A Phase 1b Study

ETAPA I:
Evaluation of Tumor Associated P30-Peptide Antigen I; A Pilot Trial of Peptide-based Tumor Associated Antigen Vaccines in Newly Diagnosed, Unmethylated, and Untreated Glioblastoma (GBM)

DUKE CANCER INSTITUTE

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Original version 1.0:	4/5/2021	
Amended version 2.0:	8/23/2021	FDA updates: Dose escalation and de-escalation added (Sections 5.5, 9.1, 9.1.3, 9.2,

		15.5, 15.5.1, 15.5.1.2). Definition of dose-limiting toxicity added (Sections 9.1.2, 12.5.3, 15.5.1.1). Added staggering of patients in initial dose level within each stratum (Sections 9.1.1, 15.5.1). Clarify 2 cohorts will be evaluated separately for the primary endpoint (Sections 5.3, 8, 9.1, 15.1, 15.5, 15.5.2). Increase in sample size (Sections 5.3, 9.1, 15.1, 15.5.1.3, 15.9). Update management of toxicities (Section 9.1.4). Updates to P30-EPS packaging/labeling (Sections 10.1.2, 15.1). Clarify antihistamine use (Section 12.6.3). Clarify details of secondary objective statistical analyses (Section 15.6). Add language regarding NSR determination of IVDs and IVD reporting (Sections 12.1.1, 12.1.2, 13.3).
Amended version 3.0:	4/5/2022	PRMC updates: Updates to P30-EPS vaccine label (Section 10.1.2). Additional information added regarding P30-EPS storage conditions (Section 10.1.3). New language regarding Hiltonol Patient Administration Guide (Section 10.2.2). Correction made to the Schedule of Events (Table 2).
Amended version 4.0	12/21/2022	The eligibility requirement regarding tumor size and HLA A*0201 positive status have been removed (see Section 11.1). Tumor size limits are not needed for this study of safety and feasibility. While HLA status is of interest, we will not exclude patients based on it. Information potentially will inform future studies. This change also affects Sections 5.1, 7.2.2, Table 3, Footnotes **Error! Bookmark not defined.**, **Error! Bookmark not defined.**, and **Error! Bookmark not defined.**, and Sections 12.1, 13.3, and 15.1. The section regarding HLA testing has been removed. In addition, we have removed the section recommending the avoidance of antihistamines. The volume of P30-EPS vaccine peptides was changed from 0.7 mL to 0.9 mL (Section 10.1.2).
Amended version 5.0	5/18/2023	Study Drug Section 10 revised to correct the net peptide concentration of the P30-EPS drug product based on a change to the drug product specification.
Amended version 6.0	10/16/2024	Updates to study team members. Inclusion of archival and future tumor tissue collection from patients' biopsies and surgeries (Sections 5.3, 9.1, 9.3 and 12) and addition of 3 new exploratory objectives to analyze tumor tissue (Sections 5.1, 8 and 15.7). Added clarification regarding collecting vital signs, windows for blood draws, Hiltonol® injections and Booster Phase Vaccines #6 and #7, and time of consent after completion of XRT/TMZ. Deletion of Pre-XRT column from Table 3. Update to language surrounding acceptable corticosteroid dose (Section 9.1, 9.1.5, and 12.5). Addition of exclusion criteria (11.2). Addition of statement reflecting the number of patients enrolled in this study so far (Section 10.1.4). Added statement about future patient enrollment not being allocated based on CMV results (Section 15.1). Inclusion of reference to ETAPA correlative lab manual throughout. General updates and clarifications throughout.

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Ab	Antibody
ACD	Acid Citrate Dextrose
AE	Adverse Event
ANC	Absolute Neutrophil Count
β-HCG	Beta-Human Chorionic Gonadotropin
CCF	Cancer Cell Fractions
CLIA	Clinical Laboratory Improvement Act
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CNS	Central Nervous System
COI	Conflict of Interest
CRS	Cytokine Release Syndrome
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T-Lymphocyte
DC	Dendritic Cell
DCI	Duke Cancer Institute
DIPC	Duke Immune Profiling Core
DLT	Dose Limiting Toxicity
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DRDL	Duke Research Data Lifecycle
DSMB	Data Safety and Monitoring Board
DSMP	Data Safety and Monitoring Plan
DUHS	Duke University Health System
DVT	Deep Vein Thrombosis
eCRF	Electronic Case Report Form
EphA2	Ephrin type-A receptor 2
EGFR	Epidermal Growth Factor Receptor
EGFRvIII	Epidermal Growth Factor Receptor variant type III
EGFRvIII-KLH	EGFRvIII conjugated to Keyhole Limpet Hemocyanin
FDA	Federal Drug Administration
GBM	Glioblastoma
GCP	Good Clinical Practice
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
GMP	Good Manufacturing Practice
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICH	International Conference on Harmonization
ICS	Investigational Chemotherapy Services
IDH	Isocitrate dehydrogenase
IDS	Investigational Drug Services
IEC	Institutional Ethics Committee
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IHC	Immunohistochemistry
IL-2	Interleukin-2
i.m.	Intramuscular
IRB	Institutional Review Board
IT	Information Technology
IV	Intravenous
IVD	*In vitro* diagnostic

KLH	Keyhole Limpet Hemocyanin
KPS	Karnofsky Performance Status
LAMP	Lysosomal-associated Membrane Protein
MAb	Monoclonal Antibody
MGMT	Methylguanine Methyltransferase
MHC	Major Histocompatibility Complex
mL	Milliliter
MMSE	Mini-Mental Status Examination
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NSR	Non-significant Risk
OARC	Office of Audit, Risk and Compliance
OTC	Oncology Treatment Center
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reactions
P30-EPS	P30- EphA2, CMV pp65, survivin
PFS	Progression Free Survival
PI	Principal Investigator
PRMC	Protocol Review and Monitoring Committee
PRTBTC	Preston Robert Tisch Brain Tumor Center
qIHC	Quantitative Immunohistochemistry
RANO	Response Assessment in Neuro-Oncology
RIO	Research Integrity Office
RNA	Ribonucleic Acid
SAE	Severe Adverse Event
SGOT	Serum Glutamic Oxaloacetic Transaminase
SIADH	Syndrome of Inappropriate Antidiuretic Hormone
snRNAseq	Single nuclei RNA sequencing
SOCOMM	Safety Oversight Committee
SOP	Standard Operating Procedure
TAA	Tumor Associated Antigen
TCR	T cell Receptor
Td	Tetanus-Diphtheria vaccine
T_H2	T helper type 2
TMB	Tumor Mutational Burden
TMZ	Temozolomide
TNF-α	Tumor Necrosis Factor-α
T_{Regs}	Regulatory T cells
ULN	Upper Limit of Normal
WES	Whole Exome Sequencing
WHO	World Health Organization

