ATTAC-II:
A Phase II Randomized, Blinded, and Placebo-controlled Trial of CMV RNA-Pulsed Dendritic Cells with Tetanus-Diphtheria Toxoid Vaccine in Patients with Newly-Diagnosed Glioblastoma

Sponsor:	University of Florida
Funding Source:	University of Florida
Protocol Source:	Chair, Duane A. Mitchell, MD, PhD
UF IRB#:	201400697
FDA IND#:	BB-16530
Coordinating Center:	Preston A. Wells Jr. Center for Brain Tumor Therapy University of Florida Brain Tumor Immunotherapy Program

Protocol Chair
Duane A. Mitchell, MD, PhD
University of Florida

Site Co-Principal Investigators – University of Florida
Maryam Rahman, MD
Ashley Ghiaseddin, MD

Site Principal Investigator - Duke University Medical Center
Katy Peters, MD, PhD

Site Principal Investigator - Orlando Health Cancer Institute
Naren Raj Ramakrishna, MD

UF Investigators and Key Personnel
Brian Cleaver, PhD
Anjelika Dechkovskaia, MD
Jeffrey Drake
Catherine Flores, PhD
Jianping Huang, MD, PhD
Tony Yachnis, MD
Oleg Yegorov, PhD
Tara Massini, MD
Jesse Kresak, MD
John Rees, MD

Lead Study Coordinator
Phuong Deleyrolle, RN
University of Florida

Regulatory Coordinator
Kristine Wynne, RN, BSN, MSHS
University of Florida

CONFIDENTIAL
The information contained in this document is regarded as confidential, and may not be disclosed to another party unless such disclosure is required to initiate the study, to conduct study-related activities, or to comply with national, state, or local laws and regulations. Written authorization from the coordinating site is required for disclosure otherwise.

Summary of Protocol Changes		
Version Date	**Sections Revised**	**Purpose**
20150525 1530 RB	N/A	Initial Protocol Release
20151026 1100 NM	Entire Document	-Revised study title -Updated investigators and key personnel -Updated FDA IND# -Deleted reference to double blinding of the study
20160506 1300 KW	Title Page	-Individuals updated to reflect current personnel. -Added 'FDA' to IND Number for clarity.
	Table of Contents	-Updated for pagination.
	2, Study Synopsis	-Based on pre-clinical data suggesting that the activation and expansion of CMV pp-65-specific T cells can be enhanced through modification of the RNA encoding pp65 with a full-length LAMP fusion protein, influenza virus matrix M1 was replaced with full-length LAMP protein. -Saline skin prep was removed and all subjects randomized to receive DCs will receive Td skin prep based on clinical data showing prolonged survival. -To serve as a true control, GM-CSF was removed from the PBMC arm. -Reference to Nature paper was added. -The primary objective was modified to evaluate the impact of Td on overall survival. -The secondary objectives were modified to evaluate progression-free survival and immunologic effects of short versus full-length LAMP. -Modifications were made to clarify inclusion criteria and timing of eligibility assessments. -Since manufacturing processes are different among the arms, the study design was changed to "single-blind". -Randomization was changed to "prior to leukapheresis" to allow for adequate manufacturing time for each arm. -MRI/MRS imaging of inguinal lymph nodes replaced SPECT/CT imaging as the method to assess the impact of DC vaccination and unpulsed PBMC vaccination at inguinal draining lymph nodes. -Immune monitoring time points were expanded to include collection around MRI/MRS. -nMR analysis of urine was added as a biological correlate. -Imaging time points were amended to align with clinical standards. Minor editorial changes for clarity.
	3, Study Schema	-Study schema was modified to reflect new study design.

Summary of Protocol Changes		
Version Date	**Sections Revised**	**Purpose**
	4, Abstract	-Reference to EGFRvIII data removed. -Data related LAMP fusion proteins added. Study aims modified to align with primary and secondary objectives.
	5, Hypothesis and Objectives	-Sections modified to align with modified treatment arms. -New section added to outline exploratory objectives.
	6, Background and Significance	-Minor editorial, formatting, and clarification changes. -Section modified to delineate between ATTAC- and ATTAC-GM trials. -Survival data added for ATTAC-GM trial.
	7, Study Rationale	-Reference to *in vitro* full-length LAMP data added.
	8, Subject Eligibility	-Modifications were made to clarify inclusion criteria and timing of eligibility assessments.
	9, Investigational Plan	-Section replaced to correlate with study design.
	9.4.2, DC Generation, Storage, and Testing 9.4.3, Preparation of DCs or PBMCs for Vaccination	-Reference to short and full-length LAMP added to correlate with study design.
	9.7, MRI/MRS Imaging of DC Migration	-Reference to SPECT/CT replaced with MRI/MRS imaging. -MRI/MRS imaging time points added. -Immune monitoring time points around MRI/MRS added. -nMR analysis of urine around MRI/MRS added.
	10, Study Requirements	-Modified to correlate with study design and DC migration requirements. -Imaging time points were amended to align with clinical standards.
	12, Immunological Response Evaluations	-Section modified to add immunologic endpoints and response evaluations.
	12.2, Polyfunctional T cell Responses, T cell Phenotype, and Tetramer Analysis	-Section modified to clearly define T cell response and data analysis.

Summary of Protocol Changes		
Version Date	**Sections Revised**	**Purpose**
	12.3, Cytokine Bead Array Analysis 12.4, Antibody Titers	-New section added to outline processes for testing and analysis.
	13.1, Dose-limiting Toxicity	-Section modified to clearly define dose-limiting toxicities and stopping criteria.
	13.2 Adverse Events	-Section revised to denote the collection of all grade 3 AEs and greater.
	13.3, Unblinding	-Section deleted to reflect change in study design.
	13.4, Reporting of SAEs	-Section modified to delineate responsibilities for reporting SAEs.
	13.5.8, SPECT Scans and Radiation Exposure	-Section deleted.
	14.1.1 DSMB	-Section modified to reflect updated review responsibilities.
	14.2, Audits	-Auditing plan included for Duke University and the University of Florida outlining reporting responsibilities to the University of Florida.
	15, Statistical Methods and Data Analysis	-Statistical evaluation methods revised to reflect new study design.
	16.1, Regulatory and Ethical Compliance	- Added reference to FDA regulations.
20160720	Title Page	-Individuals updated to reflect current personnel.
	Table of Contents	-Updated for pagination.
	2, Study Synopsis. Study Design	- Section modified to correlate with study design. Subject will be followed after disease progression.
	8, Subject Eligibility	-Modifications were made to clarify timing of eligibility assessments.
	8.1, Inclusion criteria	-Steroids replacement dose has been updated to reflect revised protocol.
	9.1, Overview	- Section modified to outline process for Recursive Partitioning Analysis class determination. - Section modified to correlate with study design. Subject will be followed after disease progression.

	10.1, Enrollment	- HPN added at Enrollment visit to correlate with study design - MMSE added to determine Recursive Partitioning Analysis class according to the protocol statistical method (Section 15.1 Study Design Overview) - Infectious Disease panel added at Enrollment visit to assess subject eligibility prior to randomization.
	10.2, Prior to Leukapheresis	- Modification was made to clarify timing of randomization
	10.4, Adjuvant TMZ Cycles	- Section modified to correlate with study design: Urine pregnancy testing added prior to Inguinal MRI/MRS procedure for subject safety; Urine for immune monitoring will be collected prior to vaccine 1 for baseline comparison; Humoral response testing material was updated
	10.6, Schedule of Study Assessments	- Schedule of procedures table layout was modified. - Footnotes section modified to reflect changes to the protocol
	15.1, Study Design Overview	- Section modified to reflect changes with study design
	18.5 Appendix E	- Section added to define performance status conversion method.
20161129	Title Page	-Updated to include current personnel.
	2, Study Synopsis	-Eligibility criteria modified to allow inclusion of patients with larger residual tumor and to define acceptable steroid maintenance dose to align with standards in brain tumor protocols.
	8.1, Inclusion Criteria	-Eligibility criteria modified to allow inclusion of patients with larger residual tumor and to define acceptable steroid maintenance dose to align with standards in brain tumor protocols.
	10.5, Study Requirements at Progression	-Updated for internal consistency.
	10.6, Concomitant Medications	-Section added to define requirements for concomitant medication collection and documentation.
	10.7, Schedule of Study Assessments	-Updated to add comprehensive metabolic panel prior to the initiation of adjuvant chemotherapy to determine ongoing eligibility.
20160328 (should have been 20170328)	Title Page	- Updated to include current personnel.

20170410	Overall Document	- Footer updated to reflect version change. - Table of Contents updated for pagination. - Additional editorial, typographical and formatting changes have been made throughout the body of the protocol, along with minor changes for document consistency, all of which are tracked but not individually listed.
	Title Page	- Roles and responsibilities for multi-site implementation delineated and investigators and key personnel updated.
	Protocol Signature Page	- Documentation of investigator and sponsor agreement added to the protocol per ICH GCP.
	List of Abbreviations	- Acronyms/Abbreviation List updated to more accurately reflect use in protocol.
	2, Study Synopsis	- Serum pregnancy test inclusion criterion clarified. - Window for study procedure leukapheresis #2 was modified to avoid further delay in cycle 2 adjuvant chemotherapy. - Criteria for assessing progression of disease prior to start of TMZ clarified. - Criteria for assessing tumor response, progression, and pseudoprogression updated to utilize criteria developed for the evaluation of neuro-oncology patients undergoing immunotherapy.
	6.2, Dendritic Cell Immunotherapy 6.3, Autoimmune Encephalomyelitis	- References to EGFRvII deleted. Results were negative.
	8, Subject Eligibility	- Serum pregnancy test inclusion criterion clarified. - Criteria for assessing progression of disease prior to start of TMZ clarified.
	9.1, Overview	- Window for study procedure leukapheresis #2 was modified to avoid further delay in cycle 2 adjuvant chemotherapy. - Criteria for assessing tumor response, progression, and pseudoprogression updated to utilize criteria developed for the evaluation of neuro-oncology patients undergoing immunotherapy.
	9.2, Registration Procedures	- Procedures for subject registration added to provide for consistent multisite implementation.
	9.3, Randomization Procedures	- Procedures for subject randomization added to provide for consistent multisite implementation.
	9.4, Radiation Therapy	- Provision for local RT added to reduced travel and financial burden on subjects.

	9.5, Temozolomide Therapy	- Section revised to provide additional guidelines for maintenance chemotherapy management. - Chemotherapy regimen was modified for patients 70 years and older. There is no safety data available on dose intensified Temozolomide for patients 70 years old and older.
	9.6, Removal of PBMCs by Leukapheresis	- Window for study procedure leukapheresis #2 was modified to avoid further delay in cycle 2 adjuvant chemotherapy.
	9.6.1, PBMC Generation, Storage, and Testing	- Reference to new PBMC clinical product Batch Record added and existing SOP reference corrected.
	9.6.2, DC Generation, Storage, and Testing	- Reference to new Batch Record for DC manufacturing added.
	9.7, Tetanus and Diphteria Toxoid Booster	- Information on site pre-treatment added for consistency with other protocol sections.
	10.1, Enrollment 10.3, Chemo-radiation	- Existing study requirements relocated to better reflect timing of protocol activities. - Timing of post-operative MRI added for clarity and flexibility. - Pregnancy test added for consistency with other protocol sections.
	10.7, Schedule of Study Assessments 10.7, Footnotes	- Window for study procedure leukapheresis #2 was modified to avoid further delay in cycle 2 adjuvant chemotherapy. - Roles and responsibilities for assessing tumor progression clarified. - Blood volume requirement for Immune monitoring samples modified to limit venous puncture for patients.
	11, Response Evaluation	- Guidelines for tumor response evaluation for patients receiving immunotherapy per the immune-related response criteria (irRC) and the response assessment in neuro-oncology (RANO) working group added. - References to RECIST and RTOG 0525 were removed from this section.
	11.1, General Methodology for Determining Tumor Measurements	- Guidelines for determining tumor measurements added for consistent methodology across all participating sites.

	11.2-11.5, Response definitions	- Response and progression language revised to reflect iRANO definitions.
	11.5, Response Evaluation after a Complete Resection	- Section removed since the protocol was revised (Version 20161129) to include patients with partial tumor resection. In this study response will be evaluated by iRANO for all patients.
	13.1, Dose Limiting Toxicity	- DLT exception added to address Cerebral Edema Adverse Event grading.
	13.2, Adverse Events	- Definitions modified to align with FDA regulations. - Roles, responsibilities, timeframes, mechanism, and requirements for reporting AEs clarified.
	13.3 (including 13.3.1-13.3.3), Adverse Event Reporting	- Procedures for AE assessment and reporting delineated for multisite implementation.
	13.4.12, Confidentiality	- Procedures to minimize risk to subject privacy and confidentiality of data expanded.
	14.1.1, Data Safety Monitoring Board	- Name of UFHCC DSMB updated to DISC as a result of Cancer Center re-structure. - Requirement for reporting to local IRB added.
	14.3.1, Study Documentation	- List of relevant study documents expanded for completeness.
	14.3.2, Site Activation	- Procedures and requirements for site activation added for multisite implementation.
	14.3.3, Case Report Forms	- Procedures for data capture, collection, correction and monitoring modified for completeness.
	16 (16.1-16.6), Regulatory Requirements and Ethical Considerations	- Ethical and regulatory requirements associated with FDA-regulated trials expanded.
	17, References	- References updated.
	Appendices C and D	- Complete blood count schedule as outlined in the appendices were updated to correlate with protocol requirements.

20170505	9.5 Temozolomide Therapy	- Standard of care Temozolomide for patients 70 and older is updated to reflect the approved conventional daily dose of 150 to 200 mg per square meter of body surface area for 5 days
20170622	2, Study Synopsis 9.1, Overview 9.6, Removal of PBMCs by Leukapheresis 10.7, Schedule of study assessments	- Provision added to repeat leukapheresis to generate the required amount of vaccines to complete the study (up to 10 vaccines).
	9.8 DC or PBMC Vaccination	- Section revised to specify that vaccination may proceed with lower dose of cells if there are less cells than the targeted dose at time of vaccination.
	13.4.7, MRI	- Section revised for consistency with inclusion criteria. Patients unable to undergo MRI will not be excluded from the study but followed by CT scans.

20170801	1, Study Synopsis 8.1, Inclusion Criteria	- Inclusion criterion #2 revised to provide clarification of the histopathologic diagnosis eligible for participation in this clinical trial. Variants of Glioblastoma WHO Grade IV are eligible for enrollment. - Inclusion criterion #3 revised to remove Cytomegalovirus (CMV) seropositive status as a requirement for participation in this trial. Serology testing remains in the protocol and will be used to stratify randomization. During the first year of study enrollment, we observed an unexpectedly low frequency of CMV seropositivity (patients with circulating antibodies to CMV) in the GBM patient population. While not used as eligibility in Phase I trials (Mitchell et al., Nature 2015 and Batich et al., Clin Can Res 2017), CMV seropositivity was included as an inclusion criterion for this Phase II trial to ensure similar immunologic exposure to the CMV virus across enrolled subjects on the vaccine and control arms. Based on published reports of the CMV exposure for individuals older than 50 years old, a frequency of 60-90% seropositivity would have been expected. Thus far, we have detected circulating CMV antibodies in only 33% of our clinic/inpatient GBM patients which is unexpectedly low for older individuals, and suggests, a possible false negative rate in patients, possibly secondary to immunosuppression. Supportive of this notion, a publication in OncoImmunology from a virology group at Karolinska Institute noted "Discordant humoral and cellular immune responses to Cytomegalovirus (CMV) in glioblastoma patients whose tumors are positive for CMV" (Rahbar et al., OncoImmunology 2015). In the conclusion of this paper the authors noted, "In GBM patients, HCMV activity is higher than in healthy controls and serology is a poor test to define previous or active HCMV infection in these patients." We plan to analyze retrospectively whether CMV seropositivity impacts on immunologic response and clinical outcomes to vaccination.
	3, Study Schema	- Modified for consistency with eligibility criteria.
	10.1, Enrollment	- Modified for consistency with eligibility criteria.
	Summary of Protocol Changes	- Previous version date corrected – changed from 20170621 to 20170622.

20171106	Addendum to the consent form document – Section 3. Will you be paid for taking part in this study?	- Patients will be offered the opportunity to have their travels covered for research visits during their participation in the study. The documents was revised to include details about travel arrangements.
	Title Page	- Updated investigators and key personnel
	2. Study Synopsis	- Section modified to correlate with revised protocol
	3. Study Schema	- Pre-treatment with Td or saline were added to the study schema to correlate with study design
	8.1 Inclusion criteria & 8.2 Exclusion criteria	- Post-op MRI requirements updated for clarity - Eligibility criteria were updated for clarity regarding contraceptive methods requirements - Progression disease will be assessed using modified RANO criteria

	9.1 Overview	- Section updated to specify randomization stratification by:
		  - Age (69 y.o. and younger or 70 y.o. and older), receiving different treatment regimen
		  - RPA class (II, IV or V)
		  - CMV serology (seropositive or seronegative) to distribute patients equally across the 3 treatment arms.
		- Criteria for assessing tumor response or progression changed from “iRANO” to “Modified RANO”. iRANO was created to assess tumor progression and response in patient undergoing immunotherapy. Since this protocol involves patients not receiving immunotherapy, the last modified RANO provides a more accurate and efficient assessment tool across the 3 treatment arms.

	10.2 Prior to Leukapheresis	- Infectious disease testing requirements updated to correlate with institutional practices at all participating sites.
	10.7 Schedule of Study Assessments	- Footnote 4 updated to reflect revised protocol - Footnote 8: Post therapy MRI requirements updated to correlate with SOC
	11 Response Evaluation	- Section revised to include the Modified RANO assessment criteria - Guidelines for determining tumor measurements updated to outlined Modified RANO requirements - Response and Progression language revised to reflect Modified RANO definitions - Guidelines added to provide guidance on clinical status assessment - Modified radiographic response assessment diagram replaced with Modified RANO algorithm
	13.2 Adverse Events	- Section updated to provide additional information on AE collection requirements

	13.4.6 Leukapheresis	- Section modified for consistency with protocol revision
	14.1.1 Data Monitoring Plan	- Data monitoring plan added
	15.1 Study Design Overview	- Section updated to specify randomization stratification by age 69 y.o and younger or 70 y.o. and older; and CMV serology status
	15.1 Study Design Overview	- Section updated to specify randomization stratification by Age group, RPA class and CMV serology
	15.2 Sample Size Justification for Vaccine Comparison	- Section revised to correct randomization timepoint. Randomization occurs on average 4 to 6 weeks after surgery and prior to chemo-radiation. 1st vaccination occurs on average 15 weeks after randomization. Power and stopping rule calculations are slightly different but inconsequently. These numbers were updated.
	15.3.1 Primary Objective and Secondary Clinical Objectives	- An intent to treat subanalysis is added to this section.
	17. References	- References updated
	Appendice G	- Guidance on contraception was added
20180104	Table of Contents	- Updated for pagination.
	13.3	- Section updated to clarify IND Safety Reporting procedures and procedures should a subject death occur per FDA during its review of Duke University's IND submission.
20180222	2, Study Synopsis	- Inclusion criteria listed in the synopsis section updated to correlate with inclusion as stated in the protocol. - Study Design section modified to correlate with protocol modification.
	8.1, Inclusion criteria to be assessed prior to adjuvant TMZ	- Inclusion criterion #1 updated to clarify that radiation dose of 1.8-2.0 Gy/fraction given 5 days per week for a total of 59.4-60.0 Gy over < 7 weeks may be given as part of this protocol per institutional practices for administration of external beam RT for patients with GBM.
	9.1, Overview	- Language related to measure of response as it applies to patients with no measurable disease at baseline or best response was removed from the study design section. Assessment of response/progression for patients with no measurable disease at baseline is described in details in Section 11, Response Evaluation.
	9.5, Temozolomide Therapy	- Post radiation chemo therapy guidelines for patients who experienced significant hematologic AEs during chemo-radiation are added.

	11.10, Clinical Judgement	- Provision added to provide allowance for clinical judgement to decide whether to discontinue study treatment during the interval of assessment for resolving tumor progression.
	14.1.2, DSMB	- Section modified to reflect that the UFHCC DISC will be the DSMB of record for this trial and that reports and correspondence from the DISC will be provided to participating investigators for distribution and review locally as required. Duke University's DSMB will defer to the UF DISC since UF will review safety data for all sites and provide applicable reports.
	17, References	- Section modified to add the article by Wu, J. and X. Xiong (2014) which was cited in the protocol text but not listed in the Reference Section.
	Appendices C, D	- Delay and dose modifications guidelines updated to reflect standard of practice in regards to absolute neutrophils count.
20180504	16.2, Informed Consent	- Provision for obtaining consent from potential participants whose primary language is not English added.
20180629	2, Study Synopsis	- Inclusion criteria and study design in the synopsis section updated to correlate with inclusion criteria and study design as stated in the protocol.
	8.1 Inclusion Criteria	- Requirement to have pre-op and post-op scan of the same type (MRI vs. CT) is removed. Eligibility is assessed based on tumor residual measurement therefore a MRI (or CT for patients unable to undergo MRI) available within 28 days of study enrollment is sufficient to assess eligibility independently of type of scan obtained pre-op.
	8.1 Inclusion criteria to be assessed prior to adjuvant TMZ	- Inclusion criterion #1 updated to clarify that dose of 59.4-60.0 Gy over < 7 weeks is the targeted total dose. Radiation may be delayed or interrupted as outlined in protocol appendix A. Patients who receive less than 59.4-60 Gy due to treatment delayed or interruption as outlined in the appendix A will remain eligible for the study unless they experienced significant toxicity that persisted over 4 weeks.
	9.1, Overview	- Language added to clarify that patients who have histopathologic confirmation of treatment effect after biopsy or surgery may remain in the study.
	9.5, Temozolomide Therapy	- Provision added to resume treatment for patients who experienced delay at the discretion of the PI in consultation with the treating physician if they believe patient may benefit from ongoing treatment.
	9.8, DC or PBMC Vaccination	- Provision added to continue vaccination with investigational drug for patients who cannot safely resume Temozolomide.

	11.11, Confirmation of Tumor Progression by Histopathology	- Section added to provide guidelines regarding confirmation of tumor progression and confirmation of treatment effect by histopathology

Version Date	**Section**	**Purpose**
20180912	Synopsis	- Updated to reflect protocol changes
	9.1 Overview, 10.5 At Progression	- Blood samples for immunologic monitoring will be obtained at progression if feasible.
	9.8 DC or PBMC Vaccination	- Provision added to allow participants to received generated vaccines when they no longer meet protocol requirement other than progression. They will be replaced for purpose of study endpoints if they do not meet criteria of an evaluable subject.
	10.7 Schedule of Study Assessments	- Footnotes updated for consistency with protocol requirements.
	13.1 Dose-limiting Toxicity	- Stopping rules revised to continuously monitor vaccine-related toxicity at an acceptable toxicity rate of 1 patient in 7.
	15.2 Sample Size Justification for Vaccine Comparison	- Section revised to state that Interim analysis will occur after 40 observed deaths. This correspond to a trial "information fraction" of 52%. Interim analysis was initially planned at 24 months. However, due to accrual rate, these numbers events were not observed at 24 months.
20181108	Synopsis and 9.1, Overview	- Statement added to clarify that the subgroup of patients who were disqualified prior to receiving the 1st vaccine, may be allowed to receive vaccines that are already generated. They will not count toward the study endpoints but will be monitored for toxicities.
20190415	Synopsis and 8.1 Inclusion Criteria	- Inclusion criteria language related to completion of radiation therapy was modified to remove target dose of radiation, to clarify that the intent is to include patients who complete standard dose therapy with allowance for routine clinical management decisions such as dose modification to allow safe standard-of-care radiation delivery and chemotherapy treatment.
	9.4, Radiation Therapy	- Revised for internal consistency.

	9.6 Removal of PBMCs by leukapheresis	- Clarification about additional leukapheresis procedures timeframe was added to limit/avoid treatment interruption/delay.
	9.8	- Clarified that missed vaccines during Cycles 1-8 may be given with subsequent cycles of Temozolomide (or monthly for patients off Temozolomide) until patients receive a total of 10 vaccines if there is no tumor progression.
	10.3, Chemoradiation	- Revised for internal consistency.
	10.7	- Footnote related to radiation therapy update for internal consistency.
	15.3, Analytic Methods	- Clarified what constitutes an evaluable subject for safety and toxicity and primary and secondary endpoints.
	18.1, Appendix A: Radiation Therapy	- Revised for internal consistency.
20190621	13.1 Dose-Limiting Toxicity	- Section revised to allow for a longer period of clinical management (changed from 48 hours to 4 weeks) of potential drug-related Grade III neurologic toxicity to more closely align with immunotherapy trials for other cancers as well as standard clinical management guidelines. Immune-related neuro toxicities are a recognized potential adverse event of immunotherapy. Symptoms may present throughout the course of treatment and involve the peripheral and central nervous system and usually respond well to steroids, if started early. Management of patients with immune-related adverse events includes treatment with a 4-week steroid taper. Resumption of therapy can occur if a neurotoxic AE is improved to Grade II or lower within 4 weeks of clinical management. However, if Grade III neurologic toxicity is observed again with subsequent study vaccines, a DLT will be declared and the patient will not receive additional study vaccines. - Surgical intervention to reverse neurological toxicity will not constitute DLT if the toxicity is reversed within 4 weeks. Immune response may induce inflammation of CNS tissue. Surgical intervention may be required for decompression and removal of necrotic tissue to reduce cerebral edema.
	13.2 Stopping Rules	- Section title added for clarity. Language related to stopping rules are unchanged.
20190820	Title Page	- UF key personnel updated for accuracy.

	13.1 Dose-Limiting Toxicity	- NCI CTCAE for AE grading revised from Version 4.0 to Version 5.0 per FDA's recommendation. - The definition of drug-related has been revised for clarity per FDA's recommendation. - In consultation with FDA following agency review of protocol version 20190621, the dose-limiting toxicity (DLT) definition, criteria and management have been modified to address FDA recommendations related to potential toxicities that may result from a longer period of clinical management. Specifically, the protocol has been revised to include a two-stage management plan for declaring a possible DLT: 1) a 7 day period for improvement of neurologic symptoms after medical intervention to grade 2 or better and 2) a 21-day period for return to baseline or improvement to grade I or better. - In consultation with FDA following agency review of protocol version 20190621, criteria for resumption of treatment has been modified to allow continued treatment only if neurotoxicity is improved to grade 1 or better or to patient's baseline status.
	13.3 Adverse Events 18.1 Appendix A	- CTCAE version updated to 5.0 for internal consistency.
20191101	Title Page	- Updated sponsor for IND 16530 to Immunomic Therapeutics, Inc (ITI). The ownership of IND 16530 was transferred from UF/Dr. Mitchell to ITI per agreement of the parties.
20200323	Synopsis, 9.1 Overview, 15.1 Study Design Overview	- Sections revised to increase total enrollment number to reach the study target of 120 evaluable subjects. The study target of 120 evaluable patients (defined as having received at least one study vaccine) as described in section 15.2 Sample Size Justification for Vaccine Comparison remained unchanged. However, total enrollment number is required to achieve this goal due to screen-failures, subject withdrawals prior to vaccination and early progression or AEs that preclude vaccination. Based on the enrollment and withdrawal numbers since study activation, we expect that up to 175 patients will be need to achieve the study goal of 120 evaluable patients.

	10.4 Adjuvant TMZ Cycles	Section updated to clarify that clinical management of patients will be done by initial assessment of disease status by the treating neuro-oncologist/study investigators, upon review of surveillance brain MRI during routine clinic visit followed by study vaccine administration or chemo cycle initiation. Clinical assessment of disease status will be followed by formal measurements of enhancing lesions for modified RANO criteria.
20200610	Synopsis; 8.1 Inclusion Criteria; References	- In view of cIMPACT-NOW, Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy, latest recommendation, based on survival data, to reclassify the designation of "Diffuse Astrocytic Glioma, IDH wildtype with molecular features of glioblastoma, WHO Grade IV" to "Glioblastoma, IDH-wildtype" if histologically lower grade but supported by molecular profiling, (Louis et al.; Brain Pathology; 2020) inclusion Criteria #2 was revised to clarify that eligibility diagnosis of GBM may be made by histopathology or molecular studies.
20201201	15.1 Study Design Overview 15.2 Sample Size Justification for Vaccine Comparison 15.3.2 Secondary Biological Objectives	-The planned interim analysis was designed to halt enrollment early if results demonstrated unlikely benefit and was anticipated after 2 years of enrollment with anticipated 40 events (deaths) to power the analysis. To date after more than four years of enrollment and a total of 132 patients enrolled on the study, we have just crossed the threshold of events for an interim analysis and anticipate enrollment for only an additional 10-12 months. The factors that have contributed to such a long interval before reaching threshold of events are slower monthly accrual than original projected but also potentially longer overall survival in the treated population of GBM patients than modeled in the interim analysis plan. Additionally, the immunotherapy treatment has been well tolerated in patients with only 2 Grade 3 or greater AEs associated with immunotherapy treatment amongst all treated subjects. Therefore, the study team and sponsor have determined that completion of enrollment on the study is the appropriate research plan for study completion and will forgo the interim analysis.
20210810	Title Page	- Funding updated from NCI to UF. NIH/NCI funding for this project has ended.
	Title Page, 14.1.1, Data Monitoring Plan	-Orlando Health name updated due to recent rebranding.

	Synopsis, 9.1 Overview, 15.1 Study Design Overview	-Sections revised to increase total enrollment number to reach the study target of 120 evaluable subjects. The study target of 120 evaluable patients (defined as having received at least one study vaccine) as described in section 15.2 Sample Size Justification for Vaccine Comparison remains unchanged. -Based on current withdrawals due to early progression, AEs that preclude vaccination, subjects requests or screen-failure rate, we plan to enroll up to 200 subject to achieve the study goal of 120 evaluable patients.
	10.6, Concomitant Medications	- Section revised to clarify the use the use of bevacizumab for patients experiencing symptomatic radiation necrosis.
20211021	9.6, Removal of PBMCs by Leukapheresis	- Section revised to add language related to placement of central venous catheter and potential use of fluoroscopy.
	13.5.7 Central Venous Catheter	- Section 13.5.7 Central Venous Catheter added to the potential risk section of the protocol to outline the potential risk associated with placement of a central venous catheter
20220119	Title Page	-Updated to reflect change in personnel. The UFH Cancer Center will provide ongoing statistical support.
20230509	Title Page	-Updated to reflect change in personnel.
	10.1 Enrollment	- Molecular diagnostics were added to the study requirements for data analysis. With recent advancements on tumor molecular aberrations significance for high-grade brain tumors diagnosis and the understanding of their impact on biological mechanism of treatment resistance and patients' prognosis, MGMT-promoter and IDH-1/2 status have become a requisite for data analysis on glioblastoma studies.
	12. Immunological Response Evaluations	- Section has been updated to include correlative studies that will be conducted for data analysis and reporting at the end of the study.
20231122	Title Page	- IND Sponsor updated to reflect change from Immunomic Therapeutics back to UF. -Update study personnel to reflect current personnel.
20241007	15.3.2 Secondary Biological Objectives	- Section updated to ensure consistency with the analysis outlined in section 12.3 Multiplex Cytokine Quantification. Cytokines array analysis results will be reported as part of the study's secondary biological objectives.

