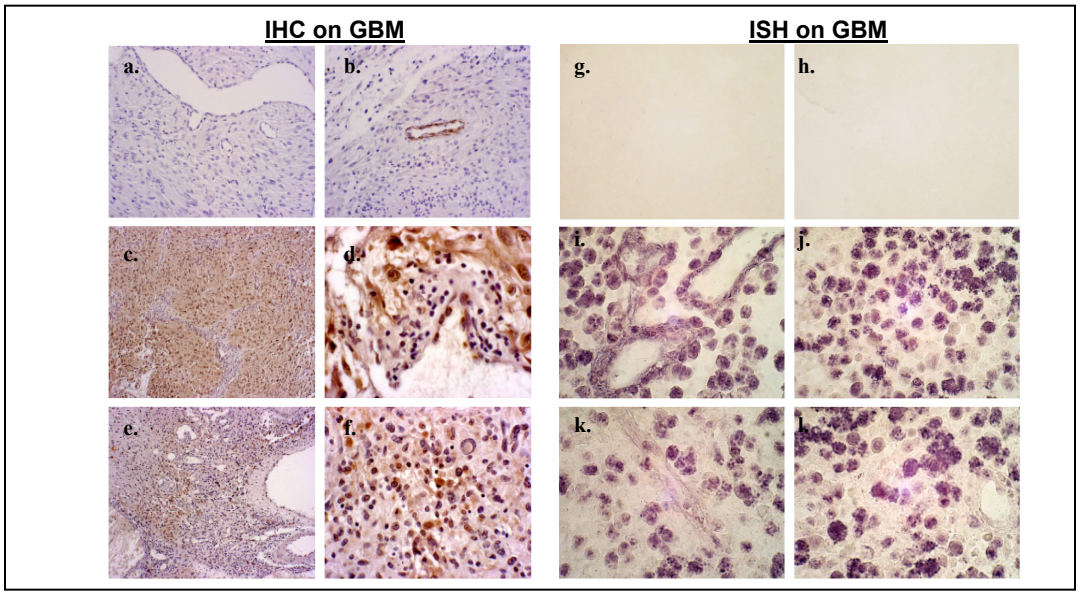


Figure 4: IHC and ISH on GBM Specimen IHC on GBM specimen: a. Isotype control antibody; b. anti-smooth muscle actin c. anti-*CMV* IE1 antibody (40x); d. anti-*CMV* IE1 antibody (200x); e. anti-*CMV* pp65 antibody (40x); f. anti-*CMV* pp65 antibody (100x). ISH on GBM specimens: Two GBM specimens were examined GBM1: g. negative control DNA probe; i. positive control DNA probe (Alu sequence); k. *CMV* genomic DNA probe; GBM2: h. negative DNA probe; j. positive control DNA probe (Alu sequence); l. *CMV* genomic DNA probe. (Figure from Mitchell et al., Neuro-Oncology, 2008, 10(1):10-18).

brain samples, meningiomas, or brains affected by ischemia, Alzheimer's disease, paraneoplastic encephalitis, or *Cryptococcal* cerebritis. Our analysis of viral strains by PCR amplification and DNA sequencing revealed that several clinical isolates of CMV were associated with these tumors, consistent with a low level systemic viral reactivation in patients with GBM[23]. While the biologic and clinical significance of the association of CMV with malignant gliomas is still under investigation, a preponderance of published data and preliminary data presented in this application verifies the expression of CMV intracellular and cell surface antigens in primary gliomas. One of the advantages of immunotherapeutic targeting of viral antigens in cancer, is that the immune system does not distinguish whether the target plays a critical role in maintaining the malignant phenotype of the cell, but rather triggers a host of mechanisms to kill the cell expressing the antigen, regardless of whether the antigen has oncogenic relevance. 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 viral-antigen targeted immunotherapy[2-7, 20, 29, 30]. Recent publications in the New England Journal of Medicine highlighted the effectiveness of synthetic peptide vaccines targeting HPV antigens in the eradication of vulvar intrapethial neoplasia in vaccinated women[20, 31]. These examples, and others, demonstrate the perhaps unique capacity of the immune system to eradicate tumors associated with strong viral antigens.

CMV has also been reported to be associated with a number of other malignancies including colorectal cancer and prostate cancer[32-39], and while this data awaits further confirmation, it suggests that an effective therapeutic CMV vaccine may have utility in disease settings outside of treatment of malignant brain tumors.

The immunodominant CMV proteins are well-conserved and induce a robust immune response that naturally eliminates cells expressing CMV proteins and confines the virus to a latent state. As a result, the immunologic responses to CMV have been well-characterized. In addition to our own work, DCs pulsed with CMV antigens have been shown to be potent inducers of CMV-specific immune responses in several studies[40-44]. There has also been a vast amount of experience with both the safety and efficacy of immunotherapy targeting CMV[45]. Vaccination against CMV has safely reduced the risk of viral infection and transmission to fetuses in animal models[46-48] and in clinical trials[49-54]. The induction of potent CMV-specific T-cell immunity has been used to safely and successfully protect against CMV reactivation and to treat acute CMV infections[55, 56] in myelodepleted bone marrow transplant patients. In addition, T-cell mediated immunotherapy has proven highly effective in the treatment of CMV-associated disease within the CNS[57] and in the treatment of acute CMV infections[55, 56].

9.4 *Polyfunctional T-cell Responses and Anti-Viral Immunity*

Protective immunity against acute and chronic viral infections requires effective T cell responses. While CD4 and CD8 T cell responses against viral infections that are rapidly cleared are characterized by polyfunctional effector functions including cytotoxicity (expression of CD107a), and the secretion of IFNγ, IL-2, and TNFα chronic infections often are typified by reactive T cells that are mono- or dual-functional, largely restricted to the secretion of IFNγ and TNFα, and unable to express and secrete IL-2[58-61]. Recent studies in HIV+ patients have demonstrated that the salient feature of T cell responses in patients who are long term non-progressors (LTNPRs) versus those who progress to AIDS, is the maintenance of polyfunctional HIV-specific T cell responses in LTNPRs[62-67]. Recent studies have demonstrated that polyfunctional T cell responses correlate with protection against CMV infection as well. Our preliminary data demonstrate that patients with GBM exhibit deficits in baseline CMV-specific polyfunctional immune responses *in vivo* but these deficits can be reversed *in vitro* by immunogenic stimulation with CMV antigens presented by autologous DCs. These findings may explain why CMV-infected tumor cells may persist in hosts with GBM and also suggests that proper vaccination *in vivo* could restore polyfunctional immune responses and mediate clearance of CMV-associated malignant gliomas (Figure 5).

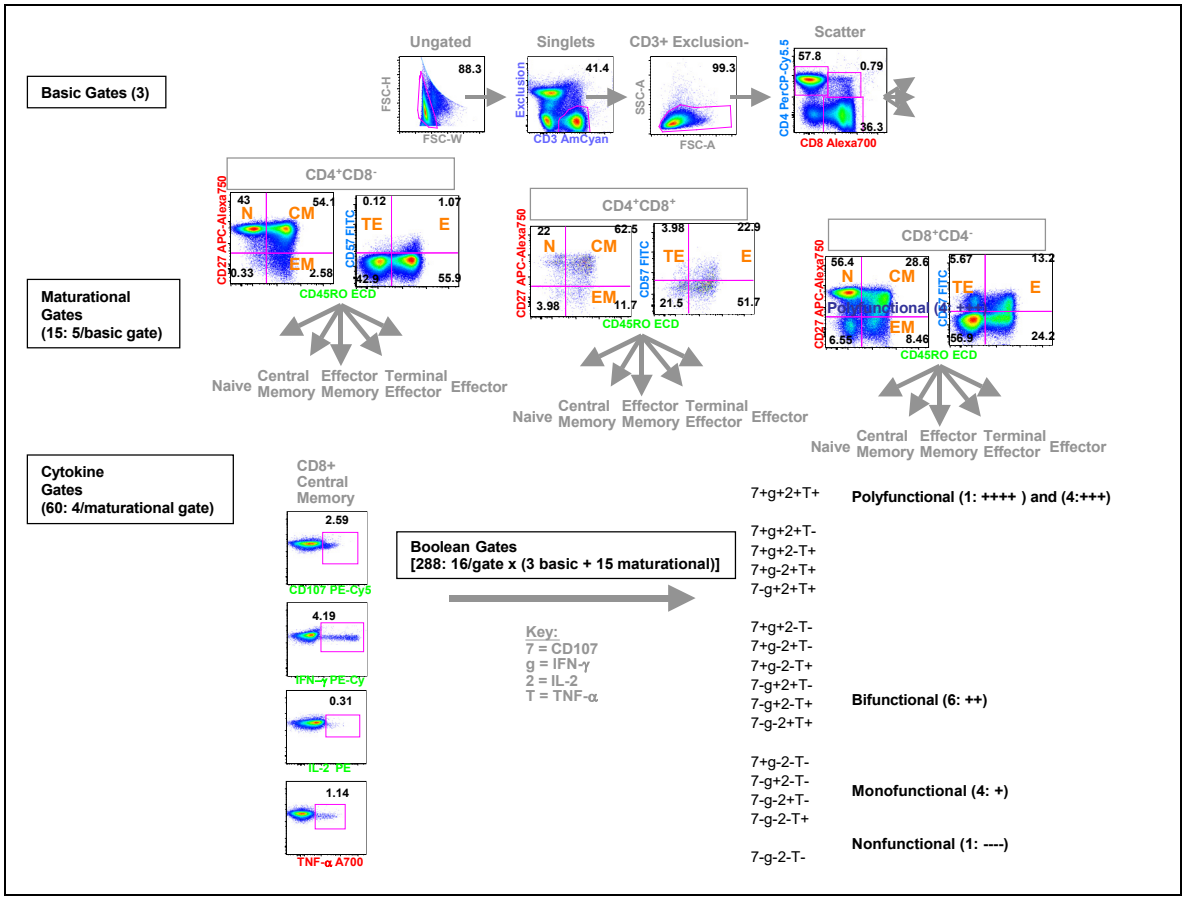


Figure 5: Polychromatic Flow Cytometry to Analyze Polyfunctional CD4+ and CD8+ T cells: T cells isolated from a normal donor were stimulated for 6h *in vitro* with CMV pp65 peptide mix. T cells were analyzed using 11-color polychromatic flow cytometry. CD4 and CD8 T cells were characterized as 1] Naive (N), 2] Central Memory (CM) and 3] Effector Memory (EM) based on the expression of CD45RO and CD27. Terminal Effector (TE) cells and Effector (E) cells were identified based on CD45RO and CD57 expression. The activation state of each T cell sub-population was analyzed by measuring 1] CD107, 2] IFN-γ, 3] IL-2 and 4] TNF-α production. Polyfunctional responses were defined as indicated in the figure.

9.5 *Temozolomide, Lymphopenia, and Homeostatic Proliferation*

TMZ is an alkylating chemotherapeutic that has recently been shown to prolong survival in patients with GBM[14]. As a result, it has become part of the standard regimen used to treat these patients. **TMZ induces a transient lymphopenia**, and although counterintuitive, we and others have shown that following periods of lymphopenia, immune responses can be markedly enhanced[9, 68-71]. Probably as a result of a surge in cytokines (IL-7, IL-15) in response to lymphopenia, lymphocytes undergoing homeostatic proliferation enjoy a reduced activation threshold[72, 73], differentiate directly into effector memory T-cells capable of rapid and intense response to antigen[74], display increased expression of anti-apoptotic molecules, and are less sensitive to immunosuppressive NK cell-mediated lysis[74]. Still, lymphocytes must encounter their cognate antigen and compete for limiting amounts of these homeostatic cytokines to proliferate under these conditions[73]. Thus, B- or T-cells specific for antigens that predominate during this recovery period, like those provided in the form of a vaccine, have a **competitive advantage and become disproportionately over-represented in the recovering lymphocyte** population both in murine models [9, 75] and in humans [70]. These skewed homeostatic responses have been shown to enhance antitumor immunity [9, 75, 76] but can also increase the risk of autoimmunity.

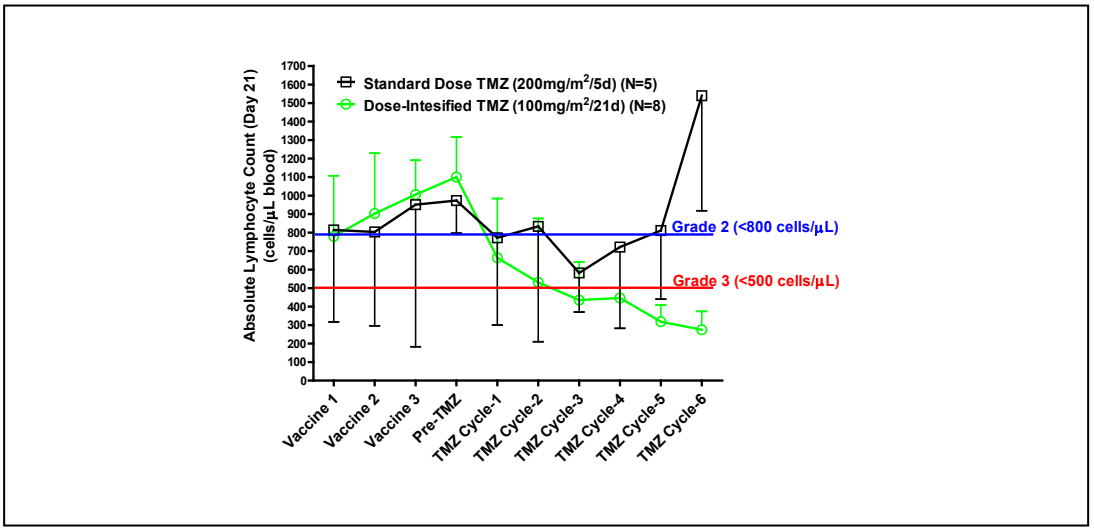


Figure 6: Lymphocyte Counts of Patients with Newly-Diagnosed GBM Receiving Standard Dose Adjuvant TMZ (200 mg/m2/5d) and Dose-Intensified TMZ (100 mg/m2/21d). Standard dose TMZ induces Grade 2 lymphopenia in 100% of patients (<800 cells/uL; blue line) and transient Grade 3 lymphopenia in 40% of patients. Sustained Grade 3 lymphopenia (<500 cells/uL; red line) was induced by dose-intensified TMZ cycle #3 in all patients.

Leveraging this principle, Rosenberg and colleagues have produced dramatic clinical responses [71, 77-80], along with some autoimmune toxicity, in patients with advanced malignant melanoma [68, 69]. After lymphodepletion, T-cells can expand dramatically and tumor-specific T-cells can **constitute up to 90% of the host's T-cell repertoire and can be maintained for months** [68, 81], and in these studies **clinical regressions correlate with the frequency of tumor-specific T-cells achieved in the peripheral blood and persistence of these cells *in vivo*** [31, 77-80, 82-84].

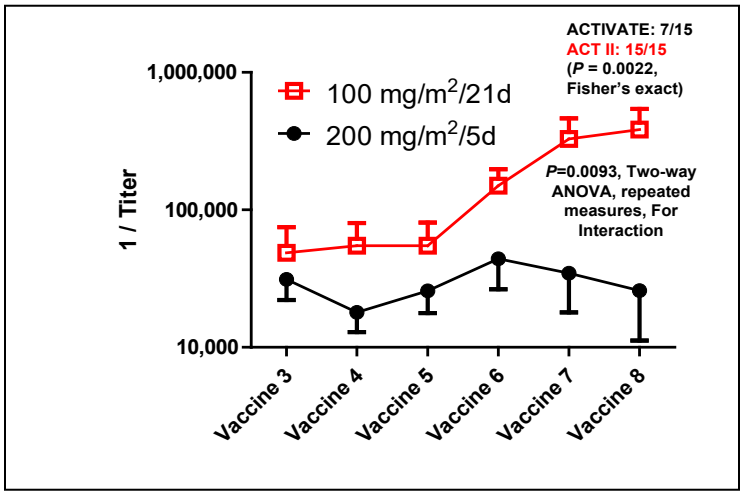


Figure 7: Antibody Titers in Patients During Standard-Dose TMZ (200 mg/m^2/5d) and Dose-Intensified TMZ (100 mg/m^2/21d) are Shown. All patients receiving the 21 day dose regimen developed EGFRvIII-specific antibodies with titers detectable at greater than 1:100,000 dilution. Humoral and cellular immune responses have been dramatically better in patients receiving the higher TMZ dose.

TMZ produces a survival benefit in patients with GBM who are MGMT methylated and has become a routine part of the therapy for these patients. Due to the known myelosuppressive and immunosuppressive effects of TMZ and its potential negative implications for immunotherapy targeting GBMs, we evaluated the impact of TMZ treatment, in the context of our EGFRvIII peptide vaccine trial (now rindopepimut, Celldex Therapeutics), on the lymphocyte compartment and immunologic responses of patients with newly-diagnosed GBM undergoing two different dose regimens of adjuvant TMZ [85]. After concurrent TMZ with EBRT, patients received TMZ for either 5 days (200 mg/m^2) or 21 days (100 mg/m^2) of each 28 day cycle. 100% of patients receiving the **standard 5 day schedule** exhibited **Grade 2 lymphopenia** (<800 cells/uL) **(**Figure 6**)** with nadirs occurring 14-21 days after the first dose (n=5). Grade 3 lymphopenia was observed in only 1 patient with this regimen. **Sustained Grade 3 lymphopenia** (<500 cells/uL) was induced in **all patients** receiving the 21 day regimen, however, by the fifth cycle of TMZ **(**Figure 6**)**. Despite the profound lymphopenia, we have been **able to induce and maintain potent EGFRvIII-specific immune responses (**Figure 7 and Figure 8**) in patients with GBM receiving serial cycles of TMZ** [86].