5 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

5.1 Purpose

A phase 1b study of P30-linked ephrin type-A receptor 2 (EphA2), cytomegalovirus (CMV) pp65, and survivin peptide vaccination (collectively called the P30-EPS vaccine) in patients with a newly diagnosed, isocitrate dehydrogenase (IDH) wildtype, O^6-methylguanine-DNA methyltransferase (MGMT) promoter unmethylated, and untreated World Health Organization (WHO) grade 4 malignant glioma at the Preston Robert Tisch Brain Tumor Center (PRTBTC) at Duke, is planned to address the following objectives.

Primary Objective:

1. Evaluate the safety profile of a cancer vaccine comprised of P30-linked EphA2, CMV pp65, and survivin peptides.

Secondary Objectives:

1. Assess whether pp65-specific polyfunctional T cells increase after priming vaccination with the P30-linked pp65 peptide.
2. Assess whether EphA2- or survivin-specific polyfunctional T cells increase after priming vaccination with P30-linked EphA2 or survivin peptides.
3. Assess whether pp65-specific polyfunctional T cells increase after booster vaccination with the P30-linked pp65 peptide.
4. Assess whether EphA2- or survivin-specific polyfunctional T cells increase after booster vaccination with P30-linked EphA2 or survivin peptides.
5. Describe the survival and progression-free survival (PFS) of patients who received a vaccine comprised of P30-linked EphA2, CMV pp65, and survivin peptides.

Exploratory Objective:

1. Assess the diversity of the P30-EPS-specific memory T cell repertoire via T cell Receptor (TCR) sequencing.
2. Define the relationship between reported biomarkers of response to immunotherapy in glioblastoma (GBM) and survival.
3. Assess resected tumor for expression of vaccine targets (i.e. EphA2, pp65, and survivin).
4. Assess changes in the tumor immune microenvironment, mutational heterogeneity/clonality, neoantigen depletion, and their association with survival.

Hypothesis:

We hypothesize that the P30-linked CMV EphA2, pp65, and survivin peptide vaccine (P30-EPS) treatment will have an acceptable toxicity profile in patients with a newly diagnosed, IDH wild type, MGMT promoter unmethylated, WHO grade 4 malignant glioma.

5.2 Background and Significance

WHO grade 4 malignant glioma, specifically GBM, accounts for 46.6% of primary malignant brain tumors, affects approximately 10,996 people annually [1], and results in a high mortality rate with a median survival ranging from 16.1 to 20.5 months for patients with newly diagnosed GBM [2-4]. Standard of care strategies for treating a newly diagnosed GBM include surgical resection, radiation therapy with concurrent temozolomide (TMZ), and adjuvant TMZ. Recently, a subset of GBM patients with an unmethylated MGMT promoter have been identified as having little to no survival benefit when treated with TMZ and radiation compared to radiation alone [5, 6]. Independent of response to TMZ, having an unmethylated MGMT promoter is linked to worse prognosis and shorter overall survival [6]. Overall, poor prognosis provides rationale to develop new therapies to treat patients with GBM, particularly those with an unmethylated MGMT promoter. Immunotherapies have come to the forefront of current research, as they elegantly harness the body's natural defense mechanisms to target tumors. We have developed a novel peptide vaccination strategy using the tetanus toxoid derived universal class II-binding P30 epitope linked to HLA-A*0201-resticted class I binding tumor-associated antigens (TAAs) from EphA2, CMV pp65, and survivin [7]. The novel combination of P30-linked EphA2, pp65, and survivin is a single vaccine (collectively called P30-EPS) targeted against antigens commonly expressed by GBM and provides a promising new therapeutic strategy for patients with a new GBM diagnosis.

5.3 Design and Procedure

A maximum of 36 patients with a newly diagnosed, previously untreated WHO grade 4 malignant glioma that is IDH wildtype, MGMT promoter unmethylated will be treated in this study after undergoing standard of care surgical resection followed by 3-6 weeks of radiation therapy (XRT) with or without concomitant TMZ and providing informed consent. Patients will consent to this study after completion of XRT/TMZ, as long as they will be able to start treatment within 8 weeks of the end of XRT/TMZ (and as long as this is felt to be in the best interest of the patient by the treating physician). Patients will receive seven P30-EPS peptide vaccines over two phases. The first 5 vaccines will be given during the Priming Phase (day 1 through day 22). The final 2 vaccines will be given during the Booster Phase (day 84 [± 7 days] and day 140 [± 7 days]). During the Booster Phase from Day 84 onwards, patients will also be given 20 μg/kg Hiltonol® injections to be administered at home every 2 weeks until they return to the clinic on Day 140. Blood will be drawn throughout the study to investigate the immune response to P30-EPS vaccination. We will also request archival tumor tissue from patients' baseline surgical resection or prior biopsy, as well as tumor tissue from future surgeries, but only in the case that the patients may undergo other clinically indicated biopsy or tumor resection while participating in this study. Available tumor tissue will be analyzed by correlative analyses as outlined in Section 9.3.

5.4 Selection of Subjects

All inclusion/exclusion criteria may be found in Section 11.