ATTAC-II:
A Phase II Randomized, Blinded, and Placebo-controlled Trial of CMV RNA-Pulsed Dendritic Cells with Tetanus-Diphtheria Toxoid Vaccine in Patients with Newly-Diagnosed Glioblastoma

PROTOCOL SIGNATURE PAGE

I have read this protocol and agree to adhere to the requirements. I will provide copies of this protocol and all pertinent information to the study personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the investigational plan and the conduct of the study according to 21 CFR parts 50, 54, 56 and 312, 45 CFR part 46, ICH Good Clinical Practices Guidelines and Institutional Review Board (IRB) requirements. The signature below constitutes the approval of this protocol and any attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Clinical Site:_____

Site Principal Investigator Printed Name:_____

Site Principal Investigator Signature and Date:_____

TABLE OF CONTENTS

1 LIST OF POSSIBLE ABBREVIATIONS

Ab	Antibody
ACTIVATE	**A** **C**omplementary **T**rial of an **I**mmunotherapy **V**accine **A**gainst **T**umor-Specific **E**GFRvIII
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
AMRIS	Advanced Magnetic Resonance Imaging and Spectroscopy
ANOVA	Analysis of Variance
ANC	Absolute Neutrophil Count
APC	Allophycocyanin
ALT	Adoptive Lymphocyte Transfer
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AT	Ambient Temperature
ATTAC	**A**nti-**T**umor Immunotherapy **T**argeted **A**gainst **C**ytomegalovirus in Patients with Newly Diagnosed Glioblastoma Multiforme during Recovery from Therapeutic Temozolomide-induced Lymphopenia
β-HCG	Beta-Human Chorionic Gonadotropin
BID	Twice Daily
BMT	Bone Marrow Transplant
BTIP	Brain Tumor Immunotherapy Program
BUN	Blood Urea Nitrogen
CA	California
CBC	Complete Blood Count
Cc	Cubic Centimeters
CDC	Centers for Disease Control
cDNA	Complimentary Deoxyribonucleic Acid
CEA	Carcinoembryonic Antigen
CFA	Complete Freund's Adjuvant
CFC	Cytokine Flow Cytometry
CLIA	Clinical Laboratory Improvement Act
cm	centimeter
CMV	Cytomegalovirus
CNS	Central Nervous System
Co-PI	Co-Primary Investigator
Cr	Serum Creatinine
CR	Complete Response
CRF	Case Report Form
CSF	Cerebro Spinal Fluid
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T Lymphocyte
CV	Curriculum Vitae
DC	Dendritic Cell

DCI	Duke Cancer Institute
DISC	Data Integrity and Safety Committee
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
DUMC	Duke University Medical Center
EAE	Experimental Autoimmune Encephalomyelitis
EBRT	External Beam Radiation Therapy
EBV	Epstein-Barr Virus
ECHO	Echocardiography
eCRF	Electronic Case Report Form
EGFR	Epidermal Growth Factor Receptor
EGFRvIII	Epidermal Growth Factor Receptor Mutation III
EGFRvIII-KLH	EGFRvIII conjugated to Keyhole Limpet Hemocyanin
ELISA	Enzyme-Linked ImmunoSorbent Assay
ELISPOT	Enzyme-linked Immunospot
EORTC	European Organization for Research and Treatment of Cancer
FACS	Fluorescence Activated Cell Sorting
FACT-accredited	Foundation for the Accreditation of Cellular Therapy
FDA	Food and Drug Administration
FDF	Financial Disclosure Form
FITC	Fluorescein Isothiocyanate
GBM	Glioblastoma Multiforme
G-CSF	Granulocyte Colony Stimulating Factor
GFP	Green Fluorescent Protein
GLP	Good Laboratory Practice
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
HABS	Human Monoclonal Antibodies
HB	Hepatitis B
HbsAg	Hepatitis B Surface Antigen
HD	High Dose
HDC	High Dose Chemotherapy
H&E	Hematoxylin and Eosin
HHS	Health and Human Services
HIPAA	The Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigens
HSC	Hematopoietic Stem Cells
IBC	Institutional Biosafety Committee
IFN	Interferon
ICH	International Conference on Harmonization
IFN-γ	Interferon-gamma
IHC	Immunohistochemistry
IL-1β	Interleukin 1B
IL-2	Interleukin-2

IL-4	Interleukin-4
IL-6	Interleukin-6
IL-12	Interleukin-12
IND	Investigational New Drug
IRB	Institutional Review Board
ISH	In Situ Hybridization
IV	Intravenous
KLH	Keyhole Limpet Hemocyanin
KPS	Karnofsky Performance Status
L	Liters
LAMP	Lysosomal-Associated Membrane Protein
Lf	Flocculation Units
LMD	Leptomeningeal Disease
M^2	Meters Squared
MA	Myeloablative
MAb	Monoclonal Antibody
MAGE	Melanoma Antigens
MD	Medical Doctor
MD	Maryland
Mcg	Micrograms
MG	Malignant Glioma
Mg	Magnesium
Mg	Milligrams
MHC	Major Histocompatibility Complex
mL	Milliliters
mm	millimeter
MMSE	Mini-Mental Status Examination
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
mRNA	Messenger Ribonucleic Acid
MTD	Maximal Tolerated Dose
Na	Sodium
NA	Non-adherent
NC	North Carolina
NEJM	New England Journal of Medicine
NCI	National Cancer Institute
NCI CTC	National Cancer Institute Common Toxicity Criteria
NIH	National Institutes of Health
NK	Natural Killer
NMA	Non-Myeloablative
nMR	Nuclear Magnetic Resonance
NY	New York
OHRP	Office for Human Research Protections
OS	Overall Survival
PBLs	Peripheral Blood Lymphocytes

PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PBSC	Peripheral Blood Stem Cell
PBSCT	Peripheral Blood Stem Cell Transplant
PCR	Polymerase Chain Reaction
PD	Progressive Disease
PEPvIII	Peptide variant III
PFS	Progression Free Survival
PFS-12	Progression Free Survival, 12 Months
PhD	Doctorate of Philosophy
PHI	Private/Protected Health Information
PI	Principal Investigator
PO	Per Os (by mouth)
PR	Partial Response
PRTBC	Preston Robert Tisch Brain Tumor Center
PS	Performance Status
®	Registered
RAC	Recombinant DNA Advisory Committee
RANO	Response Assessment in Neuro-Oncology
RDC	Remote Data Capture
RN	Registered Nurse
RNA	Ribonucleic Acid
RPA	Recursive Partitioning Analysis
RT	Radiation Therapy
RTOG	Radiation Therapy Oncology Group
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event
SD	Standard Deviation
SD	Stable Disease
SH	Subtractive Hybridization
SOC	Standard of Care
SOP	Standard Operating Procedure
SPECT	Single Photon Emission Computed Tomography
SQ	Subcutaneous
TCR	T-cell Receptor
Td	Tetanus and Diphtheria Toxoids
TGF-β	Transforming Growth Factor-β
TILs	Tumor Infiltrating Lymphocytes
TMZ	Temozolomide
TNF	Tumor Necrosis Factor
TNF-α	Tumor Necrosis Factor-Alpha
T_{regs}	Regulatory T-cells
TTP	Time to Progression
UF	University of Florida
UFHCC	University of Florida Health Cancer Center

µg	Micrograms
µl	Microliters
US	United States
USP	United States Pharmacopeia
UV	Ultraviolet
VDLN	Vaccine-site Draining Lymph Nodes
VICTORI	Dose-Finding and Safety Study of Autologous, Tumor-Specific Antigen-Pulsed Dendritic Cell Immunotherapy for Malignant Brain Tumors
WA	Washington
WBI	Whole Body Irradiation
WHO	World Health Organization

2 STUDY SYNOPSIS

Title	**ATTAC-II:** A Phase II Randomized, Blinded, and Placebo-controlled Trial of CMV RNA-Pulsed Dendritic Cells with Tetanus-Diphtheria Toxoid Vaccine in Patients with Newly-Diagnosed Glioblastoma
Study Drugs:	Autologous dendritic cells (DCs) derived from PBMC loaded with RNA encoding the human cytomegalovirus (CMV) matrix protein pp65 as a fusion protein with the short targeting peptide from lysosomal membrane associated protein (pp65-shLAMP) plus GM-CSF and tetanus-diphtheria (Td) vaccine as adjuvants; autologous DCs derived from PBMC loaded with pp65 RNA as a fusion protein with full-length LAMP protein (pp65-flLAMP) plus GM-CSF and Td as adjuvants; or autologous unpulsed PBMCs administered as a control vaccine.
Rationale:	CMV antigens have been identified in glioblastoma (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. Our prior studies examining patients with GBM have demonstrated the feasibility, safety, immunogenicity, and potential clinical efficacy of this approach (Mitchell and Batich et al., Nature 2015). Temozolomide (TMZ) has recently shown modest efficacy, but is not curative, in some patients with newly diagnosed GBM and is now routinely given to these patients during and after radiation therapy (RT). Therapeutic TMZ induces a profound lymphopenia, however, that may inhibit anti-tumor vaccination. We have demonstrated, however, that repeated vaccination during recovery from cycles of lymphodepletive temozolomide can induce potent humoral and cellular immunologic responses. Granulocyte macrophage-colony stimulating factor (GM-CSF) is a powerful adjuvant capable of stimulating macrophage function, inducing proliferation and maturation of DCs, and is able to enhance T-lymphocyte stimulatory function. Intradermal administration of GM-CSF enhances the immunization efficacy at the site of administration in a dose dependent fashion. Significant anti-tumor immunity has been demonstrated in preclinical murine studies in which irradiated, stably transfected tumor cell lines secreting GM-CSF has protected against subsequent tumor challenge, especially against intracerebral tumors. Tetanus toxoid (Td) is a routinely used vaccine in the normal human population that we have shown in pilot studies may function as a potent adjuvant to enhance DC trafficking to vaccine-site draining lymph nodes (VDLNs). Our previous studies have shown that successful DC migration to VDLNs may be a requisite for clinical activity of RNA-pulsed DCs and administration of Td prior to vaccination may improve the effectiveness of DC vaccines in patients with GBM.
Primary Objective:	1. To determine whether the addition of pp65-LAMP mRNA DC vaccine plus GM-CSF and Td to dose-intensified TMZ treatment is worthy of investigation in a large phase III study based on impact on overall survival.
Secondary Objectives:	1. Evaluate the impact of CMV pp65-LAMP RNA-pulsed DC vaccines on progression-free survival in patients with newly-diagnosed GBM. 2. Determine the immunologic effects of vaccination with pp65 RNA fusion constructs incorporating full-length LAMP vs short LAMP sequences.
Inclusion Criteria:	Enrollment and randomization must occur prior to subjects undergoing standard of care chemo-radiation.

All the following inclusion criteria need to be met in order to randomize the subject:

1. Age ≥ 18 years.
2. Confirmed diagnosis of *de novo* Glioblastoma (WHO Grade IV glioma) by histopathology or molecular studies.
   (Secondary GBM not eligible).
3. The tumor must have a supratentorial component.
4. Patient must have undergone surgical resection of tumor with less than approximately 3cm x 3cm ($9cm^2$) residual enhancing tumor as product of longest perpendicular planes by MRI.
5. Patients must have recovered from the effects of surgery, postoperative infection, and other complications.
6. A diagnostic contrast-enhanced MRI or CT scan (if MRI is not available) of the brain must be performed preoperatively and postoperatively. Post-op MRI must be performed within 28 days prior to study enrollment. If no post op MRI/CT within 28 days of enrollment is available, a new brain MRI (or CT) must be obtained in order to determine residual burden and eligibility prior to randomization.
   Patients unable to undergo MR imaging because of non-compatible devices can be enrolled, provided pre- and post-operative contrast-enhanced CT scans are obtained and are of sufficient quality.
7. Karnofsky Performance Status (KPS) ≥ 70.
8. Signed informed consent. If the patient's mental status precludes his/her giving informed consent, written informed consent may be given by the legally authorized representative.
9. For females of childbearing potential, negative serum pregnancy test at enrollment (test will be repeated within 72 hours prior to starting TMZ).
10. Women of childbearing potential (WOCBP) must be willing to use acceptable contraceptive methods to avoid pregnancy throughout the study and for at least 24 weeks after the last dose of study drug.
    Refer to Appendix G for definition of WOCBP and guidance on acceptable contraceptive methods.
11. Males with female partners of childbearing potential must agree to use physician-approved contraceptive methods (*e.g.*, abstinence, condoms, vasectomy) throughout the study and should avoid conceiving children for 24 weeks following the last dose of study drug.
    Refer to Appendix G for guidance on acceptable contraceptive methods

To be assessed prior to initiation of adjuvant TMZ:

1. Patients must have completed standard RT at the discretion of the treating Radiation Oncologist and concomitant TMZ therapy at a targeted dose of $75mg/m^2/d$ for ≤ 49 days without significant toxicity that persisted over 4 weeks. Significant toxicity is defined as one or more of the following:
   a. ANC < $0.5 \times 10^9/L$ (Grade 4)
   b. Platelet count < $10 \times 10^9/L$ (Grade 4)
   c. Grade 3 or 4 non-hematologic toxicity (except alopecia, nausea and vomiting unless the patient has failed maximal antiemetic therapy, and fatigue).
2. History & physical with neurologic examination within 14 days prior to initiation of adjuvant TMZ.

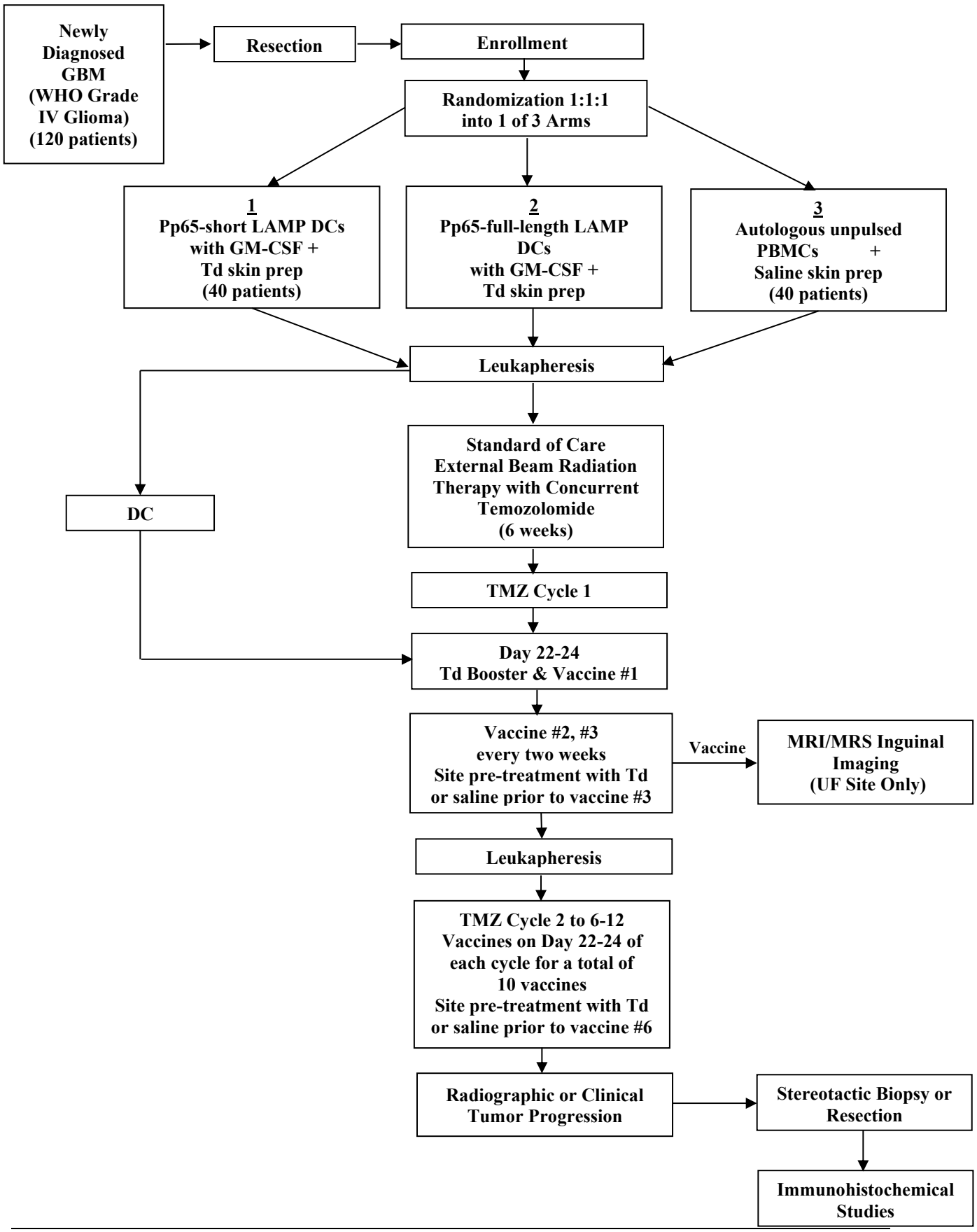
	3. For patients receiving steroids, daily dose must be ≤ 4 mg. 4. CBC with differential obtained within 14 days prior to initiation of adjuvant TMZ with adequate bone marrow function as defined below: a. Absolute neutrophil count (ANC) ≥ 1500 cells/mm^3. b. Platelet count ≥ 100,000 cells/mm^3. c. Hemoglobin ≥ 10 g/dl. (The use of transfusion or other intervention to achieve Hgb ≥ 10 g/dl is acceptable.) 5. Adequate renal function within 14 days prior to initiation of adjuvant TMZ as defined below: a. BUN ≤ 25 mg/dl b. Creatinine ≤ 1.7 mg/dl 6. Adequate hepatic function within 14 days prior to initiation of adjuvant TMZ as defined below: a. Bilirubin ≤ 2.0 mg/dl b. ALT ≤ 5 times institutional upper limits of normal for age c. AST ≤ 5 times institutional upper limits of normal for age
Exclusion Criteria:	All the following exclusion criteria need to be verified in order to randomize the subject: 1. Prior invasive malignancy (except for non-melanomatous skin cancer) unless disease free for ≥ 3 years. (For example, carcinoma in situ of the breast, oral cavity, and cervix are all permissible.) 2. Metastases detected below the tentorium or beyond the cranial vault and leptomeningeal involvement. 3. Recurrent or multifocal malignant gliomas. 4. HIV, Hepatitis B, or Hepatitis C seropositive. 5. Known active infection or immunosuppressive disease. 6. Prior chemotherapy or radiosensitizers (including Gliadel wafers) for cancers of the head and neck region, other than TMZ prescribed during radiation for GBM (prior chemotherapy for a different cancer is allowable). 7. Prior radiotherapy to the head or neck (except for T1 glottic cancer and that prescribed for GBM ≤60Gy), resulting in overlap of radiation fields. Radiosurgery is not permitted. 8. Severe, active co-morbidity, defined as follows: a. Unstable angina and/or congestive heart failure requiring hospitalization. b. Transmural myocardial infarction within the last 6 months. c. Acute bacterial or fungal infection requiring intravenous antibiotics at initiation of XRT/TMZ. d. Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at initiation of XRT/TMZ. e. Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects. f. Acquired Immune Deficiency Syndrome (AIDS) based upon current CDC definition. The need to exclude patients with AIDS from this protocol is necessary because the treatments involved in this protocol may be significantly immunosuppressive. g. Patients with autoimmune disease requiring medical management with immunosuppressants. h. Major medical illnesses or psychiatric impairments that, in the investigator's opinion, will prevent administration or completion of protocol therapy.

	i. Active connective tissue disorders such as lupus or scleroderma that, in the investigator's opinion, place the patient at high risk for radiation toxicity. 9. Pregnancy or women of childbearing potential and men who are sexually active and who are unwilling or unable to use an acceptable method of contraception for the entire study period; this exclusion is necessary because the treatment involved in this study may be significantly teratogenic. 10. Pregnant or lactating women, due to possible adverse effects on the developing fetus or infant. 11. Prior allergic reaction to TMZ, GM-CSF, or Td. 12. Prior history of brachial neuritis or Guillain-Barré syndrome. 13. Patients treated on any other therapeutic clinical protocols within 30 days prior to study entry. **To be assessed prior to initiation of adjuvant TMZ:** • Patient did not start RT and TMZ within 7 weeks of surgery. • Progression of disease as defined by modified RANO criteria. • More than 45 days after completion of RT and TMZ.
Study Design:	The eligibility criteria for this trial will be similar to those used in the RTOG 0525 study (Aldape 2009). Prior to the initiation of TMZ and external beam RT, patients will sign consent and undergo leukapheresis for PBMCs in accordance with **SOP-UFBTIP-131**. Up to 200 newly diagnosed WHO Grade IV glioma patients will be enrolled in the study. Eligible patients will be randomized, undergo leukapheresis and receive "standard-of-care" radiotherapy concurrent with TMZ under the assumption that approximately 120 patients will be vaccinated according to treatment arm in this placebo-controlled, single-blinded, randomized Phase II study. Prior to leukapheresis, patients will be randomized to receive one of three treatment regimens with dose-intensified TMZ treatment at 100 mg/m^2/day for 21 days with an allocation ratio of 1:1:1 into one of three arms to receive: 1) pp65-shLAMP mRNA DCs with GM-CSF 150 μg (Td skin prep) 2) pp65-flLAMP mRNA DCs with GM-CSF 150 μg (Td skin prep) 3) Unpulsed PBMCs (saline skin prep) Randomization will be stratified by: - Age 69 years old and younger, who will receive dose-intensified adjuvant temozolomide; or 70 years old and older who will receive standard dose adjuvant temozolomide. - Recursive Partitioning Analysis (RPA) class (III, IV, or V) - CMV seronegative or seropositive Within 7 weeks of surgery, patients will undergo standard RT with concurrent TMZ at a target dose of 75 mg/m^2/day.

	Patients will receive the initial cycle of TMZ 4 (+/- 1) weeks after completing RT. Study drugs will be given intradermally at day 22-24 after the first TMZ cycle and divided equally between both inguinal regions in accordance with **SOP-UFBTIP-128**. All patients will receive Td booster (5 Lf) with vaccine #1 regardless of booster history. Vaccine #2 and #3 will occur at 2 week intervals. All patients will undergo repeat leukapheresis 2 to 4 weeks after vaccine #3 for PBMCs to generate additional DC vaccines and for immunologic monitoring with specific assessment of baseline antigen-specific cellular and humoral immune responses. TMZ cycle 2 will occur 24 hours to 1 week after repeat leukapheresis. Patients may undergo additional leukapheresis if needed to generate up to 10 DC vaccines. Patients will then be vaccinated monthly in conjunction with subsequent TMZ cycles every 5(+/- 1) weeks for a total of 6 to 12 cycles after RT as per guidelines defined in recent phase III clinical trial evaluating TMZ regimens in patients with GBM (RTOG 0525). Vaccines will be given on day 22-24 of each TMZ cycle. Patients will continue with monthly vaccinations until a total of 10 vaccines have been administered or until tumor progression (whichever comes first). All patients will undergo vaccine site pretreatment with Td (1 Lf) or saline (depending on randomization arm) 6-24 hours prior to Vaccine #3, #6, and #9. At vaccine #3, patients at the UF site will undergo MRI/MRS imaging of inguinal vaccine-site draining lymph nodes before vaccine site pretreatment, 48hrs post vaccination, and 5 to 7 days post vaccination (optional). Patients who have been enrolled in the study and become ineligible to receive study treatment for reasons other than progression may be allowed to receive vaccines that have been generated at the discretion of the PI as outlined in section 9.8, DC or PBMC Vaccination. Blood for immune monitoring will be collected during initial leukapheresis, prior to vaccines 1-3, during MRI/MRS imaging time points surrounding vaccine #3, during the second leukapheresis, prior to vaccine 4, 7, 10, and at progression if feasible. Peripheral blood will be processed in accordance with **SOP-UFBTIP-126**. Urine samples will be collected at MRI/MRS imaging time points surrounding vaccine #3 and transferred to the UFBTIP laboratory for nMR analysis. Up to twelve cycles of TMZ may be given if the patient demonstrates continued improvement or stability on radiologic scan, decreased corticosteroid requirement, improvement in performance status, or improvement in neurologic function. Patients will be imaged bimonthly during TMZ cycles and every 3 months after the 12th TMZ cycle (or 1 year post surgery if TMZ discontinued prior to 12 cycles) without receiving any other prescribed antitumor therapy unless progression occurs. The primary measure of response will be by serial measures of the product of the two largest cross-sectional diameters. The modified RANO criteria (Ellingson, Wen, Cloughesy 2017) will be used for assessment of pseudoprogression, progression or response.

	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. As this is not a research procedure, consent for biopsy will be obtained separately. If tissue is obtained, it will be used to confirm tumor progression histologically and evaluate cellular infiltration and CMV antigen expression at the tumor site. If histopathology confirms treatment effect vs tumor progression patient may resume study treatment at the discretion of the PI in consultation with the treating physician, if the PI believes that it is safe to continue therapy and that patients may benefit from the ongoing treatment regimen as described in section 11.11 Confirmation of Tumor Progression by Histopathology. Patients will be followed until death due to any cause. MRI and clinical evaluation for assessment of disease progression will be conducted bi-monthly for the first 12 months and then every 3 months thereafter.

3 STUDY SCHEMA



4 ABSTRACT

Despite aggressive, computer-guided surgery, high-dose focused radiation, and toxic, multi-mechanistic chemotherapy, malignant gliomas (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(Imperato, Paleologos et al. 1990). The inherent biologic specificity of immunotherapy, however, offers the prospect of targeting neoplastic cells more precisely. DCs are endowed with an extraordinary ability to activate $CD4^+$ and $CD8^+$ T-cells(Banchereau and Steinman 1998; Steinman 2001), and DCs loaded with antigens derived from tumor cells have the potential to induce potent anti-tumor immunity. Unfortunately, the inherent genetic instability of tumor cells severely limits the number of conserved, homogeneously-expressed, and immunogenic tumor-specific antigens.

The discovery that MGs, but not surrounding normal brain tissue, serve as a refuge for CMV reactivation(Cobbs, Harkins et al. 2002) 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 central nervous system (CNS)(Bigger, Tanigawa et al. 1999), and antitumor immunotherapy targeting viral proteins in human CNS tumors associated with Epstein-Barr virus (EBV), another *Herpesvirus*, have been curative(Emanuel 1991; Papadopoulos, Ladanyi et al. 1994; Emanuel, Lucas et al. 1997; Rooney, Smith et al. 1998; Lucas and Barrett 1999; Liu, Savoldo et al. 2002). Similarly, vaccinations directed against the highly immunogenic antigens of human papilloma virus have recently been shown to reduce the incidence of cervical intraepithelial neoplasia in a prospective, randomized, double-blind trial(Koutsky, Ault et al. 2002).

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(Stupp, Mason et al. 2005) and is now routinely given to these patients during and after RT. Therapeutic TMZ, however, also induces a profound lymphopenia.

Human CMV antigens have been shown by a number of laboratories to be expressed in a high proportion (>90%) of malignant gliomas. We have recently completed a phase I/II clinical trial exploring the safety, immunogenicity, and potential clinical efficacy of autologous pp65 RNA-pulsed DC vaccines in patients with newly-diagnosed GBM. This trial explored the capacity to enhance DC migration to VDLNs using inflammatory skin preparations administered prior to pp65 RNA-loaded DC vaccines in a randomized pilot trial design (n=12 patients). The results of these studies demonstrated the capacity to safely expand CMV-specific cellular and humoral immunity in patients with GBM using autologous pp65 RNA-pulsed DC vaccines and demonstrated a strong correlation with successful DC migration to VDLNs and clinical outcomes *(R=0.73, P=0.007; Pearson correlation coefficient)*. Strikingly, patients randomized to receive a tetanus toxoid booster as an inflammatory stimulus at the vaccine-site showed an increased migration of DCs to VDLNs *(P=0.04)* and a corresponding increased progression-free and overall survival *(P=0.01 Logrank analysis)*. These results suggested that successful DC trafficking *in vivo* is associated

with improved outcomes in patients with GBM receiving pp65 RNA-pulsed DC vaccines and that inflammatory stimuli that enhance DC migration improve the efficacy of this treatment modality. In support of this hypothesis, we found increased levels of chemokines that facilitate DC migration in patients randomized to the tetanus group. Additionally, we have corroborated these findings using a transgenic mouse model employing GFP+ mice to evaluate DC migration. Pp65 RNA-pulsed DCs are a novel and promising therapeutic modality for patients with GBM, and our studies indicate that DC migration to VDLNs constitutes a major biological axis for potential clinical intervention in order to enhance the efficacy of this treatment strategy.

Studies within our laboratory have demonstrated that the activation and expansion of CMV pp65-specific T cells can be enhanced through modification of the RNA encoding pp65 with a full-length LAMP fusion protein. The fusion of antigenic proteins to LAMP channels antigens synthesized within the cytosol into the class II presentation pathway leading to enhanced CD4+ T cell activation and CTL induction. In the previous ATTAC trial, the CMV pp65 RNA construct encoded for a short peptide signal sequence from the LAMP protein which has been shown to channel proteins into the lysosomal processing pathway. We compared short LAMP signal sequence fusions of CMV pp65 to fusions with the full-length LAMP protein and found full-length LAMP led to superior T cell expansion.

In this trial, we will evaluate in a randomized, single-blinded phase 2 clinical trial whether the clinical efficacy of pp65 RNA-pulsed DCs is worthy of investigation in a definitive phase III clinical trial. We will also examine whether immunologic responses to the full-length LAMP and short sequence LAMP fusion constructs differ significantly in vaccinated patients.

5 HYPOTHESIS AND OBJECTIVES

5.1 Hypothesis

- Our primary hypothesis is that:
  - pp65-LAMP mRNA DC vaccine plus GM-CSF and Td in adult patients with newly-diagnosed GBM receiving dose-intensified TMZ will be worthy of investigation in a large phase III study based on impact on overall survival.
- Secondary hypotheses are that:
  - There is a relationship between CMV pp65-LAMP RNA-pulsed DC vaccines and progression-free survival in vaccinated patients.
  - Full-length LAMP vs short LAMP fusions with CMV pp65 will induce potent immune responses that mediate the safe eradication of invasive malignant disease in adult patients with newly diagnosed GBM.

5.2 Objectives

5.2.1 Primary Objective

- To determine whether the addition of pp65-LAMP mRNA DC vaccine plus GM-CSF and Td to dose-intensified TMZ treatment is worthy of investigation in a large phase III study based on impact on overall survival.

5.2.2 Secondary Objectives

- Evaluate the impact of CMV pp65-LAMP RNA-pulsed DC vaccines on progression-free survival in patients with newly-diagnosed GBM.
- Determine the immunologic effects of vaccination with pp65 RNA fusion constructs incorporating full-length LAMP vs short LAMP sequences.

5.2.3 Exploratory Objectives:

- Evaluate immunologic responses within vaccine-site draining lymph nodes using magnetic resonance spectroscopy
- Examine CCL3 and other chemokines/cytokines for utility as serum biomarkers for predicting DC migration and response to immunotherapy
- Examine urinary biomarkers of immune response using nMR spectroscopy

6 BACKGROUND AND SIGNIFICANCE

6.1 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(Kelly 1992), 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(Walker, Alexander et al. 1978; Walker, Green et al. 1980; Shapiro 1986; Salford, Brun et al. 1988; Dinapoli, Brown et al. 1993; Stupp, Mason et al. 2005). 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(Pickard, Bailey et al. 1990; Ekman and Westphal 2005). Moreover, the non-specific nature of conventional therapy for brain tumors often results in incapacitating damage to surrounding normal brain and systemic tissues(Imperato, Paleologos et al. 1990; Hall and Fodstad 1992). 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.

6.2 Dendritic Cell Immunotherapy

DCs are potent immunostimulatory cells that continuously sample the antigenic environment of the host and specifically activate $CD4^+$ and $CD8^+$ T-cells and B-cells (Banchereau and Steinman 1998; Steinman 2001). They are at the crossroads of many of the elegant networks of the immune system, and DCs represent the most promising contemporary biologic entity for realizing the promise of immunotherapy. Potent immune responses and encouraging clinical results have been seen in Phase I and II human clinical trials in systemic cancers(Hsu, Benike et al. 1996; Murphy, Tjoa et al. 1996; Nestle, Alijagic et al. 1998; Salgaller, Lodge et al. 1998; Salgaller, Tjoa et al.

1998; Tjoa, Simmons et al. 1998; Fujii, Shimizu et al. 1999; Holtl, Rieser et al. 1999; Lim and Bailey-Wood 1999; Morse, Deng et al. 1999; Thurner, Haendle et al. 1999; Lodge, Jones et al. 2000; Murphy, Tjoa et al. 2000; Rieser, Ramoner et al. 2000; Schuler-Thurner, Dieckmann et al. 2000; Heiser, Coleman et al. 2002; Su, Dannull et al. 2003).

Numerous animal studies(Liau, Black et al. 1999; Yu, Wheeler et al. 2001), including many of our own(Ashley, Faiola et al. 1997; Heimberger, Crotty et al. 2000; Heimberger, Archer et al. 2002), and a few clinical studies(Yu, Wheeler et al. 2001; Liau, Prins et al. 2005) have demonstrated potent antitumor responses using DC-based immunotherapy against MGs.

Adjuvants frequently used with vaccination include Freund's incomplete adjuvant, bacilli Calmette-Guerin, QS-21, and diphtheria toxoid. Supplemental cytokines have been used as well for the adjuvant immunological effects (Rosenberg, Yang et al. 1998). GM-CSF has been commonly used, as it is commercially available and well tolerated. GM-CSF is capable of stimulating macrophage function, inducing proliferation and maturation of dendritic cells, and is able to enhance T-lymphocyte stimulatory function. Intradermal administration of GM-CSF enhances the immunization efficacy at the site of administration in a dose dependent fashion at an optimal dose of 125 μg (Kremer, Stevens et al. 2000). Significant anti-tumor immunity has been demonstrated in preclinical murine studies in which irradiated, stably transfected tumor cell lines secreting GM-CSF have protected against subsequent tumor challenge, especially against intracerebral tumors(Sampson, Ashley et al. 1997; Mach, Gillessen et al. 2000). Furthermore, the potency of GM-CSF has been demonstrated in a Phase I clinical trial in melanoma patients vaccinated with irradiated autologous melanoma cells engineered to secrete GM-CSF(Soiffer, Lynch et al. 1998). The immunization sites were intensely infiltrated with T lymphocytes, dendritic cells, macrophages, and eosinophils in 100% of evaluable patients. Extensive tumor destruction was seen in 11 of 16 patients. Both cytotoxic T cells and antibody responses were associated with this tumor destruction. Hence, GM-CSF has an extensive track record both as a growth factor and an adjuvant, is commercially available and has an acceptable toxicity profile. The experience on the ACTIVATE study supports the use of GM-CSF and sets the precedent for this phase I study.

Td is a routinely used vaccine in the normal human population that we have shown in pilot studies may function as a potent adjuvant to enhance DC trafficking to VDLNs. Our previous studies have shown that successful DC migration to VDLNs may be a requisite for clinical activity of RNA-pulsed DCs and administration of Td prior to vaccination may improve the effectiveness of DC vaccines in patients with GBM.

6.2.1 Pre-Clinical Results against Intracerebral Tumors

In our laboratories and those of others, systemic immunization using DCs co-cultured with uncharacterized tumor homogenate(Heimberger, Crotty et al. 2000), whole tumor RNA(Ashley, Faiola et al. 1997), unidentified peptides eluted from tumor cells by gentle acid washing(Liau, Black et al. 1999), or a distinct peptide encompassing the tumor-specific EGFRvIII mutation(Heimberger, Archer et al. 2002) have been shown to induce humoral and cell mediated systemic immune responses and to prolong the survival of rodents with brain tumors.

In prior preclinical studies (Heimberger, Crotty et al. 2000), inbred VM/Dk mice received three or four weekly intraperitoneal injections of autologous bone marrow-derived DCs transiently co-cultured with tumor homogenate. The homogenate was derived from a syngeneic murine astrocytoma cell line derived from a spontaneously occurring astrocytoma in the inbred VM/Dk mouse strain. Splenocytes from mice immunized in this way were able, *in vitro*, to lyse the astrocytoma cell line that was used to generate the tumor homogenate. They were also able to lyse other astrocytoma cell lines derived from the same inbred mouse strain, but they had no effect against syngeneic fibroblasts. Similarly, these immunized mice also demonstrated a significantly increased antibody titer against the astrocytoma cell line used to generate the homogenate. In addition, mice immunized with DCs transiently co-cultured with tumor homogenate that were subsequently challenged with a lethal dose of this astrocytoma cell line intracerebrally were found to have a median survival >160% longer than those immunized with DCs cultured without tumor homogenate (P=0.016). In addition, 50% of the mice treated with the tumor homogenate-supplemented DCs survived long-term without any evidence of tumor growth and also survived a rechallenge of tumor cells indicating that a sustained anti-tumor immune response had been established. These findings are especially significant in light of the fact that the astrocytoma cell line used is known to secrete the immunosuppressive agent transforming growth factor-β (TGF-β) which is secreted by most human gliomas(Kuppner, Hamou et al. 1988; Wahl, Hunt et al. 1988; Zuber, Kuppner et al. 1988; Bodmer, Strommer et al. 1989; Maxwell, Galanopoulos et al. 1992).

In another report (Ashley, Faiola et al. 1997), C57BL/6 mice received three weekly intraperitoneal injections of autologous bone-marrow derived DCs co-cultured with tumor homogenate or whole tumor RNA derived from the poorly immunogenic B16F10 melanoma cell line. Standard *in vitro* cytotoxicity assays again revealed that splenocytes harvested from mice immunized with DCs transiently co-cultured with either tumor-derived homogenate or whole tumor RNA were able to lyse B16F10 melanoma cells but not unrelated tumor cells from the same major histocompatibility complex (MHC) background. In this experiment, mice immunized with autologous bone-marrow derived DCs co-cultured with tumor homogenate or whole tumor RNA increased median survival by >233% (P=0.0006) and 48% (P=0.0001), respectively, relative to mice immunized with DCs co-cultured with tumor homogenate or whole tumor RNA derived from an unrelated tumor with the same MHC background. In addition, 8/13 (61.5%) in the specific homogenate group and 4/10 (40%) in the specific RNA group survived beyond the endpoint of the study without evidence of tumor. Immunization of mice with pre-existing tumors with specific tumor homogenate also demonstrated the potency of this immunization approach by increasing survival by 62.5% relative to controls. In these mice an inflammatory infiltrate composed of mononuclear cells and polymorphonuclear leukocytes was identified only in mice treated with DCs co-cultured with tumor homogenate that matched the intracerebral tumor challenge.

In a report from (Liau, Black et al. 1999), the survival of tumor-bearing rats injected subcutaneously with autologous bone marrow-derive DCs co-cultured with peptides eluted from tumor cells with a gentle acid wash was significantly prolonged compared to tumor-bearing rats receiving equivalent numbers of DCs co-cultured with peptides acid-eluted from normal astrocytes (P< 0.05). Median survivals in these groups were 35 and 22 days respectively. In addition, three of the twelve rats (25%) treated with DCs co-cultured with acid-eluted tumor peptides remained alive at the end of the experiment. In addition, immunohistochemical analysis of five animals from each group in this experiment documented an increased peritumoral and intratumoral infiltration

of CD8+T-cells, and to a lesser extent $CD4^+$ T-cells and macrophages, in the group treated with DCs co-cultured with peptides acid-eluted from tumor cells when compared to controls.

6.2.2 Prior Experience in Patients with Intracerebral Tumors

The occurrence of human DCs in the peripheral blood is low (0.15% of circulating mononuclear cells), and procedures to isolate circulating DCs are cumbersome, relying on negative selection techniques to deplete the mononuclear cell fraction of contaminating monocytes and lymphocytes. Furthermore, brain tumor patients are characteristically immunosuppressed either from the use of steroids or due to the fact that malignant brain tumors secrete immunosuppressive agents like TGF-β. We have been using a simple method described previously(Romani, Reider et al. 1996), to generate human DCs by culturing peripheral blood mononuclear cells (PBMCs) in media supplemented with GM-CSF and interleukin-4 (IL-4). We have compared the ability to generate DCs from patients with malignant brain tumors and patients undergoing craniotomy for non-tumor related procedures. The phenotype of DCs from both tumor and normal populations were identical and were characterized as being highly positive for HLA-ABC and HLA-DR, the co-stimulatory molecules CD80 and CD86, and the DC/monocyte marker CD11c, but negative for the monocyte marker CD14. The cells were negative for the B and natural killer (NK) cell lineage markers, CD19 and CD56, respectively, which is consistent with published DC phenotypes.