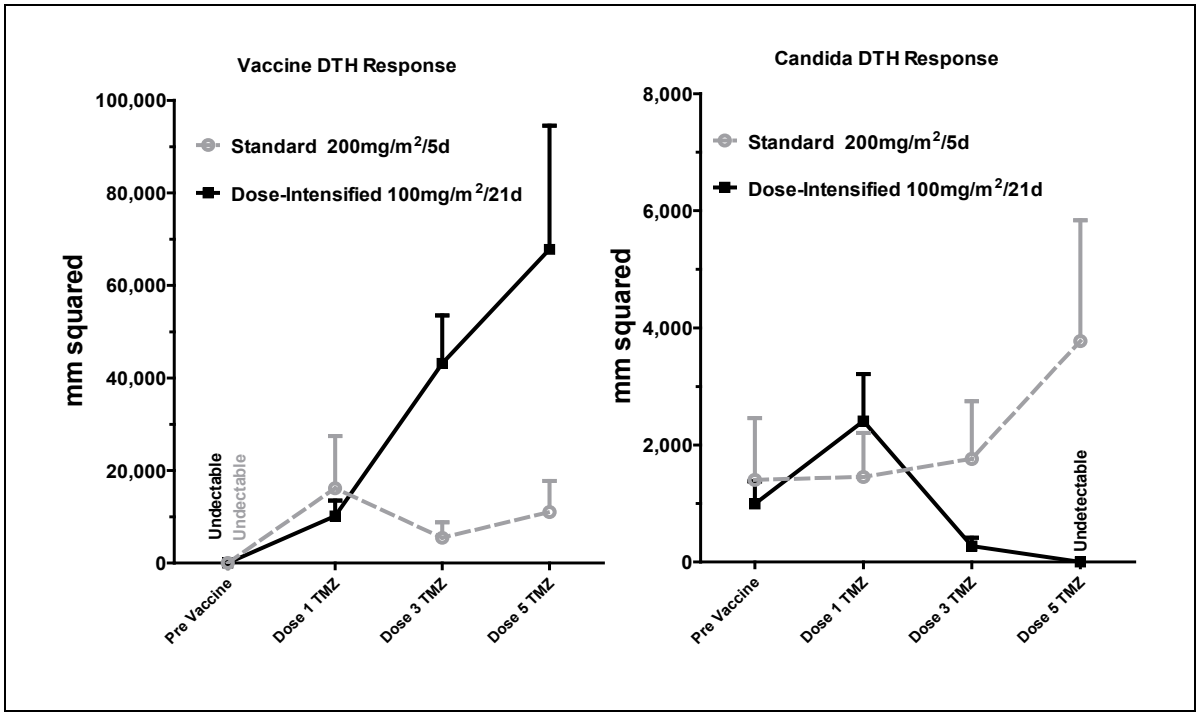


Figure 8: DTH Responses to EGFRvIII vaccination (Left) in patients receiving lymphodepletive TMZ (100 mg/m^2/d x 21 days) cycles (black) were increased when compared with standard dosing (200 mg/m^2/d x 5 days) with continued vaccination. DTH responses to the recall antigen Candida (Right) in patients receiving lymphodepletive TMZ (100 mg/m^2/d x 21 days) cycles (black) were decreased when compared with standard dosing (200 mg/m^2/d x 5 days) with continued cycles of TMZ.

9.6 *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)] [87, 88]. 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[89]. A serum diphtheria antitoxin level of 0.01 IU/mL, measured by neutralization assays, is the lowest level giving some degree of protection; a level of 0.1 IU/mL by ELISA is regarded as protective. Diphtheria antitoxin levels ≥1.0 IU/mL by ELISA have been associated with long-term protection.

Following deep s.c./i.m. administration of the tetanus toxoid vaccine, toxoid molecules are taken up at the vaccination site by immature DCs, which are professional antigen-presenting cells. Within these cells, they are processed through the endosomal pathway (involving the phagolysosome) where they are bound to MHC type II molecules on the surface of DCs. The MHC II:toxoid complex then migrates to the cell surface. While this process is happening within the cell, the now activated mature DC at the vaccine site migrates along lymph channels to the draining lymph node where they encounter naive TH2 cells, each with their own unique TCR. Identifying and then binding of the MHC II:toxoid to the specific TH2 receptor then activates the naive T cell, causing it to proliferate. Simultaneously, toxoid molecules not taken up by DCs pass along lymph channels to the same draining lymph nodes where they come into contact with B cells, each with their own unique B-cell receptor (BCR). Binding to the B cell through the specific immunoglobulin receptor that recognizes tetanus toxoid results in the internalization of toxoid, processing through the endosomal pathway and presentation on the cell surface as an MHC II:toxoid complex, similarly to DCs undergoing the same process [90].

These two processes occur in the same part of the lymph node with the result that the B cell with the MHC II:toxoid complex on its surface now comes into contact with the activated TH2 whose receptors are specific for this complex. The process, termed linked recognition, results in the TH2 activating the B cell to become a plasma cell with the production initially of IgM, with a later switch to IgG antibodies produced. Additionally, a subset of these B cells becomes memory cells [90].

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 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 [91]. 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.

Because of these findings, we have opened a trial enrolling patients to a randomized dendritic cell immunotherapy trial where the two groups receive vaccine site preconditioning with tetanus versus conditioning with non-antigen loaded DC, (Pro000054740 ELEVATE). This trial is powered to determine if greater migration to the draining lymph node correlates with overall survival. To obtain further confirmation that pre-conditioning the vaccine site with Td toxoid increases DC migration to VDLNs and improves overall survival. Due to these promising results, we have included Td pre-conditioning following Td booster in all of our immunotherapy trials including this one.

9.7 *Phase I/II Trials in GBM Targeting CMV*

A Phase I/II clinical trial of autologous pp65 RNA loaded DCs was initiated at our center (ATTAC Protocol- FDA-IND-BB-12839; Duke IRB Protocol 8108; PI: Duane A. Mitchell). This trial has enrolled 9 patients with newly diagnosed GBM who underwent gross total resection (>95%) followed by standard external beam radiation (XRT) (60 Gy) and concurrent TMZ (75 mg/m^2/d) for six weeks followed by monthly 5 day TMZ (150-200 mg/m^2/d) for six cycles. Leukapheresis harvested post-surgical resection and prior to initiation of XRT/TMZ was used to generate pp65 RNA electroporated autologous DCs. Following the first cycle of TMZ (100 mg/m^2/21 days) 2 x 10^7 DCs i.d. mixed with 150 µg of GM-CSF were administered every two weeks for the first three doses and monthly thereafter on day 22 of each cycle. Patients were monitored by MRI (every two months) for tumor progression and blood collected monthly for immunologic monitoring. Patients exhibit a median PFS of 29.2 months **(**Figure 9**)** and OS that has not reached median at 40

months of follow-up. This is favorable compared to historical controls (p = 0.0002). Immunologic responses are shown in (Figure 10 and Figure 11).

Our preliminary data indicate that the recovery phase from TMZ therapy provides an immunologic environment favoring enhanced immune responses to vaccination. This approach has been successful in targeting EGFRvIII positive tumors [91].

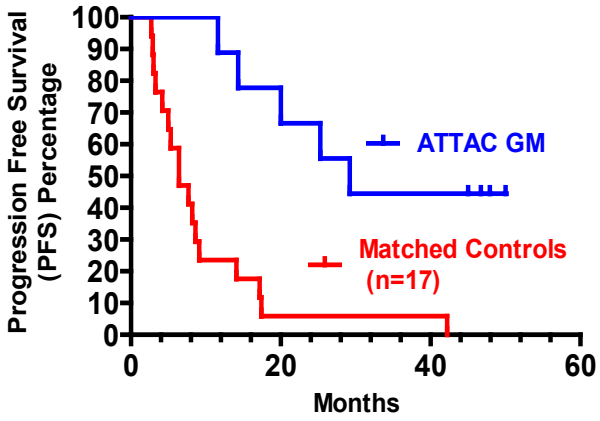


Figure 9: Progression Free Survival (PFS) Percentage Promising PFS in patients with GBM receiving DC vaccines plus GM-CSF targeting CMV pp65. 9 patients with GBM were treated with DC vaccines targeting CMV pp65 on the ATTAC GM trial (FDA IND-BB 12839; Duke IRB 3108). Progression free survival (PFS) is favorable compared to historical controls (ATTAC GM PFS 29.2 months vs Historical controls 6.7 months P=0.0002).

9.8 *Peptide Vaccines for Cancer*

Peptide vaccines encoding minimal CD8+ cytotoxic T cell (CTL) epitopes have been demonstrated in many contexts to induce protective immunologic responses in experimental animals and mediate regressions of established tumors [92, 93]. However, with few several notable exceptions[31, 94-97], vaccine-based cancer immunotherapy in humans is in need of significant improvement in immunogenicity and clinical outcomes [71].

The addition of CD4+ T cell helper epitopes to peptide vaccine formulations is widely believed to be an advantageous strategy for enhancing anti-tumor immunity [98]. This presumption is supported by the observation that CD4+ T cell directed therapy can mediate effective anti-tumor immunity in humans and experimental animals [99-101]. CD4+ T helper cells deliver assistance for CD8+ effector cells by fully activating dendritic cells through the CD40-CD40L signaling pathway [102]. However, vaccination with T helper epitope encoding peptides has yielded mixed results in human clinical trials as a method to enhance anti-tumor cytotoxic T cell responses *in vivo* [100, 103-105] and thus warrants systematic evaluation.

Recent studies have highlighted the use of longer peptides (15-30 amino acids in length) instead of minimal CTL epitopes (typically 8-10 amino acids) for induction of a more robust, long lived CD8+ cytotoxic T cell responses in experimental animals [31, 106]. The expanded length of the peptide requires it be processed by antigen-presenting cells. Minimal CTL epitopes do not require processing by antigen-presenting cells and thus are free to bind directly to T-cell receptors. This can induce tolerance due to a lack of co-

stimulatory molecules that are presented to T-cells during antigen presentation by dendritic cells, however this area of peptide formulation requires further evaluation [107].

We believe the use of serial vaccination during recovery from chemotherapy-induced lymphopenia, as described above, is a novel platform for enhancing the efficacy of tumor-specific vaccines and we have observed prolonged and potent immunologic responses in TMZ treated mice using minimal epitope vaccines.

Peptide vaccination in humans has exhibited an excellent safety profile with virtually no dose limiting toxicities [31, 104]. Most toxicities occur when peptide vaccines are used in conjunction with immunostimulatory cytokines such as IL-2, which are expected results of IL-2 therapy [108]. This feature of peptide vaccinations allows it to be used in combinatorial immunotherapeutic strategies and/or as salvage therapies in resistant disease [109, 110].

9.9 *Tumor-Specific Immunotherapy in Patients with GBM*

Our studies in adults with malignant primary brain tumors have shown that tumor-specific antigens in the form of RNA or proteins are capable of inducing T-cell proliferation, antibody induction, cytokine secretion, and specific lysis of tumor cells. We have completed a Phase II multi-institutional tumor-specific immunotherapy study in adults. In this study, patients with newly-diagnosed with GBM are vaccinated monthly after receiving standard radiation and TMZ therapy and are followed for clinical and radiographic responses. This study showed that a vaccination approach targeting the tumor-specific mutation of the epidermal growth factor receptor, EGFRvIII, was capable of universally eliminating the targeted cell population such that all patients with recurrent tumors no longer expressed the targeted antigen. These studies have also produced remarkably consistent and impressive median survival of >126 weeks which is significantly better than TMZ-treated historical controls matched for treating institution, known prognostic factors, and eligibility criteria (P<0.001). Although patients with residual disease were excluded from our Phase II studies, a small number of patients with residual disease were treated on our initial Phase I studies. All of these patients had nearly complete radiographic responses. Remarkable radiographic responses have also been seen in our recent studies using mRNA-loaded DCs in patients with GBM as well.

Using human *Cytomegalovirus* antigens as a model platform, we have demonstrated the capacity for RNA-loaded DCs to induce antigen-specific $CD8^+$ and $CD4^+$ T cell responses *in vitro* including lytic reaction against antigen expressing targets, proliferative responses (Figure 10), and cytokine-secretion in response to antigenic restimulation (Figure 11).

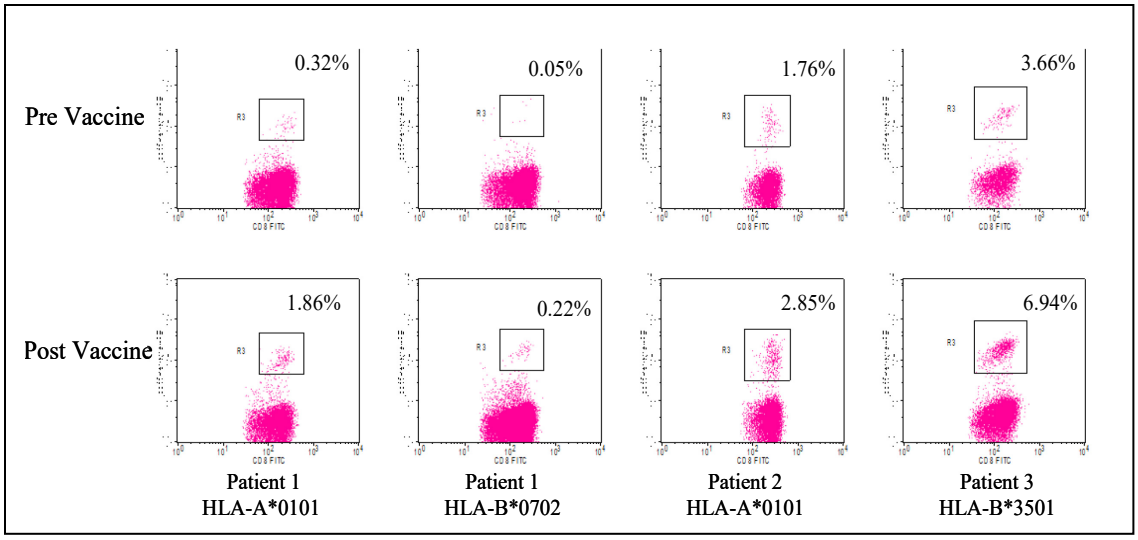


Figure 10: Increase in Frequency of pp65-Specific T cells in Patients with GBM Receiving DC Vaccination and Standard-Dose TMZ. Pre and Post vaccine PBMC from patients with GBM receiving cycles of TMZ (150-200 mg/m^2 x 5 days) and autologous pp65 RNA-pulsed DC vaccines were stained with CD8-FITC and CD3-APC in conjunction with PE conjugated CMVpp65 tetramers (Beckman Coulter, HLA-A*0201, HLA-A*2402, HLA-A*0101, HLA-B*0702, HLA-B*0801, HLA-B*3501) and analyzed by FACS. Dot plots above show percent tetramer positive of total CD3+CD8+ lymphocytes.

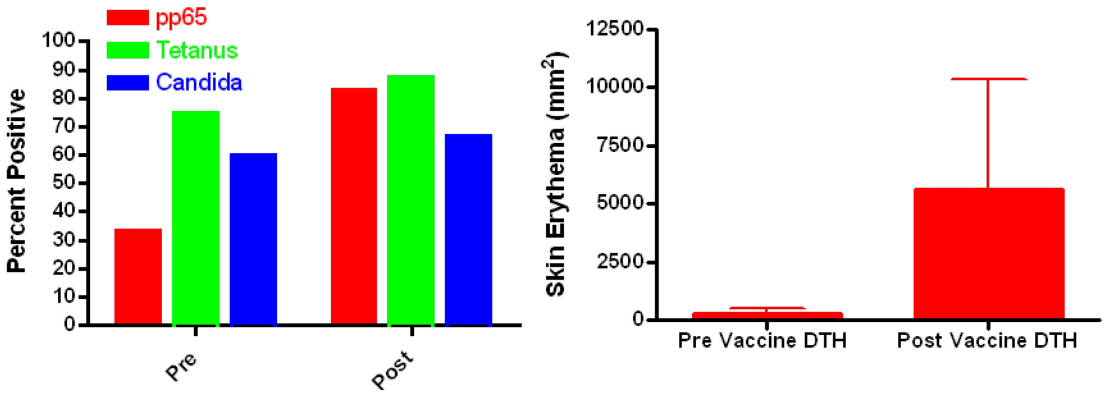


Figure 11: DTH Responses to pp65 RNA Pulsed DC Vaccines. To assess for patients' baseline and progressive status of cellular immunity, routine skin tests were performed before the first immunization, at the time of post-immunization leukapheresis. For skin testing, 1 x 10^6 pp65 RNA transfected mature DCs and 1 x 10^6 untransfected mature DCs were injected intradermally to assess for the development of a DTH reaction. In addition, in order to assess the immunocompetence of the patients they received DTH skin test to the common recall antigens *Trichophyton* and *Candida*.

10 STUDY POPULATION

10.1 *Inclusion Criteria*

1. Age ≥ 18 years.
2. Histopathologically proven newly-diagnosed primary glioblastoma with complete or partial surgical resection. Biopsy not acceptable.
3. Patients must be CMV seropositive.
4. The tumor must be supratentorial.

5. Karnofsky performance status of ≥ 70.
6. Stable or decreasing steroid dose (≤ 4 mg/day) at time of post-XRT adjuvant TMZ initiation. If patients are decreasing steroid use, once they are at 2 mg/day, they may be supplemented with physiologic hydrocortisone therapy (20-30 mg/day in divided doses), at the discretion of the treating oncologist.
7. Hematology
    - ANC ≥ 1500 cells/μL
    - Platelet count ≥ 100,000 cells/μL
    - Hemoglobin ≥ 9.0 g/dl
8. Chemistry
    - ALT/AST ≤ 3.0 times the upper limit of normal
    - Total bilirubin ≤ 1.5 x the upper limit of normal *(Exception: Patient has known Gilbert's Syndrome or patient has suspected Gilbert's Syndrome, for which additional lab testing of direct and/or indirect bilirubin supports this diagnosis. In these instances, a total bilirubin of ≤ 3.0 x ULN is acceptable.)*

10.2 *Exclusion Criteria*

1. Radiographic or cytologic evidence of leptomeningeal or multifocal disease at any time prior to study entry.
2. Prior conventional antitumor therapy, other than steroids, RT or TMZ therapy given for glioblastoma.
3. Pregnant or need to breast feed during the study period.
4. Not adhering to pregnancy prevention recommendations.
5. Active infection requiring intravenous antibiotics or an unexplained febrile (> 101.5^{o} F) illness.
6. Immunosuppressive disease or human immunodeficiency virus infection.
7. Patients with unstable or severe intercurrent medical conditions such as severe heart or lung disease.
8. Allergic or unable to tolerate TMZ for any reason. Any patient that successfully completed at least 5 weeks of Temodar during standard of care XRT/TMZ and whose blood counts meet the eligibility requirements (inclusion #7) within 4 weeks post XRT/TMZ is eligible.
9. Patients with previous inguinal lymph node dissection, radiosurgery, brachytherapy, or radiolabeled monoclonal antibodies.
10. Prior allogeneic solid organ transplant.
11. Currently receiving or ever received immunosuppressive therapy for an autoimmune disorder or an organ transplant.

11 INVESTIGATIONAL PLAN

11.1 *Overview*

If their CMV serostatus is unknown, patients will consent to CMV screening either prior to or following XRT/TMZ. Patients are enrolled to the study following XRT/TMZ and prior to initiation of post-XRT cycles of TMZ provided they meet all other eligibility criteria (if CMV status is not already known, a screening consent will be obtained for CMV screen only to confirm eligibility). After signing main consent, patients receive a Tetanus-diphtheria booster vaccination with 0.5 mL of Td (tetanus, diphtheria toxoid, adsorbed) intramuscularly. After meeting all eligibility criteria, patients are randomized to one of two arms:

- Arm 1) will receive standard TMZ (150-200 mg/m^2/day on days 1-5 of each 28-day cycle).
- Arm 2 will receive dose-intensified TMZ (75-100 mg/m^2/day on days 1-21 of each 28-day cycle).