5.5 Data Analysis and Statistical Considerations

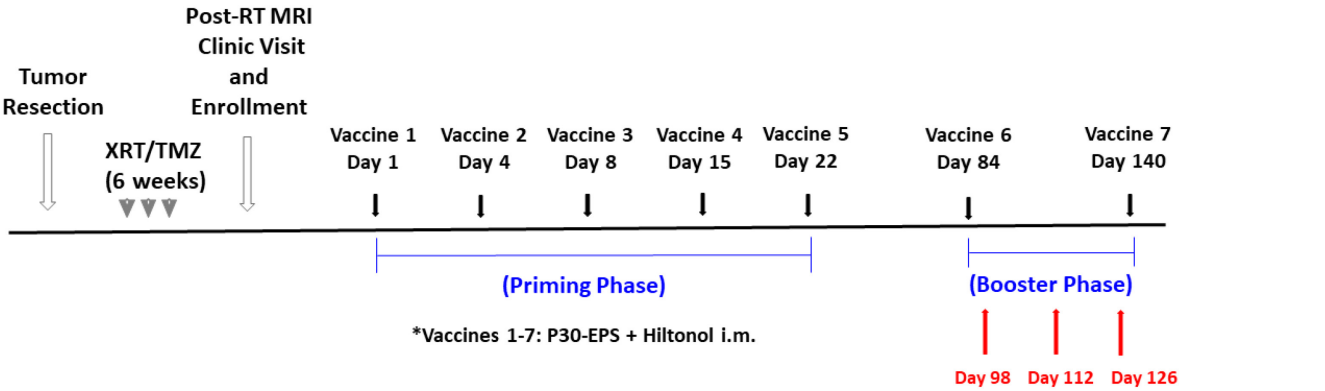
The study will enroll two patient strata: patients who are CMV seronegative and patients who are CMV seropositive. Within each stratum, cohorts of 3 patients will initially be accrued to the study to assess the toxicity associated with the P30-EPS vaccine. The starting dose of P30-EPS vaccine is 300 µg/peptide/dose, and the dose will be escalated to 400 µg/peptide/dose. In the event of unacceptable dose-limiting toxicity (DLT), the dose will be de-escalated to 200 µg/peptide/dose (dose level minus one). Acute toxicity monitoring will focus on DLTs, as defined in Section 9.1.2, occurring between vaccine #1 and 30 days after vaccine #5. Details are provided in Section 15.5.1.

Additional monitoring rules that focus on the full treatment period are provided in Section 15.5.2. Accrual will be suspended if these monitoring thresholds are surpassed. During suspension, data will be carefully reviewed to determine if accrual to a stratum should be continued or terminated, and whether elements of the treatment regimen should be modified.

Among CMV seropositive patients treated at the maximum tolerated dose (MTD) for CMV seropositive patients, a one-sample t-test will assess the mean fold increase in pp65-specific T cells between days 1 and 22, days 1 and 84 and days 84 and 140. Among CMV seronegative patients treated at their MTD, the proportion of patients with a pp65-specific response will be estimated between days 1 and 22, days 1 and 84 and days 84 and 140. A one-sample t-test will assess the mean fold increase in EphA2-, and survivin-specific T cells between days 1 and day 22, days 1 and 84 and days 84 and 140. If underlying assumptions for proposed parametric analyses are violated, alternative methods will be considered. The fold-change between day 1 and day 84 (i.e. overall fold-change due to the priming vaccination) will also be examined, as well as the fold-change between day 84 and day 140 that assesses the effect of the booster vaccination.

A Kaplan-Meier estimator will describe the survival and PFS of patients within the two CMV strata.

6 STUDY SCHEMA



7 BACKGROUND AND SIGNIFICANCE

7.1 Study Disease

Malignant primary brain tumors are more common than Hodgkin's disease and account for more human deaths than melanoma or cancer of the bladder or kidney. GBM is the most common malignant primary brain tumor and remains universally fatal with a median overall survival (OS) of less than 21 months despite aggressive, computer-guided tumor resection [8], high-dose external beam XRT or brachytherapy, and chemotherapy [9-12]. Patients who have GBM with an unmethylated MGMT promoter status have a worse prognosis, but they also do not derive a meaningful benefit from the addition of TMZ to radiation therapy [5],[6]. New therapeutic strategies for GBM patients with an unmethylated MGMT promoter is a recognized area of unmet need. Immunotherapy offers a promising opportunity for specific tumor targeted treatment for GBM patients.

7.2 Study Agents

TMZ, the most widely used alkylating agent in GBM, is cytotoxic to cells by inducing DNA damage that can be rapidly repaired by the MGMT protein [13, 14]. In a subset of GBMs, the MGMT promoter is methylated, undermining the repair mechanism and conferring chemosensitivity[15]. However, MGMT is overexpressed in 60% of GBMs providing an inherent resistance to alkylating agents like TMZ [16]. In fact, GBMs with an unmethylated MGMT promoter (i.e. GBMs that overexpress MGMT) do not show increased survival when treated with radiation and TMZ as compared with those treated with radiation alone [5]. Therefore, novel therapies are particularly needed for this TMZ-resistant population.

Peptide vaccines encoding minimal $CD8^+$ cytotoxic T-lymphocyte (CTL) epitopes have been demonstrated in many contexts to induce protective immunologic responses in experimental animals and mediate regression of established tumors [17, 18]. Tumor-associated antigens (TAAs) (P30-EPSs) offer a vast repertoire of tumor targets for cancer immunotherapy. In particular, peptide TAA vaccines derived from major histocompatibility complex (MHC) class I-restricted tumor antigens are a promising strategy for inducing potent tumor-targeted CD8+ CTL responses [19-21], but the efficacy of MHC class I-restricted peptide vaccines has yet to be realized in cancer immunotherapy [22-25]. This is likely due in part to class I focused vaccines providing insufficient $CD4^+$ T cell help for the creation of strong $CD8^+$ CTL responses. Many studies have highlighted that class II-binding helper $CD4^+$ T cells function at both the priming and effector stages of immunity to increase the magnitude and functionality of CTL responses [25]. At priming, antigen-specific $CD4^+$ T cells activate dendritic cells (DCs) through CD40:CD40 ligand interactions, causing increased antigen presentation as well as the chemokine and cytokine secretion necessary to recruit and activate T cells. Therefore, in the context of vaccination, providing $CD4^+$ T cell help through class II restricted antigens is vital for recruiting, activating, and expanding vaccine-induced $CD8^+$ CTLs.