DC immunotherapy in patients with MGs has been evaluated only in a few studies. In the published study by Yu *et al.*(Yu, Wheeler et al. 2001), patients received biweekly intradermal injections of peripheral blood derived DCs pulsed with uncharacterized peptides eluted from the surface of autologous glioma cells by gentle acid washings. All patients were required to complete a course of RT and were off steroids at the time of immunization. Toxicity was minimal and included only mild fever and lymphadenopathy. There was no clinical or radiographic evidence of autoimmune encephalomyelitis in any patient and no serious adverse events occurred. The immunization resulted in enhanced cytotoxic T lymphocyte (CTL) activity in 4/7 patients and both cytotoxic and memory T-cells were found to have infiltrated the patient's tumors whom underwent reoperation after immunization. Although this study was performed in a selected population of patients, the median survival of 455 days in the treated group compared very favorably with an institutional control group where median survival was only 257 days. Similarly, when immunized patients were compared to Curran's recursive partition analysis, which controls for known prognostic factors, the results still appeared quite favorable. Unfortunately, no clinical responses were seen and any antigen-specific immune response could not be characterized because the immunizing antigens were not characterized.

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

In another Phase I/II trial, tumor lysate pulsed DCs were given to ten patients who received immunizations every 3 weeks for a minimum of one and a maximum of 10 immunizations. There were only two minor clinical responses seen. Of 5 patients evaluated by enzyme-linked immunospot (ELISPOT) before and after vaccination T-cells reactive against tumor lysate-pulsed DCs were increased in two patients(Yamanaka R, Abe T et al., 2003). In a more recently published study, patients with GBM were treated with 1×10^6 to 1×10^7 DCs pulsed with acid eluted

autologous tumor peptides. There was no evidence of dose-limiting toxicity (DLT) or serious adverse events. One patient had an objective clinical response documented by magnetic resonance imaging, and six patients developed measurable systemic antitumor CTL responses.

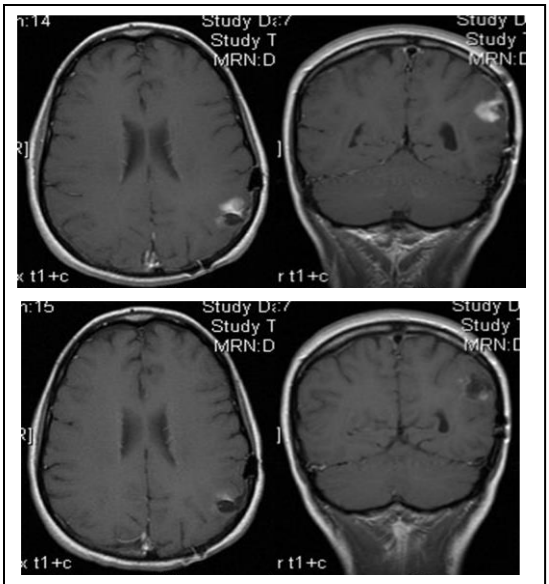


Fig. 1 Progressive MG after radiation and temozolomide (top) with nearly complete response, 3 months after PEPvIII-pulsed DC vaccine (bottom).

Kikuchi *et al.*(Kikuchi, Akasaki et al. 2004) investigated the safety and clinical response to immunotherapy using fusions of DCs and glioma cells combined with recombinant human IL-12 for the treatment of MG. Fifteen patients with MG participated in this study. Cultured autologous glioma cells were established from surgical specimens in each case. Fusion cells were prepared from DCs and glioma cells using polyethylene glycol. All patients received fusion cells intradermally on day 1. IL-12 was injected subcutaneously at the same site on days 3 and 7. No serious adverse effects were observed. In four patients, magnetic resonance imaging showed a greater than 50% reduction in tumor size. One patient had a mixed response.

In a Phase I/II clinical trial (BB IND 9944) at Duke University, patients with newly-diagnosed MGs were vaccinated with mature DCs loaded with a peptide spanning the fusion junction of EGFRvIII conjugated to keyhole limpet hemocyanin (PEPvIII-KLH) (500 mcg/immunization), mixed with GM-CSF (approximately 150 mcg/immunization). EGFRvIII is a tumor specific antigen, which is expressed on approximately 47% of all MGs. The vaccination protocol consist of 3 vaccines 2 weeks apart of PEPvIII-KLH loaded, mature DCs beginning 2 weeks following completion of post-resection RT. 19 patients were enrolled with 16 completing vaccination with no adverse events. No patient showed a positive delayed-type hypersensitivity reaction to KLH or PEPvIII before vaccination and of the evaluable patients after vaccination, 14/15 (93.3%) patients reacted to KLH and 11/15 (73.3%) reacted to PEPvIII. *In vitro* proliferation in response to PEPvIII was seen in 11/12 (92%) and to KLH in 9/12 (75%) of patients tested. Two patients, one with anaplastic astrocytoma and one with GBM with residual radiographic disease after resection, and radiation, had a nearly complete radiographic response (**Fig. 1**).

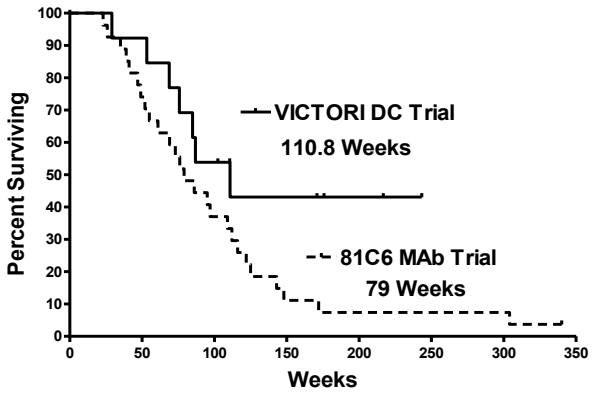


Fig. 2: Survival comparison of patients with GBM treated with PEPvIII-KLH loaded DCs (VICTORI Trial) with patients treated at Duke University with 81C6 anti-tenascin ^{131}I labeled MAb. Both patient populations had similar eligibility criteria. Published median survival for such patients treated with Gliadel™ is 59.6 weeks(Westphal, Hilt et al. 2003) and with concurrent RT and temozolomide is 58.4 weeks(Stupp, Mason et al. 2005).

These patients have remained stable for 174.9 and 217.3 weeks. Of the 14 patients without radiographically evident disease, 4/14 (28.6%) have not progressed at 102.7, 171.3, 180.7, 430.7 weeks with a median overall time to progression of 10.4 months comparing favorably with a historical unvaccinated cohort (EGFRvIII positive and gross total resection) that had a median TTP of 7.1 months (n=39). For patients with GBM, the median survival time was 20.0 months which compares favorably with recently published trials evaluating newly-diagnosed patients with GBM treated with GLIADEL® (13.9 months)(Westphal, Hilt et al. 2003); radiation and concurrent TMZ (14.6 months)(Stupp, Mason et al. 2005); or radiolabeled anti-tenascin monoclonal antibodies performed at Duke University (18.3 months)(Boskovitz, Wikstrand et al. 2004) (**Fig. 2**).

6.2.3 RNA Transfected DCs

The use of RNA to encode tumor antigens for DCs was pioneered at Duke University in Dr. Gilboa's laboratory, but the ability of RNA-loaded DCs to stimulate potent antitumor immunity has been independently confirmed in murine and human systems(Boczkowski, Nair et al. 1996; Van Tendeloo, Ponsaerts et al. 2001; Ponsaerts, Van den Bosch et al. 2002; Ponsaerts, Van Tendeloo et al. 2002; Van Meirvenne, Straetman et al. 2002; Morse, Nair et al. 2003). In fact, there is accumulating evidence that RNA transfection represents a superior method for loading antigens onto DCs(Strobel, Berchtold et al. 2000; Van Tendeloo, Ponsaerts et al. 2001). This novel and innovative approach to DC antigen loading has multiple conceptual advantages over other forms of antigen delivery as well. RNA-based antigen loading does not require knowledge of major MHC restriction, and responses are not restricted to single MHC haplotypes or to a narrow B- or T-cell repertoire. This diversity increases the likelihood of inducing effective and sustained antitumor immune responses by simultaneous activation of both CTLs and helper T-cells(Sercarz, Lehmann et al. 1993; Kundig, Bachmann et al. 1996; Kim, Trivedi et al. 1998). Furthermore, in direct comparisons, RNA-loaded DCs have been found to be better stimulators of antigen-specific T-cells than other approaches(Strobel, Berchtold et al. 2000). Finally, RNA also carries a significant safety advantage, not possessed by other nucleic acid or viral vectors, in that it cannot be integrated permanently into the host genome. In addition to the preliminary data we present below, Kobayashi *et al.*(Kobayashi, Yamanaka et al. 2003) have demonstrated that tumor mRNA-loaded DCs can elicit a specific CD8+ CTL response against autologous tumor cells in patients with MG.

6.3 Autoimmune Encephalomyelitis

DCs, however, have been shown to be quite capable of initiating significant autoimmune responses in murine models, and there has been one incident of a spontaneous generalized vitiligo that occurred after a second intravenous infusion of DCs in a patient with melanoma. Although our group and others have demonstrated that DCs loaded with unselected tumor-derived antigens induce potent, specific, and clinically effective immune responses against brain tumors in rodent models without the induction of autoimmune reactivity(Ashley, Faiola et al. 1997; Liau, Black et al. 1999; Heimberger, Crotty et al. 2000; Heimberger, Archer et al. 2002), and although no autoimmune reactions have been identified in human DC trials in patients with MGs(Yu, Wheeler et al. 2001; Kikuchi, Akasaki et al. 2004; Liau, Prins et al. 2005), immunization in preclinical studies has only been effective when given before tumor challenge or in the context of very small established tumors. These data suggest that for DC-based immunotherapy to be effective in the context of large human tumors, a very strong and sustained antitumor immune response will be required(Ochsenbein, Klenerman et al. 1999). In animal models, when such responses have been

generated against tumor-associated antigens that are shared with host cells, severe and clinically significant autoimmune disease has occasionally resulted(Ludewig, Ochsenbein et al. 2000).

Despite the fact, that no clinical or radiological evidence of experimental autoimmune encephalomyelitis (EAE) was induced by the DC-based immunization therapies in any of the preclinical experiments or the clinical trials outlined above, it must be acknowledged that one potential complication of DC-based immunotherapy, especially once optimized, is the induction of clinically significant autoimmunity. In fact, many published manuscripts have demonstrated the induction of clinically significant autoimmunity, including EAE, in preclinical studies employing DC-based or other immunization strategies(Borrow, Cornell et al. 1995; Dittel, Visintin et al. 1999; Bourquin, Iglesias et al. 2000; Ludewig, Ochsenbein et al. 2000). Although this may even be a desirable outcome for nonessential tissues, such as the prostate, when infiltrated by tumor, an autoimmune encephalomyelitis could be a lethal consequence of any non-specific active immunotherapy approach for primary intracerebral tumors where the target antigens are ill-defined and not tumor-specific.

In light of the documented expression of normal and fetal brain antigens on human glioma cell lines(Wahlstrom, Linder et al. 1973), and brain tumor tissue(Siris 1936; Slagel, Wilson et al. 1969; Wickremesinghe and Yates 1971), active, specific immunization strategies, including DC-based approaches, with uncharacterized brain tumor antigens that are not tumor-specific possess the theoretical risk of inducing an uncontrolled autoimmune response against normal CNS antigens. Such a response would be similar to EAE, which is an acute or chronic, autoimmune, inflammatory demyelinating disease mediated primarily by antigen-specific $CD4^+$ T-cells. Myelin basic protein is the most common known antigenic trigger, but myelin proteolipid protein(Waksman, Porter et al. 1954; Wikstrand and Bigner 1979), myelin oligodendrocyte glycoprotein(Tuohy, Lu et al. 1988), glial fibrillary acidic protein, and S-100β(Linington, Berger et al. 1993), are also sufficient antigens for the induction of EAE.

The susceptibility of humans to the induction of EAE was discovered accidentally when patients were immunized against rabies with spinal cords from rabbits that were infected with the rabies virus(Pasteur 1885; Remlinger 1904; Remlinger 1905; Stuart and Krikorian 1930; Wekerle, Kojima et al. 1994). The toxic component found in these immunizations was subsequently proven to be related to encephalitogenic components contained within the spinal cord preparation and not the virus(Stuart and Krikorian 1928). EAE has also been induced in monkeys after repeated injections of homogenized CNS tissue(Rivers and Schwentker 1935). It was not until the use of strong adjuvants such as complete Freund's adjuvant (CFA), that EAE could be easily and reproducibly created in animals in this manner(Kabat, Wolf et al. 1947). Since that time, it has been shown that EAE can be readily induced in the various species of rats, guinea pigs, mice, sheep, and monkeys after a single injection of CFA and either autologous or heterologous CNS tissue homogenate(Bigner, Pitts et al. 1981).

Given the range of protocols that routinely use immunization with CNS tissue for the production of lethal EAE in non-human primates(Bigner, Pitts et al. 1981), and the documented susceptibility of humans to EAE, the induction of such autoimmune responses is a legitimate concern. Some apprehension regarding EAE is also warranted on the basis of previous active, specific immunotherapy trials in humans with brain tumors. Although no cases of EAE were reported in some human studies(Ommaya 1976; Albright, Seab et al. 1977; Mahaley, Bigner et al. 1983;

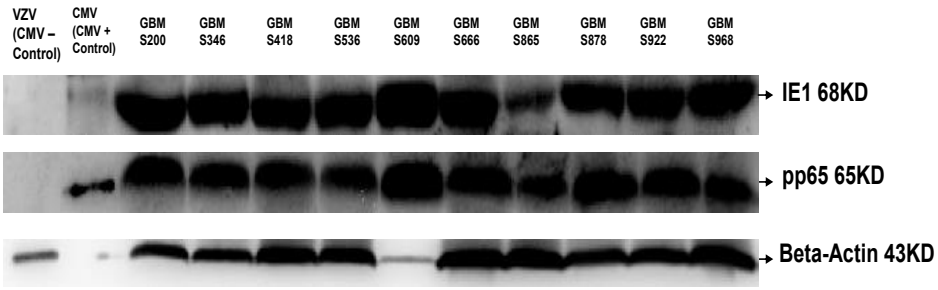
Bullard, Thomas et al. 1985), and protocols have been developed for safe active, specific immunotherapy with glioma-derived cells in primates(Wikstrand and Bigner 1981), careful review of several other studies(Bloom, Peckham et al. 1973) and(Trouillas and Lapras 1970; Trouillas 1973) reveal one possible case of EAE within each study. Thus, the risk of EAE, or other similar and potentially lethal autoimmune responses, may severely limit the efficacy of active, ***non-specific*** immunotherapy for malignant brain tumors. Thus targeting tumor-specific antigens, such as the more recently discovered viral antigens associated with CMV that are present within MGs, would enhance the safety of early trials in this field.

6.4 Presence of CMV in Malignant Astrocytomas

Human CMV is an endemic β-Herpesvirus that does not usually cause significant clinical disease(Vancikova and Dvorak 2001). During primary maternal infection, however, human CMV can cause severe encephalitis in fetuses and lead to congenital brain defects. Human CMV disease is also a significant problem in immunocompromised adults such as organ transplant recipients or patients with acquired immune deficiency syndrome (AIDS)(Vancikova and Dvorak 2001). Herpesviruses have also been implicated in a number of human malignancies including lymphoma, nasopharyngeal cancer, cervical cancer, and Kaposi's sarcoma(Rafferty 1973; Kadow, Regueiro-Ren et al. 2002). Recently, expression of proteins unique to human CMV has been reported within a large proportion of malignant tumors including colorectal carcinoma, prostate cancer, and malignant astrocytomas(Cobbs, Harkins et al. 2002; Harkins, Volk et al. 2002; Samanta, Harkins et al. 2003). Universal detection of the human CMV immunodominant protein pp65, immediate early gene 1 protein (IE1), and several other early antigens was demonstrated using immunohistochemistry (IHC) in Grade II-IV astrocytomas(Cobbs, Harkins et al. 2002). Presence of the virus in these samples was confirmed with *in situ* hybridization (ISH), polymerase chain reactions (PCR) for human CMV-specific glycoprotein B (UL55), electron microscopic detection of intact virions(Cobbs, Harkins et al. 2002). Human CMV antigens were not detected in surrounding normal brain samples, meningiomas, or brains affected by ischemia, Alzheimer's disease, paraneoplastic encephalitis, or *Cryptococcal* cerebritis.

The reliable detection of CMV with MGs requires sensitive detection modalities, but these results have now been confirmed by nine independent laboratories(Cobbs, Harkins et al. 2002; Mitchell, Xie et al. 2008; Scheurer, Bondy et al. 2008; Soderberg-Naucler 2008; Dziurzynski, Wei et al. 2011; Lucas, Bao et al. 2011; Sampson and Mitchell 2011; Soroceanu, Matlaf et al. 2011; Dziurzynski, Chang et al. 2012; Ranganathan, Clark et al. 2012). This finding offers an unparalleled opportunity to leverage immunotherapeutic approaches to target the highly immunogenic viral antigens expressed by CMV as tumor-specific targets.

A



Expression of CMV antigens in primary GBM tumors by Western blot analysis. 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 abundance of CMV proteins in laboratory strain virus infected lysates, 1/40 the amount of lysate was loaded for positive control lanes. Detection of IE1 has been observed in 14/22 (63.6%) analyzed GBM samples and pp65 detection was positive in 19/22 (86.3%) tumors analyzed by WB. gB envelope protein was detected in 16/16 (100%) tumors chosen based on positivity for pp65 (not shown). Normal brain lysates derived from autopsy specimens were negative in 5/5 cases (median age 64) not shown (0%), $p<0.0001$.

biologically plausible explanation for this unexpected specificity was also provided when it was subsequently shown that the EGFR is also the cellular receptor for CMV because the EGFR is often dramatically up-regulated in MGs, but is essentially not expressed in normal brain(Wang, Huong et al. 2003). Interestingly, astrocytic cell lines are some of the few cell lines that support CMV propagation *in vitro*(Poland, Costello et al. 1990). The presence of CMV antigens in malignant astrocytomas has been confirmed in independent tissue samples from our laboratory as well. It is not known whether CMV plays a role in the pathogenesis of MG, or whether tumor growth simply provides an environment supportive of local reactivation of the virus. Detection of CMV antigens in MG specimens has been independently confirmed in our laboratory by IHC, ISH, PCR, and reverse transcriptase polymerase chain reaction (RT-PCR).

6.5 Immunotherapy of CMV

The presence of highly-immunogenic human CMV antigens within MGs affords a unique opportunity to target these tumors immunologically. There is a vast amount of experience with both the safety and efficacy of immunotherapy targeting human CMV(Vancikova and Dvorak 2001), and the presence of this virus within brain tumors may allow this experience to be leveraged toward the effective eradication of MG expressing human CMV antigens. Adoptive T-cell therapy has been used to safely and successfully protect against CMV reactivation in myelodepleted bone marrow transplant (BMT) patients(Riddell and Greenberg 1995; Walter, Greenberg et al. 1995; Paar and Pollard 1996; Dazzi and Goldman 1998). In addition, T-cell mediated immunotherapy has proven highly effective in the treatment of CMV-associated disease within the CNS(Bigger, Tanigawa et al. 1999) and in the treatment of acute CMV infections(Walter, Greenberg et al. 1995; Numazaki, Ikehata et al. 1997). Tumors associated with other human *Herpesviruses*, such as Epstein-Barr virus-associated lymphoma, including tumors within the CNS, have also been effectively treated and even large tumors have been cured by immunotherapy(Emanuel 1991; Papadopoulos, Ladanyi et al. 1994; Emanuel, Lucas et al. 1997; Rooney, Smith et al. 1998; Lucas and Barrett 1999; Liu, Savoldo et al. 2002). More recently, a vaccine directed against the potent viral antigens of human papilloma virus has also been shown to reduce the incidence of human

papilloma virus-related cervical intraepithelial neoplasia in a prospective, randomized, double-blind trial(Koutsky, Ault et al. 2002).

The potential for non-specific targeting of normal tissues is thought to be minimal in seropositive patients. After initial infection, CMV establishes lifelong latency in the infected individual, with cells of the myeloid lineage constituting a major reservoir for persistence of the virus. Virus can be detected within myeloid progenitors in the bone marrow, with a small portion of these cells demonstrating viral DNA replication **without** any detectable gene expression(Kondo and Mocarski 1995; Prosch, Docke et al. 1999). Also a small proportion (typically 1 in 1,000 to 1 in 10,000) of peripheral blood monocytes can be found to contain CMV DNA, while detection of viral RNA (gene expression) is not detected(Sissons, Sinclair et al. 1991; Larsson, Soderberg-Naucler et al. 1998; Reeves, MacAry et al. 2005).

Vaccination specifically against CMV(Gonczol, Ianacone et al. 1989; Plotkin, Higgins et al. 1994; Britt, Fay et al. 1995; BenMohamed, Krishnan et al. 2002; Pepperl-Klindworth, Frankenberg et al. 2002) has effectively reduced the risk of viral infection and transmission to fetuses in animal models(Minamishima 1977; Morello, Ye et al. 2002; Cicin-Sain, Brune et al. 2003) and in clinical trials(Sachs, Simmons et al. 1984; Gonczol, Ianacone et al. 1989; Plotkin, Higgins et al. 1994; Adler, Shaw et al. 1995; Adler, Hempfling et al. 1998; Drulak, Malinoski et al. 2000). Human clinical trials have also demonstrated some benefit of administering neutralizing antibodies in the treatment of human CMV infection(Ohizumi, Suzuki et al. 1994; Falagas, Snydman et al. 1997; Hammond 1999; Tzakis 2001), highlighting the importance of the development of vaccination strategies that elicit both cellular and humoral immune responses. DCs strongly activate both T-cell and B-cell responses *in vivo*(Steinman 2001), and DCs pulsed *in vitro* with CMV antigens have been shown to be potent inducers of CMV-specific CTL responses in several studies(Cho, Han et al. 2001; Kleihauer, Grigoleit et al. 2001; Szmania, Galloway et al. 2001; Peggs, Verfuerth et al. 2002; Raftery, Schwab et al. 2002), in addition to our own work which is outlined below.

6.6 Targeting CMV in Patients with Malignant Gliomas

6.6.1 DCs Electroporated with pp65-LAMP mRNA

In preparation for the clinical trial outlined in this protocol, we wanted to demonstrate that DCs generated from patients with GBM, when loaded with CMV pp65-LAMP mRNA, could be used to generate antigen-specific autologous T-cells. These *in vitro* assays showed that after 15 days of co-culture with pp65 RNA-loaded DCs, the number of CD8+/pp65/A2 tetramer+ cells increased from <1% at baseline to 42.8% of all lymphocytes after 15 days while the absolute number of positive cells increased from $1.5x10^5$ to $1.17x10^7$ (78-fold expansion). Further experiments demonstrated the ability to expand CD8+/HLA-A2 tetramer+ T cells from patients with GBM by 2.51 $\pm$ 0.83 logs (n=5, range 1.73-3.74) with a purity of 54.0 $\pm$ 24.9% (n=5, range 35.9-88.4).

To establish that T-cells stimulated with pp65-LAMP mRNA transfected mature DCs reacted in an antigen specific manner against CMV pp65, we assayed for specific IFN-γ release by ELISPOT. DCs that had been previously been transfected with pp65-LAMP mRNA, matured and frozen,

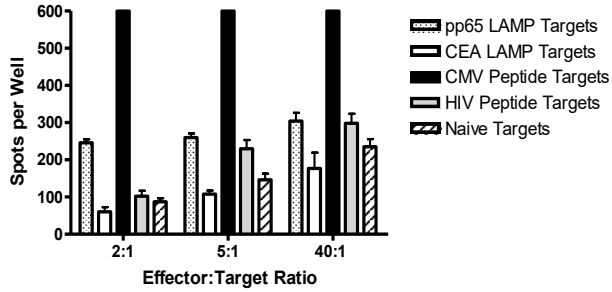


Fig. 4: T-cells stimulated by CMV pp65-LAMP electroporated DCs secrete IFN-γ specifically. DCs were mixed with T-cells in a 1:10 ratio and incubated in Aim V media supplemented with 2% HABS. IL-2 (10U/mL) was added on day 3 and the cells were grown for 14 days. After an 18 hour incubation the wells were washed and the nitrocellulose was developed and the spots counted.

were thawed, washed, counted and used to stimulate autologous T-cells. At the end of 14 days, the effector T-cells were incubated with target DCs transfected with CMV pp65-LAMP mRNA, CEA LAMP mRNA, pp65 peptide mix (138 peptides, 15 mers with 11 amino acid overlaps), *HIV* pol peptide mix, or untransfected DCs at different effector to target ratios in nitrocellulose wells coated with anti-IFN-γ MAb. Effector T-cells generated by *in vitro* stimulation with CMV pp65-LAMP transfected DCs secreted IFN-γ only in response to pp65 transfected targets, (**Fig. 4**). Cytokine flow cytometry (CFC) also demonstrated specific response to CMV pp65 peptide after a clinical scale expansion of cells from a patient with GBM. Briefly, cells were incubated for 6 hours with mixed overlapping CMV pp65 peptides (138 peptides, 15 mers with 11 amino acid overlaps) and brefeldin A (10ug/mL) before fixation and permeabilization. Samples were labeled with CD4 (or CD8)-FITC, CD69-PE, CD3-PE/Cy5.5, and IFN-γ-APC (along with appropriate isotype controls). Flow cytometry analysis of pre and post expansion indicated that after expansion CD8+/CD69+/CD3+/ IFN-γ+ increased from 3.28% to 52.47% while CD4+/CD69+/CD3+/ IFN-γ+ increased from 0.43% to 78.08%. It is also worth noting that in this particular patient sample there was greater percent of CD8 T-cells secreting IFN-γ (57.84%) than were CMV-pp65 tetramer positive (21.9%). Finally, to verify that these effector cells were capable of killing malignant astrocytes as well, HLA-A-2+ U251MG cells were ^{51}Cr-labelled and either pulsed with an HLA-A2 specific pp65 peptide (NLVPMVATV) (PEP-CMV) or the control *HIV* p17$_{77\text{-}85}$ peptide (SLYNTVATL) (PEP-*HIV*) (Anaspec), or infected with CMV. As expected, CMV infection significantly reduced MHC class I expression on U251MG target cells with mean fluorescent intensity of HLA-A2 staining decreasing from 827.5 in uninfected cells to 593.9 10 days after infection. Still effector cells were able to specifically kill the malignant astrocytes infected with CMV as well as peptide-pulsed MG targets (**Fig. 5**). These responses were dependent on CD8+ T-cells and HLA-A2. Another CLT assay demonstrated that T-cells stimulated by DCs transfected with CMV pp65-LAMP RNA and cultured for 14 days show high specific response against CMV pp65 peptide pulsed target cells. Specific killing was demonstrated by utilizing the

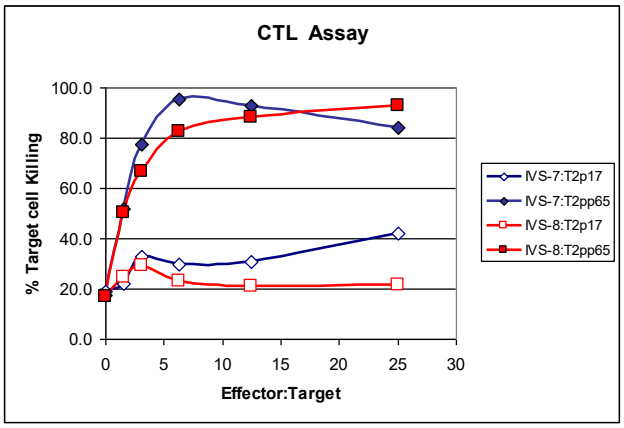


Fig. 5: T-cells stimulated by CMV pp65-LAMP electroporation generate specific CTL response against CMV-pp65 pulsed target cells. Briefly, target T2 cells are concurrently labeled with a combination of fluorescent membrane dye and either *CMV*-pp65 or *HIV* p17 peptides. Labeled targets were then co-cultured for 1 hour with effector cells in media containing a fluorogenic caspase substrate. In analysis, targets are differentiated by the fluorescent membrane dye while killing is defined by cells containing the fluorescently cleaved substrate. Figure above represents effectors IVS-7 and IVS-8 with *CMV*-pp65 or *HIV*-p17 peptide pulsed T2 cells.

highly sensitive) CyToxiLux® Plus assay kit (OncoImmunin, Inc., Gaithersburg, MD)(Liu, Chahroudi et al. 2002)(not shown).

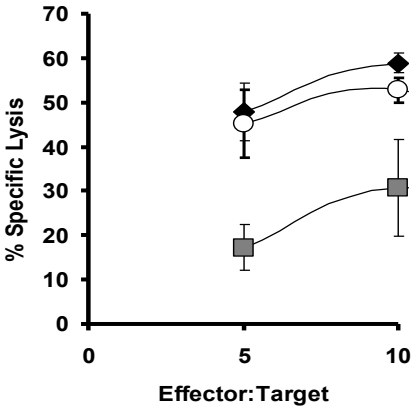


Fig. 6: T-cells stimulated by CMV pp65-LAMP electroporated DCs lysis CMV-specific targets CTL assay showing lysis of U251MG cells infected with *CMV* (open circles) or pulsed with HLA-A2 *CMV* pp65 peptide (black diamonds). or HIV p17 peptide (grey squares).(*P*=0.002, t-test)

6.6.2 Recognition of Human CMV antigens by TILs

We have recently gathered evidence that recognition of human CMV antigens may occur at a significant level in patients with GBM even without vaccination. PBLs and tumor-infiltrating lymphocytes (TILs) were isolated from patients and briefly expanded in a non-specific fashion *in vitro* with αCD3/αCD28 beads (Dynal) and IL-2. Tetramer analysis of an HLA-A.2+ TIL sample revealed a frequency of 10.26% pp65/A2 tetramer+ cells among the TILs isolated (**Fig. 6**). PBLs from the same patient, cultured in an identical fashion, had a starting frequency of 0.99% pp65/A2 tetramer+ cells, but this number instead actually declined during the same culture period to 0.46%. In an ELISPOT assay, these same TILs, when exposed to autologous tumor, HLA-A2 matched irrelevant tumor, or T2 cells loaded with HLA-A2 restricted CMV peptide epitope, also demonstrated CMV-specific secretion of IFN-γ **(Fig. 7**). Incidentally, this patient's tumor stained very strongly for CMV antigens in our IHC assay.

TIL recognition of pp65 in an HLA-A2-negative patient was also confirmed using cytokine florescent cytometry (CFC) after stimulation with CMV pp65 or *HIV* p17 peptide mix (BD) that stimulates both CD4+ and CD8+ T-cells. This patient's TILs consisted almost entirely of CD8+ T-cells and 41.12% of the T-cells became CD69+/IFN-γ+ after exposure to CMV pp65 peptide mix, indicating a strong reaction to CMV-specific peptides (**Fig. 8**). *HIV* peptide stimulation did not stimulate cytokine production above background (0.87%).

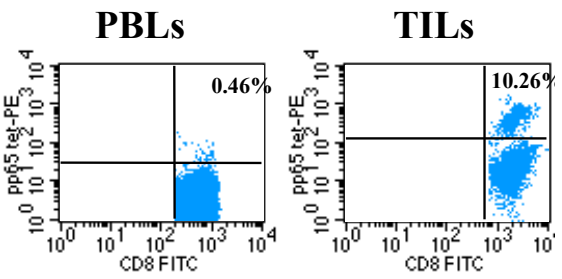


Fig. 7: Tetramer analysis showing that a disproportionate percentage of TILs from a HLA-A2+ patient with GBM are pp65-specific. HIV pol/A2 tetramer was used as negative control. (*P*<0.0001, binomial proportions)

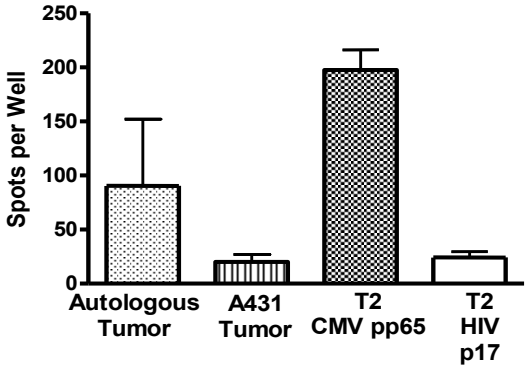


Fig. 8: ELISPOT showing that TILs from an HLA-A2+ patient with GBM recognize *CMV*. T-cells specifically secrete IFN-γ in response to autologous tumor and PEP-*CMV*-pulsed T2 cells, but not an irrelevant HLA-A2 tumor (A431) or PEP-HIV-pulsed T2 cells. (*P*=0.019, ANOVA)

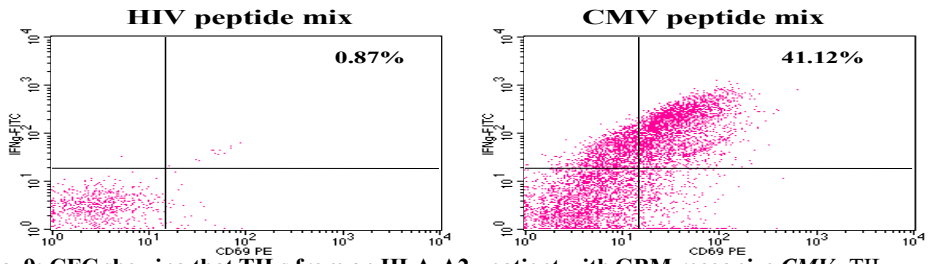


Fig. 9: CFC showing that TILs from an HLA-A2 - patient with GBM recognize *CMV*. TILs were activated (CD69+) and produced IFN-γ in response to *CMV* peptide mix, but not in response to HIV peptide mix. (*P*<0.0001, binomial proportions)

6.7 ATTAC and ATTAC-GM Clinical Trials

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). Twenty-four patients with newly-diagnosed GBM who underwent gross total resection (>95%) followed by standard external beam radiation (60Gy) and concurrent TMZ (75 mg/m^2/day) for 6 weeks followed by adjuvant monthly TMZ were enrolled into two cohorts. Cohort 1, referred to as the ATTAC trial (n=13 patients) received standard-dose TMZ (150-200 mg/m^2 x 5 days per cycle) and were randomized to receive different inflammatory skin preparations (unpulsed mature DCs or Td booster) prior to intradermal vaccination with pp65 RNA-loaded DCs. At the vaccine 4, patients received Indium-111 labeled DCs and migration to VDLNs was monitored by SPECT/CT imaging.

Cohort 2 (n=11 patients), referred to the ATTAC-GM trial, received dose-intensified TMZ and pp65 RNA-pulsed DCs mixed with GM-CSF (150ug). SPECT/CT imaging of DCs was also conducted at the fourth vaccine to evaluate the impact of GM-CSF on DC migration. Leukapheresis harvested post-surgical resection and prior to initiation of TMZ was used to generate DCs and pp65 RNA electroporated autologous DCs (2x10^7 DCs i.d.) were administered every two weeks for first three doses after first TMZ cycle and monthly thereafter on day 22-24 of each cycle. Patients in both trials were monitored by MRI (every two months) for tumor progression and blood collected monthly for immunologic monitoring. Treatment was well tolerated with no vaccine related adverse events on cohort 1 (ATTAC). A single patient on cohort 2 (ATTAC GM) experienced a Grade 3 hypersensitivity reaction that was attributed to GM-CSF. This patient received additional DC vaccines without the inclusion of GM-CSF without incident.