New patients who enroll on study after protocol version 20181218 is approved and who are MGMT unmethylated will only receive one adjuvant cycle of their assigned TMZ regimen. Patients who enroll on study before protocol version 20181218 is approved or enroll on study after protocol version 20181218 is approved and either are MGMT-methylated or their methylation status is inconclusive will continue with up to 12 cycles of TMZ.

MGMT gene promoter methylation status is obtained at Duke using validated testing from LabCorp by PCR and/or from Caris by pyrosequencing. If MGMT gene promoter methylation status has already been conducted outside of Duke, the results will be used, as long as the testing was performed by a validated method/test. If the results of testing by LabCorp, Caris, or other external, validated test is indeterminate or if the results of more than one type of testing are in disagreement, the results will be considered methylated and the patient will be treated per protocol as methylated based on their randomization. That is, Arm 1 will receive standard adjuvant dosing of temozolomide for the first 5 days of each cycle for 6 to 12 cycles. Arm 2 will receive dose-intensified temozolomide for 21 days of each cycle for 6 to 12 cycles.

Patients who are dependent on steroid supplements above immune suppressive levels (4 mg daily) at time of vaccination or who are unable to tolerate TMZ will be withdrawn from the study before vaccination therapy (see Section 10.2). In order to meet the goal of obtaining 26 evaluable patients who received Component A only (13 patients per arm) for the primary outcomes, up to 70 patients may be consented on the main informed consent.

For both study arms, the initial Duke visit will be within 3 ± 1 weeks after completion of standard of care radiation. For Arm 1, the initial cycle of TMZ will begin as soon as possible after randomization at a standard targeted dose of 150-200mg/m^2/d for 5 days. For Arm 2, the initial cycle of TMZ will begin, as soon as possible after randomization at the dose-intensified dose of 75-100 mg/m^2/d for 21 days. All patients will have immune monitoring blood drawn and then receive a tetanus pre-conditioning injection (Td 1 flocculation unit, Lf, in 0.4 mLs of saline) in the RIGHT groin (as described below) on day 22 (+1 day). Additional safety measures were implemented in this study during the latter half of 2019 and the beginning of 2020, resulting from safety events on study. Patients will receive their 1st vaccine in the Oncology Treatment Center (OTC) and will receive a normal saline bolus

by IV prior to vaccine administration. The IV saline lock will remain in place during the post-vaccine monitoring period for the purpose of quick administration of additional fluids and/or medication should either be necessary. Supportive medications for post-vaccine reactions may include the following by IV: normal saline bolus, Benadryl, Solu-medrol. On Day 23, all patients will be pre-medicated with Zofran® and Tylenol®. If a patient experiences any post-vaccine reactions (please refer to Sections 11.5.1 and 14.2.1 for a description of previous reactions), the patient also may be pre-medicated with oral prednisone at one or more subsequent vaccine visits, at the discretion of the patient and their provider. Patients on study who have experienced a significant post-vaccine reaction with previous vaccines (i.e., ≥ Grade 2 toxicity thought to be possibly, probably, or definitely related to study vaccine), but who are not removed from study, will receive a normal saline bolus by IV prior to vaccine administration for all subsequent vaccines and the IV saline lock will remain in place as a precautionary measure, in case supportive medications are needed. Patients on study who have never experienced a post-vaccine reaction or only experienced a Grade 1 toxicity with earlier vaccines will not be required to have an IV saline lock placed prior to administration of subsequent vaccines, although the treating provider may opt to proceed with this precautionary measure. If a patient enrolls after approval of protocol version 20181218 and has an MGMT unmethylated tumor, they will only receive a single cycle of TMZ.

Previously, patients received the vaccine as follows: 500 µg of PEP-CMV Component A mixed with Montanide ISA-51 intradermally administered in the RIGHT groin and 2 hours later, 500 µg of PEP-CMV Component B mixed in 150 µg of GM-CSF intradermally administered in the LEFT groin. With revisions made in protocol v. 20181218, patients will receive the vaccine as follows: 500 µg of PEP-CMV Component A mixed with Montanide ISA-51 administered intradermally with half in the RIGHT groin and half in the LEFT groin. Vaccines #2 and #3 will be given at 2 week (+3 days) intervals, the MRI visit with immune monitoring will take place within 2 weeks (+3 days) of vaccine #3. For patients who enrolled before approval of protocol version 20181218 for those who are MGMT methylated or with inconclusive MGMT methylation status, TMZ cycle 2 will begin after the aforementioned visit, but no sooner than 14 days post vaccine 3. This will result in an at least an ~35-day delay before starting TMZ cycle 2 MGMT unmethylated patients enrolled after approval of protocol version 20181218 will not receive subsequent cycles of TMZ, but will continue to receive vaccines approximately every 4 (+2) weeks.

Patients who receive more than 1 cycle of TMZ will be vaccinated in conjunction with each **subsequent** 28-day TMZ cycle for a total of 6 to 12 cycles of TMZ after RT followed by further vaccination up to 20 vaccines. Patients who receive only 1 cycle of TMZ will be vaccinated on a 28-day cycle with up to 20 vaccines. All cycles, with or without TMZ, may be given every 4 (+ 2) weeks, in order to adjust for slight delays on startup of each 28-day cycle, and the total number of cycles are given at the discretion of the treating oncologist. During vaccine (+/- TMZ) cycles, vaccinations will occur on day 23 (-1 day, + 2 days) of each cycle for each arm: (Arm 1) standard TMZ (150-200 mg/m^2/day on days 1-5 of each 28-day cycle), or (Arm 2) dose-intensified TMZ (75-100 mg/m^2/day on days 1-21 of each 28-day cycle). All vaccines will be given i.d. approximately 10 cm below the inguinal ligament bilaterally. A target of six cycles with a maximum of twelve cycles of TMZ may be given to patients enrolled prior to approval of protocol version 20181218 and those with MGMT/inconclusive methylation status if enrolled after approval of protocol version 20181218, at the discretion of the treating neuro-oncologist. After the completion of a patient's TMZ cycles, vaccines will continue to be administered every 4 (+ 2) weeks for a maximum of 20 vaccines (unless tumor progression occurs).

Patients will be imaged with contrast-enhanced MRI after vaccine 3 (at cycle 2 initiation visit) and then approximately every 8 weeks [every 2 cycles thereafter (i.e., end of cycles 3, 5, 7, 9, 11)]. RANO criteria [111] will be used for assessment of pseudoprogression and patients demonstrating definitive progression will be removed from study. Any patient removed prior to immune monitoring post vaccine 3 will be replaced for immunologic endpoints. Clinical endpoint comparisons will be made amongst patients randomized to adjuvant TMZ treatment arms who have received at least one vaccine.

Blood for immune monitoring will be obtained:

- Prior to Td pre-conditioning
- During vaccines 1 and 2 (just prior to Component A, 1 and 2 hours after Component A)
- Prior to vaccine 3
- After vaccine 3 (within 2 weeks of vaccine 3 [+ 3 days])
- Prior to vaccines 4 and 6
- At tumor progression (if feasible)

Patients seen by the Duke neuro-oncology team between these vaccine visits may have blood drawn for immune monitoring at the discretion of the study team.

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

Study Drug Safety Arm (added 5/16/17 following safety events that occurred on 4/5/17, not applicable for patients enrolled on protocol version 6/12/18 or later)

Definition of Study Drug Safety Arm:

The purpose of this 6 patient safety arm is to investigate which component or combination of components of the PEP-CMV vaccine (PEP-CMV is administered as two components: Component A and Component B) results in a hypersensitivity reaction, similar to that seen in the first 3 patients treated with the vaccine. Serious Adverse Events for the first 3 patients receiving study drug vaccine were submitted to FDA and the IRB on 4/10/17. These patients experienced rapid onset (within 2 hours of vaccine) of a combination of the following symptoms: nausea, vomiting, diarrhea, chills, fever, myalgias, dyspnea, generalized muscle weakness, back pain, and cough. We believe that the combination of the vaccine components administered at the same time resulted in this intense hypersensitivity reaction and by administering the components with a delay, we may alleviate the severity of these reactions. Additionally, by administering the components separately, we can determine if it's an individual component or a combination of the components that results in the reaction. After we determine which component or combination of components is related to the hypersensitivity reaction, we will put the study on a temporary hold and investigate how we can modify the study drug or administration procedure in order to reduce the occurrence of these reactions.

Rationale of Study Drug Safety Arm:

Our previous experience with peptide vaccines is from the RESIST study (Pro00054746) which has given vaccines to 15 patients without observing any systemic hypersensitivity reaction. This vaccine has tetanus preconditioning and a single IDH-targeting peptide that is co-administered with GM-CSF and Montanide ISA 5.1. Our current study differs from the RESIST study by using different peptides, a pp65 synthetic long peptide and human CMV glycoprotein B (gB), that are meant to target the CMV antigens inthe tumor cells. Additionally, Component B of the vaccine has the gB conjugated to KLH, a component that is not present in the RESIST peptide. Furthermore, Component B has a mixture of KLH and GM-CSF. Given that GM-CSF and Montanide administered together in the RESIST study has not led to systemic hypersensitivity reactions, it is likely that KLH alone or the combination of two or more of Montanide, GM-CSF, KLH, and/or the tetanus preconditioning results in the reaction in these patients.

11.2 *Temozolomide Therapy*

11.2.1 Temozolomide Therapy: After Radiation

With the exception to the delay between cycles 1 and 2, TMZ will be administered after standard external beam RT as part of this protocol every 4 (+ 2) weeks according to assignment arm (5-day or 21-day) at the discretion of the treating oncologist using standard guidelines as outlined in the TMZ Package Insert (uploaded in electronic IRB).

11.2.2 Temozolomide Dose Reduction

All subjects randomized to the standard dose of TMZ will start 5-day TMZ at a dose of 150-200 mg/m^2/day, at the discretion of the treating physician. All subjects randomized to dose-intensified TMZ will start 21-day TMZ at a dose of 75-100 mg/m^2/day, at the discretion of the treating physician. During 5-day or 21-day TMZ treatment (28 day cycles), dose adjustments to TMZ, if needed, may be conducted as outlined below:

Table 1. Temozolomide Dose Delay, Reduction, or Discontinuation

Toxicity	Delay TMZ Dose [a]	Reduce TMZ by 25%	Discontinue TMZ
Absolute Neutrophil Count	$\geq$0.5 and <1.0 x 10^9/L	$\geq$0.5 and <1.0 x 10^9/L	<0.5 x 10^9/L
Platelet Count	$\geq$10 and <100 x 10^9/L	$\geq$10 and <100 x 10^9/L	<10 x 10^9/L
CTC Non-hematological Toxicity (except for alopecia, nausea, vomiting)	CTC Grade 3	CTC Grade 3	CTC Grade 4 [b]
	a: If dose is delayed, treatment with TMZ can resume when the following conditions are met: absolute neutrophil count $\geq$1.0 x 10^9/L; platelet count $\geq$100 x 10^9/L; CTC non-hematological toxicity resolved to baseline (except for alopecia, nausea, vomiting). b: TMZ is to be discontinued if a 25% dose reduction is required more than 1 time or if the same Grade 3 non-hematological toxicity (except for alopecia, nausea, vomiting) recurs after dose reduction or delay.		

11.3 *Pseudoprogression*

There is now a well-documented phenomenon of pseudoprogression in patients with GBM characterized by MRI changes during radiation with temozolomide and up to 2-3 months beyond. These changes resemble tumor growth but actually are due to treatment-related changes. Based on current treatment guidelines [112], patients with radiographic changes consistent with pseudoprogression that are neurologically and clinically stable are recommended to continue standard temozolomide and be reassessed by MRI. Such patients will undergo randomization and receive vaccine as described in the protocol after consultation with the treating neuro-oncologist.

11.4 *Treatment Procedures*

11.4.1 Patient Number Assignment and Randomization

Patients will be assigned a study ID number serially once they have signed the CMV consent form or main study consent form if the CMV consent form is not required, this number will stay with them as they are checked for study eligibility. This number should be referenced on all patient-specific study-related material. A permuted-block randomization scheme developed by the study biostatistician will be used to assign patients to treatment arms. The randomization module for the Duke electronic database system (Title 21 CFR Part 11 Compliant) will be used for this purpose.

11.4.2 PEP-CMV Vaccination

As described above, patients will be randomized to one of two arms (1:1) to receive: (1) standard TMZ (150-200 mg/m^2/day on days 1-5 of each 28-day cycle), or (2) dose-intensified TMZ (75-100 mg/m^2/day on days 1-21 of each 28-day cycle).

During the first cycle of adjuvant TMZ, all patients will receive a tetanus pre-conditioning injection in the RIGHT groin on Day 22 (+1 day) and the following day receive the vaccine. Previously, patients received vaccine prepared as follows: 500 µg of PEP-CMV Component A mixed with Montanide ISA-51 i.d. administered in the RIGHT groin and 2 hours later, 500 µg of PEP-CMV Component B mixed in 150 µg of GM-CSF i.d. administered in the LEFT groin. With revisions made in protocol v. 20181218, all newly enrolled patients will have the vaccine prepared as follows: 500 µg of PEP-CMV Component A mixed with Montanide ISA-51 administered i.d. with half in the RIGHT groin and half in the LEFT groin. With the 20190318 amendment, Component B will be removed from the vaccination regimen for all patients previously receiving Component B as part of the regimen. The subsequent 2 vaccines will be administered every 2 weeks (+ 3 days) for a total of 3 vaccines. This will result in a ~35-day delay in the second cycle of TMZ. MGMT unmethylated patients, after protocol version 20181218 is approved, will not receive subsequent cycles of TMZ, but will continue to receive vaccines approximately every 4 (+2) weeks.

Patients will be vaccinated in conjunction with subsequent TMZ cycles every 4 (+2) weeks for a total of 6 to 12 cycles of TMZ after RT. Additional vaccines may be administered up to 20 vaccines, at the discretion of the treating neuro-oncologist. All vaccines will be given i.d. approximately 10 cm below the inguinal ligament bilaterally. For MGMT unmethylated patients after protocol version 20181218 is approved, only one cycle of TMZ will be provided; however, vaccines will continue every 4 (+ 2) weeks after the single TMZ cycle for a maximum of 20 (unless tumor progression occurs).

PEP-CMV is administered as Component A alone (500 µL per side). As described above, vaccines will be delivered intradermally. Details of the procedure will be recorded on the appropriate eCRF. Patients will receive their 1st vaccine in the OTC with an IV bolus of normal saline prior to vaccination, and the IV saline lock will remain in place in case quick administration of fluids or medications is needed for a reaction. Patients will be pre-medicated with Zofran® and Tylenol® and optionally with oral prednisone at the discretion of the patient and his/her provider if patient has previously experience post-vaccine reaction(s). Patients will be monitored in the clinic for approximately 4 hours post-immunization for the development of any adverse reactions after vaccines #1 and #2 for a total of 8 sets of vital signs (i.e., every 30 minutes). Supportive medications for post-vaccine reactions may include the following by IV: normal saline bolus, Benadryl, Solu-medrol. If no reaction(s) occur during vaccines #1 and #2, patients will be monitored for 2 hours for a total of 4 sets of vital signs (i.e., every 30 minutes) after vaccine #3, as long as the reason for no reactions is not pre-medication with oral prednisone. If no reaction(s) occur after vaccine #3, patients will be monitored for 30 minutes with one set of vital signs for subsequent vaccines, as long as the reason for no reactions is not pre-medication with oral prednisone. If a patient experiences a post-vaccine reaction at any point, the monitoring period will revert to 4 hours until such time as he/she have 2 consecutive vaccines with no reaction. The PI or sub-investigator may extend monitoring periods at any given visit if they feel it is clinically indicated for the patient's safety. Patients who have experienced a significant post-vaccine reaction at any previous vaccine (i.e., ≥ Grade 2 toxicity considered possibly, probably, or definitely related to the study vaccine), but who are not removed from study, will receive a bolus of normal saline by IV before study vaccine and the saline lock will remain in place as a precautionary measure. Patients who have never experienced a post-vaccine reaction or only experienced a Grade 1 toxicity with earlier vaccines will not be required to have an IV saline lock placed prior to administration of subsequent vaccines, although the treating provider may opt to proceed with this precautionary measure. The immunization procedures will be supervised by a nurse or physician that has completed Basic Life Support (BLS) course. A cardiac resuscitation cart will be available in the vicinity when performing these immunizations in case of severe allergic reactions.

11.4.3 Treatment Plan for Study Drug Safety Arm (not applicable for patients enrolled on protocol version 6/12/18 or later)

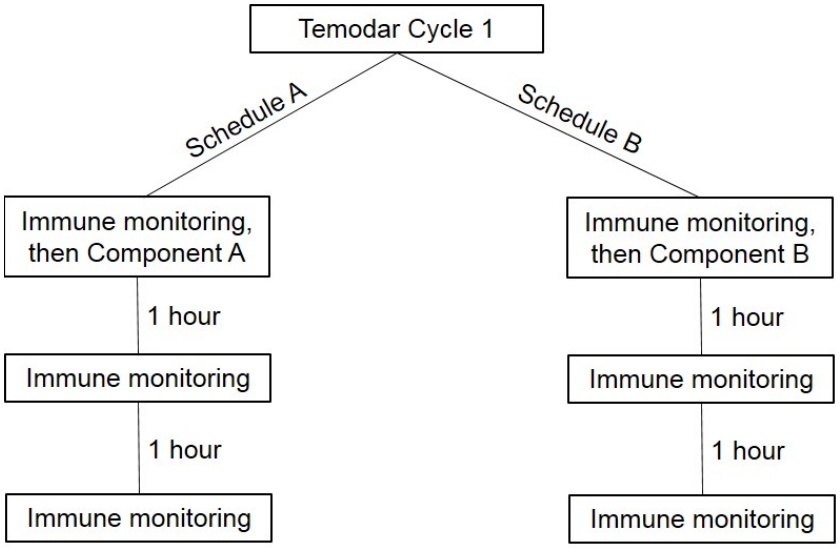


Figure 12: Study Drug Safety Arm Study Schema for Vaccine #1.