In order to provide this necessary $CD4^+$ T cell help at priming, this trial will link the tetanus derived universal class II-binding P30 epitope to minimal HLA-A*0201-resticted class I binding TAA peptides. P30 ($TT_{947-967}$) has an extensive safety profile as it is a routine part of childhood vaccination. P30 is known to promiscuously bind human class II molecules and is a well-established $CD4^+$ T cell helper epitope that boosts the immunogenicity of vaccination [26]. In this study, minimal class I epitopes restricted to HLA-A*0201 from three TAAs will be used- Ephrin type-A receptor 2 or EphA2 (TLADFDPRV) [27], *Cytomegalovirus* (CMV) pp65 (NLVPMVATV) [28, 29], and the survivin epitope (ELTLGEFLKL) [30]. Each of these peptides will be individually linked with a furin cleavage site (RVKR) to the P30 sequence (FNNFTVSFWLRVPKVSASHLE).

P30-EphA2 is a 34 amino acid synthetic peptide with the following sequence:
TLADFDPRVRVKRFNNFTVSFWLRVPKVSASHLE

P30-pp65 is a 34 amino acid peptide with the following sequence:
NLVPMVATVRVKRFNNFTVSFWLRVPKVSASHLE

P30-Survivin is a 35 amino acid peptide with the following sequence:
ELTLGEFLKLRVKRFNNFTVSFWLRVPKVSASHLE

Peptides will be mixed with carboxymethylcellulose, polyinosinic-polycytidylic acid (poly I:C), and poly-L-lysine double-stranded RNA (poly-ICLC) prior to injection, as has been safely used in other peptide vaccine trials in patients with glioma [27, 31]. Poly I:C and its clinical equivalent poly-ICLC (Hiltonol®), are TLR3 agonists responsible for inducing expression of inflammatory cytokines including IFNα, TNFα, and IL-6, which potentiates both antigen presentation by DCs and $CD8^+$ T cell responses [32]. Peptides will be produced by BaChem (Torrence, CA), which routinely produces peptides of this length with 95% purity under Good Manufacturing Practice (GMP) conditions (BaChem, Personal Communication). Peptides will be pooled for administration.

7.2.1 Pre-clinical Experience

P30-Linked Vaccination

Successful immunotherapy for GBM may require the generation of potent tumor-targeted CTLs. In the course of trying to generate such responses in preclinical models, we discovered that class I antigens located proximal to immunogenic class II antigens were more likely to generate CTLs. This is similar to many anti-viral $CD8^+$ T cell responses, in which a proximal class II epitope provides $CD4^+$ T cell help at priming to increase the magnitude of the $CD8^+$ T cell response [33]. This led us to determine that artificially positioning a natural class II immunogenic antigen near a minimal class I antigen in the context of a vaccine could significantly enhance the immune response to the class I antigen. We then demonstrated that the universal class II epitope, P30, could be substituted for natural class II antigens (Figure 1) and be used to increase both the number of immunogenic antigens and the potency of the tumor-specific $CD8^+$ T cell response (Figure 2) [34]. We also demonstrated P30-linked vaccination engenders $CD8^+$ antigen-specific CTLs dependent on $CD4^+$ T cell help mediated through CD40 ligand (CD40L). CD40L binds to DCs which upregulate CD70 to stimulate CD27 on both $CD4^+$ and $CD8^+$ T cells. For $CD8^+$ T cells, this results in increased survival, proliferation, function, migration, memory formation, and expansion upon restimulation. We show the class I epitope and P30 must be physically linked (Figure 1) and that $CD4^+$ T cells are required at priming as CD40L blockade during vaccination prevents efficacy. Finally, we showed that with P30 linkage to a class I epitope that we can even convert seemingly non-immunogenic class I epitopes (Topbp1) to epitopes that induce antitumor efficacy (Figure 2).

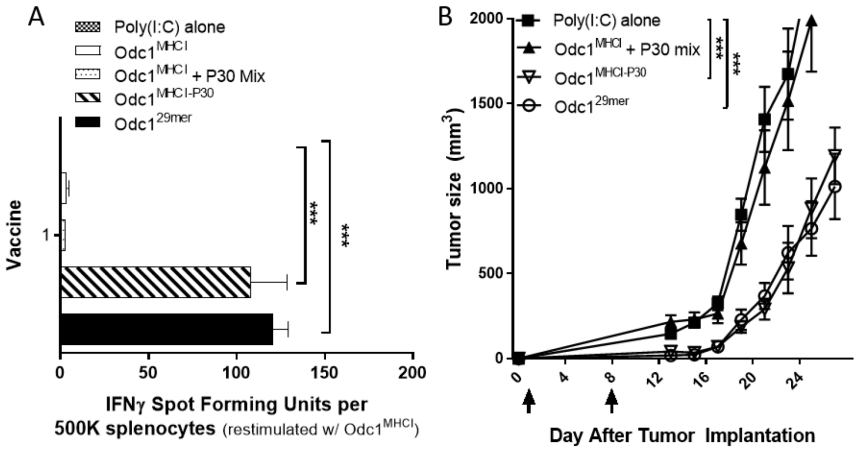


Figure 1. Class I Odc1 linked to class II P30 has equivalent immunogenicity (A) and efficacy (B) to its endogenous class II epitope.

A) Mice were immunized with Poly(I:C) alone, the minimal Odc1 class I epitope ($Odc1^{MHCI}$), $Odc1^{MHCI}$ mixed with the P30 helper epitope, $Odc1^{MHCI}$ linked to P30 ($Odc1^{MHCI-P30}$), or the Odc1 29mer long peptide (n = 3). The splenocyte response to $Odc1^{MHCI}$ was evaluated 7 days later by IFNγ ELISpot. One-way ANOVA with post-hoc Tukey's test *** $p<0.05$. (B) Subcutaneous SMA-560 tumor growth in mice following therapeutic immunization on days 1 and 8 with Poly(I:C) alone, $Odc1^{MHCI}$ mixed with P30, $Odc1^{MHCI-P30}$, or the Odc1 29mer long peptide (n = 7).

Pp65 Targeted Vaccination

We have previously demonstrated that a peptide vaccine targeting the tumor-specific epidermal growth factor receptor mutation, EGFRvIII, could induce specific immune responses in mice and humans [35-39] and was sufficient to consistently eliminate all EGFRvIII-expressing tumors cells without toxicity [35, 37, 40]. Unfortunately, EGFRvIII is heterogeneously expressed, and tumors recur as a result of outgrowth of the EGFRvIII negative tumor cells [35]. Targeting CMV antigens overcomes this shortcoming, as the nearly universal presence and homogeneous expression of CMV antigens in GBM, but not normal brain, is now well-established [41-49].