Expansion of CMV-specific T cells was observed by tetramer responses in 4 of 13 patients on cohort 1 after the third vaccine. Subsequent immune

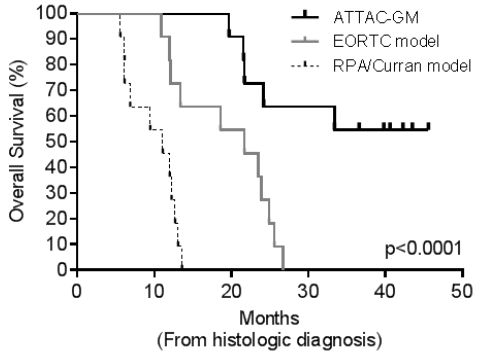


Fig. 10: Overall survival in patients with GBM receiving pp65 RNA-pulsed DC vaccines + GM-CSF during dose-intensifed TMZ. Dose-intensified TMZ has been shown to convey no treatment benefit in patients with GBM in recent phase III trial (RTOG 0525) compared to standard-dose TMZ. Overall survival of patients (n=11) with newly-diagnosed GBM receiving pp65 RNA-pulsed DCs + GM-CSF (150ug) during dose-intensified TMZ exceeds 40 months. EORTC and RPA/Curran nomogram predicted survival curves for patients enrolled are depicted. Patient survival observed significantly exceeds predicted survival and historical data for patients with GBM.

monitoring was conducted using ELISPOT analysis which detected expansion of CMV-specific T cells in 10 of 11 subjects enrolled on cohort 2.

Median overall survival for all patients enrolled on cohort 1 is 20.6 months with patients randomized to Td skin prep exhibiting greater DC migration to VDLNs and superior clinical outcomes ($P<0.01$). There was a strong correlation between successful migration of DCs to VDLNs (measured at vaccine 4) and clinical outcomes in cohort 1 and patients randomized to receive Td + DC vaccine had prolonged survival (>36.6 months) compared to patients randomized to undpulsed DC preparation (18.5 months). Cohort 2 (ATTAC-GM) has a median PFS and OS that exceeds 40 months **(Fig. 10)**.

6.8 T Cell Homeostatic Proliferation

Homeostatic proliferation is a mechanism by which the body maintains normal physiologic levels of circulating lymphocytes. Within the normal host, homeostatic proliferation involves the relatively slow replication of naïve and memory T-cells along with the generation of new lymphocytes from the bone marrow to maintain normal numbers of T-cells (and other lymphocytes).

Under conditions that induce profound lymphopenia, however, such as during non-myeloablative chemotherapy, naïve and memory T-cells enter a rapid proliferative state in order to replenish the drastically diminished lymphocyte numbers. During physiologic hematopoietic recovery, new T-cells must be regenerated from the bone marrow or by division of the few remaining T-cells in the peripheral lymphoid organs or circulation and is a prolonged process that may take weeks to months to recover normal T-cell counts. Following a period of lymphopenia, there is a spontaneous repopulation of the host's T-cells to re-establish the T-cell repertoire(Cho, Rao et al. 2000). Tanchot *et al.*(Tanchot, Rosado et al. 1997) propose that T-cells remaining after lymphodepletion compete for a finite amount of cytokine and antigenic stimulation. Adoptively transferred lymphocytes when transplanted into lymphopenic hosts, also undergo rapid proliferation and differentiation into effector and memory cells and can provide enhanced protection against tumor outgrowth in experimental animals(Wrzesinski and Restifo 2005). Furthermore, populations of tumor-specific lymphocytes when transferred into lymphopenic melanoma patients have been shown to be capable of eradicating disseminated malignant disease and maintaining high levels of tumor-specific memory T-cells in the circulation of treated patients(Dudley, Wunderlich et al. 2002; Dudley, Wunderlich et al. 2005; Hughes, Yu et al. 2005; Zhou, Dudley et al. 2005). These studies indicate that homeostatic proliferation may be leveraged to augment anti-tumor immunity by using T-cells educated to be reactive against tumor either through *ex vivo* expansion or possibly DC vaccination *in vivo*.

Recently it has also been demonstrated that depletion of the host's native lymphocyte pool with subsequent vaccination during endogenous homeostatic T-cell reconstitution enhanced the protection of mice bearing established extracranial tumors(Asavaroengchai, Kotera et al. 2002). Similarly, adoptive reconstitution with tumor-reactive T-cells after intentional non-myeloablative lymphodepletion allowed for a marked preferential expansion and maintenance of tumor-specific transferred T-cells resulting in dramatic clinical responses, along with some autoimmune toxicity, in patients with advanced malignant melanoma(Dudley, Wunderlich et al. 2002). Later studies have also indicated that *ex vivo* manipulation of the adoptively transferred cells, for example by

depletion of T_{regs}, further enhances the reconstitution of the host with tumor antigen-specific T-cells(Antony, Piccirillo et al. 2005). These approaches take advantage of still undefined homeostatic forces that provide an environment conducive to T-cell proliferation during recovery from lymphopenia. We propose to use tetanus prior to DC vaccination in patients with treatment-induced lymphopenia in order to determine whether this addition increases total uptake at 48 hours compared to those patients that received PBMCs with or without tetanus.

Our previous studies have demonstrated the safety of DC vaccination targeting the EGFRvIII mutation in patients with MGs, and we expect the targeting of CMV antigens to be well tolerated. There is considerable clinical experience with T-cell based immunotherapy targeting CMV in bone marrow and organ transplant patients and these studies have demonstrated the safety and efficacy of immunotherapy against CMV, even in CNS-involved disease. Lymphopenia will be induced in our patient population using standard doses of TMZ, an alkylating agent used routinely in the treatment of MGs at our institution and abroad as outlined below. We, therefore, expect that DC + GM-CSF pretreated with or without Td should be safe and highly efficacious in patients with MGs.

Lymphodepletion may be advantageous in patients with GBMs for several reasons. First, lymphodepletion will eliminate the defective T-cells that are well-described in patients with GBM(Brooks, Netsky et al. 1972). Second, lymphodepletion will eliminate T_{regs}, an inhibitory subset shown by us to be significantly upregulated in patients with GBMs (**Fig. 11**) and, in fact TMZ may preferentially deplete these suppressive cells(Su, Sohn et al. 2004). By transferring tumor-specific T-cells into such an environment, especially in the context of tumor-specific vaccination, the tumor-specific T-cells may have a selective advantage. It has also been shown in lymphopenic hosts, that proliferating T-cells differentiate directly into memory T-cells capable of rapid and intense response to antigen re-exposure(Tanchot, Rosado et al. 1997). Grossman and Paul propose that T-cells respond to lymphopenia by tuning their activation threshold down to a level at which T-cells are driven to proliferate, even in the absence of foreign antigens(Grossman and Paul 2000). Also in line with our thinking, others(Dudley, Wunderlich et al. 2002; Hu, Poehlein et al. 2002) propose that, if tumor antigens are present during this repopulation, the population of T-cell could be skewed to create a tumor antigen-targeted response. Homeostatic proliferation can then be augmented with autologous transfer of antigen-specific activated T-cells to skew the reconstituted pool resulting in an even more homogeneous T-cell repertoire that preferentially targets tumor antigen. In fact, in other studies, it has been shown that transferred T-cells can expand dramatically in the host to the point where they constitute up to

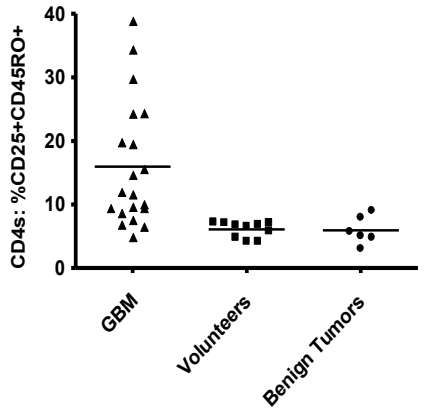


Fig. 11: Comparison of T_{reg} fraction in peripheral blood from GBM patients (n=20) with fraction in healthy volunteers (n=10) and patients with benign intracranial tumors (meningioma n=5; acoustic neuroma n=1). Data are expressed as the % of CD4+ cells that are CD25+CD45RO+. Horizontal hash bars indicate mean levels for each group (volunteers = 6.07% ± 0.39%, MG=15.94% ± 2.20%, benign=5.94% ± 0.89%). Mean fraction in patients with GBM is significantly elevated over that in both volunteers (P=0.004) and patients with benign intracranial tumors treated with equivalent doses of peri-operative steroids (P=0.023).

90% of the host's T-cell repertoire and can be maintained for months following transfer(Dudley, Wunderlich et al. 2002).

6.9 Treatment-Induced Lymphopenia from TMZ

TMZ, a methylating agent with good blood-brain barrier penetration, has recently been shown to increase survival by a small, but statistically significant, 2.5 months in a subset of patients with newly-diagnosed GBM if given in conjunction with RT following initial resection of the tumor(Stupp, Dietrich et al. 2002; Hegi, Diserens et al. 2005; Stupp, Mason et al. 2005). Leukopenia is essentially the only known human toxicity of TMZ. Although initially counter-intuitive, this TMZ-induced leukopenia may actually be advantageous in treating patients with immunotherapy due to the subsequent homeostatic proliferation it induces. In this protocol patients will receive standard of care doses of TMZ concurrent with RT following initial resection of the tumor. We believe that the myelosuppression induced by therapeutic TMZ treatment, if carefully timed ***before*** vaccination or adoptive reconstitution with CMV-specific T-cells, will actually enhance the proliferation and maintenance of these tumor-specific T-cells through the natural forces that drive T-cell homeostatic proliferative recovery. In addition, TMZ has also been shown to preferentially deplete T_{regs}(Su, Sohn et al. 2004). Thus, this combination strategy will uniquely exploit the toxicity of one effective therapy for GBMs, TMZ, to enhance another already promising therapy, immunotherapy. In preparation for this protocol, we have evaluated, in animal models, TMZ and sublethal whole body irradiation (WBI), as a positive control, as methods for induction of treatment-induced lymphopenia in order to determine the ability of TMZ-induced lymphodepletion to enhance active and adoptive immunotherapy. These studies were initially performed in a murine T-cell receptor transgenic model in which the antigen-specific T-cells can be followed *in vivo* in mice receiving adoptive transfer. In this model system, we found that ALT coupled with DC vaccination (DC + ALT) is a potent mechanism for inducing antigen-specific T-cell expansion after TMZ treatment. TMZ was found to be an effective agent for inducing homeostatic proliferation of transferred CD4+ and CD8+ T-cells and for enhancing DC or peptide vaccinations with or without ALT **(Fig. 12)**.

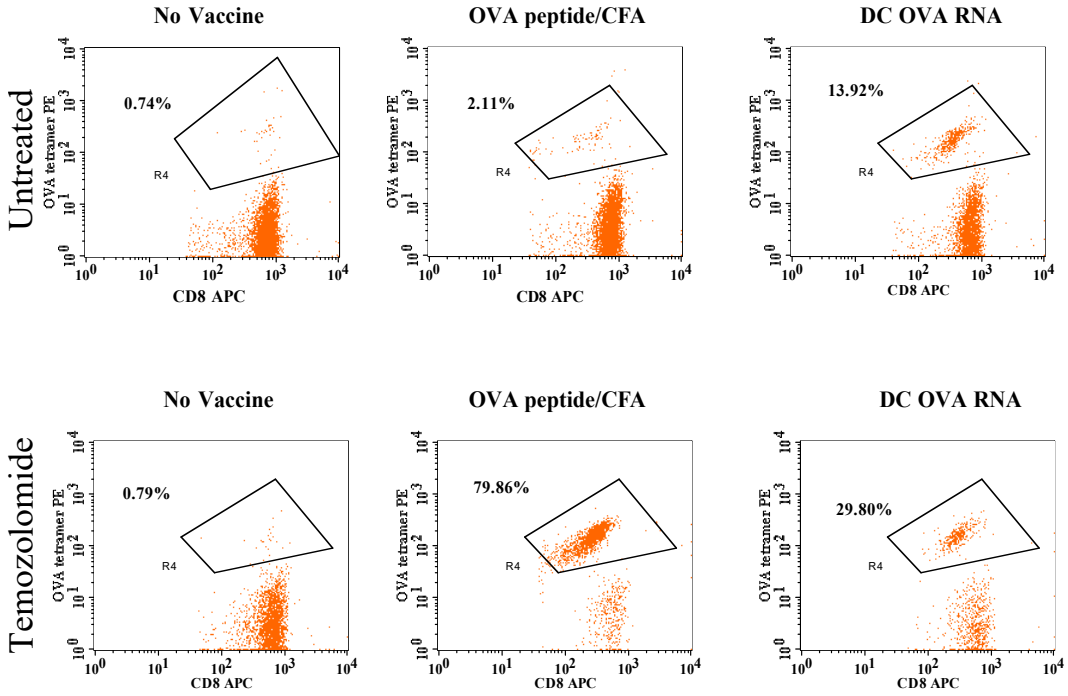


Fig. 12: Homeostatic lymphodepletion induced by temozolomide enhances antigen-specific immune responses. Untreated mice or mice receiving non-myeloablative high-dose temozolomide treatment (5 days x 60 mg/kg) were administered a mixture of OVA-specific and normal lymphocytes intravenously and received either no vaccine, vaccination with DCs transfected with OVA RNA, or OVA peptide vaccination in complete Freund's adjuvant. The peripheral blood of treated animals was collected and analyzed 6 days after vaccination for the expansion of OVA-specific T-cells in the circulation using αCD8 monoclonal antibodies and a specific tetramer. As shown above, mice treated with temozolomide prior to ALT demonstrated markedly increased T-cell expansion after vaccination compared to untreated hosts receiving ALT.

7 STUDY RATIONALE

We have demonstrated in murine models that DCs loaded with tumor-specific antigens in the form of peptides or RNA can induce potent and specific humoral and cell-mediated immune responses that are effective against murine intracerebral (i.c.) tumors, including a syngeneic murine astrocytoma, without inducing autoimmunity(Heimberger, Crotty et al. 2000; Heimberger, Archer et al. 2002; Heimberger 2003; Mitchell, Cui et al. 2011). Our previous clinical experience has also shown that DC vaccines in combination with standard of care radiation therapy and chemotherapy are capable of generating potent, tumor-specific immune responses and clinical radiographic responses in patients with MGs. We and others have also shown that antigens derived from CMV are contained within MGs and may serve as potent and specific immunotherapy targets. Vaccination and adoptive T-cell strategies targeting CMV in humans in other contexts, including the targeting of lesions within the CNS, have been safe and effective. We have also shown that DCs generated from patients with GBM and loaded with pp65-LAMP mRNA are capable of generating CD4+ and CD8+ T-cells that produce IFN-γ and kill malignant astrocytes infected with CMV in an antigen-specific fashion. We have found that TILs isolated from these patients are significantly enriched for T-cells that specifically recognize CMV antigens, suggesting that this

response may be important in the biology of these tumors. We have recently demonstrated that fusion of the immunodominant antigen CMVpp65 to full-length LAMP targeting protein enhances activation and expansion of CMV-specific T cells *in vitro*. TMZ has recently shown efficacy in some patients with newly-diagnosed GBM and is now frequently given to these patients during initial RT. Therapeutic TMZ induces a profound lymphopenia, however, that may inhibit anti-tumor vaccination. With these observations in mind, we are now proposing to determine the immunologic and clinical effects of CMV-specific vaccination after therapeutic TMZ-induced lymphodepletion with GM-CSF and with Td skin preparations in patients with newly-diagnosed GBM.

Td is a routinely used vaccine in the normal human population that we have shown in pilot studies may function as a potent adjuvant to enhance DC trafficking to VDLNs. Our previous studies have shown that successful DC migration to VDLNs may be a requisite for clinical activity of RNA-pulsed DCs and administration of Td prior to vaccination may improve the effectiveness of DC vaccines in patients with GBM.

Adjuvants frequently used with vaccination include Freund's incomplete adjuvant, bacilli Calmette-Guerin, QS-21, and diphtheria toxoid. Supplemental cytokines have been used as well for the adjuvant immunological effects(Rosenberg, Yang et al. 1998). GM-CSF has been commonly used, as it is commercially available and well tolerated. GM-CSF is capable of stimulating macrophage function, inducing proliferation and maturation of dendritic cells, and is able to enhance T-lymphocyte stimulatory function. Intradermal administration of GM-CSF enhances the immunization efficacy at the site of administration in a dose dependent fashion at an optimal dose of 125 μg(Kremer, Stevens et al. 2000). Significant anti-tumor immunity has been demonstrated in preclinical murine studies in which irradiated, stably transfected tumor cell lines secreting GM-CSF have protected against subsequent tumor challenge, especially against intracerebral tumors(Sampson, Ashley et al. 1997; Mach, Gillessen et al. 2000). Furthermore, the potency of GM-CSF has been demonstrated in a Phase I clinical trial in melanoma patients vaccinated with irradiated autologous melanoma cells engineered to secrete GM-CSF(Soiffer, Lynch et al. 1998). The immunization sites were intensely infiltrated with T lymphocytes, dendritic cells, macrophages, and eosinophils in 100% of evaluable patients. Extensive tumor destruction was seen in 11 of 16 patients. Both cytotoxic T cells and antibody responses were associated with this tumor destruction. Hence, GM-CSF has an extensive track record both as a growth factor and an adjuvant, is commercially available and has an acceptable toxicity profile. The experience on the ACTIVATE study supports the use of GM-CSF and sets the precedent for this study.

8 SUBJECT ELIGIBILITY

8.1 Inclusion Criteria

Enrollment and randomization must occur prior to subjects undergoing standard of care chemo-radiation.

All the following inclusion criteria need to be met in order to randomize the subject:

1. Age ≥ 18 years.

2. Confirmed diagnosis of de novo Glioblastoma (WHO Grade IV glioma) by histopathology or molecular studies.
   (Secondary GBM not eligible).
3. The tumor must have a supratentorial component.
4. Patient must have undergone surgical resection of tumor with less than 3cm x 3cm (9cm2) residual enhancing tumor as product of longest perpendicular planes by MRI.
5. Patients must have recovered from the effects of surgery, postoperative infection, and other complications.
6. A diagnostic contrast-enhanced MRI or CT scan (if MRI is not available) of the brain must be performed preoperatively and postoperatively. Post-op MRI must be performed within 28 days prior to study enrollment. If no post op MRI/CT within 28 days of enrollment is available, a new brain MRI (or CT) must be obtained in order to determine residual burden and eligibility prior to randomization.
   Patients unable to undergo MR imaging because of non-compatible devices can be enrolled, provided pre- and post-operative contrast-enhanced CT scans are obtained and are of sufficient quality.
7. Karnofsky Performance Status (KPS) ≥ 70.
8. Signed informed consent. If the patient's mental status precludes his/her giving informed consent, written informed consent may be given by the legally authorized representative.
9. For females of childbearing potential, negative serum pregnancy test at enrollment (test will be repeated within 72 hours prior to starting TMZ).
10. Women of childbearing potential (WOCBP) must be willing to use acceptable contraceptive method to avoid pregnancy throughout the study and for at least 24 weeks after the last dose of study drug.
    Refer to Appendix G for definition of WOCBP and guidance on acceptable contraceptive methods.
11. Males with female partners of childbearing potential must agree to practice adequate contraceptive methods throughout the study and should avoid conceiving children for 24 weeks following the last dose of study drug.
    Refer to Appendix G for guidance on acceptable contraceptive methods.

To be assessed prior to initiation of adjuvant TMZ:

1. Patients must have completed standard RT at the discretion of the treating Radiation Oncologist and concomitant TMZ therapy at a targeted dose of 75mg/m^2/d for ≤ 49 days without significant toxicity that persisted over 4 weeks. Significant toxicity is defined as one or more of the following:
   a. ANC < 0.5 x 10^9/L (Grade 4)
   b. Platelet count < 10 x 10^9/L (Grade 4)
   c. Grade 3 or 4 non-hematologic toxicity (except alopecia, nausea and vomiting unless the patient has failed maximal antiemetic therapy, and fatigue).
2. History & physical with neurologic examination within 14 days prior to initiation of adjuvant TMZ.
3. For patients receiving steroids, daily dose must be ≤ 4 mg.
4. CBC with differential obtained within 14 days prior to initiation of TMZ with adequate bone marrow function as defined below:
   a. Absolute neutrophil count (ANC) ≥ 1500 cells/mm^3.

   b. Platelet count ≥ 100,000 cells/mm^3.
   c. Hemoglobin ≥ 10 g/dl. (The use of transfusion or other intervention to achieve Hgb ≥ 10 g/dl is acceptable.)
5. Adequate renal function within 14 days prior to initiation of adjuvant TMZ as defined below:
   a. BUN ≤ 25 mg/dl
   b. Creatinine ≤ 1.7 mg/dl
6. Adequate hepatic function within 14 days prior to initiation of adjuvant TMZ as defined below:
   a. Bilirubin ≤ 2.0 mg/dl
   b. ALT ≤ 5 times institutional upper limits of normal for age
   c. AST ≤ 5 times institutional upper limits of normal for age

8.2 Exclusion Criteria

All the following exclusion criteria need to be verified in order to randomize the subject:

1. Prior invasive malignancy (except for non-melanomatous skin cancer) unless disease free for ≥ 3 years. (For example, carcinoma in situ of the breast, oral cavity, and cervix are all permissible.)
2. Metastases detected below the tentorium or beyond the cranial vault and leptomeningeal involvement.
3. Recurrent or multifocal malignant gliomas.
4. HIV, Hepatitis B, or Hepatitis C seropositive.
5. Known active infection or immunosuppressive disease.
6. Prior chemotherapy or radiosensitizers (including Gliadel wafers) for cancers of the head and neck region, other than TMZ prescribed during radiation for GBM (Prior chemotherapy for a different cancer is allowable).
7. Prior radiotherapy to the head or neck (except for T1 glottic cancer and that prescribed for GBM ≤60Gy), resulting in overlap of radiation fields. Radiosurgery is not permitted.
8. Severe, active co-morbidity, defined as follows:
   a. Unstable angina and/or congestive heart failure requiring hospitalization.
   b. Transmural myocardial infarction within the last 6 months.
   c. Acute bacterial or fungal infection requiring intravenous antibiotics at initiation of XRT/TMZ.
   d. Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at initiation of XRT/TMZ.
   e. Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects.
   f. Acquired Immune Deficiency Syndrome (AIDS) based upon current CDC definition. The need to exclude patients with AIDS from this protocol is necessary because the treatments involved in this protocol may be significantly immunosuppressive.
   g. Patients with autoimmune disease requiring medical management with immunosuppressants.
   h. Major medical illnesses or psychiatric impairments that, in the investigator's opinion, will prevent administration or completion of protocol therapy.

i. Active connective tissue disorders such as lupus or scleroderma that, in the investigator's opinion, place the patient at high risk for radiation toxicity.

9. Pregnancy or women of childbearing potential and men who are sexually active and who are unwilling or unable to use an acceptable method of contraception for the entire study; this exclusion is necessary because the treatment involved in this study may be significantly teratogenic.
10. Pregnant or lactating women, due to possible adverse effects on the developing fetus or infant.
11. Prior allergic reaction to TMZ, GM-CSF, or Td.
12. Prior history of brachial neuritis or Guillain-Barré syndrome.
13. Patients treated on any other therapeutic clinical protocols within 30 days prior to study entry.

To be assessed prior to initiation of TMZ:

1. Patients did not start RT and TMZ within 7 weeks of surgery.
2. Progression of disease as defined by modified RANO criteria.
3. More than 45 days after completion of RT and TMZ.

9 INVESTIGATIONAL PLAN

9.1 Overview

The eligibility criteria for this trial will be similar to those used in the RTOG 0525 study (Aldape 2009). Prior to the initiation of TMZ and external beam RT, patients will sign consent and undergo leukapheresis for PBMCs in accordance with **SOP-UFBTIP-131**.
Up to 200 newly diagnosed WHO Grade IV glioma patients will be enrolled in the study. Eligible patients will be randomized, undergo leukapheresis and receive "standard-of-care" radiotherapy concurrent with TMZ under the assumption that approximately 120 patients will be vaccinated according to treatment arm in this placebo controlled, single blinded, randomized Phase II study.

Prior to leukapheresis, patients will be randomized to receive one of three treatment regimens with dose-intensified TMZ treatment at 100 mg/m^2/day for 21 days with an allocation ratio of 1:1:1 into one of three arms to receive:

1) pp65-shLAMP mRNA DCs with GM-CSF 150 µg (Td skin prep)
2) pp65-flLAMP mRNA DCs with GM-CSF 150 µg (Td skin prep)
3) Unpulsed PBMCs (saline skin prep)

Randomization will be stratified by;

- Age 69 years old and younger, who will receive dose-intensified adjuvant temozolomide; or 70 years old and older who will receive standard dose adjuvant temozolomide.
- Recursive Partitioning Analysis (RPA) class (III, IV, or V).
  To determine RPA class, Performance Status (PS) will be determined from KPS conversion using the ECOG comparison scale for patients ≤ 50 years; a Mini-Mental State Examination will be performed at enrollment for patients ≥ 50 years.
- CMV seronegative or seropositive

Within 7 weeks of surgery, patients will undergo standard RT with concurrent TMZ at a target dose of 75 mg/m^2/day.

Patients will receive the initial cycle of TMZ 4 (+/- 1) weeks after completing RT. Study drugs will be given intradermally at day 22-24 after the first TMZ cycle and divided equally between both inguinal regions in accordance with **SOP-UFBTIP-128**. All patients will receive Td booster (5 Lf) with vaccine #1 regardless of booster history. Vaccine #2 and #3 will occur at 2 week intervals.

All patients will undergo repeat leukapheresis 2 to 4 weeks after vaccine #3 for PBMCs to generate additional DC vaccines and for immunologic monitoring with specific assessment of baseline antigen-specific cellular and humoral immune responses. TMZ cycle 2 will occur 24 hours to 1 week after repeat leukapheresis. Patients may undergo additional leukapheresis, if needed, to generate up to 10 DC vaccines as outlined in section 9.6., Removal of PBMCs by Leukapheresis.

Patients will then be vaccinated monthly in conjunction with subsequent TMZ cycles every 5(+/- 1) weeks for a total of 6 to 12 cycles after RT as per guidelines defined in recent phase III clinical trial evaluating TMZ regimens in patients with GBM (RTOG 0525). Vaccines will be given on day 22-24 of each TMZ cycle. Patients will continue with monthly vaccinations until a total of 10 vaccines have been administered or until tumor progression (whichever comes first).

All patients will undergo vaccine site pretreatment with Td (1 Lf) or saline (depending on randomization arm) 6-24 hours prior to Vaccine #3, #6, and #9. At vaccine #3, patients at the UF site will undergo MRI/MRS imaging of inguinal vaccine-site draining lymph nodes before vaccine site pretreatment, 48 hrs post vaccination, and 5 to 7 days post vaccination (optional). Peripheral blood and urine samples will be collected at these time points for soluble biomarker analysis.

Patients who have been enrolled in the study and become ineligible to receive study treatment for reasons other than progression may be allowed to receive vaccines that have been generated for them at the discretion of the PI as outlined in section 9.8 DC or PBMC Vaccination.

Blood for immune monitoring will be collected during initial leukapheresis, prior to vaccines 1-3, during MRI/MRS imaging time points surrounding vaccine #3, during the second leukapheresis, prior to vaccine 4, 7, 10, and at progression if feasible. Peripheral blood will be processed in accordance with **SOP-UFBTIP-126**. Urine samples will be collected at MRI/MRS imaging time points surrounding vaccine #3 and transferred to the UFBTIP laboratory for nMR analysis.

Up to twelve cycles of TMZ may be given if the patient demonstrates continued improvement or stability on radiologic scan, decreased corticosteroid requirement, improvement in performance status, or improvement in neurologic function.

Patients will be imaged bimonthly during TMZ cycles and every 3 months after the 12th TMZ cycle (or 1 year post surgery if TMZ discontinued prior to 12 cycles) without receiving any other prescribed antitumor therapy unless progression occurs.

The primary measure of response will be by serial measures of the product of the two largest cross-sectional diameters. The modified RANO criteria (Ellingson, Wen, Cloughesy 2017) will be used for assessment of pseudoprogression, progression or response.

Tumor progression should 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. As this is not a research procedure, consent for biopsy will be obtained separately. If tissue is obtained, it will be used to confirm tumor progression histologically and evaluate cellular infiltration and CMV antigen expression at the tumor site.
If histopathology confirms treatment effect vs tumor progression, the patient may resume study treatment at the discretion of the PI in consultation with the treating physician, if the PI believes that it is safe to continue therapy and that the patient may benefit from the ongoing treatment regimen as described in section 11.11 Confirmation of Tumor Progression by Histopathology.

Patients will be followed until death due to any cause. MRI and clinical evaluation for assessment of disease progression will be conducted bi-monthly for the first 12 months and then every 3 months thereafter.

9.2 Registration Procedures

To register a patient to this study, patient demographic data will be entered into the UFHCC OnCore database by the participating site coordinator after subject consent has been obtained and documented. The Oncore database will assign a study ID to the patient after eligibility is confirmed and an "On Study" Date is entered in Oncore. Patients who signed consent but are deemed not eligible to participate, will be assigned a failed-screening ID by the Coordinating Center Project Coordinator.

For patients enrolled at an outside participating site who will receive study treatment at the University of Florida, the ATTAC Registration Form will be submitted to the Protocol Coordinating Center along with applicable supporting documentation. Once the patient has been entered in the UFHCC database and the registration form has been received, the Protocol Coordinating Center will confirm eligibility and randomize eligible patients. The patient's assigned study ID will be forwarded to the participating site coordinator by email.

9.3 Randomization Procedures

Patients will be randomized by the designated site personnel where study treatment will be administered in accordance with SOP-UFBTIP-140.01.

9.4 Radiation Therapy

Standard external beam RT will be administered concomitantly with TMZ (Appendix A) according to standard guidelines which were adopted from the RTOG 0525 protocol and per the recommendation of the treating Radiation Oncologist. Institutional practices for administration of external beam RT for patients with GBM may be followed.

Patients enrolled in the study will be encouraged to receive Radiation Therapy (and concomitant chemotherapy) at their participating study site. However, to limit travel and finance-related burden

associated with daily travel to participating study site, patients should be allowed to receive standard chemo-radiation with their local treating oncologist.

9.5 Temozolomide Therapy

TMZ will be administered concomitantly with standard external beam RT.

Management of chemotherapy will be done by the patient's treating neuro-oncologist in accordance with institutional practices.

Post RT, patients will receive Adjuvant cycles of doses-intensified TMZ. Dose intensified TMZ will be administered orally once per day for 21 consecutive days (days 1-21) of a 35 (+/- 7) days cycle at 100 mg/m^2/day. Cycle #1 will last up to 12 weeks to allow time to administer 3 bi-weekly vaccines and repeat leukapheresis. Based on TMZ-associated adverse events during the previous cycles, dose reduction of dose-intense TMZ may be done at the discretion of the site treating neuro-oncologist. Patients who experienced significant hematologic toxicity will not receive dose-intense temozolomide. Patients who experienced hematologic toxicity grade $\geq$ 3 due to Temozolomide during their chemo-radiation treatment will receive standard dose Temozolomide at 150-200 mg/m2/day for 5 consecutive days of a 35 (+/7) days cycle. For these patients, one dose reduction to 100 mg/m2/day for 5 days will be allowed based on AE on previous cycle.

Appendix B, C and D are provided as guidelines for dose modifications and chemo management.

Patients age 70 and above, will receive standard dose Temozolomide at 150-200 mg/m2/day for 5 consecutive days of a 35 (+/7) days cycle. For these subjects, one dose reduction to 100 mg/m2/day for 5 days will be allowed based on AE on previous cycle.

In the event that chemo adjuvant cycle is delayed past the timeframe provided in Appendix C and D, the treatment with Temozolomide may resume when toxicity has resolved (or returned within treatment limits) if patient has not experienced tumor progression at the discretion of the PI in consultation with the treating physician, if the PI believes that it is safe to continue therapy and that the patient may benefit from additional chemo cycles. Study vaccines will be given at day 22-24 of each cycle when chemo is restarted.

9.6 Removal of PBMCs by Leukapheresis

Prior to the initiation of TMZ and external beam RT and again 2 to 4 weeks after vaccine #3, subjects will undergo leukapheresis in accordance with **SOP-UFBTIP-131.** Intravenous access will be obtained by placing two large gauge catheters into the antecubital veins bilaterally or by using a dialysis-type central venous catheter that will allow for large volume apheresis. In addition to research protocol consent, additional surgical/procedural consent will be obtained for placement of the central venous catheter by the surgeon/proceduralist. Placement of this special catheter may require use of fluoroscopy. The procedure will be conducted using the COBE®Spectra™ Apheresis System (or equivalent). A sample of each leukapheresis product will be used for immunologic monitoring.

Subjects may undergo additional leukapheresis, if needed, to generate up to 10 DC vaccines. Additional leukapheresis procedures may be performed at anytime at the discretion of the PI to

limit treatment interruption/delay as much as possible. When feasible, additional leukapheresis should be scheduled no earlier than 1 week following DC vaccination and prior to the following TMZ cycle. After the leukapheresis, TMZ cycle may occur as scheduled.

9.6.1 PBMC Generation, Storage, and Testing

PBMCs will be extracted from leukapheresis by density centrifugation in the UF cGMP facility. The cells are washed and processed according to **BR-UFBTIP-1001 and BR-UFBTIP-1001-C** for PBMC to be used as a clinical product. The PBMCs are then stored until needed in liquid nitrogen. After freezing and prior to release of PBMC as a clinical product, an aliquot of cryopreserved cells will be thawed and sent for QA/QC release testing according to **SOP-UFBTIP-122.** Testing will include sterility, aerobic and anaerobic bacterial cultures, fungal cultures, mycoplasma and endotoxin testing.

9.6.2 DC Generation, Storage, and Testing

Dendritic Cells will be manufactured according to **BR-UFBTIP-1002 or BR-UFBTIP-1002-C** from either fresh or frozen PBMC. Immature DCs will be generated from adherent cells of the PBMCs under the culture condition that contains GM-CSF and IL-4 for 7 days. At the end of the 7 day incubation, the cells are then harvested and electroporated with pp65-shLAMP mRNA or pp65-flLAMP mRNA per randomization assignment. The DCs are placed in a flask with AIM V media containing HABS + GM-CSF + IL-4+ TNF-α + IL-6 + IL-1β and incubated at 37^{O}C in 5% CO_2 for 18-24 hours for maturation. The antigen loaded mature DCs are harvested, washed, and then stored until needed in a liquid nitrogen freezer. After freezing and prior to release, an aliquot of cells will be thawed and sent for QA/QC release testing according to **SOP-UFBTIP-122**. Testing will include sterility, aerobic and anaerobic bacterial cultures, fungal cultures, and endotoxin testing. Production of PBMC product will be in accordance with BR-UFBTIP-1001-C.

9.6.3 Preparation of DCs or PBMCs for Vaccination

For each vaccination, DCs or PBMCs will be rapidly thawed at 37^{o}C, washed with PBS and counted. The cell concentration will be adjusted to $2x10^{7}$ cells per 400µL of preservative free saline containing 150 µg of GM-CSF for DC vaccines and saline only for PBMC vaccines and loaded into a 1 mL tuberculin syringe with an appropriately sized needle in accordance with **SOP-UFBTIP-1124**.

9.7 Tetanus and Diphtheria Toxoid Booster and site pre-treatment

At time of first vaccination, all patients, regardless of tetanus vaccination history, will receive a Td booster (5 Lf) intramuscularly into the deltoid muscle.