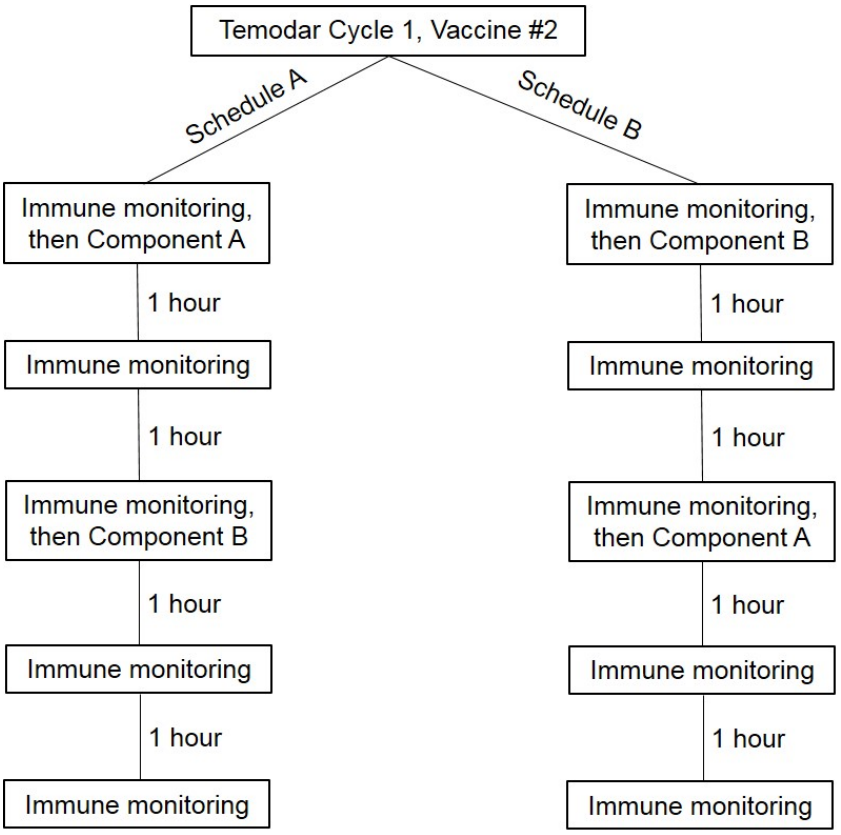


Figure 13: Study Drug Safety Arm Study Schema for Vaccine #2 if the Patient Experiences No Hypersensitivity Reaction to Vaccine #1.

Following the safety events that were initially reported on 4/10/17, 6 additional patients were consented to receive study vaccine with standard of care (SOC) 5-day TMZ at 150-200 mg/m^2/d for 28-day cycles. These patients were not randomized according to the original study design to either 21 day or 5-day Temodar; they received only SOC 5 day Temodar cycles. Of these 6 patients, 3 patients were assigned to Schedule A and 3 patients were assigned to Schedule B. They were assigned to Schedule A or B such that every other patient that receives vaccine is on a different schedule, i.e., patient 1 received Schedule A, patient 2 received Schedule B, patient 3 received Schedule A, etc. There was a planned interval of at least 48 hours between each individual patient receiving study vaccine.

Vaccine #1 was administered according to the schematic shown in Figure 12. Every patient received a tetanus preconditioning injection in the RIGHT groin on Day 22 (+1 day) of the TMZ cycle, and the following day received a single component of the vaccine. Component A consisted of 500 µg of PEP-CMV mixed with Montanide ISA-51 i.d. administered in the RIGHT groin and Component B consists of 500 µg of PEP-CMV, gB conjugated to KLH, mixed in 150 µg of GM-CSF i.d. in the LEFT groin. Patients assigned to Schedule A were infused with only Component A at the first vaccine. Patients assigned to Schedule B were infused with only Component B at the first vaccine. Blood for immune monitoring (~ 20 mL[3]) was drawn just prior to component administration, 1 hour, and 2 hours after component administration. If no hypersensitivity reaction was observed after the administration of only a single Component, the patient received both components at the subsequent vaccine administration, according to Figure 13. If a hypersensitivity reaction occurred in 2 out of 3 Schedule A patients after receiving only Component A, further administration of Component A would be stopped and the study team would evaluate potential modifications for Component A. If a hypersensitivity reaction occurred in 2 out of 3 Schedule B patients after receiving only Component B, but no hypersensitivity reactions had been experienced by Schedule A patients receiving only Component A, further administration of Component B to Schedule B patients would be stopped and all remaining vaccinations would be done with Component A alone. If 2 out of 3 patients on both Schedule A and B experienced hypersensitivity reactions after Vaccine #1, the study team would evaluate potential modifications to both components and no patient would proceed to Vaccine #2.

Those patients who received Vaccine #1 with no hypersensitivity reaction proceeded to Vaccine #2, administered according to Figure 13. Patients assigned to Schedule A had Component A administered in the RIGHT groin and were observed for 2 hours (up to 4 hours), followed by administration of Component B in the LEFT groin. Patients had to be asymptomatic for at least 2 hours before proceeding to administration of Component B. Blood draws occurred every hour, beginning at just prior to Component A administration and ending at the 2 hour observation time point following Component B administration. Blood (~ 20 mL[3]) was drawn to monitor immune activity through analysis of, but not limited to, TNFα, GM-CSF, IFNα, IFNγ, IL-6, and IL-1. Patients assigned to Schedule B had Component B administered in the LEFT groin and were observed for 2 hours (up to 4 hours), followed by administration of Component A administered in the RIGHT groin. Blood draws occurred every hour, beginning at just prior to Component B administration and ending at the 2-hour observation time point following Component A administration. These 6 patients would continue to receive study vaccine according to their assigned schedule, if

[3] One 10 mL EDTA tube, most frequently lavender top for plasma collection, such as BD-36643. One 10 mL serum tube, most frequently red top for serum collection, such as BD-VT6430.

no hypersensitivity reactions occurred, for as long as they remained on study. If patients in Schedule A experienced a hypersensitivity reaction after Vaccine #2, they would continue all further injections according to Vaccine #1, Component A only. If patients in Schedule B experienced a hypersensitivity reaction after Vaccine #2, but there was no hypersensitivity reactions to Schedule A patients with Component A alone, they would continue all further injections with Component A only. After completing Vaccine #2, the immune monitoring blood draws and MRIs followed the schedule described in Table 3 and did not include the additional immune monitoring blood draws that were drawn in Vaccine #1 and Vaccine #2 (Figure 12 and Figure 13). The timing of the first 3 vaccines could result in a potential ~35-day delay in the second cycle of TMZ.

Patients were vaccinated in conjunction with subsequent TMZ cycles every 4 (+2) weeks for a total of 6 to 12 cycles of TMZ after RT at the discretion of the treating neuro-oncologist. All vaccines were given i.d. approximately 10 cm below the inguinal ligament bilaterally (Component A in the RIGHT groin and Component B in the LEFT). Vaccines continued 4 (+ 2) weeks after TMZ cycles for a maximum of 20 (unless tumor progression occurred).

PEP-CMV was administered as two components, Component A in a total volume of 1 mL and Component B in a total volume of 800 µL, as described above, that was delivered intradermally. Patients were pre-medicated with Zofran® and were monitored in the clinic for 2 hours post-immunization for the development of any adverse effects. Vital signs were obtained every 15 minutes until discharge from the clinic. Additional follow-up with the patients was done by phone at 24 and 48 hours to inquire about additional symptoms/adverse events experienced in the 48 hours following vaccine. Vaccine administration was supervised by a nurse or physician that has completed Basic Life Support (BLS) course. A cardiac resuscitation cart was available in the vicinity when performing these immunizations in case of severe allergic reactions.

11.4.4 Drug Accountability

The investigator and pharmacist are responsible for correct storage of vaccine. The vaccine made available for this clinical trial must be used in accordance with the protocol and must only be handled by the investigator or appropriately designated individuals. Documentation of receipt and disposition of the vaccine will be managed in accordance with study operational procedures. At completion of the trial, or during routine monitoring visits, the PI or his designee will perform a full drug accountability check.

The master record must include:

- Date of receipt of vaccine shipment
- All batch numbers
- Quantities received

The dispensing record must include:

- Quantities dispensed
- Identification of the person to whom vaccine was administered
- Date of each dispensing

These master and dispensing records are separate from records kept for individual trial patients.

11.5 *Safety, Toxicity, and Adverse Events*

11.5.1 Post-Vaccine Management Plan

Following a safety event on **4/5/17** in which a patient was hospitalized for post-vaccine reactions including nausea, vomiting, fever, chills, myalgias, dyspnea, and diarrhea, A post-vaccine management plan was initiated to increase patient safety. A detailed treatment plan of the first 6 patients scheduled to receive vaccine following this safety event is described in Section 11.4.3. For these 6 patients and subsequent patients, subjects were observed for approximately 2 hours following the first 3 vaccine administrations and follow-up phone calls occurred at 24 and 48 hours (after the first 3 vaccines only). For all subsequent vaccines, Vaccine #4+, subjects were observed for approximately 30 minutes following vaccination. Monitoring included vitals approximately every 30 minutes from the previous vitals measurement.

Additional safety events were observed in two patients treated with their first vaccine in **June 2019**. Following discharge from our clinic after protocol-specified monitoring for 2 hours, both patients experienced reactions. One patient experienced grade 1 fever, chills, and sinus tachycardia. The 2nd patient experienced grade 1 chills and nausea, grade 2 dehydration, and grade 3 flu-like symptoms. Both patients received the protocol-specified pre-medications (Zofran®, Tylenol®) at their Vaccine #1 visits. At their Vaccine #2 visits, both patients were also pre-medicated with oral prednisone and did not experience similar reactions. In response to these safety events, an extended post-vaccine monitoring period is being implemented in which patients will be observed in clinic for approximately 4 hours following Vaccines #1 and #2 with regular vital signs monitoring every 30 minutes and will be asked to remain in the area overnight to lessen the chance they are travelling should they experience a reaction. Patients will continue to receive follow-up phone calls at 24 and 48 hours (after the first 3 vaccines only).

During an interim review of safety on **November 27, 2019**, the following Grade 3 unacceptable toxicities (as defined in protocol v. 20190730) were noted in 4 out of 15 patients:

Patient ID 1125	hypotension, lactic acidosis, flu-like symptoms
Patient ID 1131	hypotension
Patient ID 1149	flu-like symptoms, hypotension, lactic acidosis
Patient ID 1163	hypotension

Further safety measures were implemented in the latter half of 2019 and the beginning of 2020, resulting from safety events on study. Patients will receive their 1st vaccine in the Oncology Treatment Center (OTC) and will receive a normal saline bolus by IV prior to vaccine administration. The IV saline lock will remain in place during the post-vaccine monitoring period for the purpose of quick administration of additional fluids and/or medication should either be necessary. Supportive medications for post-vaccine reactions may include the following by IV: normal saline bolus, Benadryl, Solu-medrol.

To date (**January 10, 2020**), we have analyzed immune responses in 16 patients who received vaccine 1 and were batch assayed. We believe that the observed toxicities are related to cytokine release syndrome (CRS). The immune responses across 4 degrees of reactions (ranging from no reactions to Grade III reactions) demonstrate a significant difference between levels of cytokines (G-CSF, GM-CSF, IFN-γ, IL-10, IL-2, IL-8, MIP1-α, and TNF-α) two hours after vaccine, as compared to those levels pre-vaccination, with IL-

6 levels also nearing significance. For these cytokines, subsequent pairwise comparisons included comparisons involving those experiencing Grade III adverse events after Tukey adjustment. These data suggest that the adverse event reactions following PEP-CMV vaccination are indeed immune-related.

The cumulative data indicate that PEP-CMV vaccination is inducing the activation of T cells specific for the target antigen pp65. Furthermore, in patients with AEs, the pp65-specific T cell activation may be inducing a temporary elevation of pro-inflammatory cytokines. We believe that the vaccine reactions we have been observing in PERFORMANCE are likely indicative of vaccine potency. Patients who have demonstrable Grade III reactions also have measurable cellular responses to the PEP-CMV vaccines, which may be indicative of clinical responses.

CRS is associated with elevated circulating levels of cytokines including IL-6 and IFNγ. Commonly referred to as an infusion reaction, it results from the release of cytokines from cells targeted by the antibody, as well as immune effector cells recruited to the area. When cytokines are released into the circulation, systemic symptoms such as fever, nausea, chills, hypotension, tachycardia, asthenia, headache, rash, scratchy throat, and dyspnea can result. In most patients, the symptoms are mild to moderate in severity and are managed easily. However, some patients may experience severe, life-threatening reactions that result from massive release of cytokines. Massive cytokine release is an oncologic emergency, and special precautions must be taken to prevent life-threatening complications. A CRS Management Plan based upon the ASTCT Consensus Grading [113] is provided in Table 2 below for the mitigation of symptoms of CRS. Please note that ASTCT Consensus Grading guidelines refer to the CTCAE v.5 description of constitutional symptoms for its CRS term.

Table 2. CRS Management Plan based upon the ASTCT Consensus Grading

Grade of Toxicity	ASTCT Definition	Management Plan
1	Fever ≥ 38°C, with or without constitutional symptoms[4], not attributable to another cause	Symptomatic management only
2	Fever ≥ 38°C with hypotension not requiring vasopressors and/or hypoxia requiring the use of oxygen delivered by low-flow nasal cannula (≤6 L/minute) or blow-by	Hypotension: Clearly establish a baseline blood pressure; if hypotension develops, give fluids. Other reactions: Patients with grade 2 toxicity will be individually tailored, depending on the patient age and medical co-morbidity, and therefore clinical judgement will be crucial prior to use of immunosuppression for grade 2 toxicity. Patients with grade 2 toxicity will be monitored in the OTC with very close cardiac monitoring.

[4] *The associated constitutional symptoms may be reported, but do not affect CRS grade per ASTCT Consensus Grading Guidelines.*

3	Fever ≥ 38°C with hypotension requiring 1 vasopressor with or without vasopressin and/or hypoxia requiring high-flow nasal cannula (>6 L/minute), facemask, nonrebreather mask, or venturi mask not attributable to another cause	If grade 3 toxicity develops where fluid resuscitation and 1 low dose vasopressor are not sufficient to reverse the hypotension, the patient will be transferred and monitored closely in the emergency department. All patients with grade 3 toxicity will receive immunosuppressive agents including corticosteroids, such as 2mg/kg Solu-medrol. In severe cases, Tocilizumab, antihuman IL-6R mAb, will be administered. This drug is stocked in the Duke pharmacy.
4	Fever ≥ 38°C with hypotension requiring multiple vasopressors (excluding vasopressin) and/or hypoxia requiring positive pressure (e.g., CPAP, bilevel positive airway pressure, intubation, mechanical ventilation) not attributable to another cause	All patients with immediate, life-threatening toxicity will be treated with full support, including mechanical ventilation, immunosuppressive agents, Tocilizumab to prevent inflammatory cascade, and close comprehensive organ monitoring.

11.5.2 Special Consideration

Due to the impact of high dose steroids on the development of an optimal immune response, if a patient demonstrates neurologic or cerebral radiographic signs suggestive of a localized inflammatory reaction, secondary to the immune response triggered by PEP-CMV vaccination, that requires an increase in dexamethasone dose, every effort should be made to not increase the dose above 4 mg per day at any time. Instead, patients should be treated with bevacizumab at the reduced dose of 7.5 mg/kg IV approximately every 3 weeks. If a patient requires planned treatment for their tumor with bevacizumab > 7.5 mg/kg approximately every 3 weeks, they will be considered off study and enter the follow-up phase. Neuroimaging (MRI) will be performed according to protocol schedule and, at that time, it will be assessed whether further treatment with bevacizumab is needed to control the cerebral inflammation. Bevacizumab will not be provided by the study. Every attempt should be made to reduce or discontinue dexamethasone, when clinically possible, so as not to mitigate immune response.

If there are AEs or other circumstances prohibiting the use of bevacizumab, corticosteroids, surgery, or other interventions deemed more appropriate for the patient by the treating physician will be used, if needed, to treat any localized inflammatory reaction secondary to DC vaccination.

11.5.3 Allergy Testing

Patients who experience a hypersensitivity reaction as described in Section 15.3 may be offered optional Allergy Testing. To complete the optional Allergy Testing, patients must sign the Allergy Test Consent Form. Allergy testing will only be done on patients who have experienced a hypersensitivity reaction following vaccine administration, in an attempt to uncover which specific element of Component A and/or Component B results in the

reaction. Patients will receive intradermal injections of potential allergens to both the left and the right groin. The allergens include components that are present in Component A and Component B of the PEP-CMV vaccine, as relevant. Each intradermal injection will be administered 1 hour apart and blood draws (~20 mL) will be taken 1 hour prior to the first injection and 1 hour following the final injection. Blood (~20 mL) will be drawn to monitor immune activity through analysis of TNFα, GM-CSF, IFNα, IFNγ, IL-6, and IL-1, as appropriate. Patients will receive SLP and Montanide in the right groin and GM-CSF, gB, and KLH in the left groin. If the patient has only received either Component A or Component B, they will only receive intradermal injections of the elements that were part of the vaccine that they received. Patients will be monitored according to Allergy and Immunology standard of care practices in the clinic.

11.5.4 Unacceptable Toxicities

The original definition of unacceptable toxicities in this study was any vaccine-related or any non-neurologic ≥ Grade 3 toxicity of any duration not attributable to TMZ, bevacizumab, and/or disease progression; any Grade 4 toxicity, including neurologic events not due to progressive disease; or any life threatening-event not attributable to concomitant medication, co-morbid event, or disease progression. The definition of unacceptable toxicity was revised with v.20200117 of the protocol and will be defined now as any life-threatening ≥ Grade 3 toxicity that is possibly, probably, or definitely related to the PEP-CMV vaccine with the following exceptions (updated in the v.20200504 protocol). A portion of the Grade 3 vaccine reactions noted in prior patient experiences with the PEP-CMV vaccine, described in Sections 11.5.1 and 14.2.1, will not be considered unacceptable if they are indicative of immune response. That is, toxicities such as Grade 3 flu-like symptoms, fever, and chills/rigors will not be considered unacceptable toxicities if the duration is less than 72 hours. Please see Section 11.5.1 for guidelines on management of immune response, including cytokine release syndrome (CRS). Any ≥ Grade 3 organ toxicity (cardiac, renal, hepatic), including CRS-related toxicities such as hypotension and tachycardia, of any duration will be considered an unacceptable toxicity. Toxicities will be tracked on all subjects who received PEP-CMV vaccine using the NCI CTCAE (Version 4.03) criteria. Patients who experience unacceptable toxicities will not continue to receive vaccine therapy. Although not considered an unacceptable toxicity, any patient with ≥ Grade 2 urticaria will not receive further vaccines, will be withdrawn from the study, and will be replaced if less than 3 vaccines have been administered without unacceptable toxicity.

The prevalence of unacceptable toxicities occurring during the initial 3 bi-weekly vaccinations or the vaccinations administered concurrently with temozolomide will be continuously monitored. If more than 25% of accrued patients experience unacceptable toxicities, then accrual will be suspended and reported toxicity will be carefully reviewed to determine if modifications to the protocol treatment should occur. Peptide vaccinations employing Montanide ISA-51 as adjuvants have generally been well tolerated in human patients in numerous phase I-III trials.

11.5.5 Adverse Event Reporting and Documentation

An “Adverse Event” will be defined as any adverse change from the subject’s pre-treatment baseline condition (which is based on the physical and neurologic assessment done at the SOC clinic visit prior to initiation of post-radiation chemotherapy and vaccine therapy), including any clinical or laboratory test abnormality that occurs during the course of research **after** vaccine treatment has started. Adverse events will be categorized and graded in accordance with the NCI CTCAE (Version 4.03).