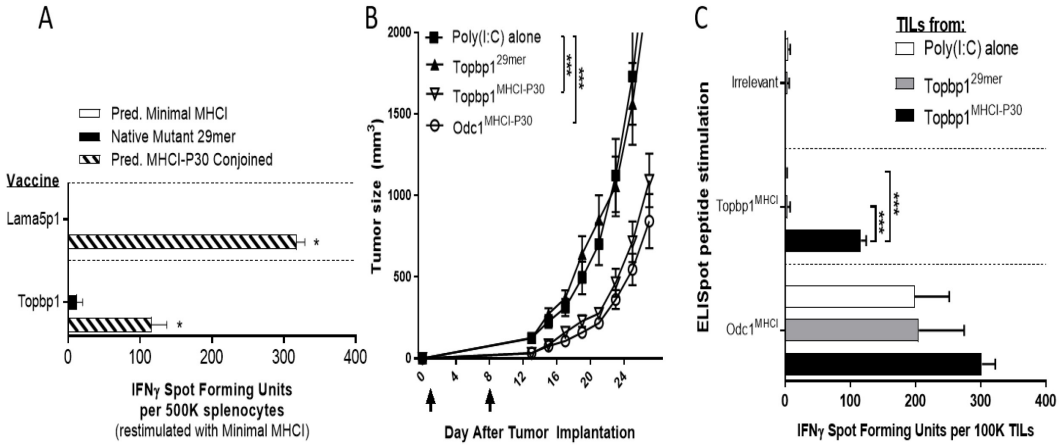


Figure 2. Linking P30 to the Lama5p1 and Topbp1 class I neoantigen epitopes increases CD8+ T cell potency (A), induces de novo efficacy with Topbp1 (B), and preserves responses to other neoantigens (C).

(A) Mice were immunized with the predicted MHCI-restricted neoantigen alone (lama5 or Topbp1), the predicted MHCI-restricted neoantigen linked to P30, or the 29mer long endogenous peptide (Native 29mer). 7 days later splenocytes were tested against the minimal class I epitope by IFNγ ELISpot (n = 3). One-way ANOVA with post-hoc Tukey's test. (B) Subcutaneous SMA-560 tumor growth in mice following vaccination on days 1 and 8 with Poly(I:C), Topbp1^{29mer}, the minimal Topbp1 class I epitope linked to P30 (Topbp1$^{MHC\ I\text{-}P30}$), or Odc1^{MHCI} linked to P30 (Odc1$^{MHCI\text{-}P30}$) (n = 7). (C) Odc1^{MHCI} and Topbp1^{MHCI}-reactive TILs from Poly(I:C), Topbp1^{29mer}, or Topbp1$^{MHCI\text{-}P30}$ vaccinated mice were assessed in day 27 subcutaneous SMA560 tumor by IFNγ ELISpot (n = 5-6). Background subtracted, One-way ANOVA with post-hoc Tukey's test.

Importantly, published data from our group demonstrate that a CMV pp65-directed approach can recognize and lyse GBM cells. Human T cells stimulated by DCs loaded with immunodominant CMV pp65 antigen, specifically lysed autologous tumor cells. This demonstrated that GBM expresses pp65 antigen that can be recognized and killed by pp65-specific CTLs (Figure 3) [50]. Though targeted through a different mechanism (DCs rather than peptides), this data indicates that CMV-specific patient effector T cells can functionally recognize autologous GBM-expressing endogenous CMV antigens.

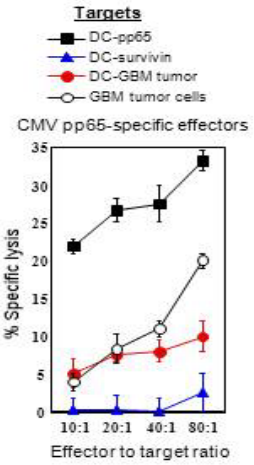


Figure 3. CMV pp65 RNA loaded DCs isolated from patients with GBM were used to stimulate autologous peripheral blood mononuclear cells (PBMC) and cells were analyzed in a standard europium release assay.

Autologous RNA loaded patient DCs or autologous GBM tumor cells were used as targets. Survivin loaded DCs were utilized as negative controls and tumor-derived RNA loaded DCs were assessed as surrogate targets.

Hiltonol® and PolyICLC Formulations

We have preclinical experience with Hiltonol and PolyICLC. Experiments on the immunogenicity potential of Hiltonol versus Poly-ICLC adjuvant with vaccination against the melanoma Tyrosinase-related protein-2 (TRP-2) antigen show that Hiltonol or PolyICLC result in increased IFN-γ response to TRP-2 over adjuvant alone (Figure 4).

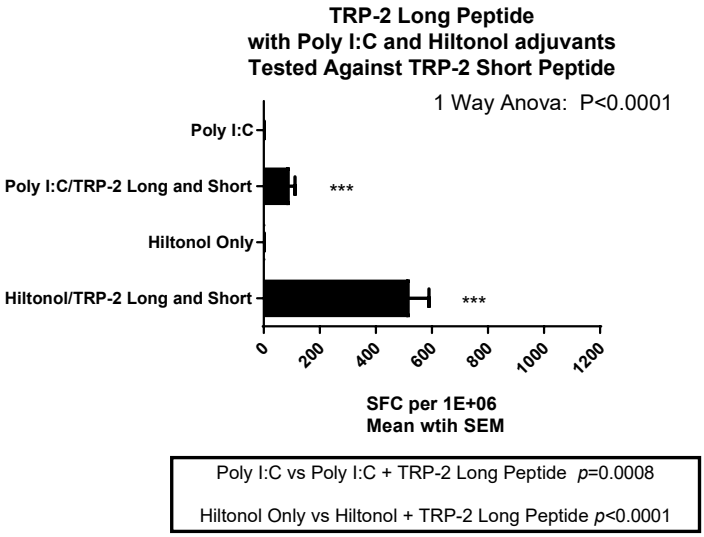


Figure 4. Hiltonol and PolyICLC adjuvants enhance IFN-γ responses to candidate melanoma antigen TRP-2.