Subjects will undergo vaccine site pretreatment with a one-fifth dose of Td (1 Lf) (treatment arms 1 and 2) or saline (arm 3) intradermally 6-24 hours prior to Vaccine #3, #6, and #9, alternating site for each pretreatment

9.8 DC or PBMC Vaccination

On day 22-24 of the first TMZ cycle, all patient groups will receive their first intradermal immunization in accordance with **SOP-UFBTIP-128** (per randomization assignment). The 2 subsequent immunizations will be given every 2 (+/- 1) weeks for a total of three doses. Each immunization will be divided equally between both inguinal regions with a total volume of 200 μL per side. Vaccines target dose is 2 x 10 ^7 cells. If less cells (DCs or PBMCs) are available at time of vaccination, injection may proceed with available cells and patients will be informed of the lower dose. Patients will be monitored for thirty minutes to one hour post-immunization for the development of any adverse effects. The immunization procedures will be supervised by a nurse or physician that has completed an Advanced Cardiac Life Support (ACLS) course. A cardiac resuscitation cart will be available in the immediate vicinity when performing these immunizations in case of severe allergic reactions.

After vaccine #3, patients will undergo a repeat leukapheresis for PBMCs and immunologic monitoring.

Subsequently, patients will be immunized on day 22-24 of every cycle of TMZ.

Monthly vaccinations will continue until a total of 10 vaccines have been administered or until tumor progression (whichever comes first). Missed vaccines during cycles 1-8 may be given with subsequent cycles of Temozolomide (or monthly for patients off Temozolomide) if there is no tumor progression until patients receive a total of 10 vaccines.

Patients who experienced toxicity during temozolomide adjuvant cycles for whom it is deemed not safe to resume chemo cycles may continue to receive monthly study vaccines at the discretion of the PI in consultation with the treating physician, if the PI believes that it is safe to continue therapy and that patients may benefit from the study treatment. Vaccination with investigational product may be given until the patient receives a total of 10 vaccines or until progression, whichever comes first.

Patients who can no longer receive Temozolomide and start other anti -cancer therapies before experiencing disease progression will not receive additional study vaccines.

Patients who have been enrolled in the study but are no longer eligible to receive study treatment for reasons other than progression are allowed to receive vaccines that have been generated for them at the discretion of the PI. They will be replaced for purposes of study endpoint if they do not meet criteria of an evaluable subject (received standard XRT/TMZ and received at least one DC vaccine). Such subjects would include: subjects who do not complete standard radiation treatment with concomitant TMZ, have prolonged interruption of treatment beyond allowable study guidelines. These patients will be included in the intent to treat analysis but excluded from the evaluable subjects analysis if disqualified prior to receiving first vaccine. Such subjects may receive QA/QC qualified DC vaccines according to study schedule with or without TMZ at discretion of the PI and in consultation with the treating neuro-oncologist (i.e. subjects removed for intolerance of TMZ treatment may discontinue or reduce TMZ dose as clinical management would dictate).

Given absence of curative treatment for GBM, release of vaccines to patients who have consented to the study but are unable to meet study requirements (most often chemotherapy intolerance) is deemed in best interest of the enrolled subjects and best utilization of cGMP manufactured autologous products. While these patients may be excluded from primary endpoint analysis of evaluable subjects, all toxicity monitoring and DLT stopping rules will apply to any subjects receiving DC vaccination.

9.9 MRI/MRS Imaging of DC Migration

All patients will undergo vaccine site pre-treatment with Td (1Lf) (Martin-Fontecha, Sebastiani et al. 2003) or saline 6-24 hours prior to Vaccine #3, #6, and #9.

At vaccine #3, patients at the University of Florida site will undergo MRI/MRS imaging before vaccine site pretreatment, 48hrs post vaccination, and 5 to 7 days post vaccination (optional) to evaluate impact of DC vaccination and unpulsed PBMC vaccination at inguinal draining lymph nodes. MRI/MRS imaging 5 to 7 days post vaccination may be performed when feasible for patients. Regions of interest will be analyzed in a blinded fashion by qualified radiologists within the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility and UF Health Department of Radiology. Peripheral blood and urine will be collected at these time points for soluble biomarker analysis. These correlative studies are designed to determine whether changes in VDLNs can be assessed by MRI/MRS and correlation between changes and clinical outcome.

10 STUDY REQUIREMENTS

10.1 Enrollment

- Signed informed consent.
- History, physical & neurological exam
- MMSE for subject ≥ 50 years.
- Histopathology report confirming GBM (WHO Grade IV Glioma) and molecular diagnostics.
  If molecular testing were not performed at time of diagnosis, promoter hypermethylation of the *O*6-methylguanine-methyl-transferase (*MGMT*) gene, and the mutation status of the *IDH1* and *IDH2* genes will be obtained for study data analysis.
- Preoperative MRI (or CT).
- Postoperative MRI (or CT) within 28 days of study enrollment. If the postoperative MRI is older than 28 days, a new MRI will be obtained prior to patient randomization. The new MRI will serve as baseline MRI.
- Karnofsky Performance Status.
- CBC with differential and CD4+/CD8+ T cell ratio.
- Infectious disease testing to include: HIV, Hepatitis B, Hepatitis C and CMV.
- For females of child-bearing potential, β-HCG serum pregnancy test.
- Pathology slides (if available) consisting of 1 hematoxylin and eosin (H&E) and 19 unstained slides, from all prior resections, prepared on Fischer Plus glass or Histostix coated slides.

- Confirm eligibility.

10.2 Prior to Leukapheresis (Vaccine Cell Generation)

- Perform randomization prior to leukapheresis #1.
- CBC with differential and CD4+/CD8+ T cell ratio.
- Comprehensive metabolic panel
- For females of child-bearing potential, β-HCG serum pregnancy test.
- Infectious disease testing in accordance with institutional practices.
- Blood for immune monitoring.
- Dialysis type central venous catheter for patients with poor venous access.
- Oral Tums, 2 tablets, BID for 2 days prior to the leukapheresis procedure (if standard-of-care) to prevent the development of hypocalcemia from the citrate.
- Document adverse events.
- Document concomitant medications.

10.3 Chemoradiation

- For females of child-bearing potential, β-HCG serum pregnancy test within 72 hours prior to starting TMZ.
- Administer TMZ continuously from day 1 of RT to the last day of RT for a maximum of 49 days.
- Standard RT at the discretion of the treating Radiation Oncologist
- Radiation Therapy summary to include start and end dates, number of fractions, any interruptions, and total dose delivered.

10.4 Adjuvant TMZ Cycles

- History, physical & neurological exam
- Confirm eligibility prior to TMZ cycle 1.
- Administer Td booster with vaccine 1.
- Administer TMZ.
- Administer study vaccines.
- Perform vaccine site pretreatment with Td (1Lf) or saline intradermal prior to Vaccine #3, #6, and #9.
- Inguinal MRI/MRS imaging with Vaccine 3 (University of Florida site only). For women of childbearing potential a urine pregnancy test will be obtained prior to imaging.
- Blood for immune monitoring:
    - Leukapheresis 1
    - Prior to vaccine 1
    - Prior to vaccine 2
    - Prior to vaccine 3
    - At MRI/MRS imaging time points (UF site only)
    - Leukapheresis 2
    - Prior to vaccine 4
    - Prior to vaccine 7
    - Prior to vaccine 10

  - At progression
- Urine for immune monitoring
  - Prior to vaccine 1
  - At MRI/MRS imaging time points (UF site only)

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

  For immune monitoring, up to 80 mLs of blood will be collected in 10 yellow top tubes and processed in accordance with **SOP-UFBTIP-126**. For humoral response, approximately 3.5 mLs will be collected in a gold top tube.

- CBC with differential and CD4+/CD8+ T cell ratio at each vaccine visit and, if clinically indicated, at routine clinic visits.
- MRI (or CT) at the following time points:
  - bimonthly during TMZ cycles and every 3 months after the 12th TMZ cycle (or 1 year post surgery if TMZ discontinued prior to 12 cycles)
  - Response and progression will be assessed following Modified RANO Criteria as described in Section 11.Response Evaluation. Initial assessment of disease status will be done by the treating neuro-oncologist/study investigators, upon review of surveillance brain MRI during routine clinic visit followed by study vaccine administration or chemo cycle initiation. Clinical assessment of disease status will be followed by formal measurements of enhancing lesions for modified RANO criteria.
- Review and document adverse events at each visit.
- Review and document concomitant medications at each visit.

10.5 At Progression

- MRI (or CT) documenting progression.
- Blood for immunologic monitoring if feasible.
- Pathology slides (if available) consisting of 1 hematoxylin and eosin (H&E) and 19 unstained slides, prepared on Fischer Plus glass or Histostix coated slides.

10.6 Concomitant Medication

The following medications will be recorded with start date, stop date, and indication:

- Steroids
- Anticonvulsants
- Bevacizumab
- Any medication used in the treatment of a study drug related AE/SAE
- Any medication deemed to possibly be interactive with study drug by PI or designee.

Concomitant medication will be recorded at the time of vaccine #1 and continue until 30 days after the last dose of study drug and at leukapheresis time point.

Use of bevacizumab:

Bevacizumab is not a study drug on this trial. However, for patients who develop radiation necrosis symptoms, a short trial of bevacizumab can be considered at the discretion of the PI in consultation with the treating physician, if the PI believes that the patient may benefit from the treatment.
Dose and frequency of bevacizumab treatment will be at the discretion of the investigator in keeping with standard of practice (bevacizumab 5-10 mg/kg every 2-3 weeks) for up to 3 months treatment.

NOTE: Bevacizumab is to be used for participants who develop symptomatic radiation necrosis, and is not intended to be used to treat the underlying tumor.

10.7 Schedule of Study Assessments

Visits / Study Requirements	Pre-Treatment				Treatment Period																				Follow-up
	Enrollment	Randomization	Leukapheresis #1	Chemo-radiation [19]	Adjuvant TMZ Cycle 1	Vaccine #1	Vaccine #2	Vaccine #3	Leukapheresis #2[23]	Adjuvant TMZ Cycle 2	Vaccine #4	Adjuvant TMZ Cycle 3	Vaccine #5	Adjuvant TMZ Cycle 4	Vaccine #6	Adjuvant TMZ Cycle 5	Vaccine #7	Adjuvant TMZ Cycle 6	Vaccine #8	Adjuvant TMZ Cycle 7	Vaccine #9	Adjuvant TMZ Cycle 8	Vaccine #10	Up to 12 Cycles of TMZ	Progression
					4 wks after RT completion +/-1	Day 22-24 of Cycle 1	2 weeks after Vaccine #1 +/-1	2 weeks after Vaccine #2 +/-1	2 to 4 weeks after vaccine #3	1 day to 1 week after 2nd leukapheresis	Day 22-24 of Cycle 2	5 weeks after Cycle #2 +/-1	Day 22-24 of Cycle 3	5 weeks after Cycle #3 +/-1	Day 22-24 of Cycle 4	5 weeks after Cycle #4 +/-1	Day 22-24 of Cycle 5	5 weeks after Cycle #5 +/-1	Day 22-24 of Cycle 6	5 weeks after Cycle #6 +/-1	Day 22-24 of Cycle 7	5 weeks after Cycle #7 +/-1	Day 22-24 of Cycle 8	Every 5 weeks +/-1	Follow-up until tumor progression or death
Inclusion and Exclusion criteria	X				X																				
Informed Consent	X																								
Brain MRI or CT scan	X[1]				<----------X[8]---------->																			X[8]	
Karnofsky Performance Status	X																								
Serum pregnancy test	X		X	X[2]				X[2]	X																
Tumor Tissue	X[3]																							X[3]	
Randomization		X[12]																							
CBC w/ differential	X		X			X[9]	X[9]	X[9]			X[9]		X[9]		X[9]		X[9]		X[9]		X[9]		X[9]		
Immunophenotyping CD4+/CD8+ T cell ratio	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Peripheral blood for immune monitoring			X[14]			X[14]	X[14]	X[14]	X[14]		X[14]						X[14]						X[14]		X[14]
Comprehensive metabolic panel			X		X				X																
Infectious disease testing	X[22]		X[4]						X[4]																
Oral Tums			X[5]						X[5]																
Leukapheresis			X						X[16]																
Temozolomide				X[6]	X[10]					X[10]		X[10]		X[10]		X[10]		X[10]		X[10]		X[10]		X[10]	
External Beam RT				X[7]																					
History, Physical and Neurological Exam	X[21]				X[11]					X[11]		X[11]		X[11]		X[11]		X[11]		X[11]		X[11]		X[11]	
Td Booster						X[15]																			
Blinded vaccine site pre-treatment								X[17]							X[17]						X[17]				
Blinded study vaccine						X[13]	X[13]	X[13]			X[13]		X[13]		X[13]		X[13]		X[13]		X[13]		X[13]		
MRI/MRS Inguinal imaging (UF site only)								X[18]																	
Urine Immune monitoring (UF site only)						X[18]		X[18]																	
Concomitant medications			X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Adverse event assessment			X[20]			X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]	X[20]		

Footnotes on next page

Footnotes for Schedule of Study Assessments:

1 A diagnostic contrast-enhanced MRI or CT scan of the brain must be performed preoperatively and postoperatively.If no post op MRI/CT within 28 days of enrollment is available, a new brain MRI (or CT) must be obtained in order to determine residual burden and eligibility prior to randomization.

2 Negative serum pregnancy test within 72 hours prior to starting TMZ and negative urine pregnancy test prior to Inguinal MRI/MRS (UF site only).

3 1 H&E and 19 unstained slides from original surgery and at progression, if applicable.

4 Pre-leukapheresis testing in accordance with institutional practices.

5 Oral Tums, 2 tablets, for 2 days prior to leukapheresis (if standard-of-care).

6 Administer TMZ continuously from day 1 of RT to the last day of RT at a daily oral dose of 75 mg/m^2 for a maximum of 49 days.

7 Standard RT at the discretion of the treating Radiation Oncologist

8 Bimonthly during TMZ cycles and every 2 to 3months after the 12th TMZ cycle (or 1 year post surgery if TMZ discontinued prior to 12 cycles). Measurements for all imaging will be recorded. Prior to vaccination, assessment for tumor progression can be made by the treating investigator, but all CT/MRI measurements for study endpoints must be made by designated study personnel.

9 CBC with differential and CD4+/CD8+ T cell ratio at each vaccine visit and, if clinically indicated, at routine clinic visits.

10 Up to 12 cycles of dose-intensified TMZ may be given. Cycle #1 should be started 4 (+/- 1) weeks after completing chemoradiation. Administer once per day for 21 consecutive days (days 1-21) of a 35 (+/- 7) day cycle. The starting dose for the first cycle will be 75 mg/m^2/day, with a single dose escalation to 100 mg/m^2/day in subsequent cycles if no adverse events are noted.

11 Within 14 days prior to initiation of TMZ and prior to subsequent TMZ cycles.

12 Randomization will occur prior to leukapheresis.

13 Administer vaccine #1 intradermally at day 22-24 after the first TMZ cycle. Vaccines #2 and #3 will occur at 2 (+/- 1) week intervals. Vaccines #4-10 will be given on day 22-24 of each TMZ cycle. Continue until a total of 10 vaccines have been administered or until tumor progression (whichever comes first). Each vaccination will be equally divided between both inguinal regions. Monitor for 30-60 minutes post-immunization for any adverse effects.

14 Up to 10 yellow top tubes (ACD-A) and 1 gold top tube (SST) during initial leukapheresis, prior to vaccines 1-3, during second leukapheresis, prior to vaccine 4, 7, 10, at MRI/MRS imaging time points (UF site only) and at progression if feasible. Peripheral blood will be processed in accordance with **SOP-UFBTIP-126**.

15 All patients will receive IM Td booster (5 Lf) with vaccine #1.

16 2 to 4 weeks after vaccine #3, repeat leukapheresis for DC or PBMC generation.

17 All patients will undergo vaccine site pretreatment with Td (1 Lf) or saline 6-24 hours prior to Vaccine #3, #6, and #9.

18 Patients at University of Florida site will undergo MRI/MRS imaging of inguinal draining lymph nodes before vaccine #3 site pretreatment, 48hrs post vaccination, and 5 to 7 days post vaccination (optional). Urine samples will be collected prior to vaccine #1 and at MRI/MRS imaging time points.

19 Within 7 weeks of surgery.

20 AE collection will begin at the time of administration of vaccine #1 and continue until 30 days after the last dose of study drug. Any adverse events associated with leukapheresis will also be recorded.

21 Including MMSE for patients age ≥ 50 years.

22 Results from infectious disease markers performed within 30 days of enrollment can be used to satisfy criteria.

23 Additional leukapheresis may be performed if needed to generate additional vaccines.

11 RESPONSE EVALUATION

The modified RANO provides clinical guidelines for continuing therapy **beyond** suspected radiographic progression if the treating physician believes there may be a therapeutic benefit and provides criteria for defining progression and early drug failure while also allowing for the possibility of pseudoprogression (PsP) and pseudoresponse (PsR). An update to the RANO criteria proposes strategies to establish a general framework for response assessment in neuro-oncology that is agnostic to the mechanism of action of the particular therapy (anti-angiogenic, immunotherapy...) to address the challenges associated with interpretation of radiographic changes in patients with glioblastoma in clinical trials. The modified RANO proposes to **use the post-radiation time point as the baseline** for response evaluation and to consider only objectively defined, measurable enhancing disease in the definition of response and progression (i.e. exclusion of qualitative assessed T2/FLAIR changes)

11.1 General Methodology for Determining Tumor Measurements

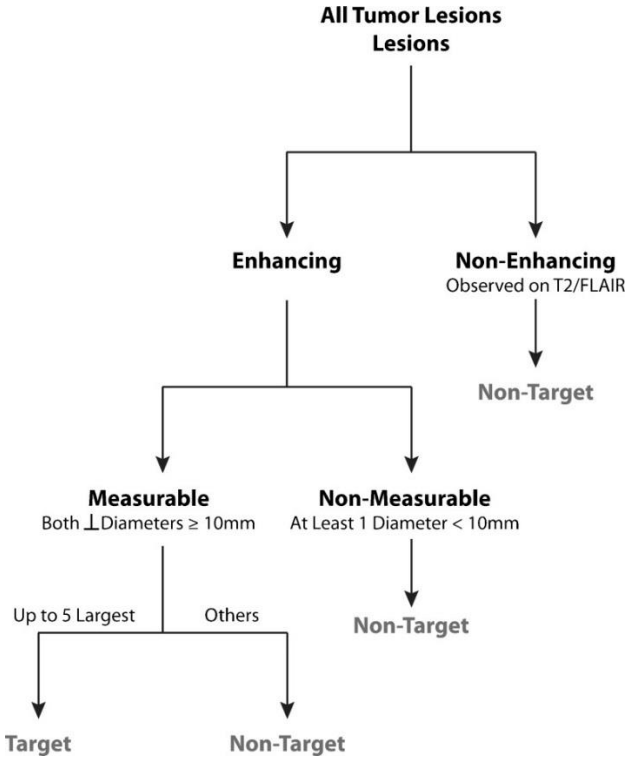
Newly diagnosed GBM patients will initially undergo a pre-entry MRI scan for initial diagnosis prior to entry in the study and prior to therapy. The post-operative scan will determine residual enhancing disease which may be used as a covariate in survival analyses. Patients will then start on standard therapy, Temozolomide with concurrent radiation therapy (RT). **The Post-RT scan will be required and used as the baseline scan** for which response will be determined. Following the first cycles of adjuvant therapy, patients will receive additional required MRI scans.

All radiographic responses will be based on MRI scans of the brain obtained with and without contrast. Tumor size will be based on the product of the maximal diameters in 2 different planes. Response will be assessed as a percentage change in tumor size from baseline. Tumor measurements in the follow-up scans will be compared to those of the post-RT scan to document response.

<u>Definition of Measurable Disease, Non-Measurable Disease, and Target Lesions</u>

Measurable disease should be defined as contrast enhancing lesions with a minimum size of **both** perpendicular measurements greater than or equal to 10mm (if the largest diameter is greater than 10mm but the perpendicular diameter is less than 10mm, this would constitute non-measurable disease). Up to a total of five target measurable lesions should be defined and ranked from largest to smallest.

Algorithm for identifying measurable and target lesions:



11.2 Complete Response (CR)

Requires ***all*** of the following:

1. Disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks. The first scan exhibiting disappearance of all enhancing measurable and non-measurable disease is considered "preliminary CR". If the second scan exhibits measurable enhancing disease with respect to the "preliminary CR" scan, then the response is not sustained, noted as pseudoresponse, PsR, and is now considered "preliminary PD" (note: confirmed PD requires at least two sequential increases in tumor volume). If the second scan continues to exhibit disappearance of enhancing disease or emergence of non-measurable disease (less than 10mm bidimensional product), it is considered a *durable CR* and the patient should continue on therapy until confirmed PD is observed.
2. Patients must be off corticosteroids (or on physiologic replacement doses only).
3. Stable or improved clinical assessments.

Note: Patients with non-measurable disease only at baseline cannot have CR; the best response possible is stable disease (SD).

11.3 Partial Response (PR)

Requires *all* of the following:

1. ≥50% decrease in sum of products of perpendicular diameters of all measurable enhancing lesions compared with baseline, sustained for at least 4 weeks. The first scan exhibiting ≥50% decrease in sum of products of perpendicular diameters of all measurable enhancing lesions compared with baseline is considered "preliminary PR". If the second scan exhibits PD with respect to the "preliminary PR" scan, then the response is not sustained, noted as pseudoresponse, PsR, and is now considered "preliminary PD" (note confirmed PD requires at least two sequential increases in tumor volume). If the second scan exhibits SD, PR, or CR, it is considered a *durable PR* and the patient should continue on therapy until confirmed PD is observed.
2. Steroid dose should be the same or lower compared with baseline scan.
3. Stable or improved clinical assessments.

Note: Patients with non-measurable disease only at baseline cannot have PR; the best response possible is stable disease (SD).

11.4 Stable Disease (SD)

Requires ***all*** of the following:

1. Does not qualify for complete response, partial response, or progressive disease. Note this also applies to patients that demonstrate PsR when the confirmation scan does not show PD or PsP when the confirmation scan does not show PR/CR.

In the event that corticosteroid dose was increased (for new symptoms/signs) without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that the steroid increase was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

11.5 Progressive Disease

Defined by *any* of the following:

1. At least two sequential scans separated by at ≥4 weeks both exhibiting ≥25% increase in sum of products of perpendicular diameters of enhancing lesions. The first scan exhibiting ≥25% increase in sum of products of perpendicular diameters of enhancing lesions should be compared to the smallest tumor measurement obtained either at baseline (if no decrease) or best response (on stable or increasing steroid dose) and is noted as "preliminary PD." If the second scan at least 4 weeks later exhibits a subsequent ≥25% increase in sum of products of perpendicular diameters of enhancing lesions relative to the "preliminary PD" scan, it is considered "confirmed PD" and the patient should discontinue therapy. If the second scan at least 4 weeks later exhibits SD or PR/CR, this scan showing "preliminary PD" is noted as "pseudoprogression", PsP, and the patient should continue on therapy until a second increase in tumor size relative to the PsP scan is observed. Note that any new *measurable* (>10mm x 10mm) enhancing lesions should *not* be immediately considered PD, but instead should be added to the sum

of bidimensional products or total volume representing the entire enhancing tumor burden.

2. In the case where the baseline or best response demonstrates no measurable enhancing disease (visible or not visible), then any new *measurable* (>10mm x 10mm) enhancing lesions are considered PD *after* confirmed by a subsequent scan ≥4 weeks exhibiting ≥25% increase in sum of products of perpendicular diameters of enhancing lesions relative to the scan first illustrating new measurable disease. The first scan exhibiting new measurable disease is noted as "preliminary PD." If the second scan at least 4 weeks later exhibits a subsequent ≥25% increase in sum of products of perpendicular diameters of enhancing lesions relative to the "preliminary PD" scan it is considered "confirmed PD" and the patient should discontinue therapy. If the second scan at least 4 weeks later exhibits SD, CR, PR, or becomes non-measurable, this scan showing "preliminary PD" is noted as "pseudoprogression", PsP, and the patient should continue on therapy until a second increase in tumor size relative to the "preliminary PD", or PsP, scan is observed. Note that any new *measurable*(>10mm x 10mm) enhancing lesions on the subsequent scan following the preliminary PD scan should *not* be immediately considered confirmed PD, but instead should be added to the sum of bidimensional products or total volume representing the entire enhancing tumor burden.
3. Clear clinical deterioration not attributable to other causes apart from tumor (e.g. seizures, medication adverse effects, therapy complications, stroke, infection) or attributable to changes in steroid dose.
4. Failure to return for evaluation as a result of death or deteriorating condition.

11.6 Symptomatic Deterioration & Reporting Clinical Status

Patients with global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time, and not either related to study treatment or other medical conditions, should be reported as PD due to "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment due to symptomatic deterioration.

Neurological exam data should be provided to the study designated neuro-radiologists as "stable, improved, declined" in case report forms. Clinical status should be recorded as "declined" if the neurological exam is worse, otherwise the clinical status should be set to "improved" or "stable". In the event that necessary clinical data is not available, clinical status should be recorded as "not available" and that particular time point can only be reviewed for PD (otherwise "non-evaluable"). Neurological data must be within ±7 days of the time-point response date, otherwise the data is considered "not available".

11.7 Steroid Use and Dose

Steroid use should be derived from the concomitant medications on the case report forms and recorded as "Yes", "No", or "not available". A value of "No" should be assigned if, at the time-point, the subject is not on steroids or on physiologic replacement doses only (<2 mg dexamethasone or equivalent per day).

Average steroid dose no greater than 2 mg change from baseline should be abstracted to "stable". If outside this range the steroid dose should be abstracted to "increased" or "decreased" accordingly. Steroid data should be within ±5 days of the time-point response date, otherwise the data is considered "not available".

11.8 Overall Objective Status

The overall objective status for an evaluation should be determined by combining the patient's radiographic response on target lesions, new disease, neurological status, and steroid dose/usage as defined in the table below for patients with *measurable* (>10mm x 10mm) disease. Note that patients with possible PsP or pseudoresponse should be given the Objective Status of "Preliminary Progression" or "Preliminary Response", respectively. Once PsP, pseudoresponse, or true progression/response are confirmed, the Objective Status can be changed accordingly.

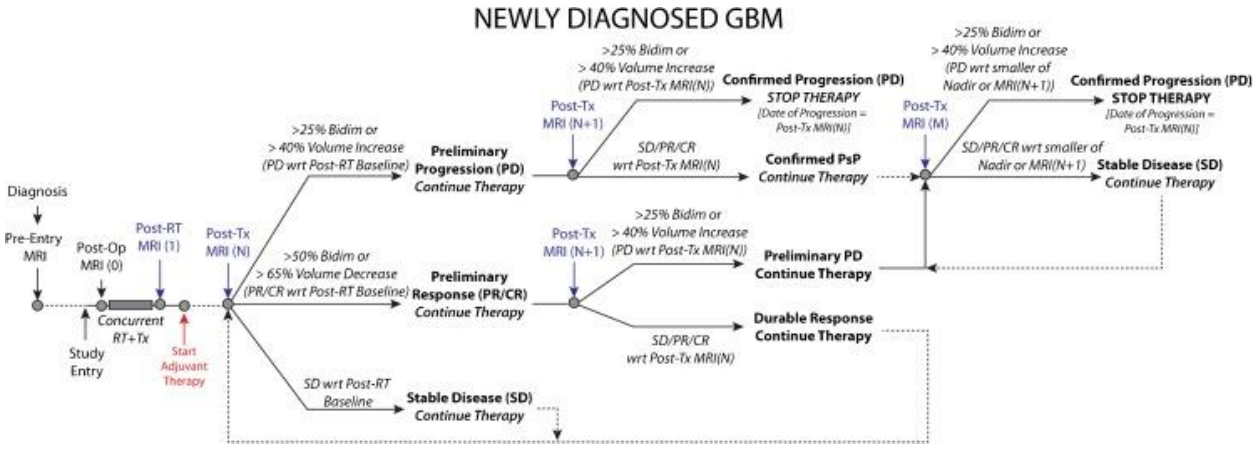
Guidelines for determining comprehensive objective status

Target lesions (current scan)	Target lesions (previous scan)	New sites of measurable disease[a]	Neurological status	Steroid usage	Steroid dose	Overall objective status
CR	Not Evaluated	No	Stable/Better	No	N/A	Preliminary CR
PR	Not Evaluated	No	Stable/Better	Any	Stable/Decreasing	Preliminary PR
PD	Not Evaluated	Yes or No	Stable/Better	Any	Stable/Increasing	Preliminary PD
PD	Preliminary or Confirmed PR/CR	No	Stable/Better	Any	Stable/Increasing	Preliminary PD
SD	Preliminary or Confirmed CR/PR or SD/NE	No	Stable/Better	Any	N/A	SD
PR	Preliminary PR	Yes or No	Stable/Better	Any	Stable/Decreasing	Confirmed PR
SD	Preliminary PR	Yes or No	Stable/Better	Any	Stable/Decreasing	SD (Preliminary PR →Confirmed PR)
SD	Preliminary CR	Yes or No	Stable/Better	Any	Stable/Decreasing	SD (Preliminary CR →Confirmed CR)
CR	Preliminary CR	No	Stable/Better	No	N/A	Confirmed CR
SD	Preliminary PD	No	Stable/Better	Any	Stable/Decreasing	SD (Confirmed PsP)
CR/PR/SD PD/NE	CR/PR/SD/PD/NE	Yes or No	Worse	Any	Stable/Increasing	Confirmed PD
PD	Preliminary PD	Yes or No	Any	Yes	Stable/Increasing	Confirmed PD

[a]Note that new sites of measurable disease are added to the sum of bidimensional products or total lesion volume, or constitutes preliminary PD in the case of no measurable disease at baseline or best response

11.9 Detailed Modified Radiographic Response Assessment

The diagram below indicates the modififed RANO treatment algorithm for the assessment of modified radiographic response in newly diagnosed glioblastoma:



Preliminary Radiographic Progression

If the lesion size has increased ≥25% sum of bidirectional product between MRI Scan 1 and N, these patients should be categorized as "preliminary radiographic progression". If the investigator believes the patient can safely continue on therapy, then they should continue to treat and acquire a follow-up confirmatory scan [MRI(N + 1)] at the next scan interval (8 weeks ± 4 weeks from MRI Scan (N) or no less than 4 weeks minimum duration between preliminary PD and confirmed PD scans) to verify tumor growth and progression. For patients with gross-total resection (GTR) and no measurable enhancing disease, *preliminary* radiographic progression is defined as a transition from *no measurable disease* to *non-measureable* (*but present*) *disease*(*<10mm x 10mm*) or *measurable disease* (*>10mm x 10mm*). If the investigator feels it is safe to keep the patient on, a confirmatory scan at MRI(N + 1) should be obtained to verify tumor progression.

Confirmed Progression

If the patient has an increase ≥25% sum of bidirectional product between MRI Scan N and N + 1, this is "Confirmed Progression", *the patient should stop therapy* and the date of radiographic progression is the date of suspected progression, MRI(N). If the patient has SD/PR/CR on MRI(N + 1) with respect to MRI(N), PsP is confirmed and the patient should continue on therapy. Patients will then continue on therapy and receive additional follow-up MRI scans [MRI(M)]. If the lesion size has increased ≥25% sum of bidirectional product on MRI(M) relative to the smaller of Nadir or MRI(N + 1), then the patient has "Confirmed Progression", *the patient should stop therapy* and the date of radiographic progression is the new date, MRI(M). For patients with no measurable disease at the Post-RT baseline, "Confirmed Progression" will be defined as a transition from *non-measurable* (*but present*) *disease* (*<10mm x <10mm*) on MRI(N) to *measurable disease* (*>10mm x 10mm*) on MRI(N + 1). For patients with confirmed PsP and no measurable disease at Nadir, "Confirmed Progression" should be defined as a transition from *no measurable disease* to *measurable disease* (*>10mm x 10mm*). In all cases, patients with confirmed progression should stop therapy.

Preliminary Radiographic Response
If a measurable lesion has decreased ≥50% sum of bidirectional product between MRI(1) and MRI(N), these patients should be categorized as "preliminary radiographic responders" and will be monitored for an additional time point and/or treatment cycle. After an additional cycle of therapy (8 weeks ± 4 weeks from MRI(N)), patients will receive a confirmatory MRI(N + 1).

Confirmed Radiographic Response
If the lesion(s) have increased ≥25% sum of bidirectional product between MRI Scan N and N + 1, (indicating radiographic progression from MRI(N)), this is considered an "unsustained radiographic response" or "pseudoresponse". These patients should be categorized as "preliminary radiographic progression". If the investigator believes the patient can safely continue on therapy, then they should continue to treat and acquire a follow-up confirmatory scan [MRI(M)] at the next scan interval (8 weeks ± 4 weeks from MRI Scan (N+1) or no less than 4 weeks minimum duration between preliminary PD and confirmed PD scans) to verify tumor growth and progression. If the patient has an increase ≥25% sum of bidirectional product between MRI Scan N+1 and M, this is "Confirmed Progression", *the patient should stop therapy* and the date of radiographic progression is the date of suspected progression, MRI(N+1). If the patient has SD/PR/CR on MRI(M) with respect to MRI(N+1), PsP is confirmed and the patient should continue on therapy. Patients will then continue on therapy and receive additional follow-up MRI scans [MRI(M)].

Alternatively, if the lesion has not increased from MRI(N), this is considered a "durable radiographic response," the patient will continue on therapy, and the date of *preliminary* radiographic progression is the time point of an increase ≥25% sum of bidirectional product (from Nadir) during the remainder of the study. The investigator can then decide whether to continue safely on therapy until progression has been confirmed and at the subsequent time point stop therapy if they feel the patient cannot safely continue therapy.

Stable Disease
If the lesion size has not increased or decreased beyond the set thresholds between Scan 1 and N, the patient is considered "stable." Such patients will continue on therapy, and the date of *preliminary* progression is the time point of an increase ≥25% sum of bidirectional product (from Nadir) during the remainder of the study. Upon preliminary progression the investigator can choose to either continue therapy and confirm progression or discontinue therapy. For cases with significant neurologic decline at the time of imaging progression as determined from MRI(N), a confirmatory scan at time point MRI(N + 1) may not be possible or necessary. For these cases, it is appropriate to define MRI(N) as the progression time point.

11.10 Clinical Judgement

Treatment effects, necrosis, vasogenic edema and true tumor progression may appear similar on post contrast brain MRI. There is a continued effort to develop new tools and method to characterize these different outcomes. However, differentiating treatment effects from true progression in patients with high-grade glioma remains an ongoing challenge.
Consequently, we propose to provide some allowance for clinical judgement when deciding whether to discontinue DC vaccination when there is a reasonable doubt regarding disease progression versus treatment effects.

In the event of radiographic changes that meet modified RANO criteria for disease progression in clinically stable or improved patients, vaccination treatment may be continued at the discretion of the PI in consultation with the treating physician, if the PI believes that it is safe to continue therapy and that patients may benefit from the ongoing treatment regimen. Subsequent evaluation by radiographic imaging, clinical evaluation, and/or surgery/biopsy that declares true tumor progression will result in establishing the date of tumor progression at the time when the patient first met modified RANO criteria for documented tumor progression. Therefore, it will not be deemed a protocol deviation for patients to have received subsequent DC vaccines during the interval of assessment for resolving tumor progression. Given the primary endpoint of overall survival for this phase 2 study and above described difficulties in radiographic assessment in this patient population, an allowance for clinical judgement on treatment discontinuation is warranted.

11.11 Confirmation of Tumor Progression by Histopathology

Due to the challenge of recognizing treatment effect from tumor progression, histological confirmation of tumor progression should be obtained when clinically indicated to avoid premature termination of effective treatment or continuing ineffective treatment. Tissue obtained by biopsy or resection will be used to confirm tumor progression histologically. If tumor progression is confirmed, ongoing treatment will be stopped and best of available care at the time of confirmed progression will be discussed with the patient. If obtained in sufficient quantity, available tissue will be used to evaluate cellular infiltration and CMV antigen expression at the tumor site.

In the event that histopathology reveals inflammatory response, indicating toxic or therapeutic responses and not tumor progression, the patient may resume ongoing treatment at the discretion of the PI in consultation with the treating physician after the patient has recovered from the effect of biopsy/surgery.