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) is medically significant such that it may jeopardize the subject, and may require medical or surgical intervention to prevent one of the outcomes listed above.

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

All adverse events which are serious and unexpected (as defined by 21CRF312.32[a]) should be reported immediately to Dr. David Ashley (Pager: 919-206-3433) or his designee (919-684-5301). Fatal or life-threatening, unexpected adverse events that are related or possibly related to the research 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 related or possibly related to the research and are not fatal or life-threatening will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the sponsor.

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

11.6 *Treatment Period*

Patients that have consented onto this study will receive vaccines as indicated above. Once patients have received vaccine therapy, they will be considered as “treated” on this study.

Treatment procedures for the Study Drug Safety Arm are described in Section 11.4.3. Further details of potential study drug modification and treatment period pausing criteria is described in Section 15.3.

11.7 *End of Treatment*

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

11.8 *Definition of Evaluable Subjects, On Study, and End of Study*

Subjects evaluable for the analysis of the primary endpoints will include all randomized patients who undergo immune monitoring post vaccine #3, prior to 2nd vaccine/TMZ cycle. Survival analyses will include all randomized patients evaluable for the primary outcome.

Safety analyses will include all patients who receive vaccine treatment.

Once the patient signs an ICF, that subject will be considered "on study". Rationale for taking patient off protocol treatment (see Section 11.10.1 below) will be documented.

11.9 *Follow-up Period*

Patients evaluable for the primary outcome will be followed for progression and survival and data recorded by the study team.

11.10 *Early Withdrawal of Subject(s)*

11.10.1 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 withdrawal may include at the PI's discretion may include, but are not limited to, the following:

- Progressive disease as documented by MRI or physical examination at any time after the initiation of immunotherapy
- Development of unacceptable toxicity
- Pregnancy
- Patients requiring an increase in corticosteroids, with the exception of nasal or inhaled steroid, such that at the time of first vaccination they require a dose above immune suppressive levels, will be removed from the study and replaced. For the purposes of this study, immune suppressive dose will be defined as ≤4 mg of dexamethasone / day or equivalent. 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
- Abnormal laboratory values
- Upon request of the subject
- If, in the investigator's medical judgment, further participation would be injurious to the subject's health or wellbeing
- Development of intolerable symptoms
- Protocol deviation
- Administrative reasons, such as a major violation of the clinical trial protocol
- Non-compliance of the subject
- Clinical decline
- Allergic or hypersensitivity reaction to Td vaccine
- Allergic or hypersensitivity reaction to PEP-CMV
- Failure to tolerate TMZ therapy for any reason
- Development of any of the following co-morbidities:
    - Unstable angina and/or congestive heart failure requiring hospitalization.

- Transmural myocardial infarction within the last 6 months.
- Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects; note, however, that laboratory tests for liver function and coagulation parameters are not required for entry into this protocol.
- Acquired Immune Deficiency Syndrome (AIDS) based upon current CDC definition; note, however, that HIV testing is not required for entry into this protocol. The need to exclude patients with AIDS from this protocol is necessary because the treatments involved in this protocol may be significantly immunosuppressive.
- Major medical illnesses or psychiatric impairments that in the investigator's opinion will prevent administration or completion of protocol therapy.

11.10.2 Follow-up Requirements for Early Withdrawal

Subjects that are withdrawn by the PI prior to randomization will be considered eligibility failures and thus not followed for survival.

Randomized subjects treated on this study that are withdrawn by the PI for any of the aforementioned reasons after the immune monitoring post-vaccine 3 will continue to be followed for progression and survival by the study team until death or are lost to follow up unless the subject voluntarily withdrew permission to follow for survival.

Randomized subjects that withdraw before the immune monitoring post-vaccine 3 will be followed for survival by the study team unless permission to do so has been withdrawn.

11.10.3 Replacement of Early Withdrawal(s)

Subjects who voluntarily withdraw prematurely or who are withdrawn by the PI prior to immune monitoring post vaccine #3 will be replaced. However, the data from these patients will be included in all appropriate analyses.

11.10.4 Off Study

Subjects evaluable for the primary outcome are considered off study if lost to follow up or deceased.

12 DESCRIPTION OF STUDY EVALUATIONS

12.1 *Schedule of Events*

Table 3: Schedule of Events

	Pre-XRT/TMZ Screening[5]	Post-XRT/TMZ screening[6]	TMZ Cycle 1[7]	Td pre-conditioning[8]	Vaccines 2 & 3	TMZ Cycles 2-12[9]	Vaccines 4-20[10]	Progression
CMV consent, if needed	X	X						
Main study consent		X						
Td booster		X						
Medical History		X						
History and Physical		X		X	X		X	
Neurological Exam		X		X	X		X	
KPS		X		X	X		X	
Curran		X						
CMV screen	X	X						
MGMT methylation		X						
CBC w/ differential[11]		X	X	X	X	X	X	
CMP[11]		X	X	X	X	X	X	
Beta HCG (quantitative)		X						
Cortisol Levels[11]		X	X		X		X	

[5] If patients have yet to undergo XRT/TMZ when they are presented with the study, they will undergo the pre-XRT/TMZ screening. If the patient is CMV positive, they will undergo standard of care XRT/TMZ and then return post XRT/TMZ for the next screening portion. If patients have already been tested for CMV, they will not need to sign the CMV consent or have the CMV test repeated.
[6] If patients have already undergone XRT/TMZ when they are presented with the study, they will undergo both pre- and post-XRT/TMZ screening after completing XRT/TMZ, prior to Cycle 1 of adjuvant TMZ. If patients have already been tested for CMV, they will not need to sign the CMV consent or have the CMV test repeated.
[7] Temodar cycle 1 should start 2-6 weeks post the end of XRT/TMZ and will last until 2 weeks post Vaccine #3. Patients will only take either 5 days of Temodar on days 1-5 (Arm 1) or 21 days on days 1-21 (Arm 2) based on the randomization results.
[8] Td pre-conditioning occurs on day 22 (+1 day) and is given in the RIGHT groin for all patients. Vaccine #1 is given on day 23 (+2 days).
[9] Temodar cycles 2 – 12, if applicable, are 28-day cycles (Patients who enrolled prior to approval of protocol version 20181217 and patients who enrolled after approval of protocol version 20181218 who are MGMT-methylated. Cycles may be given every 4 (+2) weeks, in order to adjust for slight delays on startup of each 28-day cycle, and the total number of cycles are given at the discretion of the treating oncologist.
[10] Vaccines #4 - #20 will be given every 4-6 weeks or on day 23 (-1 day/+2 days) of each TMZ cycle depending on MGMT methylation status.
[11] CBC w/diff and CMP to be drawn at post XRT/TMZ screening, days 14, 21, and 28 (+/- 2 days) of each TMZ cycle, and at the discretion of the treating oncologist.
[11] At post-XRT/TMZ screening for baseline and prior to each vaccine.

	Pre-XRT/TMZ Screening[5]	Post-XRT/TMZ screening[6]	TMZ Cycle 1[7]	Td pre-conditioning[8]	Vaccines 2 & 3	TMZ Cycles 2-12[9]	Vaccines 4-20[10]	Progression
Immune Monitoring			X[13]	X[14]	X[13]	X[15]	X[16]	X[17]
Vaccine				X	X		X	
Vitals[18]		X		X	X		X	
Randomization		X						
MRI		X				X[19]	X[20]	
Pathology testing[21]		X						X[17]
Adverse Events[22]		Ongoing						
Con Meds[22]		Ongoing						

12.2 *Medical History*

The medical history is to include any current active diagnoses and any previously treated illnesses, including the approximate date of onset (at minimum indicate the year or approximate year) and date of resolution if applicable, and description of any prior surgeries. The body systems to be reviewed and any concurrent or past diagnoses documented include but are not limited to: eyes, ears, nose, and throat; respiratory; cardiovascular; gastrointestinal; musculoskeletal; neurological; endocrine/metabolic; hematologic/lymphatic; dermatological; genitourinary; psychiatric and allergies. In addition, the patient's date of birth, gender, race, and ethnicity should be documented. If serum CMV testing was not done and/or results are not available, a screening consent will be obtained and CMV testing will be performed.

12.3 *Physical and Neurological Examination*

Physical examinations are to be completed by the investigator or designated participating clinician. The minimum body systems to be examined for any abnormalities and symptoms include: general appearance; skin; lymphatic; head, eyes, ears, nose, throat; extremities; respiratory; cardiovascular; abdominal; musculoskeletal; neurological and genitourinary.

General neurological assessments are to be completed by the investigator or designated participating clinician. The assessment includes but it not limited to: a general

[13] During vaccine 1: just prior to Component A, 1 and 2 hours after Component A (~20 mL, 1 EDTA lavender top tube and 1 serum red top tube at each blood draw). Just prior to vaccine 2 (1 EDTA lavender top tube, 1 serum red top tube, and 6 yellow ACD tubes). During vaccine 2: 1 and 2 hours after Component A (~20 mL, 1 EDTA lavender top tube and 1 serum red top tube at each blood draw). Just prior to vaccine 3 (9 yellow ACD tubes).
[14] Prior to Td pre-conditioning (~90 mL, 9 yellow ACD tubes and 2 red serum tubes)
[15] At the clinic visit 2 weeks after vaccine 3 [+3 days] (~90 mL, 9 yellow ACD tubes and 2 red serum tubes)
[16] Prior to vaccines #4 and #6 (~90 mL, 9 yellow ACD tubes and 2 red serum tubes)
[17] Immune monitoring blood and pathology testing performed at progression, if possible
[18] Vital signs will be measured at all protocol-indicated visits before and approximately every 30 minutes after vaccination during the post-vaccine monitoring times described in Sections 11.4.2 and 12.6, until discharged from the clinic. Measurements to be reported include temperature in Celsius or Fahrenheit, pulse (beats per minute), respiratory rate (breaths per minute) and blood pressure.
[19] MRI at prior to TMZ cycle 2 clinic visit (within 2 weeks +3 days after vaccine 3)
[20] MRI visits take place at the vaccine visit that coincides with the end of TMZ/vaccine cycles 1, 3, 5, 7, 9, 11 and then after every other vaccine visit during the post-TMZ period.
[21] Pathology testing is performed per BTC standard of care practice and is obtained at the time of new patient evaluation
[22] Adverse events and con meds will be collected throughout the study starting with the post-XRT/TMZ screening visit.

mental status examination (orientation, memory, attention/concentration, and language), assessment of cranial nerves, motor, coordination and gait, reflexes, and sensory evaluation.

12.4 *Karnofsky Performance Status Score*

Patients will all be graded according to the Karnofsky performance scale at enrollment and recorded in the electronic medical record.

12.5 *Curran Group Status*

Patients will be assessed for baseline Curran Group Status of I-IV at Screening (see Appendices for Curran and Eligibility Forms).

12.6 *Vital Signs*

Vital signs will be measured at all protocol-indicated visits before and approximately every 30 minutes after each vaccination and documented in the patient's electronic medical record. Patients will be monitored for approximately 4 hours after vaccines 1 and 2 for a total of 8 sets of vital signs. This monitoring time can be reduced to approximately 2 hours, for a total of 4 vital signs, after vaccine #3, if the patient experiences no reactions to the first 2 vaccines, as long as the reason for no reactions is not pre-medication with oral prednisone. If no reaction(s) occur after vaccine #3, patients will be monitored for 30 minutes with one set of vital signs for subsequent vaccines, as long as the reason for no reactions is not pre-medication with oral prednisone. If a patient experiences a post-vaccine reaction at any point, the monitoring period will revert to 4 hours until such time as the patient has 2 consecutive vaccines with no reaction. The PI or sub-investigator may extend monitoring periods at any given visit if they feel it is clinically indicated for the patient's safety. Each ~30 minute interval will be counted from the time of the previous vitals measurement. It will not be considered a deviation if the vitals are obtained within 10 minutes of the 30 minute interval. Measurements to be reported include temperature in Celsius or Fahrenheit, pulse (beats per minute), respiratory rate (breaths per minute) and blood pressure.

12.7 *Clinical Laboratory Assessments*

Whole blood samples will be collected and submitted to the institution's local laboratory according to the institution's standard procedures. Standard laboratory procedures and institutional guidelines will be followed to analyze and report findings for hematology, coagulation times and serum biochemistry.

Normal ranges with the units of measure for all required parameters will be provided to the study sponsor by the local laboratory conducting the clinical laboratory analysis. All results may be reported in conventional units if this is the typical clinic report method for the local laboratory; otherwise, the International System of units may be used.

12.8 *Measurement of Radiographic Response*

Patients with newly-diagnosed GBM will be imaged by MRI as per standard of care for eligibility and baseline measurements, to assess progression after vaccine 3 (at the TMZ cycle 2 initiation visit, if applicable) and then approximately every 8 weeks (or every other vaccine), coinciding with every 2 cycles of TMZ, if applicable (i.e., end of cycles 3, 5, 7, 9, 11). Although the purpose of this study is not to detect tumor responses, any evidence of

tumor response will be determined according to the Duke PRTBTC SOP (see Appendix D – SOPs and FORMs). RANO criteria [111] will be used for overall assessment of tumor response and 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. If tissue is obtained, it will be used to confirm tumor progression histologically and to assess immunologic cell infiltration and antigen escape using IHC. Upon progression, patients will be taken off study treatment and may be treated on other therapies as directed by the treating oncologist.

13 IMMUNOLOGICAL RESPONSE AND SAFETY EVALUATIONS

Immunological response will be evaluated from peripheral blood obtained prior to Td pre-conditioning, during vaccine #1 (just prior to Component A, 1 and 2 hours after Component A), during vaccine #2 (just prior to Component A, 1 and 2 hours after Component A), prior to vaccine #3, after vaccine #3 (at the clinic visit prior to starting the 2nd cycle of TMZ), prior to vaccines #4 and #6, and at progression (if feasible). Patients seen by the Duke neuro-oncology team between these vaccine visits may have blood drawn for immune monitoring at the discretion of the study team. The total amount of blood required for this purpose will be ~90 mL, blood draw amounts for the other time points mentioned above are described in Table 3. A comparison of pre-therapy lymphocyte functions and antibody levels to those at intervals after each immunization will be made. These tests may provide evidence for the development of immune responses following PEP-CMV immunization and will play an important role in the design of future clinical trials.

13.1 *Serum Sample and PBMC Collection, Processing and Storage*

For cellular immune assays, PBMC will be separated from blood at the clinical site within 8 h of blood draw, using a standard SOP, frozen to -70 ˚C at -1 ˚C/min (“Mr. Frosty” freezing container, Thermo Fisher Scientific, Rochester, NY) and stored in liquid nitrogen. For detection of antibodies, serum will be separated from whole blood in a red top tube using a standard SOP and aliquoted at 0.2 mL per tube and stored at -135˚C.

13.2 *Enzyme-Linked Immunospot (ELISPOT) Assay*

The ELISPOT assay, our primary assay to detect cellular immune responses, is a sensitive detection assay for evaluation of antigen-specific cytokine producing T-cells. The IFN-γ ELISPOT allows for the direct visualization of human γ -interferon cytokine release from a single cell, which has been widely reported to be an indicator of activation of an antigen-specific immune response. Testing will include, but not be limited to, interferon gamma detection.

On the day of testing, PBMC will be thawed quickly, washed, resuspended in R-10 medium and cell counts and viability measured by Guava Counter (Guava Technologies, Inc., Hayward, CA). Cells will then be rested overnight at 37˚C, 5% CO_2, washed, and cell counts and viability measured by Guava Counter. PBMCs (250,000/well) will be stimulated overnight with synthetic peptide pp65 in Component A of the PEP-CMV vaccine. Aliquots of the peptide will be resuspended to a final concentration of 1 µg/mL for each peptide. Each assay will include PBMC cultured with no peptide or PHA (2.5 µg/mL, 0.25 ug/mL) and positive and negative control PBMC for each antigen if available. PBMC are added to duplicate wells of 96-well ELISPOT assay plates coated with mouse IgG_1 anti-human IFN-

γ monoclonal antibody (MAb) will be incubated overnight at 37 °C, 5% CO_2, washed with PBS/Tween 20, incubated with biotinylated mouse IgG_1 anti-human IFN-γ MAb for 1 h at room temperature, washed with PBS, incubated with avidin-peroxidase complex for 1 h at room temperature, washed, incubated with substrate (3-amino-9-ethylcarbazole) for 4 min at room temperature and spot development stopped by distilled water rinse. Plates will be dried and shipped to Zellnet Consulting (New York, NY) for spot enumeration by automated analysis with a Zeiss KS ELISPOT system. Results will be expressed as the mean spot-forming cells (SFC)/10^6 PBMC after subtraction of counts from cells cultured with no peptide. If a response above background is detected to the peptide, responses will be measured by polyfunctional T cell assay to the pp65 peptide and to the gB peptide depending on availability of cells.

13.3 *TReg Levels*

T_{Regs} levels will be determined from PBMCs collected from blood obtained prior to starting post-RT TMZ therapy[114], then prior to Td pre-conditioning, at the clinic visit prior to 2nd cycle of TMZ, if applicable, (~2 weeks after vaccine #3), prior to vaccines 4, 6, and at progression, as described by us previously[114], using combinations of titrated antibodies against CD4 (RPA-T4), CD8 (RPA-T8), CD127 (IL-2r), and CD25 (BD Biosciences, San Jose, California). Following incubation, fixation/permeabilization buffer (eBioscience) is added to each sample. Cells are then washed in 1X permeabilization buffer (eBioscience), pelleted, and stained with foxp3-APC (e Bioscience, clone PCH101) for 30 minutes in the dark at 4°C in the presence of Permeabilization Buffer (eBioscience). Samples are washed and analyzed on a FACSCaliber LSRII Fortessa flow cytometer (BD Biosciences). Data analysis will be performed using BD FloJo software. CD25+foxp3+ will be gated from CD4+ lymphocytes to enumerate T_{Reg} lymphocytes.