7.2.2 Clinical Experience

Due to routine childhood vaccination with tetanus toxoid, universal class II binding peptides from tetanus toxoid (P30 and P2 epitopes) have a well-established safety record and pre-existing immunity to the P2 and P30 epitopes is highly likely in all patients. The tetanus toxoid-derived P2 and P30 class II helper epitopes have long history of being used as $CD4^+$ helper epitopes and have been combined with other antigens as separate peptides [51] and as a fusion peptides in clinical trials [26, 52].

pp65

Our group has 10 years of experience in vaccinating subjects with CMV pp65-LAMP loaded DCs. While the type of vaccination is different, our experience with CMV pp65-LAMP loaded DC vaccines is pertinent to this protocol. In the ATTAC trial (NCT00639639), patients were randomized to receive CMV pp65-LAMP DC vaccination following tetanus toxoid or unpulsed DC pre- conditioning at the vaccine site. In the ATTAC GM trial, patients were vaccinated with CMV pp65-LAMP DCs mixed with GM-CSF (Granulocyte macrophage colony-stimulating factor) [53]. In the ERADICATE study (NCT00693095), patients received CMV pp65-LAMP DCs and CMV-specific adoptive lymphocyte transfer [54]. In the REGULATE study (NCT00626483), patients received CMV pp65-LAMP DCs in combination with anti-CD25 monoclonal antibody (mAb) treatment (Basiliximab) to remove regulatory T cells (T_{Regs}). In the completed ELEVATE study under IND 16,301 (NCT02366728), subjects were treated with CMV pp65-LAMP DCs in combination with TMZ and either tetanus diptheria toxoid (Td) or unpulsed DC pre-conditioning. In the completed AVERT study under IND 16,301 (NCT02529072), patients received CMV pp65-LAMP DCs in combination with the anti-PD-1 mAb Nivolumab. The ongoing phase 2 “ATTAC II” trial (NCT02465268), a multi-site trial at the University of Florida and Duke, is investigating whether the addition of pp65-LAMP DC vaccines plus GM-CSF and Td to dose-intensified TMZ treatment is worthy of investigation in a larger phase 3 trial. In aggregate, we have given CMV pp65-LAMP DC vaccines to over 115 patients in combination with a number of agents. The CMV pp65-LAMP DCs have been well tolerated with no serious or off-target toxicities to date.

In addition to DC vaccination, vaccination targeting pp65 has also been given as live-attenuated virus, protein, DNA, and peptide vaccines [55]. The pp65 epitope in this trial ($pp65_{495-503}$) is an immune dominant HLA-A*0201-restricted epitope that is nine amino acids long (NLVPMVATV) and has been included in clinical trials as a minimal peptide [56] and within additional longer CMV sequences [55]. Our group has administered a 26 amino acid long CMV pp65 peptide vaccine (PEP-CMV) that includes the NLVPMVATV epitope (not tethered to P30) in two separate trials that are currently ongoing – PERFORMANCE and PRiME. The PERFORMANCE study (NCT02864368) was designed to assess the safety of the long CMV pp65 vaccine in combination with TMZ in patients with newly diagnosed GBM. The PRiME study (NCT03299309) deploys the same CMV pp65 peptide as PERFORMANCE in the recurrent disease setting for both children and young adults with recurrent malignant glioma or medulloblastoma. Combined, we have treated 31 patients with long CMV pp65 peptide vaccination here at Duke.

During an interim review of safety events in PERFORMANCE in November 2019, the following Grade 3 unacceptable toxicities (as defined in protocol v. 7/30/2019) were noted in 4 out of 15 patients:

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

Patient ID 1163 hypotension

We analyzed immune responses in 16 patients in PERFORMANCE to date (January 10, 2020). We believe that the observed toxicities in this study are related to cytokine release syndrome (CRS). The immune responses across 4 degrees of reactions (ranging from no reactions to Grade 3 reactions) demonstrate a significant difference between levels of cytokines (G-CSF, GM-CSF, IFN-γ, IL-10, IL-2, IL-8, MIP1-α, and TNF-α) two hours after vaccine, as compared to those levels pre-vaccination, with IL-6 levels also nearing significance. For these cytokines, subsequent pairwise comparisons included comparisons involving those experiencing Grade 3 adverse events (AEs) after Tukey adjustment.

These data suggest that the AE reactions following PEP-CMV vaccination are indeed immune-related. The cumulative data indicate that PEP-CMV vaccination is inducing the activation of T cells specific for the target antigen pp65. Furthermore, in patients with AEs, the pp65-specific T cell activation may be inducing a temporary elevation of pro-inflammatory cytokines. We believe that the vaccine reactions we have been observing in PERFORMANCE are likely indicative of vaccine potency. Patients who have demonstrable Grade 3 reactions also have measurable cellular responses to the PEP-CMV vaccines, which may be indicative of clinical responses, and can be easily managed through standard premedication with Tylenol, Benadryl, or Zofran as has been used in other trials with similar immune-related AEs. The reactions typically occurred 2-4 hours after vaccine administration, and most patients were generally discharged the same day or the following day with a few patients who were discharged 2-4 days post reaction. The most significant reactions in some patients included grade 3 hypotension, grade 1 tachycardia, grade 3 lactic acidosis, and grade 3 flu-like symptoms with fever (up to 39°C).

It is important to note that the T cell responses in PERFORMANCE may be solely mediated by pp65-specific $CD4^+$ T cells activated by class II epitopes found in the long pp65 vaccine. These class II epitopes are not present in the pp65-P30 linked vaccine that contains only the class I pp65 HLA-A*0201 restricted NLVPMVATV epitope. Therefore, the pp65-P30 peptide has important differences from the long pp65 peptide used in PERFORMANCE.

A peptide very similar to what we are proposing was used in two phase 1b clinical trials and contained the NLVPMVATV epitope linked to a different universal class II binding sequence from the P2 epitope of tetanus toxoid ($TT_{830-843}$ QYIKANSKFIGITE) and included adaptor (KSS) and linker (AAA) sequences (KSSQYIKANSKFIGITEAAANLVPMVATV). When given with the adjuvant PF03512676 in both CMV seropositive and seronegative HLA-A*0201 positive healthy volunteers, this peptide had an acceptable safety profile and induced the expansion of CMV-specific T cells [57]. Volunteers received escalating doses of peptide with most AEs being Grade 1 or 2 including injection site reactions or systemic mild flu-like symptoms, which resolved within 1 to 2 days. One patient (7.7%) experienced a Grade 3 AE including malaise, fever and urticaria, which

resolved with non-prescription analgesics within 8 days. When given with the adjuvant PF03512676 in 18 CMV seropositive HLA-A*0201 positive patients undergoing hematopoietic stem cell transplantation, this peptide was safe, expanded CMV-specific T cells, and only one patient experienced an AE related to vaccination (Grade 1 fever) [58]. It is expected that the vaccine will be even safer in the HLA-A*0201 subjects who will be included in this trial.