12 IMMUNOLOGICAL RESPONSE EVALUATIONS

Immunological response evaluations for baseline values will be conducted on PBMCs collected at the time of the initial leukapheresis sample. Immunologic response evaluations will also be performed from peripheral blood obtained prior to select vaccinations. The primary comparison for immunologic endpoints will be baseline response (pre-leukapheresis or P1) compared to response post vaccine #3 (post-leukapheresis or P2). Our previous studies demonstrated a significant increase in immunologic responses in vaccinated patients that peaked after the third vaccine in most subjects. A comparison of pre-therapy lymphocyte function to additional vaccine time points may be conducted on an exploratory basis. Arms 1 and 2 (pp65-shLAMP RNA-pulsed DCs and pp65-flLAMP RNA-pulsed DCs) will be analyzed separately for immunologic endpoint comparisons. Studies examining DNA vaccines using full-length LAMP1 fusion antigens compared to truncated versions of the LAMP protein have demonstrated that increased antigen expression, secretion of LAMP fusion proteins, enhanced antibody titers, increased cytokine release by CD4+ and CD8+ T cells, improved central memory T cell differentiation, and enhanced

polyfunctional T cell responses, all were dependent on full-length LAMP1 fusion antigens(Godinho, Matassoli et al. 2014). Truncated LAMP fusions that did not contain the luminal domain of LAMP were much less efficient in this wide spectrum of immunologic analyses. As a primary biologic analysis and secondary endpoint of this study, we will compare the immunologic responses in patients vaccinated with CMV pp65-flLAMP RNA-pulsed DCs (Arm 1) to patients vaccinated with CMV pp65-shLAMP RNA-pulsed DCs (Arm 2) across this spectrum of responses.

12.1 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.

On the day of testing, PBMCs will be thawed quickly, washed, resuspended in R-10 medium and cell counts and viability measured PBMCs (250,000/well) will be stimulated overnight with synthetic peptides consisting of a peptide cocktail derived from human CMV proteins. Single-use aliquots of the peptide pools 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 IgG1 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 IgG1 anti-human IFN-γ MAb for 1 hour at room temperature, washed with PBS, incubated with avidin-peroxidase complex for 1 hour at room temperature, washed, incubated with substrate (3-amino-9-ethylcarbazole) for 4 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.

12.2 Polyfunctional T cell Responses, T cell Phenotype, and Other Type of Immune Cell Phenotype

The laboratory of UFBTIP will conduct a flow cytometric analysis that aims to quickly detect the activation of T cells through CD69 and exhaustion via PD-1. A 13-color assay will be used to monitor the polyfunctional immune response of patients' CD4 and CD8 cells. The analysis will also include an examination of the phenotypic distribution of T cells that respond to the assay, including naive, effector, central memory, effector memory, and terminal effector cells. Additionally, the same panel will be used to analyze regulatory T cells (identified as CD3+CD4+CD25+FoxP3+), natural killer cells (CD56+), natural killer T cells (CD56+CD3+), invariant natural killer T cells (CD56+CD3+TCR-va24+), and cell viability (measured through the Live/Dead Fixable Blue Viability Assay) (as seen in Figure 13).

Immediately preceding select vaccinations peripheral blood will be drawn into vacutainer tubes containing ACD. PBMC will be separated by density gradient centrifugation on ficoll-Hypaque and then cryopreserved until the day of immune monitoring operation..

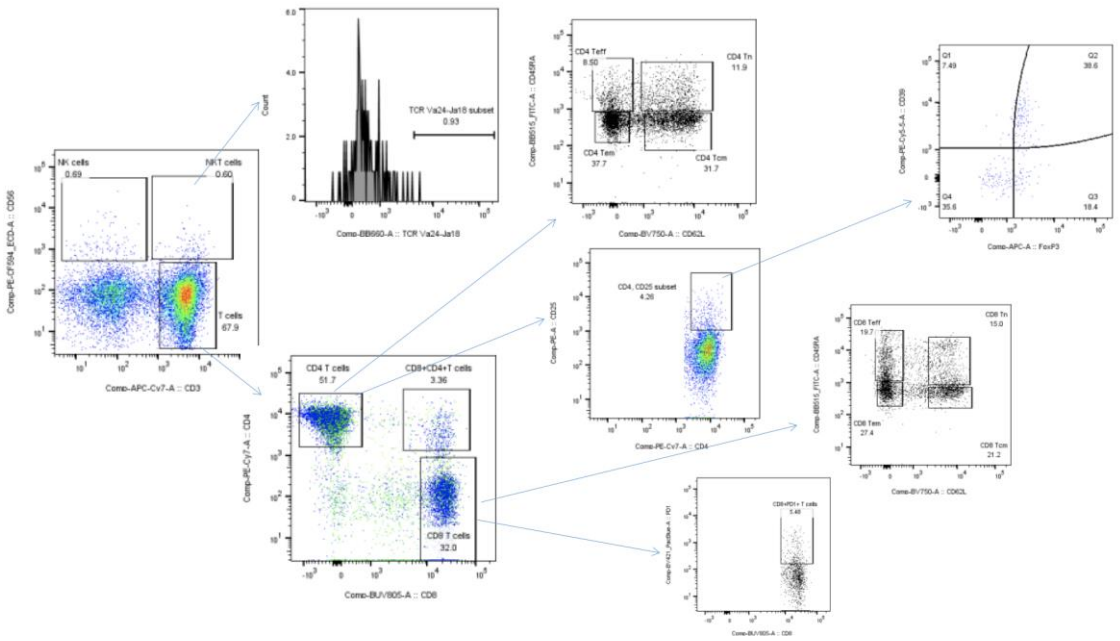


Fig. 13: Polyfunctional Lymphocytes Gating Strategy. Lymphocytes can be expanded and phenotyped to identify T cells and NK cells. Within the T cells population, CD4 and CD8 T cells are further identified and their phenotypic distribution, along with regulatory T cells, is determined. These findings demonstrate that CMV pp65-LAMP RNA-pulsed DCs can expand polyfunctional T cells in patients with GBM.

12.3 Multiplex Cytokine Quantification

The UFBTIP laboratory will perform a Multiplex cytokine analysis to detect multiple cytokines secreted by lymphocytes. This analysis will use a multiplex kit on the Meso Scale Discovery platform to simultaneously examine Type 0,1,2, and 3 cytokines (IFN-g, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL12p70, IL-13, and TNF-a) secreted by T cells. Prior to select vaccinations, peripheral blood will be drawn into vacutainer tubes containing silicone and micronized silica particales (SST) to isolate patient serum. This serum will be cryopreserved until the day of the Multiplex cytokine assay.

12.1 Single Cell Gene Expression and Immune Profiling For Monitoring Changes in Glioblastoma Patients with Sustained Radiographic Response to CMV pp64-LAMP RNA-Pulsed Dendritic Cell-Based Vaccines.

The application of single cell sequencing as a novel immune monitoring platform can be used to identify the molecular mechanisms of immune response to dendritic cell- based vaccines, trace the cell types and states involved, and uncover novel biomarkers for immunotherapy. We will perform

single-cell RNA Seq analysis of longitudinal peripheral blood mononuclear cells (PBMCs) in selected patients.

Cell preparation: Frozen cell vials with PBMCs collected at different time points following DC vaccination of selected ATTAC patients will rapidly be thawed in a 37°C water bath. Thawed PBMCs will washed and filtered with a cell drainer. Cells will be collected by centrifugation at and subsequently counted with hemocytometer. For Single cell RNA-seq, single cell suspension will be loaded onto Chromium Controller according to the manufactures specifications and TCR-seq libraries will be prepared via PCR amplification with primers specific to the TCR and for 5' gene expression library construction. Pre-processing of scRNA-seq data will be performed using specific software for identification of cell types.

13 SAFETY MONITORING AND REPORTING

13.1 Dose-limiting Toxicity

DLTs will be evaluated according to NCI CTCAE (Version 5.0) criteria.

A DLT will be defined as a possibly, probably or definitely **drug-related**:

- Grade IV toxicity (with the exception of grade IV cerebral edema) or any non-neurologic toxicity ≥ Grade III toxicity of any duration.
- Grade III neurologic toxicity if not improved to Grade ≤ II within 7 days of clinical management will be declared a DLT.
  Neurotoxicity that improves to Grade II within 7 days will be allowed an additional 21-day management period to resolve to a Grade I or return to patient baseline. If the event does not improve to a Grade I or return to patient's baseline within 21 days of management, it will then be declared a DLT

Immune-related neuro toxicities are a recognized adverse event associated with immunotherapy. Symptoms may present throughout the course of treatment and involve the peripheral and central nervous system, and usually respond well to steroids, if started early. Early recognition of neurological toxicity is critical for the initiation of clinical management or surgical intervention to prevent or potentially reverse neurological sequelae. If neurological toxicity improved to ≤ Grade I within 28 days of clinical management, patient may resume treatment.

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

If a **neurologic Grade III NCI CTC toxicity** is seen that is not attributable to a concomitant medication, co-morbid event, or disease progression that has been documented radiographically or clinically, the next immunization for that patient will be withheld for up to 2 months or until the NCI CTC toxicity improves to a Grade ≤ I or until the KPS score returns to within 10 points of

baseline. Grade III neurologic toxicities will be declared irreversible if they cannot be reversed within 4 weeks of clinical management. If the event has not improved to a Grade ≤ II within 7 days of clinical management (then to ≤ Grade I or baseline within 21 days), a DLT will be declared for that patient and no further vaccinations or study-related procedures will be performed. If the event is reversed, but Grade III NCI CTC neurologic toxicity is again seen with subsequent vaccinations, all further vaccinations and study-related procedures will be withheld and a DLT will be declared. Medical therapy or surgical intervention, may be used to reverse any toxicity if necessary.

In the case of hypersensitivity reactions, treatment measures deemed medically appropriate will be initiated and the PI notified of the event. Although not considered a DLT, any patient with ≥ Grade II urticaria will not receive further vaccines and will be withdrawn from the study.

Cerebral edema toxicity exception:

Cerebral edema normally presents in glioblastoma patients as part of the disease process and can be exacerbated by standard of care chemotherapy and radiation. Furthermore, an effective anti-tumor immune response may involve inflammatory response and edema in infiltrative tumor cells.

Therefore, we propose that worsening cerebral edema (noted by MRI) that is not associated with changes in neurological symptoms will not constitute a DLT, but worsening cerebral edema in association with Grade 3 or greater other neurologic toxicities will be managed with above outlined criteria (improvement to ≤ Grade 2 within 7 days and to Grade 1 or baseline within 21 days or will be declared a DLT). Radiographic improvement of cerebral edema will be noted, but since NCI CTCAE v5.0 guidelines do not allow for an improvement in cerebral edema Grade < 3, the CTCAE grading criteria for cerebral edema will be exempted from DLT assessment.

Grade III or greater toxicities associated with DC-based immunotherapy have been rare. Adoptive T cell therapy using tumor-infiltrating lymphocytes and high-dose IL-2 in patients with melanoma and treatment with anti-CLTA-4 monoclonal antibody blockade have been the immunotherapy regimens most often associated with treatment related toxicities. To take the most conservative approach to assessing possible toxicities associated with this treatment, we will be vigilant for any similar toxicity associated with this therapy, in addition to autoimmune toxicity specific to the CNS. Possible immune-mediated disorders that have been observed in patients who have received immunotherapy in early phase trials have involved the skin (vitiligo and cutaneous leukocytoclastic vasculitis), the thyroid gland (autoimmune thyroiditis), the liver (autoimmune hepatitis) and the pituitary (hypophysitis). Abnormal lab results, which may be immune-mediated, include elevations of serum lipase and amylase and liver function tests. If a patient has an AE that is thought to be possibly related to autoimmune antibodies (eg, thyroiditis, hepatitis, thrombocytopenia) the PI will send a blood sample for appropriate autoimmune antibody testing. If specific autoantibodies are present, the serum sample taken for storage at baseline will be tested for the presence of those autoantibodies. Further immunotherapy treatment in that patient will be held until the etiology of the event is established.

13.2 Stopping Rules

To continuously monitor vaccine-related toxicity, we will use a one-sided Pocock-type sequential boundary (Ivanova et al. 2005) to repeatedly test whether a maximum acceptable DLT rate T=14.3% (1 patient in 7) has been exceeded. The sequential boundary will consist of the minimum cumulative number of patients experiencing DLTs per concurrent number of patients enrolled that would result in a rejection of the null hypothesis T≤14.3% and halting of the trial. The sequential boundary will be adjusted so that the overall probability of stopping the trial across the accrual of all 80 patients in the two treatment arms of the trial (i.e. the overall Type 1 error rate) is 0.05 when T=14.3%. If the trial runs to completion without being halted for excessive toxicity, insight into the magnitude of the true DLT rate can be gained by noting that the sequential boundary established for T=14.3% has a >80% overall chance of stopping the trial if in fact T is actually greater than 27.1%. The sequential boundary for T=14.3% and stopping probability 0.05 is displayed in the following table:

N	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
PB	**-**	**-**	**3**	**4**	**4**	**4**	**4**	**5**	**5**	**5**	**6**	**6**	**6**	**6**	**7**	**7**	**7**	**7**	**8**	**8**
N	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
PB	**8**	**8**	**9**	**9**	**9**	**9**	**9**	**10**	**10**	**10**	**10**	**11**	**11**	**11**	**11**	**11**	**12**	**12**	**12**	**12**
N	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**
PB	**12**	**13**	**13**	**13**	**13**	**13**	**14**	**14**	**14**	**14**	**15**	**15**	**15**	**15**	**15**	**16**	**16**	**16**	**16**	**16**
N	**61**	**62**	**63**	**64**	**65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**74**	**75**	**76**	**77**	**78**	**79**	**80**
PB	**16**	**17**	**17**	**17**	**17**	**17**	**18**	**18**	**18**	**18**	**18**	**19**	**19**	**19**	**19**	**19**	**20**	**20**	**20**	**20**

N = Number of patients enrolled
PB = Pocock boundary, the minimum cumulative number of patients experiencing vaccine-related toxicity that should stop the trial when the concurrent number of patients enrolled in the trial is N

The safety monitoring rule described for vaccine-related toxicity was derived using methods described by Ivanova et al. (Ivanova A, BF Qaqish, and MJ Schell, 2005. Continuous toxicity monitoring in phase II trials in oncology. Biometrics 61: 540-545). Pocock boundaries were calculated using an online calculator maintained by one of the authors at the UNC Lineberger Comprehensive Cancer Center website:
(http://cancer.unc.edu/biostatistics/program/ivanova/ContinuosMonitoringForToxicity.aspx).

13.3 Adverse Events

An AE is any untoward medical occurrence associated with use of a drug, whether or not considered drug related. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drug, whether or not related to use of the study drug. Abnormal laboratory findings without clinical significance (based on the PI's judgment) should not be recorded as AEs. But laboratory value changes that require therapy or adjustment in prior therapy are considered adverse events. Elective and semi-elective surgery in asymptomatic patients should not be recorded as AEs.

An adverse drug reaction is any adverse event caused by a drug. A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.

AE collection will begin at the time of administration of vaccine #1 and continue until 30 days after the last dose of study drug. Grade 2 suspected adverse reactions and all Grade 3 or greater AEs must be recorded in the subject's research record and entered into the OnCore eCRF within 72 hours of PI discovery/notification of the event. Any Grade 3 or greater adverse events associated with leukapheresis will also be recorded. AEs must also be reported to the Coordinating Center as described in 13.3.

AEs will be assessed according to the CTCAE version 5.0 (term and grade) and documented. If CTCAE term does not exist, the PI should select the most appropriate CTC category and indicate "other". If CTCAE grading does not exist for an AE, the severity of the AE will be graded as (1) mild, (2) moderate, (3) severe, (4) life-threatening, or (5) fatal.

Attribution of AEs will be assigned by the PI as follows:

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

An adverse event is considered a Serious Adverse Event if it results in any of the following outcomes: 1) fatal or life threatening; 2) inpatient hospitalization or a prolongation of existing hospitalization; 3) a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life; 4) congenital anomaly or a birth defect and/or; 5) medically significant such that it may jeopardize the subject, and may require medical or surgical intervention to prevent one of the outcomes listed above.

An unexpected adverse event or unexpected suspected adverse reaction is an adverse event or suspected adverse reaction that is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed or is not consistent with the risk information described in the general investigational plan or elsewhere in the IND application (if there is no investigator brochure).

A summary of all Grade 3 or greater adverse events (not just those considered related to the vaccine) will be maintained. Events will be categorized by organ system, relationship to treatment, grade or severity, and outcome. The Protocol Chair will periodically review the adverse event summary with the intention of identifying any trends or patterns in toxicity. If any such trends are identified, depending on their severity, frequency, and timing with respect to treatment, the Protocol Chair may request that the events be evaluated by a convened data safety monitoring board.

13.4 Adverse Event and Serious Adverse Event (SAE) Reporting

All adverse events grade 2 or greater where there is evidence to suggest a causal relationship (possible, probable or definite) between the AE and investigational drug or study participation and all serious adverse events (regardless of causality) occurring at participating sites during the AE reporting periods require expedited reporting to the Coordinating Center.

Adverse events meeting the definition of an SAE and any grade 2 or greater AEs where there is a possible, probable or definite relationship between the AE and investigational drug must be reported to the Coordinating Center within 24 hours of the investigator's discovery. Initial notification may be via e-mail. A formal written initial report using **UFBTIP Serious Adverse (SAE) Report** must be sent to the Coordinating Center per the reporting timeframes outlined above. SAEs and AE requirings expedited reporting must be entered into the OnCore eCRF as soon as possible but no later than 72 hours after discovery.

All other AEs grade 3 or greater will be documented in the OnCore database for Protocol Chair review during the investigation.

The Protocol Chair will promptly review expedited investigator reports and communicate the AE assessment to the reporting investigator and IND sponsors, as applicable. If the AE meets FDA reporting requirements as described in 13.3.2, site PIs and IND sponsors will be notified and the IND sponsors will be responsible for reporting to FDA under the INDs regardless of which site initially reported the AE.

If a death occurs within 30 days of DC vaccine administration, that is not attributable to progressive disease or other obvious non-study related cause (i.e. motor vehicle accident), enrollment of new subjects will be suspended until review by the Protocol Chair and IND Sponsor has been completed and the FDA notified. If attribution of death is determined by the Protocol Chair, IND Sponsor or FDA to be possibly, probably or definitely related to trial drug, continued treatment of all enrolled subjects with trial drug will be suspended until the review is completed and recommendations for study continuation have been issued and approved by FDA. Any death attributed to trial drug, regardless of the timeline with respect to last treatment, will result in suspension of enrollment and suspension of continued treatment for enrolled subjects until review by FDA is complete, and recommendations for study continuation issued and approved. The IRB and DSMB will be notified of subject deaths per local policy. Deaths or life-threatening adverse events will be reported to FDA by the IND sponsors as soon as possible but no later than 7 calendar days after the sponsor's initial receipt of the information (21 CFR 312.32).

Grade	Relation to IP	Report to Coordinating Center	Report to IRB and DSMB	Report to FDA
AE Grade 3	No	Enter data in OnCore	Report per local policy	No
AE ≥ Grade 2	Possible, probable or definite	Report within 24 hours via email	Report per local policy	IND sponsor will report to FDA if

				meets definition below
SAE	All	Report within 24 hours via email	Report per local policy	IND Sponsor will report to FDA within 15 calendar days if meets FDA requirement
Fatal or life threatening	All	Report within 24 hours by phone and via email	Report per local policy	IND Sponsor will report to FDA within 7 calendar days if meets FDA requirement

13.4.1 Adverse Event Reporting to IRB

Adverse events and SAEs will be reported to the IRB of record per local IRB policy.

13.4.2 Adverse Event Reporting to the DSMB

Adverse events and SAEs will be reported to the DSMB of record per local policy. The UFHCC DISC will be notified of serious adverse events within 5 business days of Coordinating Center notification.

13.4.3 Adverse Event Reporting to the FDA

Safety reporting will be in accordance with FDA requirements. It is the responsibility of the IND sponsor to report to the FDA in accordance with 21 CFR 312.32 as follows:

- Report any unexpected fatal or life-threatening suspected adverse reactions associated with use of the drug by telephone or fax as soon as possible but no later than **7 calendar days** after initial receipt of the information.
- Report any serious and unexpected suspected adverse reactions if there is evidence to suggest a causal relationship between the drug and the adverse event, as well as results from animal studies that suggest significant clinical risk within **15 calendar days** after receipt of this information.

A summary of Grade II (related) and Grade III toxicities, all IND safety reports and subject deaths during participation in the investigation will be submitted by the sponsor with the IND annual report.

13.4.4 Adverse Event Reporting to Sites

The Coordinating Center will notify site PIs of any adverse events associated with the use of the investigational vaccine that is both serious and unexpected within **15 calendar days** of the notification of the event. These reports should be submitted to the IRB of record in accordance with local policy. Any changes to the protocol and/or consent will be initiated by the Coordinating Center and distributed to participating sites for review by the IRB.

13.5 Potential Risks

13.5.1 Allergic Reactions to DC Immunization

Injection of antigen presenting cells may result in an allergic reaction, which could include redness and swelling at the injection site, itching, hives, low blood pressure, difficulty breathing, or in the most extreme circumstances, death. In addition, if the immune system becomes overly activated, potential discomforts may include pain, redness and swelling at the injection site.

13.5.2 Cerebral Edema

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

13.5.3 Infection

The DC or PBMC injections may include the risk of infection due to potential contamination 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 and the fact that the DCs and PBMCs will be strictly tested for sterility. The risk of infection due to potential contamination of the DCs and PBMCs in the laboratory will be minimized by biosafety quality assurance and testing. All cell cultures will be handled in a core tissue culture facility dedicated to the processing of human cells. Following injections, patients will be monitored throughout the course of the study for any signs and symptoms of infection. If an active infection is suspected, patients will be cultured and treated with appropriate antibiotics.

13.5.4 Delayed Autoimmune Diseases

It is possible that delayed autoimmune disease(s) may develop as a result of injection with DCs or PBMCs. This means that the immune system may be stimulated to attack natural tissue in the body. Animal studies have reported the development of autoimmunity in the context of vaccination and recovery from lymphopenia. However, our current experience with DC and PBMC vaccination in glioma patients has not demonstrated evidence of autoimmunity in treated patients. It therefore, is unknown what the risk of delayed autoimmune disease is for this study.

13.5.5 Phlebotomy

Drawing blood or inserting an intravenous catheter into an arm vein may result in bruising or swelling in the area of the insertion, bleeding at the site of the needle puncture, light headedness, fainting and very rarely, local infection, which may be severe.

13.5.6 Leukapheresis

As with any donation of blood, a variety of minor reactions may occur with leukapheresis, which include fainting, dizziness, or nausea. Uncommon but serious complications may also result, which include bleeding, infection, an adverse reaction to the anticoagulant or replacement fluids, hypocalcemia, hypotension, shock, convulsions, air emboli, heart failure, or the inability to transfuse blood back into the patient. These risks are reduced by the fact that the procedure will be performed by qualified staff at a specialized clinical hemapheresis unit. Patients will be carefully monitored throughout the procedure by trained nursing and medical staff. Calcium gluconate (2 mg PO) may be given to minimize the risks of hypocalcemia, fluid supplementation may be given to minimize hypotension, and blood will be routinely screened for infectious disease in accordance with institutional practices to minimize the risk of transmitting infection.

13.5.7 Central Venous Catheter

Some patient may require placement of a dialysis-type central venous catheter for the leukapheresis procedure. Separate operative/procedural consent will be obtained from the surgeon/proceduralist placing the central line. The surgeon/proceduralist may use fluoroscopy to place the central venous catheter and ensure that it is in a satisfactory position. Fluoroscopy involves low dose radiation and is low risk. Risk of central line placement include (but not limited to): bruising, bleeding, injury to the vessel which may be permanent, possible injury to adjacent structures/organs, pneumothorax, infection and postoperative pain/discomfort. Removal of the central line is low risk but does include possible thrombosis or stenosis of the vessel. This may prevent future use of this vessel for additional leukapheresis.

13.5.8 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 imaged by CT scan. 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.

13.5.9 GM-CSF

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

13.5.10 Td

The following adverse events have been identified during post-approval use of MassBiologics' Td. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequencies or to establish a causal relationship to vaccination. The following adverse events were included because of seriousness or frequency of reporting: injection site reactions, including pain, tenderness, erythema, induration, pruritis,

swelling and warmth; peripheral edema, pyrexia, malaise; dizziness, headache, convulsions, myalgia, musculoskeletal stiffness or pain, arthralgia; rash; nausea; and cellulitis.

13.5.11 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.

13.5.12 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 Principal Investigator and primary physician will titrate therapy based on standard clinical guidelines.

13.5.13 Confidentiality

Participation in research may result in a loss of privacy if confidentiality is breached. Only designated members of the study team will have access to individually identifiable private information. Data collected from clinical records or patients will be recorded in OnCORE that uses Gatorlink authentication. Clinical records containing PHI will be maintained as source documentation and stored in secure, limited access, locked offices. Records will be made available to individuals involved with the study, the clinical staff administering the study, and regulatory representatives such as the IRB, FDA, OHRP and funding agency. Any publications resulting from this study will not include patient identifying data.

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Data and Safety Monitoring Plan

14.1.1 Data Monitoring Plan

Adverse events meeting the definition of an SAE and any grade 2 or greater AEs where there is a possible, probable or definite relationship between the AE and investigational drug must be reported to the Coordinating Center within 24 hours of the investigator's discovery. These will be assessed by the Protocol Chair and reported to the IRB, DSMB, FDA and other participating investigators as outlined in the protocol.

The UFHCC SRMC conducts initial review of IITs and determines the level of risk which corresponds with DISC review requirements. The UFHCC DISC will conduct monitoring and audit activities as outlined in the DISC Charter. The results of such activities will be provided to the PI, IND sponsor and IRB.

The UF study team will conduct annual QA self-assessments that include review of regulatory documentation (e.g., IRB documentation, FDA IND records, IND Sponsor-Investigator responsibilities), recruitment procedures, informed consent process/form documentation, subject selection, AE and SAE reporting, CRF and source documents.

The Coordinating Center will periodically review data entry and source documents uploaded into OnCore or other electronic source, and notify sites of missing, incomplete or inconsistent data as applicable.

Orlando Health Cancer Institute site data will be monitored by the coordinating center lead coordinator and regulatory manager by reviewing source documents uploaded in the study database (UF OnCore).

Duke will manufacture their own IP under their own IND and will perform site-specific monitoring that will be reported to the Coordinating Center/Protocol Chair as described in the protocol.

14.1.2 Data Safety Monitoring Board

Oversight and monitoring of study related activities such as the safety of research participants, appropriateness of the study, and integrity of the data, will be provided by the University of Florida Health Cancer Center DSMB, now called the Data Integrity and Safety Committee (UFHCC DISC), in accordance with established policies, procedures and timeframes. DISC reports and correspondence will be provided to participating investigators for review and submission to local oversight committees as required.. All reports generated by Duke pertaining to study conduct, will be sent to the University of Florida PI and Protocol Chair. DSMB reports should be submitted to the IRB per local policy.

14.2 Audits

An IRB or other local oversight group may conduct audits to evaluate compliance with the protocol and the principles of GCP. The PI will provide the auditor(s) with direct access to all relevant documents and to allocate his/her time and the time of the study team to the auditor(s) in order to discuss findings and any relevant issues.

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

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

Duke University and the University of Florida have an auditing mechanism in place and each institution will be responsible for auditing their respective data. Additionally, Duke University will send copies of all audit findings to the University of Florida who, based on these findings, can request additional documentation or conduct an on-site visit.

14.3 Data Management and Processing

14.3.1 Study Documentation

Study documentation includes but is not limited to source documents, case report forms, screening/enrollment, delegation of authority and monitoring logs (as applicable), appointment schedules, correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the "Regulatory Binder" (electronic or hard copy), which includes but is not limited to signed protocol, amendments, and investigator's brochure (as applicable), approved and signed informed consent forms, IRB submission documents, approvals and correspondence, CVs for investigators and sub-investigators, FDA Form 1572, financial disclosure forms, laboratory certifications and reference ranges, and clinical supplies receipts and distribution records.

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

14.3.2 Site Activation

A site is considered activated when the site has received official written documentation from the Protocol Coordinating Center that the site has been approved to begin enrollment. At a minimum, each participating clinical site must have the following documentation on file at UF prior to study activation:

- IRB approval of the study in the form of a letter or other official document from the participating institution's IRB of record. This documentation must show the version of the protocol approved by the IRB.
- IRB approval of an informed consent form. The consent must include a statement that data will be shared with UF, including the UFHCC, the DSMB (if applicable), and the

UF study team.
- Protocol signature page signed and dated by the investigator at each participating site.

The applicable IND sponsor is responsible for obtaining and maintaining signed and dated FDA Form 1572, and the CVs, applicable licenses and FDFs of all investigators listed on the 1572.

The Coordinating Center is responsible for disseminating study updates, protocol and/or investigator brochure amendments, reportable adverse events, and informed consent modifications to all participating sites. Protocol/consent modifications and IB updates will be forwarded electronically to participating sites within 4 weeks of UF IRB approval. Activated participating sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted. Documentation of IRB approval should be provided to the Coordinating Center within 2 weeks of receipt of approval. The Coordinating Center should review all revised consent forms prior to submission to the IRB.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, SAE reports, and any protocol violations/deviations/exceptions must be on file at UF.

The investigator or a designee from each institution must participate in conference calls, as scheduled, to update and provide information regarding the progress of the trial.

14.3.3 Case Report Forms

Electronic case report forms (eCRFs) will be utilized for this trial. Only the PI and key study personnel listed on the study delegation log are permitted to make entries, changes, or corrections in the case report forms. Source documents that support data entry should be available for monitoring or audit. The Coordinating Center will periodically review data entry and notify sites of missing, incomplete or inconsistent data.

14.3.4 Data Management Procedures and Data Verification

Users of the electronic CRF will have access based on their delegated specific roles on the study. Completeness of entered data will be checked and cross-referenced to verify accuracy. Missing or implausible data will be highlighted for the PI requiring appropriate responses (i.e. confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

The database will be reviewed and discussed prior to database closure, and will be closed only after resolution of all remaining queries. An audit trail will be kept of all subsequent changes to the data.

15 STATISTICAL METHODS AND DATA ANALYSIS

15.1 Study Design Overview

The primary goal of this randomized phase II study is to determine whether the addition of pp65-LAMP mRNA DC vaccination in adult patients with newly-diagnosed GBM receiving dose-intensified TMZ will be a treatment worthy of investigation in a large phase III study. A secondary

objective will be examination of the immunologic responses induced by full-length vs short LAMP sequences.

Although this randomized phase II study is comparative, it will not be definitive as the assessment will be conducted with a large false-positive rate. We have included a concurrently followed control arm in the trial design in lieu of a historical control group to ensure that appropriate comparisons can be made without concern that the patients receiving standard treatment differ in prognostic factors or standards of care (Rubinstein, 2009; Ratain and Sargent, 2009; Rubinstein et al., 2005).

We will randomize eligible patients, after enrollment, to one of 3 study arms in which they will receive 21-day TMZ concurrently with one of the following treatments:

Arm #	Vaccine	Skin Preparation
1	**pp65-shLAMP mRNA DCs with GM-CSF**	**Tetanus**
2	**pp65-flLAMP mRNA DCs with GM-CSF**	**Tetanus**
3	**PBMCs**	**Saline**

Randomization will be stratified by:
- Age 69 years old and younger; or 70 years old and older
- RPA Class (III, IV, or V)
- CMV seronegative or seropositive

We will use a permuted block randomization scheme with a 1:1:1 study arm allocation ratio.and a block size of 6 within each stratum.

Adapted RPA Stages For Malignant Gliomas (Survival With Concurrent Temozolomide and Radiation Therapy vs Radiation Therapy alone)				
Stage	**Characteristics**	**Median OS**	**2-year OS**	**p-value vs. control**
III	Age <50, PS 0	21 vs. 15 mo	43% vs. 20%	p<0.0001
IV	Age <50, PS 1-2 Age >=50, Surgery, MMSE >=27	16 vs. 13 mo	28% vs. 11%	p<0.01
V	Age >=50 and either Biopsy only *or* MMSE <27	10 vs. 9 mo	17% vs. 6%	p=0.05

Approximately 200 patients will be enrolled in the study, randomized and receive "standard-of-care" radiotherapy concurrent with TMZ under the assumption that approximately 120 patients will be vaccinated according to treatment arm.

A Data Sharing Agreement has been established with Immunomic Therapeutics, Inc. and the University of Florida. A Limited Data Set will be sent to evaluate immunogenicity and clinical efficacy of the vaccine products used within this clinical trial (for patients treated at the UF site

only) in which Immunomic Therapeutics, Inc. has scientific and potential commercial interest. Data will be provided within 6 months of study completion. Applicable patient identifiers will be removed before sending the limited data set. A separate data sharing agreement may be established by Immunomic Therapeutics, Inc. with Duke University.

15.2 Sample Size Justification for Vaccine Comparison

The primary focus of this trial will be to assess the worthiness of pp65-LAMP mRNA DC vaccine for further investigation by comparing overall survival (OS) between the active treatment group (Arms 1 and 2 combined) and the control group (Arm 3). Because this test for efficacy will be made within the framework of a phase II trial, we have constrained sample size requirements to yield adequate power to detect a clinically relevant improvement in OS at the expense of the false positive rate (α-level or Type I error rate). Adopting guidelines proposed by Ratain and Sargent (2009) and Rubinstein et al (2005), our proposed sample size of 40 + 40 = 80 patients in the treatment group and 40 patients in the control group will allow detection of a clinically relevant improvement in the median OS time of the treatment group with 90% power at a one-sided α-level of 0.2.

In determining the effect size that can be detected by our proposed sample size, we considered using as a control reference level the median OS estimate of 14.6 months (95% CI: 13.2 to 16.8) reported by Stupp et al (2005) for patients receiving radiotherapy plus TMZ (RT+TMZ) for newly-diagnosed GBM (40% of patients in the Stupp trial were grossly resected). In the Stupp trial, randomization occurred prior to any post-surgical treatment, and the timing of randomization in our study will be similar. However, in our study, patients will not begin to experience potential differential effects due to treatment until after their first vaccination, which will occur approximately 15 weeks after randomization. To determine an appropriate control reference level for median OS for an index day 15 weeks after randomization, we carefully transferred the coordinates of multiple points along the Stupp RT+TMZ OS curve into a statistical software package. We determined that median OS relative to a starting time 15 weeks after the Stupp index day would be 13.8 months. We also observed that RT+TMZ OS times appeared to approximately follow a Weibull distribution with a shape parameter = 1.20, indicating a slightly accelerating baseline hazard function. Upon examining in a similar manner the RT+TMZ OS curve reported in the paper by Gilbert et al (2013), we also observed approximately Weibull-distributed OS times with shape parameter = 1.16.

We therefore used 13.8 months as the median OS reference level for our control group and assumed Weibull-distributed OS times with a common shape parameter value for both the treatment and control groups. This is equivalent to assuming Weibull-distributed OS times with proportional hazards between the treatment and control groups.

Using methods adapted from Wu and Xiong (2014), we calculated improvement in median OS (or equivalently, the largest treatment vs. control hazard ratio less than 1) that could be detected by a log rank test with 90% power at 1-sided α=0.20, for selected Weibull shape parameter values ranging from 1.00 (equivalent to exponential survival with constant hazard) to 1.25 (accelerating hazard). We assumed 120 patients would be randomized and proceed to vaccination at a uniform rate over a 3-year accrual period. These patients would then be followed after the end of accrual for one additional year. As summarized in the following table, 77 deaths (expected by follow-up

times ranging from 45.1 to 48.9 months, depending on the degree of hazard acceleration assumed) will allow a treatment vs. control hazard ratio of 0.6 to be detected with sufficient power. Equivalently, improvement in median OS ranging from 7.0 to 9.2 months (again, depending on the degree of hazard acceleration assumed) can be detected with sufficient power.