13.4 *Polyfunctional Analysis*

Using our tiered approach to immune monitoring, if a CMV pp65 specific immune response is detectable by ELISpot assay, a polyfunctional analysis of 10-12 markers of T-cell phenotype and function will be performed. PBMCs will be stained for the surface markers CD3 (to identify it as a T cell), CD4 (to define as specific helper cell), CD8 (to define antigen specific cytotoxic T cells). The maturation state and the activation status of samples will be detected by polychromatic flow cytometry using optimized panels which will include CCR7, CD45RA, HLA-DR, and CD69. Levels of naïve (TN – CD45RA+CCR7+), central memory (TCM – CD45RA-CCR7+), effector memory (TEM – CD45RA-CCR7-), or terminally differentiated effector memory (TEMRA – CD45RA+CCR7+). To determine T-cell reactivity to *the vaccine* we will include antibodies specific against the intracellular cytokines IFN-γ, TNF-a, IL-2 and CCL3, CD107a along with a vital-dye reagent. Briefly, cryopreserved PBMC samples will be thawed and rested overnight at 37^0C/5% CO_2 in RPMI media containing 10% fetal calf serum. Cells are adjusted to 2x10^6/well with or without stimulation with a mixture of the peptides to Component A in the vaccine in the presence of Brefeldin A (5 µg/ml Sigma-Aldrich, St. Louis, MO) monensin (1µg/ml; Golgistop, BD Biosciences, San Diego, CA), and antibodies against CCL3 and CD107a for 5-6 hr at 37^0C and 5% CO2. Following stimulation, cells will be treated with EDTA for 15 minutes at ambient temperature (AT, 18-22ºC). The cells will be washed, and stained with MAbs specific for CD4, CD8, CCR7, CD45RA, and HLA-DR. and a vital-dye reagent (LIVE/DEAD) for 20 minutes at AT. After two washes, 1x BD FACS Lysing solution (BD Biosciences, San Jose, CA) will be added and samples will be incubated for 10 minutes at AT. After one wash, 1x BD FACS Permeabilizing Solution 2 (BD Biosciences, San Jose, CA) will be added and samples incubated for 10 minutes at AT. After one wash, cells will

be stained with CD3, CD69, IFN-γ, TNF-α, and IL-2 for 30 minutes on ice, washed, and fixed in PBS containing 1% formaldehyde (Sigma-Aldrich, St. Louis, MO). In all experiments, a negative control (cells alone), and a positive control (SEB, 10 μg/ml, Sigma-Aldrich) will be included. The samples are acquired on a custom LSRII polychromatic flow cytometer (BD Immunocytometry System, San Jose, CA) equipped for detection of 17 fluorescent parameters. We are planning to collect a minimum of 500,000 total lymphocytes from each sample, because we expect the frequency of responding cells to be between 0.05 and 1.0%. This number of events is required based on calculations performed by Dr. Holden Maecker (BD Bioscience, personal communication) to detect a statistically significant number of positive events that can be used for the analysis of the data and the characterization of the different populations.

13.5 *Tetramer Analysis*

CMV pp65- conjugated tetramers will be available (Beckman Coulter) that cover immunodominant CD8+ T-cell epitopes from HLA haplotypes A*0201, A*0101, A*2402, B*0702, B*3501, and B*0801. PBMC from patients with GBM will be labeled with CD8-FITC (BD Bioscience) and CD3-APC (BD Bioscience) in conjunction with the appropriate PE-conjugated CMVpp65 or HIV gag tetramers for 30 minutes in dark. Cells will be incubated with FACS Lyse (BD Bioscience) for 30 minutes in the dark, washed, and analyzed on a BD FACS Calibur. A minimum of 30,000 CD3+CD8+ events will be collected and sample probe will be rinsed between samples to avoid carry-over.

14 SAFETY MONITORING

14.1 *Potential Benefits*

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

Most recently, we examined immune data on sixteen study participants who had received vaccine 1 and had been batch assayed. These data indicate that PEP-CMV vaccination is inducing the activation of T cells specific for the target antigen pp65. Likely, this is a result of vaccine potency against CMV.

14.2 *Potential Risks*

14.2.1 Allergic Reactions to PEP-CMV Immunization

Injection of the PEP-CMV vaccine may result in an allergic reaction, which could include redness and swelling at the injection site, itching, hives, low blood pressure, difficulty breathing, or in the most extreme circumstances, death. In addition, if the immune system becomes overly activated, potential discomforts locally may include pain, redness and swelling at the injection site. Grade 3 vaccine reactions have been experienced by patients

who have received the PEP-CMV vaccine in this study and include hypotension, flu-like symptoms, and lactic acidosis. Patients also have experienced Grade 1 and 2 vaccine reactions, such as fever, chills, headache, sinus tachycardia, nausea, and dehydration. Our recent analysis of patients with AEs on this study indicates that pp65-specific T cell activation may be inducing a temporary elevation of pro-inflammatory cytokines. We believe that the vaccine reactions we have been observing in PERFORMANCE are likely indicative of vaccine potency. Patients who have demonstrable Grade III reactions also have measurable cellular responses to the PEP-CMV vaccines, which may be indicative of clinical responses.

14.2.2 Injection Site

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

14.2.3 Cerebral Edema

Cerebral edema may be secondary to the disease process itself, the surgical procedure, necrosis from previous radiation, or inflammation due to immune infiltration of the brain or destruction of tumor cells. Symptoms may include, but are not limited to, severe headache, confusion, lethargy, unresponsiveness, coma, or focal neurological deficits. Patients will be monitored throughout the course of the study and those patients with any signs or symptoms of cerebral edema may need their steroid doses increased, treatment with an osmotic diuretic, or surgical decompression. Edema that fails to respond to aggressive therapy may lead to permanent neurological impairment. The probability of this risk can be predicted to some degree based upon tumor size, location, pre-operative neurological impairment, and post-operative course prior to PEP-CMV injections. Patients will be monitored throughout the course of the study.

14.2.4 Steroid Induced Adrenal Suppression

It is widely recognized that many brain tumor patients will suffer from steroid induced adrenal suppression. This is often related to chronic systemic therapy with dexamethasone on a daily or twice daily regimen.

It is well supported that the best predictor of adrenal suppression is the patient's current glucocorticoid dosage. Patients who receive doses of dexamethasone of more than 2 mg per day for periods of more than three weeks and those who appear to have cushingoid features are highly likely to have adrenal suppression.

As patients suffering from adrenal suppression are at risk of acute adrenal crisis in association with an acute medical event such as allergic reactions, it is strongly recommended that patients on this study receive physiologic replacement hydrocortisone therapy (20-30 mg/day in divided doses) if a wean of dexamethasone is considered indicated.

14.2.5 Infection

The PEP-CMV injections may include the risk of infection due to potential contamination of the peptides in the laboratory. This may result in localized redness, swelling, or induration at the injection site. In the most extreme situation, this may lead to systemic bacterial/fungal sepsis and possibly death. The probability of this risk is relatively low, given the small injection volume (1 mL divided between >2 intradermal locations) and the

fact that the peptides will be strictly tested for sterility prior to each injection. The risk of infection due to potential contamination of the peptides in the laboratory will be minimized by biosafety quality assurance and testing. All cell cultures will be handled under sterile conditions in a core tissue culture facility dedicated to the processing of human cells. Prior to injection into patients, peptides must pass sterility tests in thiglycolate broth, tryptic soy blood agar, and inhibitory Sabouraud agar. Following injections, patients will be monitored throughout the course of the study for any signs and symptoms of infection. There have been no infections to date in the recent VICTORI (IRB #3108-05-9R4) or ACTIVATE (IRB #5421-05-1R1) clinical trials testing similar approaches in a similar patient population. If an active infection is suspected, patients will be cultured and treated with appropriate antibiotics.

14.2.6 Delayed Autoimmune Diseases

It is possible that delayed autoimmune disease(s) may develop as a result of injection with PEP-CMV. This means that the immune system may be stimulated to attack natural tissue in the body. Animal studies have reported the development of autoimmunity in the context of vaccination and recovery from lymphopenia. However, our current experience with peptide vaccination in glioma patients has not demonstrated evidence of autoimmunity in treated patients. Furthermore, the doses of TMZ used in this study for induction of lymphopenia are standard doses administered to patients with GBM. It, therefore, is unknown what the risk of delayed autoimmune disease is for this study.

14.2.7 Phlebotomy

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

14.2.8 MRI

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

14.2.9 Allergic Reactions to Contrast Agents

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

Patients with mild allergies (i.e., rash only) will be pretreated with Tylenol and Benadryl prior to injection of the contrast agent.

14.2.10 Temozolomide

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

14.2.11 Allergic Reactions to KLH

Injection of KLH (applies only to patients who received Component B) may result in an allergic reaction, which could include redness and swelling at the injection site, itching, hives, low blood pressure, difficulty breathing, or in the most extreme circumstances, death. In addition, if the immune system becomes overly activated, potential discomforts may include pain, redness and swelling at the injection site.

14.2.12 GM-CSF

Injection of GM-CSF (applies only to patients who received Component B) may increase the risk of infection, lower platelets, or cause fluid retention. GM-CSF also may result in an allergic reaction, which could include redness and swelling at the injection site, itching, hives, flushing, syncope, low blood pressure, difficulty breathing, or in the most extreme circumstances, death. In addition, if the immune system becomes overly activated, potential discomforts may include pain, redness and swelling at the injection site.

14.2.13 Montanide ISA 51

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

14.2.14 Td Risks

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

14.2.15 Unknown Risks

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

14.2.16 Confidentiality

Participation in research investigations may result in a loss of confidentiality. However, all data from preoperative and postoperative evaluations will be coded to protect the

patient's identity. The coding, and the results of these studies will be available only to the individuals involved with the study, the clinical staff administering the study, representatives of the National Institutes of Health, and representatives of the U.S. Food and Drug Administration. Any publications resulting from this study will not use patient identifying data.

14.2.17 Treatment Alternatives & Financial Reimbursement

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

14.3 *Data and Safety Monitoring Plan*

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 operative 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.

The Safety Oversight Committee (SOC) will perform annual reviews on findings from the DCI Monitoring Team visit and additional safety and toxicity data submitted by the Principal Investigator.

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 CPC, the Safety Oversight Committee (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.

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

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

15 STATISTICAL CONSIDERATIONS

15.1 *Study Design and Conduct*

A total of 70 patients may be consented on the main informed consent, in order to randomize a sufficient number of patients with newly diagnosed GBM who completed standard of care radiotherapy with temozolomide into one of two treatment arms. Patients will receive 3 bi-weekly (every other week) vaccinations during the first cycle of either: (Arm 1) 5-day standard of care temozolomide at 150-200 mg/m^2/day, or (Arm 2) 21-day temozolomide at 75-100 mg/m^2/day post chemo-radiation (please refer to study overview for timing of vaccine administration). We will target the treatment of 26 patients (13 per arm) who are evaluable for the primary immune endpoint. All patients will continue to receive PEP-CMV vaccine as described in the Treatment Plan at 4 (+2) week intervals for up to 20 vaccines, these vaccine administrations will coincide with TMZ cycles for those who are receiving TMZ.

With the 20190312 protocol amendment, the study's primary analysis has focused on patients who receive Component A only. Hence, we target the treatment of 26 patients (13 per arm) who received only Component A and are evaluable for the immune endpoints.

Once 6 subjects have been accrued to each arm of this study and the last patient has received the third vaccination and has been followed for approximately 2 weeks, toxicities up to that point will be formally monitored. Further accrual will be dependent upon the prevalence of unacceptable toxicity within each arm. See Section 15.4 for further details of monitoring guidelines.

Following the safety events of vaccine reactions that were initially reported on 20170407, the study was amended to investigate, in a separate unrandomized drug safety cohort of 6 patients, the etiology of these events. Within this cohort, patients were consented to receive study vaccine with standard of care (SOC) 5-day TMZ at 150-200 mg/m^2/d for 28-day cycles with various sequences of vaccine components A and B. Details of this safety assessment plan are provided in Section 11.4.3. Upon completion of this unrandomized portion of the study, the randomized study was allowed to reinitiate with changes to vaccine administration procedures based on observations made in the safety cohort (protocol v. 20180719). Subsequently, Component B was removed from the vaccine for future patients due to problems with availability and supply (protocol v.20181218). On 20190130, Component B was removed from the vaccine for all patients (current and future) due to two new safety events observed and reported in January 2019.

As of 20190312, 13 patients have been enrolled on the study:

Periods of Study Conduct	Patient Enrollment Description as of 20190312
Initial – Randomized	3 patients randomized and treated with 1-2 vaccinations that included Component A and B as originally planned before study suspension: • 2 patients assigned to standard TMZ on days 1-5 • 1 patient assigned to dose-intensified TMZ on days 1-21
Safety Cohort (See Section 11.4.3)	6 patients treated with standard TMZ with different sequences of Component A and B
Resumed – Randomized	Once randomization resumed, the 1st 4 patients randomized:

	• 2 patients assigned to standard TMZ with vaccine that included Component B; Component B was dropped for these 2 patients due to safety events (one patient received only one vaccine containing Component B, and the other patient received 3 vaccines containing Component B). • 2 patients assigned to dose-intensified TMZ – Component B was dropped before these patients started treatment; hence, only Component A was part of vaccine. For all subsequent patients enrolled, the vaccine includes Component A only.

As of protocol version 20190312, the primary analysis of this study will focus on only those patients who receive Component A alone.

As of 20200110, 15 patients have received only Component A of which 8 are currently evaluable for the immune response primary endpoint.

15.2 *Sample Size Justification for Immunologic Response Outcome*

Of primary interest is a comparison of the 2 arms with respect to the peak immune response (ELISPOT) in the immune monitoring blood that will be collected as described in the treatment plan. A patient will be considered evaluable and included in these analyses if the patient had their immune monitoring blood work drawn prior to initiation of post-RT TMZ cycle 2, if applicable.

Given that humoral responses are known to be surrogates for cellular immune responses (ELISPOT), we will use antibody titer data from the ACT II study, a study conducted at Duke, to estimate an appropriate sample size for pairwise comparison of peak immune response (ELISPOT). The ACT II dataset provides peak antibody titer to EGFRvIII data for 5 patients in arm A and 10 patients in arm B. Given that the distribution of peak titers in the two arms is not normally distributed, resampling methods were used to estimate the power of a comparison using a one-tailed Wilcoxon rank sum test. The median peak titer (and range) in arm A and B were 51200 (2560 – 204800) and 385000 (20480, 20480000) respectively. Each of 10000 simulations for a fixed sample size per arm involved randomly sampling with replacement the peak antibody data separately within arm A and B, and then computing the Wilcoxon rank sum test. The percentage of tests that was statistically significant estimated the power of the statistical test. With 2 primary endpoints, power calculations assumed a type I error rate of 0.025. With 13 evaluable patients in each arm, there is 81% to detect a difference similar to that observed in ACT II. In order that there are 26 evaluable patients, we anticipate a need to consent up to 70 patients on the main informed consent form.

With the 20190312 protocol amendment, the study has focused on patients who receive Component A only. Hence, we target the treatment of 26 patients who have received only Component A and are evaluable for the assessment of immunologic response. As of 20200110, 15 patients have received only Component A of which 8 are evaluable for immune response.

15.3 *Toxicity Monitoring*

After the 20190312 protocol amendment, only the monitoring described below in the section entitled “Overall Adverse Event Monitoring” is applicable.

Initial Study Monitoring for Adverse Events: Once 6 subjects have been accrued to each arm of this study and the last patient has received the third vaccination and has been followed for approximately 2 weeks, toxicities up to that point will be formally monitored. A patient who does not complete this follow-up period without experiencing an unacceptable toxicity will be replaced for this initial monitoring of adverse events. If 1 or less of the 6 subjects within each arm experience an unacceptable toxicity, accrual will continue. Otherwise, if 2 or more of the 6 subjects within an arm experience unacceptable toxicity, the study will be carefully reviewed to determine if modifications to the protocol or treatment plan need to occur.

Study Drug Safety Arm Monitoring for Hypersensitivity Reactions (not applicable for patients enrolled on protocol version 6/12/18 or later): For the *study drug safety arm* (Section 11.4.3), unacceptable toxicity is defined as any hypersensitivity reaction within 6 hours after vaccine reaction that requires intervention to prevent life threatening symptoms, according to the PI and the study team. The PI will review the reaction to determine if it is unacceptable.

Corrective Action for Hypersensitivity Reactions in Study Drug Safety Arm (not applicable for patients enrolled on protocol version 6/12/18 or later): If 2 out of 3 Schedule A patients experience hypersensitivity reactions after Component A injection (Vaccine #1), the study will be put on hold in order to evaluate potential modifications to Component A to increase patient safety.

If 2 out of 3 Schedule A patients experiences hypersensitivity reactions after Component B injection (Vaccine #2), we will discontinue the use of Component B and continue all further vaccinations with Component A alone.

If 2 out of 3 Schedule B patients experience hypersensitivity reactions after Component B injection (Vaccine #1), but Schedule A patients have not experienced hypersensitivity reactions after Vaccine #1, we will discontinue the use of Component B in Schedule B patients and continue all further vaccinations with Component A alone.

If 2 out of 3 Schedule B patients experience hypersensitivity reactions after Component A injection during Vaccine #2, but Schedule A patients have not experienced hypersensitivity reactions after Vaccine #1, Schedule B patients will discontinue the use of Component B and continue all further vaccinations with Component A alone.

If 2 out of 3 patients from both Schedule A and B experience hypersensitivity reactions after Vaccine #1, the study will be put on hold and the study team will work on modifying the study drug to decrease the hypersensitivity reaction.

Overall Adverse Event Monitoring: The prevalence of unacceptable toxicities occurring during the initial 3 bi-weekly vaccinations administered concurrently with temozolomide will be monitored. If more than 25% of accrued patients experience unacceptable toxicities, then accrual will be suspended and reported toxicity will be carefully reviewed to determine

if accrual should be terminated or modifications to the protocol treatment should occur. This review will consider that patients will have different lengths of follow-up and duration of vaccination treatment.

As described in Section 11.5.3, the definition of unacceptable toxicity has been modified with v.20200117 of the protocol, and now excludes vaccine reactions indicative of immune response. Therefore, as of the time of this amendment, no patient had experienced an unacceptable toxicity under the new definition.

15.4 *Analytic Methods*

15.4.1 Primary Analyses

Safety: To assess the safety of PEP-CMV vaccination in combination with adjuvant TMZ, the percentage of patients with unacceptable toxicity will be estimated within each arm. All patients who received any PEP-CMV vaccine will be included in these safety analyses.

As described in Section 15.1, the vaccination treatment regimen has been modified over time due to safety issues. Hence, toxicity will be summarized for each period of study conduct as follows:

- Initial randomization period where patients were assigned to one of two TMZ regimens with both Component A and B. The 2 patients assigned to standard TMZ after randomization resumed who received both Component A and B will also be included in this summary.
- Safety Cohort Period in which all patients received standard TMZ with vaccine Component A and B
- Randomization period in which patients received at least one cycle of Component B prior to discontinuation
- Randomization period in which patients are assigned to one of two TMZ regimens and receive only vaccine Component A

Immunologic Response: The primary analysis will focus on patients who have follow-up immunologic monitoring after the 3rd vaccination and before initiation of the second TMZ/vaccine cycle. Such a patient is considered “evaluable” for the immunologic response primary analyses.

The Wilcoxon rank sum test will compare treatment groups with regard to the median peak number of T cells that secrete IFNγ by ELISPOT in response to component A of PEP-CMV. Analyses will include only those patients who have an assessment of immune response after receiving 3 vaccinations.

Though the original vaccination included Component A and B, the primary immunologic analysis will focus on only those patients who received Component A alone. Additional exploratory analyses may include data from the few “evaluable” patients who received both vaccine Components A and B.