EphA2

The $EphA2_{883–891}$ (TLADFDPRV) epitope as a separate peptide has been used in several trials in patients with glioma [27, 31]. In one trial, over 70% of the HLA-A*0201 positive patients demonstrated EphA2-specific T cells after vaccination [27]. In this prior study, a synthetic peptide representing the same $EphA2_{883–891}$ epitope was given with other peptides at a dose of 300 µg/peptide/dose via subcutaneous vaccinations in combination with 20 µg/kg intramuscular (i.m.) injections of poly-ICLC to HLA-$A2^+$ adults with low grade glioma [27]. AEs included grade 1 and 2 injection site reactions (100%) and flu-like symptoms (fatigue, myalgias, fever, headache), which were usually limited to 48 hours after each vaccine and were controlled with acetaminophen or ibuprofen. Three patients developed grade 1 leukopenia and one patient developed a grade 3 fever after the seventh vaccine that resolved with a nonsteroidal anti-inflammatory drug the next day.

The same research group also gave the $EphA2_{883–891}$ epitope as part of a similar TAA multipeptide vaccine to HLA-A*0201 positive pediatric patients with newly diagnosed malignant brainstem and non-brainstem glioma [59]. Twenty-six patients were enrolled to receive the TAA vaccine that included the P2 epitope ($TT_{830–845}$) in Montanide ISA-51, in combination with 30 µg/kg i.m. injections of Hiltonol®. Vaccines were given three weeks apart for eight courses of vaccination followed with booster vaccination every six weeks. Toxicities were mild and included injection site reactions (100%), flu-like symptoms (92%), Grade 1 GI symptoms (31%), Grade 1 leukopenia (15.4%), and no Grade 3 or higher toxicities. Eleven children showed cellular immune responses to the EphA2 epitope after vaccination.

Survivin

The TAA survivin has been shown to be overexpressed in a variety of human cancers including malignant glioma [60]. In patients with breast cancer, melanoma and chronic lymphocytic leukemia, T cell responses to the class-I HLA-A*0201 restricted survivin epitope that we will use ($Survivin_{95-104}$ ELTLGEFLKL) have been detected [61]. These data demonstrate that endogenous immunity to the $survivin_{95-104}$ epitope spontaneously develop in patients with cancer. Furthermore, the $survivin_{95-104}$ epitope has been shown to expand T cells from HLA-A*0201 healthy donors [61].

A highly similar artificial epitope ($survivin_{96-104:M2}$ LMLGEFLKL) was given along with the $EphA2_{883–891}$ epitope in the same study published by Okada *et al* described above. In this study $survivin_{96-104:M2}$ LMLGEFLKL was given with other TAA peptides (including $EphA2_{883–891}$) at a dose of 300 µg/peptide/dose via subcutaneous

vaccinations in Montanide ISA-51, in combination with 20 µg/kg i.m. injections of Hiltonol® to HLA-A2^{+} adults with low grade glioma [27]. In comparison to survivin$_{95-104}$, survivin$_{96-104:M2}$ is one amino acid shorter and was created to include a modified second amino acid (T to M) to further increase binding strength and T cell activation. In this multi-peptide vaccine, the tetanus derived P2$_{830-845}$ epitope was also included as a separate class II-binding helper peptide. Immune responses to survivin were detectable and AEs included grade 1 and 2 injection site reactions (100%) and flu-like symptoms (fatigue, myalgias, fever, headache), which were usually limited to 48 hours after each vaccine and were controlled with acetaminophen or ibuprofen. Three patients developed Grade 1 leukopenia and one patient developed a Grade 3 fever after the seventh vaccine that was resolved with a nonsteroidal anti-inflammatory drug the next day. This study indicates that a tetanus-derived helper peptide when given separately with a modified Survivin$_{95-104}$ (survivin$_{96-104:M2}$) I epitope was safe and could generate survivin-specific T cells. As outlined above for EphA2, the same research group also gave the modified survivin$_{96-104:M2}$ peptide as part of a similar TAA multipeptide vaccine to HLA-A*0201 positive pediatric patients with newly diagnosed malignant brainstem and non-brainstem glioma [59]. No Grade 3 or higher toxicities were observed and immune responses to survivin were detected in 3 of 21 children.

7.3 Study Purpose/Rationale

This safety study will also be a proof of concept trial design. Patient numbers can be increased if replacements are needed.

The primary objective for the trial described here is to evaluate the safety profile of a cancer vaccine comprised of P30-linked EphA2, CMV pp65, and survivin peptides, and secondarily to assess if P30-linked vaccination increases the number of P30-EPS-specific polyfunctional T cells.

8 OBJECTIVES AND ENDPOINTS

	Objective	Endpoint	Analysis
Primary	Evaluate the safety profile of a cancer vaccine comprised of P30-linked CMV pp65, EphA2 and survivin peptides	Percent of patients who experience DLT within each stratum at each dose level	See Section 15.5
Secondary	Assess whether pp65-specific polyfunctional T cells increase after priming vaccination with the P30-linked pp65 peptide.	Among CMV seropositive patients, mean fold increase in pp65-specific T cells between day 1 and day 22 in patients, and day 1 and 84. Among CMV seronegative patients, percent of patients who develop pp65-specific polyfunc-tional T cells between day 1 and 22, and day 1 and 84.	See Section 15.6.1
Secondary	Assess whether EphA2- or survivin-specific polyfunctional T cells increase after priming vaccination with P30-linked EphA2 or survivin peptides.	Among all patients, mean fold increase in EphA2- or survivin-specific T cells between day 1 and day 22, and day 1 and 84	See Section 15.6.2
Secondary	Assess whether pp65-specific polyfunctional T cells increase after booster vaccination with the P30-linked pp65 peptide.	Among CMV seropositive patients, mean fold increase in pp65-specific T cells between day 84 and day 140. Among CMV seronegative patients, percent of patients who develop pp65-specific polyfunc-tional T cells between day 84 and day 140.	See Section 15.6.3
Secondary	Assess whether EphA2- or survivin-specific polyfunctional T cells increase after booster vaccination with P30-linked EphA2 or survivin peptides.	Among all patients, mean fold increase in EphA2- or survivin-specific T cells between day 1 and day 84 and 140.	See Section 15.6.4