Improvement in median overall survival (Surv50) that can be detected at 90% power and a one-sided alpha-level of 0.20, for possible Weibull shape parameters characterizing the degree of hazard acceleration:

TRT: CTL N	Weibull Shape parameter	Baseline hazard function trend	Expected # of months to observe 77 deaths	Detectable effect at 90% power, 1-tail alpha=0.20: Surv50 (months)	Detectable effect at 90% power, 1-tail alpha=0.20: Hazard ratio
80:40	1.00	Constant (exponential survival)	48.9	+ 9.2	0.60
80:40	1.16	Accelerating (Gilbert et al)	46.3	+ 7.6	0.60
80:40	1.20	Accelerating (Stupp et al)	45.7	+ 7.3	0.60
80:40	1.25	Accelerating	45.1	+ 7.0	0.60

15.3 Analytic Methods

15.3.1 Primary Objective and Secondary Clinical Objectives

We will use Kaplan-Meier survival curves and the log rank test to characterize and compare PFS and OS in patients who received pp65-LAMP mRNA DC vaccine (Arms 1 and 2) and in control patients (Arm 3). PFS is defined as the time between first vaccination and first documentation of either disease progression/recurrence, or death without prior progression/recurrence. Patients remaining alive without disease progression will have PFS censored at last follow-up. OS will be defined as the time between first vaccination and death, and will be censored at the last follow-up if death has not occurred. We will also perform a stratified analysis incorporating the three stratification variables (age, RPA class, and CMV sero-status) using Cox proportional hazards regression. The index day for all of our primary comparisons will be the time of the first vaccination. Because patients who fail to proceed to the first vaccination because of GBM recurrence or for other reasons cannot experience differential effects due to treatment arm membership, we will not perform an intent-to-treat analysis that includes these patients. We will perform an intent-to-treat analysis of OS and PFS that includes patients who begin the vaccination sequence but who do not receive at least three vaccinations because of recurrence or other reasons. OS and PFS times for adequately vaccinated patients who are lost to follow-up will be considered right-censored at the time of loss and will be included in all of our survival comparisons.

All subjects who receive at least one dose of DC vaccine will be considered evaluable for purposes of safety and toxicity.

All subjects who complete chemoRT and receive dose-intense adjuvant TMZ and at least 3 DC vaccines will be considered evaluable for purposes of the primary and secondary endpoints.

The impact of pp65-LAMP mRNA DC vaccine on PFS and OS in patients with gross total resection will also be explored using the log rank test and Kaplan-Meier curves, although our trial design may not necessarily achieve sufficient power to detect a clinically meaningful effect within this patient subgroup.

15.3.2 Secondary Biological Objectives

We will estimate within-patient changes in immune response (ELISPOT, cytokines array, flow cytometric analysis and NK cell activity) from baseline to vaccine #3 and compare these changes between treatment and control groups using the Wilcoxon rank sum test. To assess the effect of these changes in immune response on OS and PFS subsequent to vaccine #3, we will conduct a landmark analysis within the framework of Cox proportional hazards models of post-baseline OS and PFS. These models will incorporate Indicators for treatment arm, change in immune response parameters from baseline to vaccine #3, interaction between treatment arm and immune response changes, and potential confounders.

16 REGULATORY REQUIREMENTS AND ETHICAL CONSIDERATIONS

The study will be conducted in accordance with the protocol, FDA Regulations 21 CFR parts 50, 54, 56 and 312, HHS Regulations 45 CFR part 46, the International Conference on Harmonization guidelines for Good Clinical Practice (ICH E6), and applicable institutional, local and state requirements. All personnel involved in the conduct of this study have completed human subjects protection training in accordance with local institutional requirements.

16.1 Institutional Review Board

Each site will obtain Institutional Review Board (IRB) approval of the protocol and protocol related documents including consent forms, prior to initiating the study. The study may be initiated only after the Principal Investigator has received written and dated approval from the IRB and notification of activation from the Coordinating Center. Amendments to the protocol must originate with the Protocol Chair and be approved by the IRB prior to implementation. The FDA will be notified as required under 21 CFR 312.30.

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form in accordance with 21 CFR 56 and local policy prior to implementation except where necessary to eliminate apparent immediate hazard to the subject. If a change is made to eliminate an apparent immediate hazard, the PI must notify the IRB in accordance with local policy, the Protocol Chair and the sponsor within 24 hours. The IND sponsor will notify the FDA of such change in accordance with 21 CFR 312.30. Each investigator will follow the requirements of their local IRB for periodic reporting of study progress, reporting of serious adverse events, unanticipated problems, and protocol deviations or violations, safety monitoring reports and study completion. Protocol exceptions must have approval from the Protocol Chair, FDA (as applicable through the sponsor) and IRB prior to implementation.

The Principal Investigator must obtain protocol re-approval as required by the IRB, but not less than once per year.

16.2 Informed Consent

Informed consent will be obtained and documented in accordance with 21 CFR 50 and the requirements of the IRB. 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. For potential subjects whose primary language is not English, investigators should follow the requirements and determinations of the reviewing IRB and any applicable institutional procedures.

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 or a legally authorized representative. The original informed consent form will be maintained with the subject's study records, and a copy of the informed consent form will be provided to the subject. The Principal Investigator is responsible for asking the subject whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Principal Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

16.3 Privacy, Confidentiality, and Data Storage

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained. 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. After agreeing to participate, some patient identifiers will be collected, used and/or recorded, including, but not limited to name, date of birth, telephone number, address, dates from health records and a unique patient number. This information may be kept in patient binders as well as recorded in the respective eCRF. Images collected will be as de-identified as possible. Only approved study personnel and those included in the HIPAA Authorization will have access to identified, partially identified, and/or coded data. Data and specimens will be collected, stored and transported with consideration for subject privacy and confidentiality. Electronic records of subject data will be maintained in a password-protected computer. Access to electronic databases will be limited to the Principal Investigator and key study personnel. Data stored on portable memory devices will be de-identified.

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

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

16.4 Protocol Amendments

All protocol amendments (including consent form changes) must be initiated by the Protocol Chair and approved by the IRB prior to implementation. The FDA will be notified as required under 21 CFR 312.30. 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, the Protocol Chair and the sponsor as described in 16.1.

16.5 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; or
- in compliance with local record retention policies.

16.6 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. Investigators and key personnel must provide financial disclosures prior to trial participation and as interests change and complete any applicable institutional conflict of interest documentation per local policy.

17 REFERENCES

Adler, S. P., S. H. Hempfling, S. E. Starr, S. A. Plotkin and S. Riddell (1998). "Safety and immunogenicity of the Towne strain cytomegalovirus vaccine." Pediatric Infectious Disease Journal **17**(3): 200-206.

Adler, S. P., K. V. Shaw, M. McVoy, R. L. Burke and H. Liu (1995). "Guinea pig and human cytomegaloviruses do not share cross-reactive neutralizing epitopes." Journal of Medical Virology **47**(1): 48-51.

Albright, L., J. A. Seab and A. K. Ommaya (1977). "Intracerebral delayed hypersensitivity reactions in glioblastoma multiforme patients." Cancer **39**(3): 1331-1336.

Aldape, K. D. (2009). "MGMT methylation testing in RTOG 0525: A phase III trial of newly diagnosed glioblastoma." J Clin Oncol **27**.

Antony, P. A., C. A. Piccirillo, A. Akpinarli, S. E. Finkelstein, P. J. Speiss, D. R. Surman, D. C. Palmer, C.-C. Chan, C. A. Klebanoff, W. W. Overwijk, S. A. Rosenberg and N. P. Restifo (2005). "CD8+ T Cell Immunity Against a Tumor/Self-Antigen Is Augmented by CD4+ T Helper Cells and Hindered by Naturally Occurring T Regulatory Cells." Journal of Immunology **174**(5): 2591-2601.

Asavaroengchai, W., Y. Kotera and J. J. Mule (2002). "Tumor lysate-pulsed dendritic cells can elicit an effective antitumor immune response during early lymphoid recovery." Proceedings of the National Academy of Sciences of the United States of America **99**(2): 931-936.

Ashley, D. M., B. Faiola, S. Nair, L. P. Hale, D. D. Bigner and E. Gilboa (1997). "Bone marrow-generated dendritic cells pulsed with tumor extracts or tumor RNA induce antitumor immunity against central nervous system tumors." Journal of Experimental Medicine **186**(7): 1177-1182.

Banchereau, J. and R. M. Steinman (1998). "Dendritic cells and the control of immunity." Nature **392**(6673): 245-252.

BenMohamed, L., R. Krishnan, C. Auge, J. F. Primus and D. J. Diamond (2002). "Intranasal administration of a synthetic lipopeptide without adjuvant induces systemic immune responses." Immunology **106**(1): 113-121.

Bigger, J. E., M. Tanigawa, C. A. Thomas, 3rd and S. S. Atherton (1999). "Protection against murine cytomegalovirus retinitis by adoptive transfer of virus-specific CD8+ T cells." Investigative Ophthalmology & Visual Science **40**(11): 2608-2613.

Bigner, D. D., O. M. Pitts and C. J. Wikstrand (1981). "Induction of lethal experimental allergic encephalomyelitis in nonhuman primates and guinea pigs with human glioblastoma multiforme tissue." Journal of Neurosurgery **55**(1): 32-42.

Bloom, H. J., M. J. Peckham, A. E. Richardson, P. A. Alexander and P. M. Payne (1973). "Glioblastoma multiforme: a controlled trial to assess the value of specific active immunotherapy in patients treated by radical surgery and radiotherapy." British Journal of Cancer **27**(3): 253-267.

Boczkowski, D., S. K. Nair, D. Snyder and E. Gilboa (1996). "Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo." Journal of Experimental Medicine **184**(2): 465-472.

Bodmer, S., K. Strommer, K. Frei, C. Siepl, N. de Tribolet, I. Heid and A. Fontana (1989). "Immunosuppression and transforming growth factor-beta in glioblastoma. Preferential production of transforming growth factor-beta 2." Journal of Immunology **143**(10): 3222-3229.

Borrow, P., J. L. Cornell, M. D. Ruppe and L. Mucke (1995). "Immunization-induced inflammatory infiltration of the central nervous system in transgenic mice expressing a microbial antigen in astrocytes." Journal of Neuroimmunology **61**(2): 133-149.

Boskovitz, A., C. J. Wikstrand, C. T. Kuan, M. R. Zalutsky, D. A. Reardon and D. D. Bigner (2004). "Monoclonal antibodies for brain tumour treatment." Expert Opinion in Biological Therapy **4**(9): 1453-1471, 2004 Sep.

Bourquin, C., A. Iglesias, T. Berger, H. Wekerle and C. Linington (2000). "Myelin oligodendrocyte glycoprotein-DNA vaccination induces antibody-mediated autoaggression in experimental autoimmune encephalomyelitis." European Journal of Immunology **30**(12): 3663-3671.

Britt, W., J. Fay, J. Seals and C. Kensil (1995). "Formulation of an immunogenic human cytomegalovirus vaccine: responses in mice." Journal of Infectious Diseases **171**(1): 18-25.

Brooks, W. H., M. G. Netsky, D. E. Normansell and D. A. Horwitz (1972). "Depressed cell-mediated immunity in patients with primary intracranial tumors. Characterization of a humoral immunosuppressive factor." Journal of Experimental Medicine **136**(6): 1631-1647.

Bullard, D. E., D. G. Thomas, J. L. Darling, C. J. Wikstrand, J. V. Diengdoh, R. O. Barnard, J. G. Bodmer and D. D. Bigner (1985). "A preliminary study utilizing viable HLA mismatched cultured glioma cells as adjuvant therapy for patients with malignant gliomas." British Journal of Cancer **51**(2): 283-289.

Cho, B. K., V. P. Rao, Q. Ge, H. N. Eisen and J. Chen (2000). "Homeostasis-stimulated proliferation drives naive T cells to differentiate directly into memory T cells.[see comment]." Journal of Experimental Medicine **192**(4): 549-556.

Cho, H. I., H. Han, C. C. Kim and T. G. Kim (2001). "Generation of Cytotoxic T Lymphocytes Specific for Human Cytomegalovirus Using Dendritic Cells In Vitro." Journal of Immunotherapy **24**(3): 242-249.

Cicin-Sain, L., W. Brune, I. Bubic, S. Jonjic and U. H. Koszinowski (2003). "Vaccination of mice with bacteria carrying a cloned herpesvirus genome reconstituted in vivo." Journal of Virology **77**(15): 8249-8255.

Cobbs, C. S., L. Harkins, M. Samanta, G. Y. Gillespie, S. Bharara, P. H. King, L. B. Nabors, C. G. Cobbs and W. J. Britt (2002). "Human cytomegalovirus infection and expression in human malignant glioma." Cancer Research **62**(12): 3347-3350.

Dazzi, F. and J. M. Goldman (1998). "Adoptive immunotherapy following allogeneic bone marrow transplantation." Annual Review of Medicine **49**: 329-340.

Dinapoli, R. P., L. D. Brown, R. M. Arusell, J. D. Earle, J. R. O'Fallon, J. C. Buckner, B. W. Scheithauer, J. E. Krook, L. K. Tschetter, J. A. Maier, D. M. Pfeifle and D. H. Gesme, Jr. (1993). "Phase III comparative evaluation of PCNU and carmustine combined with radiation therapy for high-grade glioma." Journal of Clinical Oncology **11**(7): 1316-1321.

Dittel, B. N., I. Visintin, R. M. Merchant and C. A. Janeway, Jr. (1999). "Presentation of the self antigen myelin basic protein by dendritic cells leads to experimental autoimmune encephalomyelitis." Journal of Immunology **163**(1): 32-39.

Drulak, M. W., F. J. Malinoski, S. A. Fuller, S. S. Stewart, S. Hoskin, A. M. Duliege, R. Sekulovich, R. Burke and S. Winston (2000). "Vaccination of seropositive subjects with CHIRON CMV gB subunit vaccine combined with MF59 adjuvant for production of CMV immune globulin." Viral Immunology **13**(1): 49-56.

Dudley, M. E., J. R. Wunderlich, P. F. Robbins, J. C. Yang, P. Hwu, D. J. Schwartzentruber, S. L. Topalian, R. Sherry, N. P. Restifo, A. M. Hubicki, M. R. Robinson, M. Raffeld, P. Duray, C. A. Seipp, L. Rogers-Freezer, K. E. Morton, S. A. Mavroukakis, D. E. White and S. A. Rosenberg (2002). "Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes." Science **298**(5594): 850-854.

Dudley, M. E., J. R. Wunderlich, J. C. Yang, R. M. Sherry, S. L. Topalian, N. P. Restifo, R. E. Royal, U. Kammula, D. E. White, S. A. Mavroukakis, L. J. Rogers, G. J. Gracia, S. A. Jones, D. P. Mangiameli, M. M. Pelletier, J. Gea-Banacloche, M. R. Robinson, D. M. Berman, A. C. Filie, A. Abati and S. A. Rosenberg (2005). "Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma." Journal of Clinical Oncology **23**: 2346-2357, 2005 Apr 2341.

Dziurzynski, K., S. M. Chang, A. B. Heimberger, R. F. Kalejta, S. R. McGregor Dallas, M. Smit, L. Soroceanu and C. S. Cobbs (2012). "Consensus on the role of human cytomegalovirus in glioblastoma." Neuro Oncol **14**(3): 246-255.

Dziurzynski, K., J. Wei, W. Qiao, M. A. Hatiboglu, L. Y. Kong, A. Wu, Y. Wang, D. Cahill, N. Levine, S. Prabhu, G. Rao, R. Sawaya and A. B. Heimberger (2011). "Glioma-associated cytomegalovirus mediates subversion of the monocyte lineage to a tumor propagating phenotype." Clin Cancer Res **17**(14): 4642-4649.

Ekman, M. and M. Westphal (2005). "Cost of brain tumour in Europe." European Journal of Neurology **12**: 45-49.

Ellingson, Benjamin M., Patrick Y. Wen, and Timothy F. Cloughesy. "Modified Criteria for Radiographic Response Assessment in Glioblastoma Clinical Trials." *Neurotherapeutics* 14.2 (2017): 307–320. *PMC*. Web. 17 Oct. 2017.

Emanuel, D. J. (1991). "Uses of immunotherapy for control of human cytomegalovirus-associated diseases." Transplantation Proceedings. **23**(3 Suppl 3): 144-146.

Emanuel, D. J., K. G. Lucas, G. B. Mallory, Jr., M. K. Edwards-Brown, K. E. Pollok, P. D. Conrad, K. A. Robertson and F. O. Smith (1997). "Treatment of posttransplant lymphoproliferative disease in the central nervous system of a lung transplant recipient using allogeneic leukocytes." Transplantation **63**(11): 1691-1694.

Falagas, M. E., D. R. Snydman, R. Ruthazer, J. Griffith, B. G. Werner, R. Freeman and R. Rohrer (1997). "Cytomegalovirus immune globulin (CMVIG) prophylaxis is associated with increased survival after orthotopic liver transplantation. The Boston Center for Liver Transplantation CMVIG Study Group." Clinical Transplantation **11**(5 Pt 1): 432-437.

Fujii, S., K. Shimizu, K. Fujimoto, T. Kiyokawa, T. Shimomura, M. Kinoshita and F. Kawano (1999). "Analysis of a chronic myelogenous leukemia patient vaccinated with leukemic dendritic cells following autologous peripheral blood stem cell transplantation." Japanese Journal of Cancer Research **90**(10): 1117-1129.

Galanis, E., J. C. Buckner, M. J. Maurer, R. Sykora, R. Castillo, K. V. Ballman and B. J. Erickson (2006). "Validation of neuroradiologic response assessment in gliomas: measurement by RECIST, two-dimensional, computer-assisted tumor area, and computer-assisted tumor volume methods." Neuro-Oncology **8**(2): 156-165.

Gonczol, E., J. Ianacone, G. Furlini, W. Ho and S. A. Plotkin (1989). "Humoral immune response to cytomegalovirus Towne vaccine strain and to Toledo low-passage strain." Journal of Infectious Diseases **159**(5): 851-859.

Grossman, Z. and W. E. Paul (2000). "Self-tolerance: context dependent tuning of T cell antigen recognition." Seminars in Immunology **12**(3): 197-203; discussion 257-344.

Hall, W. A. and O. Fodstad (1992). "Immunotoxins and central nervous system neoplasia." Journal of Neurosurgery **76**(1): 1-12.

Hammond, D. (1999). "CytoGam infusions at home." Journal of Intravenous Nursing **22**(6): 331-335.

Harkins, L., A. L. Volk, M. Samanta, I. Mikolaenko, W. J. Britt, K. I. Bland and C. S. Cobbs (2002). "Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer." Lancet **360**(9345): 1557-1563.

Hegi, M. E., A. C. Diserens, T. Gorlia, M. F. Hamou, N. de Tribolet, M. Weller, J. M. Kros, J. A. Hainfellner, W. Mason, L. Mariani, J. E. Bromberg, P. Hau, R. O. Mirimanoff, J. G. Cairncross, R. C. Janzer and R. Stupp (2005). "MGMT gene silencing and benefit from temozolomide in glioblastoma." New England Journal of Medicine **352**: 997-1003, 2005 Mar 1010.

Heimberger, A. B., G. E. Archer, L. E. Crotty, R. E. McLendon, A. H. Friedman, H. S. Friedman, I. Herndon, J.E., D. D. Bigner and J. H. Sampson (2002). "Dendritic cells pulsed with a tumor-specific peptide induce long-lasting immunity and are effective against murine intracerebral melanoma." Neurosurgery **50**: 158-166.

Heimberger, A. B., L. E. Crotty, G. E. Archer, R. E. McLendon, A. Friedman, G. Dranoff, D. D. Bigner and J. H. Sampson (2000). "Bone marrow-derived dendritic cells pulsed with tumor homogenate induce immunity against syngeneic intracerebral glioma." Journal of Neuroimmunology **103**(1): 16-25.

Heimberger, A. B., Crotty, L.E., Archer, G.E., Hess, K.R., Wikstrand, C.J., Friedman, A.H., Friedman, H.S., Bigner, D.D., Sampson, J.H. (2003). "Epidermal growth factor receptor vIII peptide vaccination is efficacious against established intracerebral tumors." Clinical Cancer Research. **9**: 4247-4254.

Heiser, A., D. Coleman, J. Dannull, D. Yancey, M. A. Maurice, C. D. Lallas, P. Dahm, D. Niedzwiecki, E. Gilboa and J. Vieweg (2002). "Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors." Journal of Clinical Investigation. **109**(3): 409-417.

Holtl, L., C. Rieser, C. Papesh, R. Ramoner, M. Herold, H. Klocker, C. Radmayr, A. Stenzl, G. Bartsch and M. Thurnher (1999). "Cellular and humoral immune responses in patients with metastatic renal cell carcinoma after vaccination with antigen pulsed dendritic cells." Journal of Urology **161**(3): 777-782.

Hsu, F. J., C. Benike, F. Fagnoni, L. T.M., D. Czerwinski, B. Taidi, E. G. Engleman and R. Levy (1996). "Vaccination of patients with B-cell lympoma using autologous antigen-pulsed dendritic cells." Nature Medicine **2**: 52-58.

Hu, H. M., C. H. Poehlein, W. J. Urba and B. A. Fox (2002). "Development of antitumor immune responses in reconstituted lymphopenic hosts." Cancer Research **62**(14): 3914-3919.

Hughes, M. S., Y. Y. Yu, M. E. Dudley, Z. Zheng, P. F. Robbins, Y. Li, J. Wunderlich, R. G. Hawley, M. Moayeri, S. A. Rosenberg and R. A. Morgan (2005). "Transfer of a TCR gene derived from a patient with a marked antitumor response conveys highly active T-cell effector functions." Human Gene Therapy **16**: 457-472, 2005 Apr.

Imperato, J. P., N. A. Paleologos and N. A. Vick (1990). "Effects of treatment on long-term survivors with malignant astrocytomas." Annals of Neurology **28**(6): 818-822.

Kabat, E. A., A. Wolf and A. E. Bezer (1947). "The rapid production of acute disseminated encephalomyelitis in rhesus monkeys by injection of heterologous and homologous brain tissue with adjuvants." Journal of Experimental Medicine **85**: 117-130.

Kadow, J. F., A. Regueiro-Ren and S. P. Weinheimer (2002). "The role of viruses in human cancer development and antiviral approaches for intervention." Current Opinion in Investigational Drugs **3**(11): 1574-1579.

Kelly, P. J. (1992). "Stereotactic resection and its limitations in glial neoplasms." Stereotactic and Functional Neurosurgery **59**(1-4): 84-91.

Kikuchi, T., Y. Akasaki, T. Abe, T. Fukuda, H. Saotome, J. L. Ryan, D. W. Kufe and T. Ohno (2004). "Vaccination of glioma patients with fusions of dendritic and glioma cells and recombinant human interleukin 12." Journal of Immunotherapy with Emphasis on Tumor Immunology **27**: 452-459.

Kim, J. J., N. N. Trivedi, D. M. Wilson, S. Mahalingam, L. Morrison, A. Tsai, M. A. Chattergoon, K. Dang, M. Patel, L. Ahn, J. D. Boyer, A. A. Chalian, H. Schoemaker, T. Kieber-Emmons, M. A. Agadjanyan, D. B. Weiner and H. Shoemaker (1998). "Molecular and immunological analysis of genetic prostate specific antigen (PSA) vaccine." Oncogene **17**(24): 3125-3135.

Kleihauer, A., U. Grigoleit, H. Hebart, A. Moris, P. Brossart, A. Muhm, S. Stevanovic, H. G. Rammensee, C. Sinzger, S. Riegler, G. Jahn, L. Kanz and H. Einsele (2001). "Ex vivo generation of human cytomegalovirus-specific cytotoxic T cells by peptide-pulsed dendritic cells." British Journal of Haematology **113**(1): 231-239.

Kobayashi, T., R. Yamanaka, J. Homma, N. Tsuchiya, N. Yajima, S. Yoshida and R. Tanaka (2003). "Tumor mRNA-loaded dendritic cells elicit tumor-specific CD8(+) cytotoxic T cells in patients with malignant glioma." Cancer Immunology Immunotherapy **52**: 632-637.

Kondo, K. and E. S. Mocarski (1995). "Cytomegalovirus latency and latency-specific transcription in hematopoietic progenitors." Scand J Infect Dis Suppl **99**: 63-67.

Koutsky, L. A., K. A. Ault, C. M. Wheeler, D. R. Brown, E. Barr, F. B. Alvarez, L. M. Chiacchierini and K. U. Jansen (2002). "A Controlled Trial of a Human Papillomavirus Type 16 Vaccine." New England Journal of Medicine **347**(21): 1645-1651.

Kremer, I. B., S. R. Stevens, J. W. Gould, J. DiCarlo, G. E. Quinby and K. D. Cooper (2000). "Intradermal granulocyte-macrophage colony-stimulating factor alters cutaneous antigen-presenting cells and differentially affects local versus distant immunization in humans." Clinical Immunology **96**(1): 29-37.

Kundig, T. M., M. F. Bachmann, S. Oehen, U. W. Hoffmann, J. J. Simard, C. P. Kalberer, H. Pircher, P. S. Ohashi, H. Hengartner and R. M. Zinkernagel (1996). "On the role of antigen in maintaining cytotoxic T-cell memory." Proceedings of the National Academy of Sciences of the United States of America **93**(18): 9716-9723.

Kuppner, M. C., M. F. Hamou, S. Bodmer, A. Fontana and N. de Tribolet (1988). "The glioblastoma-derived T-cell suppressor factor/transforming growth factor beta 2 inhibits the generation of lymphokine- activated killer (LAK) cells." International Journal Cancer **42**(4): 562-567.

Larsson, S., C. Soderberg-Naucler, F. Z. Wang and E. Moller (1998). "Cytomegalovirus DNA can be detected in peripheral blood mononuclear cells from all seropositive and most seronegative healthy blood donors over time." Transfusion **38**(3): 271-278.

Liau, L., K. L. Black, R. M. Prins, C. N. Sykes, P. L. DiPatre, T. F. Cloughesy, D. P. Becker and J. M. Bronstein (1999). "Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens." Journal of Neurosurgery **90**(6): 1115-1124.

Liau, L. M., R. M. Prins, S. M. Kiertscher, S. K. Odesa, T. J. Kremen, A. J. Giovannone, J. W. Lin, D. J. Chute, P. S. Mischel, T. F. Cloughesy and M. D. Roth (2005). "Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment." Clinical Cancer Research **11**: 5515-5525.

Lim, S. H. and R. Bailey-Wood (1999). "Idiotypic protein-pulsed dendritic cell vaccination in multiple myeloma." International Journal of Cancer **83**(2): 215-222.

Linington, C., T. Berger, L. Perry, S. Weerth, D. Hinze-Selch, Y. Zhang, Lu, HC, H. Lassmann and H. Wekerle (1993). "T cells specific for the myelin oligodendrocyte glycoprotein mediate an unusual autoimmune inflammatory response in the central nervous system." European Journal of Immunology **23**(6): 1364-1372.

Liu, L., A. Chahroudi, G. Silvestri, M. E. Wernett, W. J. Kaiser, J. T. Safrit, A. Komoriya, J. D. Altman, B. Z. Packard and M. B. Feinberg (2002). "Visualization and quantification of T cell-mediated cytotoxicity using cell-permeable fluorogenic caspase substrates.[see comment]." Nature Medicine **8**(2): 185-189.

Liu, Z., B. Savoldo, H. Huls, T. Lopez, A. Gee, J. Wilson, M. K. Brenner, H. E. Heslop and C. M. Rooney (2002). "Epstein-Barr virus (EBV)-specific cytotoxic T lymphocytes for the prevention and treatment of EBV-associated post-transplant lymphomas." Recent Results Cancer Research **159**: 123-133.

Lodge, P. A., L. A. Jones, R. A. Bader, G. P. Murphy and M. L. Salgaller (2000). "Dendritic cell-based immunotherapy of prostate cancer: immune monitoring of a phase II clinical trial." Cancer Research **60**(4): 829-833.

Louis, D. N., Wesseling, P, Aldape, K., et al (2020). "cIMPACT-NOW update 6: new entity and diagnostic principle recommendations of the cIMPACT-Utrecht meeting on future CNS tumor classification and grading." Brain Pathology 30:844-856.

Lucas, K. G., L. Bao, R. Bruggeman, K. Dunham and C. Specht (2011). "The detection of CMV pp65 and IE1 in glioblastoma multiforme." J Neurooncol **103**(2): 231-238.

Lucas, K. G. and J. C. Barrett (1999). "Adoptive immunotherapy for EBV-associated malignancies." Cancer Treat Research **101**: 203-232.

Ludewig, B., A. F. Ochsenbein, B. Odermatt, D. Paulin, H. Hengartner and R. M. Zinkernagel (2000). "Immunotherapy with dendritic cells directed against tumor antigens shared with normal host cells results in severe autoimmune disease." Journal of Experimental Medicine **191**(5): 795-804.

Macdonald, D. R., T. L. Cascino, S. C. Schold, Jr. and J. G. Cairncross (1990). "Response criteria for phase II studies of supratentorial malignant glioma." Journal of Clinical Oncology **8**(7): 1277-1280.

Mach, N., S. Gillessen, S. B. Wilson, C. Sheehan, M. Mihm and G. Dranoff (2000). "Differences in dendritic cells stimulated in vivo by tumors engineered to secrete granulocyte-macrophage colony-stimulating factor or Flt3-ligand." Cancer Research **60**(12): 3239-3246.

Mackensen, A., B. Herbst, J. L. Chen, G. Kohler, C. Noppen, W. Herr, G. C. Spagnoli, V. Cerundolo and A. Lindemann (2000). "Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells." International Journal of Cancer **86**(3): 385-392.

Mahaley, M. S., Jr., D. D. Bigner, L. F. Dudka, P. R. Wilds, D. H. Williams, Bouldin, TW, J. N. Whitaker and J. M. Bynum (1983). "Immunobiology of primary intracranial tumors. Part

7: Active immunization of patients with anaplastic human glioma cells: a pilot study." Journal of Neurosurgery **59**(2): 201-207.

Martin-Fontecha, A., S. Sebastiani, U. E. Hopken, M. Uguccioni, M. Lipp, A. Lanzavecchia and F. Sallusto (2003). "Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming." Journal of Experimental Medicine **198**: 615-621, 2003 Aug 2018.

Maxwell, M., T. Galanopoulos, J. Neville-Golden and H. N. Antoniades (1992). "Effect of the expression of transforming growth factor-beta 2 in primary human glioblastomas on immunosuppression and loss of immune surveillance." J.Neurosurg. **76**(5): 799-804.

Minamishima, Y. (1977). "Immunoprophylaxis of experimental cytomegalovirus infection." Annales de Microbiologie (Paris) **128**(3): 399-407.

Mitchell, D. A., X. Cui, R. J. Schmittling, L. Sanchez-Perez, D. J. Snyder, K. L. Congdon, G. E. Archer, A. Desjardins, A. H. Friedman, H. S. Friedman, J. E. Herndon, 2nd, R. E. McLendon, D. A. Reardon, J. J. Vredenburgh, D. D. Bigner and J. H. Sampson (2011). "Monoclonal antibody blockade of IL-2 receptor alpha during lymphopenia selectively depletes regulatory T cells in mice and humans." Blood **118**(11): 3003-3012.

Mitchell, D. A., W. Xie, R. Schmittling, C. Learn, A. Friedman, R. E. McLendon and J. H. Sampson (2008). "Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma." Neuro Oncol **10**(1): 10-18.

Morello, C. S., M. Ye and D. H. Spector (2002). "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 **76**(10): 4822-4835.

Morse, M. A., Y. Deng, D. Coleman, S. Hull, E. Kitrell-Fisher, S. Nair, J. Schlom, M. E. Ryback and H. K. Lyerly (1999). "A Phase I study of active immunotherapy with carcinoembryonic antigen peptide (CAP-1)-pulsed, autologous human cultured dendritic cells in patients with metastatic malignancies expressing carcinoembryonic antigen." Clinical Cancer Research **5**(6): 1331-1338.

Morse, M. A., S. K. Nair, P. J. Mosca, A. C. Hobeika, T. M. Clay, Y. Deng, D. Boczkowski, A. Proia, D. Neidzwiecki, P. A. Clavien, H. I. Hurwitz, J. Schlom, E. Gilboa and H. K. Lyerly (2003). "Immunotherapy with autologous, human dendritic cells transfected with carcinoembryonic antigen mRNA." Cancer Investigation. **21**(3): 341-349.

Murphy, G., B. Tjoa, H. Ragde, G. Kenny and A. Boynton (1996). "Phase I clinical trial: T-cell therapy for prostate cancer using autologous dendritic cells pulsed with HLA-A0201-specific peptides from prostate-specific membrane antigen." Prostate **29**(6): 371-380.

Murphy, G. P., B. A. Tjoa, S. J. Simmons, M. K. Rogers, G. M. Kenny and J. Jarisch (2000). "Higher-dose and less frequent dendritic cell infusions with PSMA peptides in hormone-refractory metastatic prostate cancer patients." Prostate **43**(1): 59-62.

Nestle, F. O., S. Alijagic, M. Gilliet, Y. Sun, S. Grabbe, R. Dummer, G. Burg and D. Schadendorf (1998). "Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells." Nature Medicine **4**(3): 328-332.

Numazaki, K., M. Ikehata, S. Yanai, M. Umetsu, H. Motoya, S. Chiba and T. Sekine (1997). "Adoptive immunotherapy for interstitial pneumonia associated with cytomegalovirus infection." Clinical Infectious Diseases **25**(5): 1246-1247.

Ochsenbein, A. F., P. Klenerman, U. Karrer, B. Ludewig, M. Pericin, H. Hengartner and R. M. Zinkernagel (1999). "Immune surveillance against a solid tumor fails because of

immunological ignorance." Proceedings of the National Academy of Sciences of the United States of America **96**(5): 2233-2238.

Okada Hideho, Weller M, Huang R, Finocchiaro G, Gilbert MR, Wick W, Ellingson BM, Hashimoto N, Pollack IF, Brandes AA, Franceschi E, Herold-Mende C, Nayak L, Panigrahy A, Pope WB, Prins R, Sampson JH, Wen PY, Reardon DA. (2015). Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. Lancet Oncol, 16, 534-42. doi: 10.1016/S1470-2045(15)00088-1

Ohizumi, Y., H. Suzuki, Y. Numazaki, M. Imaizumi, Y. Koisumi, K. Tada, M. Minegishi, S. Tsuchiya and T. Konno (1994). "Human cytomegalovirus neutralizing antibody response in Japanese children with bone marrow transplantation." Tohoku Journal of Experimental Medicine **174**(1): 11-17.

Ommaya, A. K. (1976). "Immunotherapy of gliomas: a review." Advances in Neurology **15**: 337-359.

Paar, D. P. and R. B. Pollard (1996). "Immunotherapy of CMV infections." Advances in Experimental Medicine & Biology. **394**: 145-151.

Papadopoulos, E. B., M. Ladanyi, D. Emanuel, S. Mackinnon, F. Boulad, M. H. Carabasi, H. Castro-Malaspina, B. H. Childs, A. P. Gillio, T. N. Small, J. W. Young, N. A. Kernan and R. J. O'Reilly (1994). "Infusions Of Donor Leukocytes To Treat Epstein-Barr Virus-Associated Lymphoproliferative Disorders After Allogeneic Bone Marrow Transplantation." New England Journal of Medicine **330**(17): 1185-1191.

Pasteur, L. (1885). "Methode pour prevenir la rage apres morsure." Comptes rendus de l'academie des sciences (Paris) **101**: 765-774.