15.4.2 Secondary Analyses

CMV Status at Recurrence: Among patients who provide pathology at the time of tumor recurrence, a chi-square test will be used to compare treatment arms with respect to the

percentage of patients observed to be positive for antigen escape outgrowth at tumor recurrence. A paired t-test will be used to compare within each treatment arm the mean intensity of CMV antigen expression for primary and recurrent tumors. Alternative nonparametric methods may be considered if assumptions for parametric analytic methods are not satisfied. Though the original vaccination included Component A and B, the primary analysis of CMV status at recurrence will focus on only those patients who received Component A alone. However, additional exploratory analyses may include data from the few patients who received both vaccine Components A and B for whom we have info about CMV status at recurrence.

15.4.3 Exploratory Immunologic Analyses

The following immunologic analyses will focus on patients who are evaluable for the immunologic monitoring primary analysis, i.e., patients who had immunologic monitoring conducted at baseline and prior to the second cycle of TMZ. These analyses will focus primarily upon patients who received only Component A. However, additional analyses may be conducted besides those described below.

Quality of Immune Response: The quality of the immune response will be assessed by polyfunctional flow cytometry. The analysis used here for polyfunctionality will be one that has been successful at distinguishing HIV progressors and non-progressors based on T-cell phenotype [115, 116]. All data will be background subtracted. For each measure, a lower threshold corresponding to two standard deviations above background is set to 0 based on a Poisson model essentially allowing T-cells to be designated as positive or negative for a certain phenotypic marker. The number of positive phenotypic markers post-vaccination will be calculated for each patient. The Kruskal-Wallis test will be used to compare treatment arms with respect to the number of phenotypic markers observed post-vaccination 3, as well as at vaccination 6. Additional exploratory analyses will be conducted using a Fisher's exact test that will compare treatment groups with respect to the proportion of patients with 5 positive markers, and 4 or more positive markers. Analyses similar to those described for the primary immunologic analyses will be used to examine the percentage of tetramer positive cells.

Analysis of covariance will be used to compare treatment groups with respect to the change between baseline and post-vaccination time-points in mean proliferative response and cytokine production, with baseline levels included in the model as a covariate. If assumptions of normality are not satisfied, the Kruskal-Wallis test will be used to compare treatment arms. Analyses will focus on the change between baseline and post-vaccination 3 assessments, as well as the change between baseline and vaccine 6.

Effect on Tregs: The mean levels of Tregs at baseline and at each follow-up assessment will be estimated within each treatment group. A mixed model that accounts for the correlation of repeated measurements within a patient will be used to compare treatment groups with respect to changes in Treg levels over time.

Effect of HLA haplotypes: Initially the distribution of HLA haplotypes (A, B, C, DR) will be described. Depending up the distribution of these haplotypes, the effect of HLA haplotype on outcome may be explored using the proportional hazards model (for OS and PFS) or linear regression (for some immune response outcomes). Analyses will adjust for treatment group. If possible, analyses will appropriately adjust for confounding variables.

Immunologic Cell Infiltrate: Within each treatment arm, changes in levels of infiltrating lymphocytes detected in primary and recurrent tumor specimens will be assessed using a paired t-test or its nonparametric analogue among patients who provide pathology at the time of tumor recurrence. When appropriate, a two-sample t-test will compare arms with respect to the change.

15.4.4 Other Exploratory Analyses

Progression-Free Survival and Overall Survival: The Kaplan-Meier estimator will be used to describe the progression-free survival (PFS) and overall survival (OS) experience of patients in each treatment group. These analyses will focus on those patients who have received at least 3 vaccines. PFS is defined as the time between the third vaccine and first documentation of death or disease progression/recurrence. Patients remaining alive without disease progression will have PFS censored at their last follow-up. OS is defined as time between the third vaccine and death. It should be noted that these analyses will focus on only those patients who received Component A alone as we have with other analyses. Additional analyses will describe OS and PFS from randomization.

Radiographic Response: Among the subset of patients with residual disease, the proportion of patients with radiographic response will be estimated with 95% confidence interval.

16 DATA MANAGEMENT AND PROCESSING

16.1 *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.

16.2 *Data Management*

The subject’s medical records will be the primary source document for the study. Source documents include all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical investigation used for reconstructing and evaluating the investigation. Source documentations may also include paper eligibility checklists, data flowsheets, patient reported outcomes and other

paper documents. The PI, the study coordinator, the data management team, and the clinical trials manager 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.

16.3 *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.

16.4 *Coding*

All medical terms will be coded using CTCAE (version 4.03).

16.5 *Study Closure*

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

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

17 ADMINISTRATIVE AND ETHICAL CONCERNS

17.1 *Regulatory and Ethical Compliance*

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

17.2 *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.

17.3 *Informed Consent*

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

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

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

17.4 *Privacy, Confidentiality, and Data Storage*

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

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

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by

a unique study number and subject initials. Electronic records of subject data will be maintained using an electronic database, which is housed by the DCI. Access to electronic databases will be limited to the Principal Investigator, key personnel, statisticians, 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.

17.5 *Data and Safety Monitoring*

Data and Safety Monitoring will be performed in accordance with the DCI Data and Safety Monitoring Plan. The DCI Safety Oversight Committee (SOC) is responsible for annual data and safety monitoring of DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent DSMB. The primary focus of the SOC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. For a more detailed description of the DSMP for this protocol, refer to Section 16.

17.6 *Protocol Amendments*

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

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

17.7 *Records Retention*

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

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

17.8 *Conflict of Interest*

The Principal Investigator and Sub-Investigators must comply with applicable federal, state, and local regulations regarding reporting and disclosure of conflict of interest. Conflicts of interest may arise from situations in which financial or other personal considerations have the potential to compromise or bias professional judgment and objectivity. Conflicts of interest include but are not limited to royalty or consulting fees,

speaking honoraria, advisory board appointments, publicly-traded or privately-held equities, stock options, intellectual property, and gifts.

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

Due to potential for COI in relation to proprietary interest in the PEP-CMV vaccine, a Data Safety and Monitoring Board (DSMBplus) will be established. Please see Appendix C – DSMBplus Charter for detail on the Duke PRTBTC DSMBplus Charter.

17.9 *Registration Procedure*

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

18 REFERENCES

1. Imperato JP, Paleologos NA, Vick NA. Effects of treatment on long-term survivors with malignant astrocytomas. Annals of Neurology. 1990;28(6):818-22.
2. Papadopoulos EB, Ladanyi M, Emanuel D, Mackinnon S, Boulad F, Carabasi MH, et al. Infusions Of Donor Leukocytes To Treat Epstein-Barr Virus-Associated Lymphoproliferative Disorders After Allogeneic Bone Marrow Transplantation. New Engl J Med. 1994;330(17):1185-91.
3. Emanuel DJ. Uses of immunotherapy for control of human cytomegalovirus-associated diseases. Transplantation Proceedings. 1991;23(3 Suppl 3):144-6.
4. Emanuel DJ, Lucas KG, Mallory GB, Jr., Edwards-Brown MK, Pollok KE, Conrad PD, et al. Treatment of posttransplant lymphoproliferative disease in the central nervous system of a lung transplant recipient using allogeneic leukocytes. Transplantation. 1997;63(11):1691-4. PubMed PMID: 9197369.
5. Liu Z, Savoldo B, Huls H, Lopez T, Gee A, Wilson J, et al. Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for the prevention and treatment of EBV-associated post-transplant lymphomas. Recent results in cancer research Fortschritte der Krebsforschung Progres dans les recherches sur le cancer. 2002;159:123-33. PubMed PMID: 11785836.
6. Lucas KG, Barrett JC. Adoptive immunotherapy for EBV-associated malignancies. Cancer treatment and research. 1999;101:203-32. PubMed PMID: 10800651.
7. Rooney CM, Smith CA, Ng CY, Loftin SK, Sixbey JW, Gan Y, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood. 1998;92(5):1549-55. PubMed PMID: 9716582.
8. Koutsky LA, Ault KA, Wheeler CM, Brown DR, Barr E, Alvarez FB, et al. A Controlled Trial of a Human Papillomavirus Type 16 Vaccine. New England Journal of Medicine. 2002;347(21):1645-51.
9. Asavaroengchai W, Kotera Y, Mule JJ. Tumor lysate-pulsed dendritic cells can elicit an effective antitumor immune response during early lymphoid recovery. Proc Natl Acad Sci U S A. 2002;99(2):931-6. doi: 10.1073/pnas.022634999. PubMed PMID: 11792864; PubMed Central PMCID: PMC117408.
10. Kelly PJ. Stereotactic resection and its limitations in glial neoplasms. Stereotactic and Functional Neurosurgery. 1992;59(1-4):84-91.
11. Dinapoli RP, Brown LD, Arusell RM, Earle JD, O'Fallon JR, Buckner JC, et al. Phase III comparative evaluation of PCNU and carmustine combined with radiation therapy for high-grade glioma. Journal of Clinical Oncology. 1993;11(7):1316-21.
12. Salford LG, Brun A, Nirfalk S. Ten-year survival among patients with supratentorial astrocytomas grade III and IV. Journal of neurosurgery. 1988;69(4):506-9.
13. Shapiro WR. Therapy of adult malignant brain tumors: what have the clinical trials taught us?. Seminars in oncology. 1986;13(1):38-45.
14. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. New England Journal of Medicine. 2005;352(10):987-96.
15. Walker MD, Alexander E, Jr., Hunt WE, MacCarty CS, Mahaley MS, Jr., Mealey J, Jr., et al. Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. Journal of neurosurgery. 1978;49(3):333-43. doi: 10.3171/jns.1978.49.3.0333. PubMed PMID: 355604.

16. Walker MD, Green SB, Byar DP, Alexander E, Jr, Batzdorf, Brooks WH, et al. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. New England Journal of Medicine. 1980;303(23):1323-9.
17. Hall WA, Fodstad O. Immunotoxins and central nervous system neoplasia. Journal of Neurosurgery. 1992;76(1):1-12.
18. Choi BD, Archer GE, Mitchell DA, Heimberger AB, McLendon RE, Bigner DD, et al. EGFRvIII-targeted vaccination therapy of malignant glioma. Brain Pathol. 2009;19(4):713-23. Epub 2009/09/12. doi: 10.1111/j.1750-3639.2009.00318.x. PubMed PMID: 19744042; PubMed Central PMCID: PMC2846812.
19. Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Bigner DD. Tumor-specific immunotherapy targeting the EGFRvIII mutation in patients with malignant glioma. Seminars in immunology. 2008;20(5):267-75. doi: 10.1016/j.smim.2008.04.001. PubMed PMID: 18539480; PubMed Central PMCID: PMC2633865.
20. Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Herndon JE, 2nd, Lally-Goss D, et al. An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. Molecular cancer therapeutics. 2009;8(10):2773-9. doi: 10.1158/1535-7163.MCT-09-0124. PubMed PMID: 19825799; PubMed Central PMCID: PMC2991139.
21. Schmittling RJ, Archer GE, Mitchell DA, Heimberger A, Pegram C, Herndon JE, 2nd, et al. Detection of humoral response in patients with glioblastoma receiving EGFRvIII-KLH vaccines. J Immunol Methods. 2008;339(1):74-81. PubMed Central PMCID: PMCNIHMS75456.
22. Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH, et al. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Research. 2002;62(12):3347-50.
23. Mitchell DA, Xie W, Schmittling R, Learn C, Friedman A, McLendon RE, et al. Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma. Neuro-oncology. 2008;10(1):10-8. Epub 2007/10/24. doi: 10.1215/15228517-2007-035. PubMed PMID: 17951512; PubMed Central PMCID: PMC2600830.
24. Soderberg-Naucler C. HCMV microinfections in inflammatory diseases and cancer. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 2008;41(3):218-23. doi: 10.1016/j.jcv.2007.11.009. PubMed PMID: 18164235.
25. Scheurer ME, Bondy ML, Aldape KD, Albrecht T, El-Zein R. Detection of human cytomegalovirus in different histological types of gliomas. Acta neuropathologica. 2008;116(1):79-86. PubMed PMID: 18351367.
26. Prins RM, Cloughesy TF, Liau LM. Cytomegalovirus immunity after vaccination with autologous glioblastoma lysate. The New England journal of medicine. 2008;359(5):539-41. doi: 10.1056/NEJMc0804818. PubMed PMID: 18669440; PubMed Central PMCID: PMC2775501.
27. Rafferty KA, Jr. Herpes viruses and cancer. Scientific American. 1973;229(4):26-33. PubMed PMID: 4353788.
28. Kadow JF, Regueiro-Ren A, Weinheimer SP. The role of viruses in human cancer development and antiviral approaches for intervention. Current Opinion in Investigational Drugs. 2002;3(11):1574-9. PubMed PMID: 12476955.

29. Rooney CM, Roskrow MA, Smith CA, Brenner MK, Heslop HE. Immunotherapy for Epstein-Barr virus-associated cancers. J Natl Cancer Inst Monogr. 1998;(23):89-93. PubMed PMID: 9709309.
30. Rooney CM, Heslop HE, Brenner MK. EBV specific CTL: a model for immune therapy. Vox sanguinis. 1998;74 Suppl 2:497-8. PubMed PMID: 9704488.
31. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. The New England journal of medicine. 2009;361(19):1838-47. doi: 10.1056/NEJMoa0810097. PubMed PMID: 19890126.
32. Harkins L, Volk AL, Samanta M, Mikolaenko I, Britt WJ, Bland KI, et al. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet. 2002;360(9345):1557-63. doi: 10.1016/S0140-6736(02)11524-8. PubMed PMID: 12443594.
33. Samanta M, Harkins L, Klemm K, Britt WJ, Cobbs CS. High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. Journal of Urology. 2003;170(3):998-1002. PubMed PMID: 12913758.
34. Doniger J, Muralidhar S, Rosenthal LJ. Human cytomegalovirus and human herpesvirus 6 genes that transform and transactivate. Clinical microbiology reviews. 1999;12(3):367-82. PubMed PMID: 10398670; PubMed Central PMCID: PMC100243.
35. Eizuru Y, Hyman RW, Nahhas WA, Rapp F. Herpesvirus RNA in human urogenital tumors. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine. 1983;174(2):296-301. PubMed PMID: 6314345.
36. Huang ES, Roche JK. Cytomegalovirus D.N.A. and adenocarcinoma of the colon: Evidence for latent viral infection. Lancet. 1978;1(8071):957-60. PubMed PMID: 76890.
37. Hashiro GM, Horikami S, Loh PC. Cytomegalovirus isolations from cell cultures of human adenocarcinomas of the colon. Intervirology. 1979;12(2):84-8. PubMed PMID: 231587.
38. Geder L, Sanford EJ, Rohner TJ, Rapp F. Cytomegalovirus and cancer of the prostate: in vitro transformation of human cells. Cancer treatment reports. 1977;61(2):139-46. PubMed PMID: 68820.
39. Sanford EJ, Geder L, Laychock A, Rohner TJ, Jr., Rapp F. Evidence for the association of cytomegalovirus with carcinoma of the prostate. The Journal of urology. 1977;118(5):789-92. PubMed PMID: 199749.
40. Kleihauer A, Grigoleit U, Hebart H, Moris A, Brossart P, Muhm A, et al. Ex vivo generation of human cytomegalovirus-specific cytotoxic T cells by peptide-pulsed dendritic cells. British journal of haematology. 2001;113(1):231-9.
41. Cho HI, Han H, Kim CC, Kim TG. Generation of Cytotoxic T Lymphocytes Specific for Human Cytomegalovirus Using Dendritic Cells In Vitro. Journal of immunotherapy. 2001;24(3):242-9. PubMed PMID: 11395640.
42. Raftery MJ, Schwab M, Diesner S, Egerer G, Schonrich G. Dendritic cells cross-presenting viral antigens derived from autologous cells as a sensitive tool for visualization of human cytomegalovirus-reactive CD8+ T cells. Transplantation. 2002;73(6):998-1002.
43. Peggs KS, Mackinnon S. Clinical trials with CMV-specific T cells. Cytotherapy. 2002;4(1):21-8. doi: 10.1080/146532402317251491. PubMed PMID: 11953038.

44. Szmania S, Galloway A, Bruorton M, Musk P, Aubert G, Arthur A, et al. Isolation and expansion of cytomegalovirus-specific cytotoxic T lymphocytes to clinical scale from a single blood draw using dendritic cells and HLA-tetramers. Blood. 2001;98(3):505-12.
45. Vancikova Z, Dvorak P. Cytomegalovirus infection in immunocompetent and immunocompromised individuals--a review. Current Drug Targets Immune Endocrine & Metabolic Disorders. 2001;1(2):179-87. PubMed PMID: 12476798.
46. Minamishima Y. Immunoprophylaxis of experimental cytomegalovirus infection. Annales de Microbiologie (Paris). 1977;128(3):399-407. PubMed PMID: 203216.
47. Morello CS, Ye M, Spector DH. Development of a vaccine against murine cytomegalovirus (MCMV), consisting of plasmid DNA and formalin-inactivated MCMV, that provides long-term, complete protection against viral replication. Journal of Virology. 2002;76(10):4822-35. PubMed PMID: 11967299.
48. Cicin-Sain L, Brune W, Bubic I, Jonjic S, Koszinowski UH. Vaccination of mice with bacteria carrying a cloned herpesvirus genome reconstituted in vivo. Journal of Virology. 2003;77(15):8249-55. PubMed PMID: 12857893.
49. Adler SP, Hempfling SH, Starr SE, Plotkin SA, Riddell S. Safety and immunogenicity of the Towne strain cytomegalovirus vaccine. Pediatric Infectious Disease Journal. 1998;17(3):200-6. PubMed PMID: 9535246.
50. Adler SP, Starr SE, Plotkin SA, Hempfling SH, Buis J, Manning ML, et al. Immunity induced by primary human cytomegalovirus infection protects against secondary infection among women of childbearing age. Journal of Infectious Diseases. 1995;171(1):26-32. PubMed PMID: 7798679.
51. Plotkin SA, Higgins R, Kurtz JB, Morris PJ, Campbell DA, Jr., Shope TC, et al. Multicenter trial of Towne strain attenuated virus vaccine in seronegative renal transplant recipients. Transplantation. 1994;58(11):1176-8. PubMed PMID: 7992358.
52. Gonczol E, Ianacone J, Furlini G, Ho W, Plotkin SA. Humoral immune response to cytomegalovirus Towne vaccine strain and to Toledo low-passage strain. Journal of Infectious Diseases. 1989;159(5):851-9. PubMed PMID: 2540246.
53. Sachs GW, Simmons RL, Balfour HH, Jr. Cytomegalovirus vaccine: persistence of humoral immunity following immunization of renal transplant candidates. Vaccine. 1984;2(3):215-8. PubMed PMID: 6099649.
54. Drulak MW, Malinoski FJ, Fuller SA, Stewart SS, Hoskin S, Duliege AM, et al. Vaccination of seropositive subjects with CHIRON CMV gB subunit vaccine combined with MF59 adjuvant for production of CMV immune globulin. Viral Immunology. 2000;13(1):49-56. PubMed PMID: 10733168.
55. Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. New Engl J Med. 1995;333(16):1038-44.
56. Numazaki K, Ikehata M, Yanai S, Umetsu M, Motoya H, Chiba S, et al. Adoptive immunotherapy for interstitial pneumonia associated with cytomegalovirus infection. Clinical Infectious Diseases. 1997;25(5):1246-7.
57. Bigger JE, Tanigawa M, Thomas CA, 3rd, Atherton SS. Protection against murine cytomegalovirus retinitis by adoptive transfer of virus-specific CD8+ T cells. Investigative Ophthalmology & Visual Science. 1999;40(11):2608-13.