Peggs, K., S. Verfuerth, A. Pizzey, J. Ainsworth, P. Moss and S. Mackinnon (2002). "Characterization of human cytomegalovirus peptide-specific CD8(+) T-cell repertoire diversity following in vitro restimulation by antigen-pulsed dendritic cells." Blood **99**(1): 213-223.

Pepperl-Klindworth, S., N. Frankenberg and B. Plachter (2002). "Development of novel vaccine strategies against human cytomegalovirus infection based on subviral particles." Journal of Clinical Virology **25 Suppl 2**: S75-85.

Pickard, J. D., S. Bailey, H. Sanderson, M. Rees and J. S. Garfield (1990). "Steps towards cost-benefit analysis of regional neurosurgical care." British Medical Journal **301**(6753): 629-635.

Plotkin, S. A., R. Higgins, J. B. Kurtz, P. J. Morris, D. A. Campbell, Jr., T. C. Shope, S. A. Spector and W. M. Dankner (1994). "Multicenter trial of Towne strain attenuated virus vaccine in seronegative renal transplant recipients." Transplantation **58**(11): 1176-1178.

Poland, S. D., P. Costello, G. A. Dekaban and G. P. Rice (1990). "Cytomegalovirus in the brain: in vitro infection of human brain-derived cells." Journal of Infectious Diseases **162**(6): 1252-1262.

Ponsaerts, P., G. Van den Bosch, N. Cools, A. Van Driessche, G. Nijs, M. Lenjou, F. Lardon, C. Van Broeckhoven, D. R. Van Bockstaele, Z. N. Berneman and V. F. Van Tendeloo (2002). "Messenger RNA electroporation of human monocytes, followed by rapid in vitro differentiation, leads to highly stimulatory antigen-loaded mature dendritic cells." J Immunol **169**(4): 1669-1675.

Ponsaerts, P., V. F. Van Tendeloo, N. Cools, A. Van Driessche, F. Lardon, G. Nijs, M. Lenjou, G. Mertens, C. Van Broeckhoven, D. R. Van Bockstaele and Z. N. Berneman (2002).

"mRNA-electroporated mature dendritic cells retain transgene expression, phenotypical properties and stimulatory capacity after cryopreservation." Leukemia **16**(7): 1324-1330.

Prosch, S., W. D. Docke, P. Reinke, H. D. Volk and D. H. Kruger (1999). "Human cytomegalovirus reactivation in bone-marrow-derived granulocyte/monocyte progenitor cells and mature monocytes." Intervirology **42**(5-6): 308-313.

Rafferty, K. A., Jr. (1973). "Herpes viruses and cancer." Scientific American **229**(4): 26-33.

Raftery, M. J., M. Schwab, S. Diesner, G. Egerer and G. Schonrich (2002). "Dendritic cells cross-presenting viral antigens derived from autologous cells as a sensitive tool for visualization of human cytomegalovirus-reactive CD8+ T cells." Transplantation **73**(6): 998-1002.

Ranganathan, P., P. A. Clark, J. S. Kuo, M. S. Salamat and R. F. Kalejta (2012). "Significant association of multiple human cytomegalovirus genomic Loci with glioblastoma multiforme samples." J Virol **86**(2): 854-864.

Reeves, M. B., P. A. MacAry, P. J. Lehner, J. G. Sissons and J. H. Sinclair (2005). "Latency, chromatin remodeling, and reactivation of human cytomegalovirus in the dendritic cells of healthy carriers." Proc Natl Acad Sci U S A **102**(11): 4140-4145.

Remlinger, P. (1904). "Contribution a l'etude de la toxine rabique (faits experimentaux et clinique)." Comptes rendus des seances de la Societe de Biologie **56**: 348-350.

Remlinger, P. (1905). "Accidents paralytiques au cours du traitement antirabique." Annales de l'Institut Pasteur **19**: 625-646.

Riddell, S. R. and P. D. Greenberg (1995). "Cellular adoptive immunotherapy after bone marrow transplantation." Cancer Treatment & Research **76**: 337-369.

Rieser, C., R. Ramoner, L. Holtl, H. Rogatsch, C. Papesh, A. Stenzl, G. Bartsch and M. Thurnher (2000). "Mature dendritic cells induce T-helper type-1-dominant immune responses in patients with metastatic renal cell carcinoma." Urologia Internationalis **63**(3): 151-159.

Rivers, T. M. and F. F. Schwentker (1935). "Encephalomyelitis accompanied by myelin destruction experimentally produced in monkeys." Journal of Experimental Medicine **61**: 689-702.

Romani, N., D. Reider, M. Heuer, S. Ebner, E. Kampgen, B. Eibl, D. Niedrwieser and G. Schuler (1996). "Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability." Journal of Immunological Methods **196**: 137-151.

Rooney, C. M., C. A. Smith, C. Y. Ng, S. K. Loftin, J. W. Sixbey, Y. Gan, D. K. Srivastava, L. C. Bowman, R. A. Krance, M. K. Brenner and H. E. Heslop (1998). "Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients." Blood **92**(5): 1549-1555.

Rosenberg, S. A., J. C. Yang, D. J. Schwartzentruber, P. Hwu, F. M. Marincola, S. L. Topalian, N. P. Restifo, M. E. Dudley, S. L. Schwarz, P. J. Spiess, J. R. Wunderlich, M. R. Parkhurst, Y. Kawakami, C. A. Seipp, J. H. Einhorn and D. E. White (1998). "Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma [see comments]." Nature Medicine **4**(3): 321-327.

Sachs, G. W., R. L. Simmons and H. H. Balfour, Jr. (1984). "Cytomegalovirus vaccine: persistence of humoral immunity following immunization of renal transplant candidates." Vaccine **2**(3): 215-218.

Salford, L. G., A. Brun and S. Nirfalk (1988). "Ten-year survival among patients with supratentorial astrocytomas grade III and IV." Journal of Neurosurgery **69**(4): 506-509.

Salgaller, M. L., P. A. Lodge, J. G. McLean, B. A. Tjoa, D. J. Loftus, H. Ragde, G. M. Kenny, M. Rogers, A. L. Boynton and G. P. Murphy (1998). "Report of immune monitoring of prostate cancer patients undergoing T-cell therapy using dendritic cells pulsed with HLA-A2-specific peptides from prostate-specific membrane antigen (PSMA)." Prostate **35**(2): 144-151.

Salgaller, M. L., B. A. Tjoa, P. A. Lodge, H. Ragde, G. Kenny, A. Boynton and G. P. Murphy (1998). "Dendritic cell-based immunotherapy of prostate cancer." Critical Reviews in Immunology **18**(1-2): 109-119.

Samanta, M., L. Harkins, K. Klemm, W. J. Britt and C. S. Cobbs (2003). "High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma." Journal of Urology **170**(3): 998-1002.

Sampson, J. H., D. M. Ashley, G. E. Archer, H. E. Fuchs, G. Dranoff, L. P. Hale and D. D. Bigner (1997). "Characterization of a spontaneous murine astrocytoma and abrogation of its tumorigenicity by cytokine secretion." Neurosurgery **41**(6): 1365-1373.

Sampson, J. H. and D. A. Mitchell (2011). "Is cytomegalovirus a therapeutic target in glioblastoma?" Clin Cancer Res **17**(14): 4619-4621.

Scheurer, M. E., M. L. Bondy, K. D. Aldape, T. Albrecht and R. El-Zein (2008). "Detection of human cytomegalovirus in different histological types of gliomas." Acta Neuropathol **116**(1): 79-86.

Schuler-Thurner, B., D. Dieckmann, P. Keikavoussi, A. Bender, C. Maczek, H. Jonuleit, C. Roder, I. Haendle, W. Leisgang, R. Dunbar, V. Cerundolo, P. von Den Driesch, J. Knop, E. B. Brocker, A. Enk, E. Kampgen and G. Schuler (2000). "Mage-3 and influenza-matrix peptide-specific cytotoxic T cells are inducible in terminal stage HLA-A2.1+ melanoma patients by mature monocyte-derived dendritic cells." Journal of Immunology **165**(6): 3492-3496.

Sercarz, E. E., P. V. Lehmann, A. Ametani, G. Benichou, A. Miller and K. Moudgil (1993). "Dominance and crypticity of T cell antigenic determinants." Annual Review of Immunology **11**: 729-766.

Shapiro, W. R. (1986). "Therapy of adult malignant brain tumors: what have the clinical trials taught us?." Seminars in Oncology **13**(1): 38-45.

Siris, J. H. (1936). "Concerning the immunological specificity of glioblastoma multiforme." Bulletin of Neurology of New York **4**: 597-601.

Sissons, J. G., J. H. Sinclair and L. K. Borysiewicz (1991). "Pathogenesis of human cytomegalovirus disease and the kidney." Kidney Int Suppl **35**: S8-12.

Slagel, D. E., C. B. Wilson and P. B. Simmons (1969). "Polyacrylamide electrophoresis and immunodiffusion studies of brain tumor proteins." Annals of the New York Academy of Sciences **159**: 490-496.

Soderberg-Naucler, C. (2008). "HCMV microinfections in inflammatory diseases and cancer." J Clin Virol **41**(3): 218-223.

Soiffer, R., T. Lynch, M. Mihm, K. Jung, C. Rhuda, J. C. Schmollinger, F. S. Hodi, L. Liebster, P. Lam, S. Mentzer, S. Singer, K. K. Tanabe, A. B. Cosimi, R. Duda, A. Sober, A. Bhan, J. Daley, D. Neuberg, G. Parry, J. Rokovich, L. Richards, J. Drayer, A. Berns, S. Clift and G. Dranoff (1998). "Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma." Proceedings of the National Academy of Sciences of the United States of America **95**(22): 13141-13146.

Soroceanu, L., L. Matlaf, V. Bezrookove, L. Harkins, R. Martinez, M. Greene, P. Soteropoulos and C. S. Cobbs (2011). "Human cytomegalovirus US28 found in glioblastoma promotes an invasive and angiogenic phenotype." Cancer Res **71**(21): 6643-6653.

Steinman, R. M. (2001). "Dendritic cells and the control of immunity: enhancing the efficiency of antigen presentation." Mount Sinai Journal of Medicine **68**(3): 106-166.

Strobel, I., S. Berchtold, A. Gotze, U. Schulze, G. Schuler and A. Steinkasserer (2000). "Human dendritic cells transfected with either RNA or DNA encoding influenza matrix protein M1 differ in their ability to stimulate cytotoxic T lymphocytes." Gene Therapy **7**(23): 2028-2035.

Stuart, G. and K. Krikorian (1928). "The neuro-paralytic accidents of anti-rabies treatment." Annals of Tropical Medicine **22**: 327-377.

Stuart, G. and K. Krikorian (1930). "A fatal neuro-paralytic accident of anti-rabies treatment." Lancet **1**: 1123-1125.

Stupp, R., P. Y. Dietrich, S. Ostermann Kraljevic, A. Pica, I. Maillard, P. Maeder, R. Meuli, R. Janzer, G. Pizzolato, R. Miralbell, F. Porchet, L. Regli, N. de Tribolet, R. O. Mirimanoff and S. Leyvraz (2002). "Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide." Journal of Clinical Oncology. **20**(5): 1375-1382.

Stupp, R., W. P. Mason, M. J. van den Bent, M. Weller, B. Fisher, M. J. B. Taphoorn, K. Belanger, A. A. Brandes, C. Marosi, U. Bogdahn, J. Curschmann, R. C. Janzer, S. K. Ludwin, T. Gorlia, A. Allgeier, D. Lacombe, J. G. Cairncross, E. Eisenhauer, R. O. Mirimanoff and G. the European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups and the National Cancer Institute of Canada Clinical Trials (2005). "Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma." New England Journal of Medicine. **352**(10): 987-996.

Su, Y. B., S. Sohn, S. E. Krown, P. O. Livingston, J. D. Wolchok, C. Quinn, L. Williams, T. Foster, K. A. Sepkowitz and P. B. Chapman (2004). "Selective CD4+ lymphopenia in melanoma patients treated with temozolomide: a toxicity with therapeutic implications." Journal of Clinical Oncology **22**(4): 610-616.

Su, Y. B., S. Sohn, S. E. Krown, P. O. Livingston, J. D. Wolchok, C. Quinn, L. Williams, T. Foster, K. A. Sepkowitz and P. B. Chapman (2004). "Selective CD4+ lymphopenia in melanoma patients treated with temozolomide: a toxicity with therapeutic implications.[see comment][erratum appears in J Clin Oncol. 2004 May 15;22(10):2038]." Journal of Clinical Oncology **22**(4): 610-616.

Su, Z., J. Dannull, A. Heiser, D. Yancey, S. Pruitt, J. Madden, D. Coleman, D. Niedzwiecki, E. Gilboa and J. Vieweg (2003). "Immunological and clinical responses in metastatic renal cancer patients vaccinated with tumor RNA-transfected dendritic cells." Cancer Research **63**(9): 2127-2133.

Szmania, S., A. Galloway, M. Bruorton, P. Musk, G. Aubert, A. Arthur, H. Pyle, N. Hensel, N. Ta, L. Lamb, Jr., T. Dodi, A. Madrigal, J. Barrett, J. Henslee-Downey and F. van Rhee (2001). "Isolation and expansion of cytomegalovirus-specific cytotoxic T lymphocytes to clinical scale from a single blood draw using dendritic cells and HLA-tetramers." Blood **98**(3): 505-512.

Tanchot, C., M. M. Rosado, F. Agenes, A. A. Freitas and B. Rocha (1997). "Lymphocyte homeostasis." Seminars in Immunology **9**(6): 331-337.

Thurner, B., I. Haendle, C. Roder, D. Dieckmann, P. Keikavoussi, H. Jonuleit, A. Bender, C. Maczek, D. Schreiner, P. von den Driesch, E. B. Brocker, R. M. Steinman, A. Enk, E. Kampgen and G. Schuler (1999). "Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma." Journal of Experimental Medicine **190**(11): 1669-1678.

Tjoa, B. A., S. J. Simmons, V. A. Bowes, H. Ragde, M. Rogers, A. Elgamal, G. M. Kenny, O. E. Cobb, R. C. Ireton, M. J. Troychak, M. L. Salgaller, A. L. Boynton and G. P. Murphy (1998). "Evaluation of phase I/II clinical trials in prostate cancer with dendritic cells and PSMA peptides." Prostate **36**(1): 39-44.

Trouillas, P. (1973). "Immunology and immunotherapy of cerebral tumors. Current status." Revue Neurologique (Paris) **128**(1): 23-38.

Trouillas, P. and C. Lapras (1970). "[Active immunotherapy of cerebral tumor. 20 cases]. [French]." Neurochirurgie. **16**(2): 143-170.

Tuohy, V. K., Z. J. Lu, R. A. Sobel, R. A. Laursen and M. B. Lees (1988). "A synthetic peptide from myelin proteolipid protein induces experimental allergic encephalomyelitis." Journal of Immunology **141**(4): 1126-1130.

Tzakis, A. G. (2001). "Cytomegalovirus prophylaxis with ganciclovir and cytomegalovirus immune globulin in liver and intestinal transplantation." Transplant Infectious Disease **3 Suppl 2**: 35-39.

Van Meirvenne, S., L. Straetman, C. Heirman, M. Dullaers, C. De Greef, V. Van Tendeloo and K. Thielemans (2002). "Efficient genetic modification of murine dendritic cells by electroporation with mRNA." Cancer Gene Ther **9**(9): 787-797.

Van Tendeloo, V. F., P. Ponsaerts, F. Lardon, G. Nijs, M. Lenjou, C. Van Broeckhoven, D. R. Van Bockstaele and Z. N. Berneman (2001). "Highly efficient gene delivery by mRNA electroporation in human hematopoietic cells: superiority to lipofection and passive pulsing of mRNA and to electroporation of plasmid cDNA for tumor antigen loading of dendritic cells." Blood **98**(1): 49-56.

Vancikova, Z. and P. Dvorak (2001). "Cytomegalovirus infection in immunocompetent and immunocompromised individuals--a review." Current Drug Targets Immune Endocrine & Metabolic Disorders **1**(2): 179-187.

Wahl, S. M., D. A. Hunt, H. L. Wong, S. Dougherty, N. McCartney-Francis, L. M. Wahl, L. Ellingsworth, J. A. Schmidt, G. Hall, A. B. Roberts and M. B. Sporn (1988). "Transforming growth factor-beta is a potent immunosuppressive agent that inhibits IL-1-dependent lymphocyte proliferation." Journal of Immunology **140**(9): 3026-3032.

Wahlstrom, T., E. Linder and E. Saksela (1973). "Glia-specific antigens in cell cultures from rabbit brain, human foetal and adult brain, and gliomas." Acta Pathologica et Microbiologica Scandinavica [B] Microbiology and Immunology **81**(6): 768-774.

Waksman, B. H., H. Porter, M. D. Lees, R. D. Adams and J. Folch (1954). "A study of the chemical nature of components of bovine white matter effective in producing allergic encephalomyelitis in the rabbit." Journal of Experimental Medicine **100**: 451-471.

Walker, M. D., E. Alexander, Jr, W. E. Hunt, C. S. MacCarty, Mahaley, M. S. Jr, J. Mealey, Jr, H. A. Norrell, G. Owens, J. Ransohoff, C. B. Wilson, Gehan, EA and T. A. Strike (1978). "Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial." Journal of Neurosurgery **49**(3): 333-343.

Walker, M. D., S. B. Green, D. P. Byar, E. Alexander, Jr, Batzdorf, W. H. Brooks, W. E. Hunt, C. S. MacCarty, M. S. Mahaley, Jr, J. Mealey, Jr, Owens, J. Ransohoff, J. T. Robertson, W. R. Shapiro, K. R. Smith, Jr, C. B. Wilson and T. A. Strike (1980). "Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery." New England Journal of Medicine **303**(23): 1323-1329.

Walter, E. A., P. D. Greenberg, M. J. Gilbert, R. J. Finch, K. S. Watanabe, E. D. Thomas and S. R. Riddell (1995). "Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor." New England Journal of Medicine **333**(16): 1038-1044.

Wang, X., S. M. Huong, M. L. Chiu, N. Raab-Traub and E. S. Huang (2003). "Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus." Nature **424**(6947): 456-461.

Wekerle, H., K. Kojima, J. Lannes-Vieira, H. Lassmann and C. Linington (1994). "Animal models." Annals of Neurology **36 Suppl**: S47-53.

Westphal, M., D. C. Hilt, E. Bortey, P. Delavault, R. Olivares, P. C. Warnke, I. R. Whittle, J. Jaaskelainen and Z. Ram (2003). "A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma." Neuro-oncology **5**: 79-88.

Wickremesinghe, H. R. and P. O. Yates (1971). "Immunological properties of neoplastic neural tissues." British Journal of Cancer **25**(4): 711-720.

Wikstrand, C. J. and D. D. Bigner (1979). "Surface antigens of human glioma cells shared with normal adult and fetal brain." Cancer Res. **39**(8): 3235-3243.

Wikstrand, C. J. and D. D. Bigner (1981). "Hyperimmunization of non-human primates with BCG-CW and cultured human glioma-derived cells. Production of reactive antisera and absence of EAE induction." Journal of Neuroimmunology **1**(3): 249-260.

Wrzesinski, C. and N. P. Restifo (2005). "Less is more: lymphodepletion followed by hematopoietic stem cell transplant augments adoptive T-cell-based anti-tumor immunotherapy." Current Opinion in Immunology **17**: 195-201.

Wu, J. and X. Xiong (2014). "Single-arm phase II group sequential design with survival endpoint at a fixed time point." Statistics in Biopharmaceutical Research **6**(4): 289-301.

Yu, J. S., C. J. Wheeler, P. M. Zeltzer, H. Ying, D. N. Finger, P. K. Lee, W. H. Yong, F. Incardona, R. C. Thompson, M. S. Riedinger, W. Zhang, R. M. Prins and K. L. Black (2001). "Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration." Cancer Research **61**(3): 842-847.

Zhou, J., M. E. Dudley, S. A. Rosenberg and P. F. Robbins (2005). "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 With Emphasis on Tumor Immunology **28**: 53-62, 2005 Jan-Feb.

Zuber, P., M. C. Kuppner and N. de Tribolet (1988). "Transforming growth factor-beta 2 down-regulates HLA-DR antigen expression on human malignant glioma cells." European Journal of Immunology **18**(10): 1623-1626.

18 APPENDICES

18.1 Appendix A: Radiation Therapy

The total targeted RT dose will be at the discretion of the treating Radiation Oncologist. Optimally, one treatment of 1.8-2.0 Gy/fraction will be given daily 5 days per week for a total targeted dose of 59.4-60.0 Gy over ≤ 7 weeks. 3D conformal and intensity-modulated RT is permitted. All portals should be treated during each treatment session. Doses are specified as the target dose that shall be to the center of the target volume.

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

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

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

Radiation will be delayed or interrupted if the platelet count is < 20,000. Radiation will not begin or resume until the platelet count is ≥ 20,000. Hematologic toxicities should be rated on a scale of 0-5 as defined in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

If radiotherapy has to be temporarily interrupted for technical or medical reasons unrelated to the temozolomide administration, then treatment with daily temozolomide should continue. If radiotherapy has to be permanently interrupted then treatment with daily temozolomide should stop.

18.2 Appendix B: Temozolomide Therapy during Radiation

TMZ should be administered continuously from day 1 of radiotherapy to the last day of radiation at a daily oral dose of 75 mg/m^2 for a maximum of 49 days. The drug will be administered orally 1 hour before each session of radiotherapy during weekdays (Monday through Friday). During weekends without radiotherapy (Saturday and Sunday), the drug will be taken in the morning. The dose will be determined using the body surface area (BSA) calculated at the beginning of the concomitant treatment. The BSA will be calculated from the height obtained at the pretreatment visit and the weight obtained at the visit immediately before the first day of treatment. Capsules of TMZ are available in 5, 20, 100, 140, 180 and 250 mg. The daily dose will be rounded to the nearest 5 mg. Patients will be instructed to swallow the capsules whole, in rapid succession, without chewing them. If vomiting occurs during the course of treatment, no re-dosing of the patient is allowed before the next scheduled dose. The capsules should be taken on an empty stomach, therefore a minimum of 2 hours after a meal and with no food ingestion for 1 hour after TMZ administration. Antiemetic prophylaxis is usually not required for the continuous daily dosing schedule. However, prophylaxis with a 5-HT3 antagonist is recommended prior to administration of the first few TMZ doses and should be administered orally 30 to 60 minutes before TMZ treatment.

No dose reduction should be made, but delay or discontinuation of TMZ administration should be decided weekly according to hematologic and non-hematologic AEs. If one or more of the following are observed:

- ANC < 1.0 x 10^9/ L
- Platelet count < 75 x 10^9/L
- Grade 3 non-hematologic AE (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy, and fatigue if controlled by pharmacologic or non-pharmacologic therapy)

then treatment with concomitant TMZ will be withheld until all of the following conditions are met:

- ANC ≥ 1.0 x 10^9/L
- Platelet count ≥ 75 x 10^9/L
- Grade ≤ 1 non-hematologic AE(except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy, and fatigue if controlled by pharmacologic or non-pharmacologic therapy)

In case of hematologic AE as defined above, a CBC should be performed at least twice weekly. In case of non-hematologic AE, the patient should be assessed at least weekly with relevant laboratory test(s). As soon as all of the above conditions are met, the administration of TMZ will resume at the same dose as used initially.
If one or more of the following are observed:

- ANC < 0.5 x 10^9/L (Grade 4)
- Platelet count < 10 x 10^9/L (Grade 4)
- Grade 3 or 4 non-hematologic AE (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy, and fatigue if controlled by pharmacologic or non-pharmacologic therapy)

then treatment with concomitant TMZ should be **stopped**.

If the duration of radiotherapy exceeds 7 weeks, then concomitant treatment with TMZ should be stopped after 49 days of TMZ treatment. If the administration of TMZ has to be interrupted, the radiotherapy will proceed normally. Missed doses of TMZ will not be made up at the end of radiotherapy. The total number of days and total dose of TMZ will be recorded.

Table 1: Summary of TMZ Delay or Discontinuation during Concomitant Radiation Therapy AE

	Value	Grade	Action
ANC	≥ 0.5 and $< 1.0 \times 10^9$/L	2, 3	**Delay** TMZ until: • ANC $\geq 1.0 \times 10^9$/L • Plt $\geq 75 \times 10^9$/L • Non-hem AE ≤ 1
Platelet count	≥ 10 and $< 75 \times 10^9$/L	2, 3	
Non-hematologic (except for alopecia, nausea/vomiting if controlled by maximal antiemetic therapy)	NA	3	
ANC	$< 0.5 \times 10^9$/L	4	**Stop** concomitant TMZ
Platelet count	$< 10 \times 10^9$/L	4	
Non-hematologic (except for alopecia, nausea/vomiting if controlled by maximal antiemetic therapy)	NA	4	

18.3 Appendix C: Temozolomide Therapy after Radiation

TMZ will be administered orally once per day for 21 consecutive days (days 1-21) of a 35 (+/- 7) day cycle. The starting dose for the first cycle will be 75 mg/m^2/day, with a single dose escalation to 100 mg/m^2/day in subsequent cycles if no adverse events ≥ grade 2 are noted.

Twelve cycles of TMZ may be given if the patient demonstrates continued improvement on MR scan, decreased corticosteroid requirement, improvement in performance status, or improvement in neurologic function.

Dosing Modifications

Dosing is based on adverse events (AEs) during the prior treatment cycle. If multiple AEs are seen, the dose administered should be based on the dose reduction required for the most severe grade of any single AE as shown in Table 2.

Table 2: Dosing Modification Schedule for TMZ per RTOG 0525 Regimen

Dose Level	Dose, mg/m^2/day x 21 days	Remarks
-2	35	Reduction if prior AE
-1	50	Reduction if prior AE
0	75	Starting dose for cycle 1, increase to 100 mg/m^2 for cycle 2 and beyond if no hematologic toxicity ≥ grade 2 and non-hematologic toxicity > grade 2
+1	100	Highest possible dose level (adjuvant)

First cycle

TMZ will be started at a dose of 75 mg/m^2/day.

Second cycle

The dose of TMZ will be determined according to (1) non-hematologic AE during the preceding treatment cycle, as well as (2) the nadir (lowest/worst) ANC and platelet counts.

Cycles 3-12

Any dose reductions of TMZ will be determined according to (1) non-hematologic AE during the preceding cycle, as well as (2) the nadir (lowest/worst) ANC and platelet counts. No dose escalation should be attempted. The same dose reductions as for the second cycle should be applied. If the dose was reduced or delayed for adverse events, there will be no dose escalation.

Delay

On day 1 of each cycle (within the prior 72 hours), ANC ≥ 1 x 10/L, platelet count ≥ 100 x 10/L and all grade 3 or 4 non-hematologic AEs (except for alopecia, nausea, and vomiting if controlled by maximal antiemetic therapy) must have resolved (to grade ≤ 1).

If AEs persists, treatment should be delayed by 1 week for up to 4 consecutive weeks. If, after 4 weeks of delay, all AEs have still not resolved: then any further adjuvant treatment with TMZ should be stopped.

Dose escalation
If, during the first cycle, all non-hematologic AEs observed were grade ≤ 2 (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) and with platelets ≥ 100 x 10^9/L and ANC ≥ 1 x10^9/L: then the TMZ dose should be escalated to dose level +1 and this dose should be used as the maintenance dose for subsequent cycles. If treatment after cycle 1 has to be delayed by any length of time because of ongoing hematologic AEs ≥2 and/or non-hematologic AEs of grade > 2 (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy), then no escalation is possible. If the dose was not escalated at cycle 2, then the dose should not be escalated in further cycles (3-12).

Dose reductions
If any non-hematologic AE observed was grade > 2 (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) and/or if platelets < 100 x 10^9/L and/or ANC < 1 x 10^9/L, then the dose should be reduced by one dose level. For patients who would require dose reductions to a dose level < 35 mg/m^2/day (dose level -2), TMZ will be stopped. Also, if any of the same non-hematologic grade 3 AE recurs (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) after reduction for that AE, then TMZ will be stopped. If any treatment-related non-hematologic AE observed was grade 4 (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) then adjuvant TMZ treatment should be stopped. Please see Tables 3-6 for a summary of dose modification or discontinuation.

The reason(s) for dose reduction and/or delay must be documented in the CRF.

Table 3: Summary of Dose Modification or Discontinuation During Post-Radiation Temozolomide Worst Non-Hematologic AE (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) During the Previous Cycles

Grade	Dose Modification
0-2	No dose modifications for non-hematologic AEs. Dose escalations (only for cycle 2) or reductions based on ANC and platelet counts are applicable.
3	Reduce by one dose level (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy). Dose modifications (escalations or reductions) based on ANC and platelet counts are not applicable. No further escalation is possible. If the same non-hematologic grade 3 AE recurs (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) after reduction for that AE, then stop.
4	Stop (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy). Dose modifications (escalations or reductions) based on ANC and platelet counts are not applicable.

Table 4: Worst Treatment-Related Hematologic AE during the Previous Cycles

Worst AE		**Platelets**	
		≥100 x 10^9/L	**< 100 x 10^9/L**
ANC	**≥ 1.5 x 10^9/L**	Dose unchanged	Reduce by 1 dose level
	≥1 & <1.5 x 10^9/L	Dose unchanged	Reduce by 1 dose level
	< 1 x 10^9/L	Reduce by 1 dose level	Reduce by 1 dose level

Note: A complete blood count will be performed prior to each vaccine and if clinically indicated on days 7, 14, 21 and 28 (+/- 72 hours) after the first daily dose of each adjuvant treatment cycle.

Table 5: Hematological AE on Day 1 of Each Cycle (within the prior 72 hours before Day 1

AE	Delay
ANC< 1 x 10^9/L and/or Platelet count < 100 x 10^9/L	Delay up to 4 weeks until all resolved. If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on hematologic and non-hematologic AEs are applicable.

Table 6: Non-Hematologic AE (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) on the day 1 of Each Cycle (within the prior 72 hours)

Grade	Delay
2-3	Delay up to 4 weeks until all resolved (to grade ≤ 1). If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on hematologic and non-hematologic AEs are applicable.
4	Stop (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy).

18.4 Appendix D: Alternative Temozolomide Therapy after Radiation

If dose-intensified TMZ is delayed for more than 4 weeks or stopped due to any hematologic or non-hematologic AEs that were not grade 4, and less than 6 cycles of TMZ have been achieved, once the AEs have resolved to ≤ grade 1 but not more than 8 weeks after the previous dose-intensified TMZ cycle, TMZ can be restarted at the standard Stupp protocol dosing schedule at 150mg/m^2 orally daily for 5 days on a 28+/-14 days cycle to achieve a maximal total of 12 cycles including the previous dose-intensified TMZ cycles. No dose escalation will be allowed, but 1 dose reduction to 100mg/m^2 daily x 5 days on a 28 (+/-14) day cycle will be allowed.

Table 7: Dosing Modification Schedule for Maintenance TMZ per Stupp Regimen

Dose Level	Dose, mg/m^2/day x 5 days	Remarks
-1	100	Reduction if prior AEs
0	150	Starting dose for cycle 1

Delay
On day 1 of each cycle (within the prior 72 hours), ANC ≥ 1 x 10/L, platelet count ≥ 100 x 10/L and all grade 3 or 4 non-hematologic AEs (except for alopecia, nausea, and vomiting if controlled by maximal antiemetic therapy) must have resolved (to grade ≤ 1).

If AEs persists, treatment should be delayed by 1 week for up to 4 consecutive weeks. If, after 4 weeks of delay, all AEs have still not resolved: then any further adjuvant treatment with TMZ should be stopped.

Dose escalation: No dose escalation is allowed

Dose reductions
If any non-hematologic AE observed was grade > 2 (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) and/or if platelets < 100 x 10^9/L and/or ANC < 1 x 10^9/L, then the dose should be reduced by one dose level. For patients who would require dose reductions to a dose level < 100 mg/m^2/day (dose level -1), TMZ will be stopped. Also, if any of the same non-hematologic grade 3 AE recurs (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) after reduction for that AE, then TMZ will be stopped. If any treatment-related non-hematologic AE observed was grade 4 (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) then adjuvant TMZ treatment should be stopped. Please see Tables 8-11 for a summary of dose modification or discontinuation.

The reason(s) for dose reduction and/or delay must be documented in the CRF.

Table 8: Summary of Dose Modification or Discontinuation during Post-Radiation alternative (Stupp) Temozolomide Worst Non-Hematologic AE (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) During the Previous Cycles

Grade	Dose Modification
0-2	No dose modifications for non-hematologic AEs. Dose reductions based on ANC and platelet counts are applicable.
3	Reduce by one dose level (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy. Dose reduction based on ANC and platelet counts is not applicable. If the same non-hematologic grade 3 AE recurs (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) after reduction for that AE, then stop.
4	Stop (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy. Dose reduction based on ANC and platelet counts is not applicable.

Table 9: Worst Treatment-Related Hematologic AE during the Previous Cycles

Worst AE		**Platelets**	
		≥100 x 10^9/L	**< 100 x 10^9/L**
ANC	**≥ 1.5 x 10^9/L**	Dose unchanged	Reduce by 1 dose level
	≥1 & <1.5 x 10^9/L	Dose unchanged	Reduce by 1 dose level
	< 1 x 10^9/L	Reduce by 1 dose level	Reduce by 1 dose level

Note: A complete blood count will be performed prior to each vaccine and if clinically indicated on days 7, 14, 21 and 28 (+/- 72 hours) after the first daily dose of each adjuvant treatment cycle.

Table 10: Hematological AE on Day 1 of Each Cycle (within the prior 72 hours before Day 1

AE	**Delay**
ANC< 1 x 10^9/L and/or Platelet count < 100 x 10^9/L	Delay up to 4 weeks until all resolved. If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on hematologic and non-hematologic AEs are applicable.

Table 11: Non-Hematologic AE (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy) On the day 1 of Each Cycle (within the prior 72 hours)

Grade	**Delay**
2-3	Delay up to 4 weeks until all resolved (to grade ≤ 1). If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on hematologic and non-hematologic AEs are applicable.
4	Stop (except for alopecia, nausea and vomiting if controlled by maximal antiemetic therapy)

18.5 Appendix E: ECOG Performance Status to Karnofsky Performance Status Comparison Table

Performance Status (PS) will be determined from KPS conversion using the ECOG comparison scale for patients ≤ 50 years

ECOG PERFORMANCE STATUS	KARNOFSKY PERFORMANCE STATUS
0—Fully active, able to carry on all pre-disease performance without restriction	100—Normal, no complaints; no evidence of disease 90—Able to carry on normal activity; minor signs or symptoms of disease
1—Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work	80—Normal activity with effort, some signs or symptoms of disease 70—Cares for self but unable to carry on normal activity or to do active work
2—Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours	60—Requires occasional assistance but is able to care for most of personal needs 50—Requires considerable assistance and frequent medical care
3—Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours	40—Disabled; requires special care and assistance 30—Severely disabled; hospitalization is indicated although death not imminent
4—Completely disabled; cannot carry on any selfcare; totally confined to bed or chair	20—Very ill; hospitalization and active supportive care necessary 10—Moribund
5—Dead	0—Dead

18.6 Appendix G: GUIDANCE ON CONTRACEPTION

Prior to study enrollment, women of childbearing potential must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy.

Women of childbearing potential (WOCBP) include any woman who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or who is not post-menopausal.
Post-menopause is defined as:

- Amenorrhea that has lasted for ≥ 12 consecutive months without another cause, or

For the purposes of the proposed study, **medically acceptable forms of contraceptive methods** include the following:

- Surgical sterilization at least 6 months before Study Drug administration
- Implants
- Levonorgestrel (LNG) and Copper T IUDs
- Sexual abstinence
- Injectable hormone depos
- Oral contraceptive pill
- Hormone patch
- Vaginal ring
- Condom

Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are NOT acceptable methods of contraception.

http://www.cdc.gov/reproductivehealth/unintendedpregnancy/pdf/contraceptive_methods_508.pdf for a list of contraceptive methods and effectiveness.