58. Harari A, Dutoit V, Cellerai C, Bart PA, Du Pasquier RA, Pantaleo G. Functional signatures of protective antiviral T-cell immunity in human virus infections. Immunological reviews. 2006;211:236-54. doi: 10.1111/j.0105-2896.2006.00395.x. PubMed PMID: 16824132.
59. Freeman GJ, Wherry EJ, Ahmed R, Sharpe AH. Reinvigorating exhausted HIV-specific T cells via PD-1-PD-1 ligand blockade. J Exp Med. 2006;203(10):2223-7. doi: 10.1084/jem.20061800. PubMed PMID: 17000870; PubMed Central PMCID: PMC2118103.
60. Pantaleo G, Harari A. Functional signatures in antiviral T-cell immunity for monitoring virus-associated diseases. Nat Rev Immunol. 2006;6(5):417-23. doi: 10.1038/nri1840. PubMed PMID: 16622477.
61. Harari A, Vallelian F, Meylan PR, Pantaleo G. Functional heterogeneity of memory CD4 T cell responses in different conditions of antigen exposure and persistence. Journal of immunology. 2005;174(2):1037-45. Epub 2005/01/07. PubMed PMID: 15634928.
62. Sun Y, Santra S, Schmitz JE, Roederer M, Letvin NL. Magnitude and quality of vaccine-elicited T-cell responses in the control of immunodeficiency virus replication in rhesus monkeys. J Virol. 2008;82(17):8812-9. doi: 10.1128/JVI.00204-08. PubMed PMID: 18579590; PubMed Central PMCID: PMC2519645.
63. Almeida JR, Price DA, Papagno L, Arkoub ZA, Sauce D, Bornstein E, et al. Superior control of HIV-1 replication by CD8+ T cells is reflected by their avidity, polyfunctionality, and clonal turnover. J Exp Med. 2007;204(10):2473-85. Epub 2007/09/26. doi: 10.1084/jem.20070784. PubMed PMID: 17893201; PubMed Central PMCID: PMC2118466.
64. Douek DC, Betts MR, Brenchley JM, Hill BJ, Ambrozak DR, Ngai KL, et al. A novel approach to the analysis of specificity, clonality, and frequency of HIV-specific T cell responses reveals a potential mechanism for control of viral escape. Journal of immunology. 2002;168(6):3099-104. PubMed PMID: 11884484.
65. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, Abraham J, et al. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood. 2006;107(12):4781-9. Epub 2006/02/10. doi: 10.1182/blood-2005-12-4818. PubMed PMID: 16467198; PubMed Central PMCID: PMC1895811.
66. Betts MR, Price DA, Brenchley JM, Lore K, Guenaga FJ, Smed-Sorensen A, et al. The functional profile of primary human antiviral CD8+ T cell effector activity is dictated by cognate peptide concentration. Journal of immunology. 2004;172(10):6407-17. PubMed PMID: 15128832.
67. Saez-Cirion A, Lacabaratz C, Lambotte O, Versmisse P, Urrutia A, Boufassa F, et al. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection ex vivo and peculiar cytotoxic T lymphocyte activation phenotype. Proc Natl Acad Sci U S A. 2007;104(16):6776-81. doi: 10.1073/pnas.0611244104. PubMed PMID: 17428922; PubMed Central PMCID: PMC1851664.
68. Dudley ME, Wunderlich JR, Robbins PF, Yang JC, Hwu P, Schwartzentruber DJ, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science. 2002;298(5594):850-4. doi: 10.1126/science.1076514. PubMed PMID: 12242449; PubMed Central PMCID: PMC1764179.
69. Dudley ME, Wunderlich JR, Yang JC, Sherry RM, Topalian SL, Restifo NP, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting

chemotherapy for the treatment of patients with refractory metastatic melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2005;23(10):2346-57. doi: 10.1200/JCO.2005.00.240. PubMed PMID: 15800326; PubMed Central PMCID: PMC1475951.

70. Rapoport AP, Stadtmauer EA, Aqui N, Badros A, Cotte J, Chrisley L, et al. Restoration of immunity in lymphopenic individuals with cancer by vaccination and adoptive T-cell transfer. Nature medicine. 2005;11(11):1230-7. doi: 10.1038/nm1310. PubMed PMID: 16227990.

71. Rosenberg SA, Dudley ME. Cancer regression in patients with metastatic melanoma after the transfer of autologous antitumor lymphocytes. Proc Natl Acad Sci U S A. 2004;101 Suppl 2:14639-45. doi: 10.1073/pnas.0405730101. PubMed PMID: 15381769; PubMed Central PMCID: PMC521998.

72. Grossman Z, Paul WE. Self-tolerance: context dependent tuning of T cell antigen recognition. Seminars in immunology. 2000;12(3):197-203; discussion 57-344. PubMed PMID: 10910740.

73. Tanchot C, Rosado MM, Agenes F, Freitas AA, Rocha B. Lymphocyte homeostasis. Seminars in Immunology. 1997;9(6):331-7. PubMed PMID: 9405261.

74. Cho BK, Rao VP, Ge Q, Eisen HN, Chen J. Homeostasis-stimulated proliferation drives naive T cells to differentiate directly into memory T cells. J Exp Med. 2000;192(4):549-56. PubMed PMID: 10952724; PubMed Central PMCID: PMC2193235.

75. Dummer W, Niethammer AG, Baccala R, Lawson BR, Wagner N, Reisfeld RA, et al. T cell homeostatic proliferation elicits effective antitumor autoimmunity.[see comment]. Journal of Clinical Investigation. 2002;110(2):185-92. PubMed PMID: 12122110.

76. Hu HM, Poehlein CH, Urba WJ, Fox BA. Development of antitumor immune responses in reconstituted lymphopenic hosts. Cancer Res. 2002;62(14):3914-9. PubMed PMID: 12124318.

77. Robbins PF, Dudley ME, Wunderlich J, El-Gamil M, Li YF, Zhou J, et al. Cutting edge: persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. Journal of immunology. 2004;173(12):7125-30. PubMed PMID: 15585832; PubMed Central PMCID: PMC2175171.

78. Rosenberg SA. Cancer immunotherapy comes of age. Nature clinical practice Oncology. 2005;2(3):115. doi: 10.1038/ncponc0101. PubMed PMID: 16264884; PubMed Central PMCID: PMC2656369.

79. Zhou J, Dudley ME, Rosenberg SA, Robbins PF. Persistence of multiple tumor-specific T-cell clones is associated with complete tumor regression in a melanoma patient receiving adoptive cell transfer therapy. Journal of immunotherapy. 2005;28(1):53-62. PubMed PMID: 15614045; PubMed Central PMCID: PMC2175172.

80. Zhou J, Shen X, Huang J, Hodes RJ, Rosenberg SA, Robbins PF. Telomere length of transferred lymphocytes correlates with in vivo persistence and tumor regression in melanoma patients receiving cell transfer therapy. Journal of immunology. 2005;175(10):7046-52. PubMed PMID: 16272366; PubMed Central PMCID: PMC1351312.

81. Dudley ME, Rosenberg SA. Adoptive-cell-transfer therapy for the treatment of patients with cancer. Nature reviews Cancer. 2003;3(9):666-75. Epub 2003/09/03. doi: 10.1038/nrc1167. PubMed PMID: 12951585; PubMed Central PMCID: PMC2305722.

82. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nature medicine. 2004;10(9):909.
83. Restifo NP, Rosenberg SA. Use of standard criteria for assessment of cancer vaccines. Lancet Oncol. 2005;6(1):3-4. doi: 10.1016/S1470-2045(04)01693-6. PubMed PMID: 15629270; PubMed Central PMCID: PMC1484507.
84. Khoruts A, Fraser JM. A causal link between lymphopenia and autoimmunity. Immunology letters. 2005;98(1):23-31. doi: 10.1016/j.imlet.2004.10.022. PubMed PMID: 15790505.
85. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, et al. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31(32):4085-91. doi: 10.1200/JCO.2013.49.6968. PubMed PMID: 24101040; PubMed Central PMCID: PMC3816958.
86. Heimberger AB, Sun W, Hussain SF, Dey M, Crutcher L, Aldape K, et al. Immunological responses in a patient with glioblastoma multiforme treated with sequential courses of temozolomide and immunotherapy: case study. Neuro-oncology. 2008;10(1):98-103. doi: 10.1215/15228517-2007-046. PubMed PMID: 18079360; PubMed Central PMCID: PMC2600844.
87. Food, Drug Administration HHS. Biological products; bacterial vaccines and toxoids; implementation of efficacy review. Final rule and final order. Fed Regist. 2004;69(2):255-67. PubMed PMID: 14968793.
88. Atkinson WL, Pickering LK, Schwartz B, Weniger BG, Iskander JK, Watson JC, et al. General recommendations on immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Family Physicians (AAFP). MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control. 2002;51(RR-2):1-35. PubMed PMID: 11848294.
89. Myers MG, Beckman CW, Vosdingh RA, Hankins WA. Primary immunization with tetanus and diphtheria toxoids. Reaction rates and immunogenicity in older children and adults. JAMA : the journal of the American Medical Association. 1982;248(19):2478-80. PubMed PMID: 7131703.
90. Baxter D. Active and passive immunity, vaccine types, excipients and licensing. Occupational medicine. 2007;57(8):552-6. doi: 10.1093/occmed/kqm110. PubMed PMID: 18045976.
91. Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. Nature. 2015;519(7543):366-9. doi: 10.1038/nature14320. PubMed PMID: 25762141; PubMed Central PMCID: PMCPMC4510871.
92. Pilla L, Rivoltini L, Patuzzo R, Marrari A, Valdagni R, Parmiani G. Multipeptide vaccination in cancer patients. Expert opinion on biological therapy. 2009;9(8):1043-55. doi: 10.1517/14712590903085109. PubMed PMID: 19591629.
93. Slingluff CL, Jr., Speiser DE. Progress and controversies in developing cancer vaccines. Journal of translational medicine. 2005;3(1):18. doi: 10.1186/1479-5876-3-18. PubMed PMID: 15862126; PubMed Central PMCID: PMC1142519.

94. Harzstark AL, Small EJ. Immunotherapy for prostate cancer using antigen-loaded antigen-presenting cells: APC8015 (Provenge). Expert opinion on biological therapy. 2007;7(8):1275-80. doi: 10.1517/14712598.7.8.1275. PubMed PMID: 17696825.
95. Schmitt M, Casalegno-Garduno R, Xu X, Schmitt A. Peptide vaccines for patients with acute myeloid leukemia. Expert review of vaccines. 2009;8(10):1415-25. doi: 10.1586/erv.09.90. PubMed PMID: 19803762.
96. Rezvani K. PR1 vaccination in myeloid malignancies. Expert review of vaccines. 2008;7(7):867-75. doi: 10.1586/14760584.7.7.867. PubMed PMID: 18767937.
97. Kono K, Mizukami Y, Daigo Y, Takano A, Masuda K, Yoshida K, et al. Vaccination with multiple peptides derived from novel cancer-testis antigens can induce specific T-cell responses and clinical responses in advanced esophageal cancer. Cancer science. 2009;100(8):1502-9. Epub 2009/05/23. doi: 10.1111/j.1349-7006.2009.01200.x. PubMed PMID: 19459850.
98. Knutson KL, Disis ML. Augmenting T helper cell immunity in cancer. Current drug targets Immune, endocrine and metabolic disorders. 2005;5(4):365-71. PubMed PMID: 16375690.
99. Kennedy R, Celis E. Multiple roles for CD4+ T cells in anti-tumor immune responses. Immunological reviews. 2008;222:129-44. doi: 10.1111/j.1600-065X.2008.00616.x. PubMed PMID: 18363998.
100. Odunsi K, Qian F, Matsuzaki J, Mhawech-Fauceglia P, Andrews C, Hoffman EW, et al. Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer. Proc Natl Acad Sci U S A. 2007;104(31):12837-42. doi: 10.1073/pnas.0703342104. PubMed PMID: 17652518; PubMed Central PMCID: PMC1937553.
101. Perez-Diez A, Joncker NT, Choi K, Chan WF, Anderson CC, Lantz O, et al. CD4 cells can be more efficient at tumor rejection than CD8 cells. Blood. 2007;109(12):5346-54. doi: 10.1182/blood-2006-10-051318. PubMed PMID: 17327412; PubMed Central PMCID: PMC1890845.
102. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature. 1998;393(6684):480-3. doi: 10.1038/31002. PubMed PMID: 9624005.
103. Phan GQ, Touloukian CE, Yang JC, Restifo NP, Sherry RM, Hwu P, et al. Immunization of patients with metastatic melanoma using both class I- and class II-restricted peptides from melanoma-associated antigens. Journal of immunotherapy. 2003;26(4):349-56. PubMed PMID: 12843797; PubMed Central PMCID: PMC1679660.
104. Slingluff CL, Jr., Petroni GR, Chianese-Bullock KA, Smolkin ME, Ross MI, Haas NB, et al. Randomized multicenter trial of the effects of melanoma-associated helper peptides and cyclophosphamide on the immunogenicity of a multipeptide melanoma vaccine. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011;29(21):2924-32. doi: 10.1200/JCO.2010.33.8053. PubMed PMID: 21690475; PubMed Central PMCID: PMC3138719.
105. Slingluff CL, Jr., Petroni GR, Olson W, Czarkowski A, Grosh WW, Smolkin M, et al. Helper T-cell responses and clinical activity of a melanoma vaccine with multiple peptides from MAGE and melanocytic differentiation antigens. Journal of clinical oncology : official journal of the American Society of Clinical Oncology.

2008;26(30):4973-80. doi: 10.1200/JCO.2008.17.3161. PubMed PMID: 18809608; PubMed Central PMCID: PMC2652084.
106. Bijker MS, van den Eeden SJ, Franken KL, Melief CJ, Offringa R, van der Burg SH. CD8+ CTL priming by exact peptide epitopes in incomplete Freund's adjuvant induces a vanishing CTL response, whereas long peptides induce sustained CTL reactivity. Journal of immunology. 2007;179(8):5033-40. PubMed PMID: 17911588.
107. Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nature reviews Cancer. 2008;8(5):351-60. doi: 10.1038/nrc2373. PubMed PMID: 18418403.
108. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. The New England journal of medicine. 2011;364(22):2119-27. doi: 10.1056/NEJMoa1012863. PubMed PMID: 21631324; PubMed Central PMCID: PMC3517182.
109. Noguchi M, Moriya F, Suekane S, Matsuoka K, Arai G, Matsueda S, et al. Phase II study of personalized peptide vaccination for castration-resistant prostate cancer patients who failed in docetaxel-based chemotherapy. The Prostate. 2012;72(8):834-45. doi: 10.1002/pros.21485. PubMed PMID: 21932426.
110. Weber JS, Vogelzang NJ, Ernstoff MS, Goodman OB, Cranmer LD, Marshall JL, et al. A phase 1 study of a vaccine targeting preferentially expressed antigen in melanoma and prostate-specific membrane antigen in patients with advanced solid tumors. Journal of immunotherapy. 2011;34(7):556-67. doi: 10.1097/CJI.0b013e3182280db1. PubMed PMID: 21760528; PubMed Central PMCID: PMC3709852.
111. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010;28(11):1963-72. Epub 2010/03/17. doi: 10.1200/JCO.2009.26.3541. PubMed PMID: 20231676.
112. Brandsma D, Stalpers L, Taal W, Sminia P, van den Bent MJ. Clinical features, mechanisms, and management of pseudoprogression in malignant gliomas. Lancet Oncol. 2008;9(5):453-61. doi: 10.1016/S1470-2045(08)70125-6. PubMed PMID: 18452856.
113. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. Biol Blood Marrow Tr. 2019;25(4):625-38. doi: 10.1016/j.bbmt.2018.12.758. PubMed PMID: WOS:000465364900001.
114. Fecci PE, Mitchell DA, Whitesides JF, Xie W, Friedman AH, Archer GE, et al. Increased regulatory T-cell fraction amidst a diminished CD4 compartment explains cellular immune defects in patients with malignant glioma. Cancer research. 2006;66(6):3294-302. doi: 10.1158/0008-5472.CAN-05-3773. PubMed PMID: 16540683.
115. Betts MR, Exley B, Price DA, Bansal A, Camacho ZT, Teaberry V, et al. Characterization of functional and phenotypic changes in anti-Gag vaccine-induced T cell responses and their role in protection after HIV-1 infection. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(12):4512-7. PubMed PMID: 15753288.
116. Betts MR, Gray CM, Cox JH, Ferrari G. Antigen-specific T-cell-mediated immunity after HIV-1 infection: implications for vaccine control of HIV development. Expert review of vaccines. 2006;5(4):505-16. PubMed PMID: 16989631.

19 APPENDICES

19.1 *Appendix A - Pneumocystis Carinii Management*

***Pneumocystis Carinii* Prophylaxis**

Both corticosteroid therapy and continuous temozolomide therapy induce lymphocytopenia. Patients receiving any of these drugs or both concomitantly are at an increased risk for opportunistic infections. Therefore, a prophylaxis against *P. carinii* pneumonia is recommended for all patients receiving temozolomide during radiotherapy: trimethoprim-sulfamethoxazole (Bactrim forte®, Bactrim DS®) 1 tablet 3 times per week or monthly pentamidine inhalations (300 mg via aerosol monthly) or dapsone 100 mg p.o. each day (except in patients with G6-PD deficiency). Prophylaxis is recommended to continue for the duration of radiotherapy, regardless of the lymphocyte count and will be up to the discretion of the treating neuro-oncology team. After completion of the chemoradiation, patients with a lymphocyte count < 500/mm^2 should have CD4 quantification. If the CD4 is < 200, then prophylaxis is recommended to continue and the CD4 should be quantified on a monthly basis. If the lymphocyte count is ≥ 500 or the CD4 is > 200, then prophylaxis can be stopped.

19.2 *Appendix B – TMZ Package Insert*

Uploaded separately in electronic IRB.

19.3 *Appendix C – DSMBplus Charter*

Uploaded separately in electronic IRB.

19.4 *Appendix D – SOPs and FORMs*

Uploaded separately in electronic IRB.