	Objective	Endpoint	Analysis
Other Secondary	Describe the survival and PFS of patients who received a vaccine comprised of P30-linked pp65, EphA2 and survivin peptides.	Median survival Median PFS	See Section 15.6.5
Exploratory	Assess the diversity of the P30-EPS-specific memory T cell repertoire via TCR sequencing.	Median change in normalized entropy between baseline (pre-vaccine #1) and after vaccine #5. Entropy is a measure of clonal expansion, or the extent of TCR repertoire expansion.	See Section 15.7.1
Exploratory	Define the relationship between reported biomarkers of response to immunotherapy in GBM and survival.	Association between the following: tumor mutational burden (TMB), tumor-intrinsic Erk1/2 signaling; TMEM119+ (microglia) cell density; peripheral Ki67+ CD8 T cells; and gene expression signatures of MHC-class II, IFN-γ, and cell cycle/proliferation.	See Section 15.7.2
Exploratory	Assess resected tumor for the expression of vaccine targets (i.e. EphA2, pp65, and survivin).	Association between the vaccine targets (EphA2, pp65, and survivin) and clinical outcomes	See Section 15.7.3
Exploratory	Assess changes in the tumor immune microenvironment, mutational heterogeneity/clonality, neoantigen depletion, and their association with survival.	Association between putative neoantigen depletion, mutational heterogeneity, gene expression signatures of T cell inflammation, and their relationship with survival.	See Section 15.7.4

9 INVESTIGATIONAL PLAN

9.1 Study Design

The proposed study is designed to assess the safety of administering a cocktail of three TAA peptides (P30-EPS) mixed with Hiltonol® in patients with newly diagnosed WHO grade 4 malignant glioma. In addition to assessing safety, blood draws will be performed at baseline and throughout the study to investigate how the immune system changes in response to P30-EPS peptide vaccination. We will also request archival tumor tissue from patients' baseline surgical resection or prior biopsy, as well as tumor

tissue from future surgeries, but only in the case that the patients may undergo other clinically indicated biopsy or tumor resection while participating in this study. Available tumor tissue will be analyzed by correlative analyses as outlined in Section 9.3.

A maximum of 36 patients will be accrued to the study after providing informed consent. Patients will consent to this study after completion of XRT/TMZ, as long as they will be able to start treatment within eight weeks of the end of XRT/TMZ (and as long as this is felt to be in the best interest of the patient by the treating physician). After providing informed consent, patients will undergo screening to confirm study eligibility. Both CMV seropositive and seronegative patients will be allowed on study. Because CMV seropositive patients have previously been infected with CMV, they will have pp65-specific memory T cells. CMV seronegative patients will only have naïve pp65-specific T cells. We will evaluate the impact of vaccination on both pp65-specific memory or pp65-specific naïve T cell expansion.

Regardless of when they enroll, all patients will undergo the same vaccination schedule. Dose escalation will be performed separately among CMV seropositive and seronegative patients. Within each of these strata, vaccines will initially contain 300 μg of each peptide (P30-linked EphA2, pp65, and survivin peptides) pooled in 10% dimethyl sulfoxide (DMSO) in water and mixed with 20 μg/kg of Hiltonol®. Within each stratum, the first 3 patients will be treated with 300 μg/peptide/dose. The enrollment of the first 2 patients treated with 300 μg/peptide/dose within each CMV stratum will be staggered by 2 weeks. Dose escalation to 400 μg of each peptide is planned; however, there are contingencies to reduce the dose to 200 μg of each peptide if 300 μg is not safe. If further dose reduction is needed, the pp65 peptide will be removed from the vaccine combination (See Section 15.5.1.2). Patients will undergo standard of care XRT with or without concomitant TMZ for 3-6 weeks, before beginning vaccination. All P30-EPS vaccines are given initially in a Priming Phase (on days 1, 4 (± 1 day), 8 (± 1 day), 15 (± 2 days), and 22 (± 2 days)), and then in a Booster Phase (on day 84 and day 140, both ± 7 days). During the Booster Phase on Day 84, patients will also be given 20 μg/kg Hiltonol® injections to administer at home every 2 weeks (± 2 days) until they return on Day 140. Patients will be contacted and/or sent a reminder prior to each injection at home to help ensure that these doses are not missed. Further details are available in Section 12.2.

Patients with evidence of radiographic progression according to Response Assessment in Neuro-Oncology (RANO) 2.0 criteria after radiation will be allowed to enroll on study as long as there is no evidence of new disease outside of the radiation field. Patients should continue to receive vaccines unless they experience unacceptable toxicity, decide not to continue receiving vaccines, or have disease progression.

For patients who require treatment with corticosteroids > 4 mg per day of dexamethasone (or equivalent) for mitigation of inflammatory response while they are on study, it will be recommended that patients initiate bevacizumab (7.5 mg/kg IV every 3 weeks) instead of increasing the steroid dose, if possible, as steroids may

interfere with the desired immune response to the investigational vaccine. It is strongly recommended that patients should not be receiving bevacizumab prior to vaccine 1. If patients are started on the radiation necrosis dose of bevacizumab for the purpose of managing intracranial inflammatory responses, the vaccine/return to clinic schedule for this study will not change. The bevacizumab schedule will be independent of the vaccine schedule, since it will be provided clinically, and will be managed by the oncology office administering this medication.

Baseline bloodwork for subclinical manifestations of autoimmunity as outlined in Section 12.6.5 will be taken on all subjects prior to vaccine #1 and then only repeated if clinically indicated. Bloodwork for immunological monitoring will be taken at baseline, at vaccinations 1, 5, 6, and 7 before the vaccine is given (days 1, 22, 84, and 140). The total amount of blood required for this purpose will be about 90 mL. Peripheral blood for immunologic immune monitoring will be delivered to the dedicated lab for processing. Further details are available in the ETAPA correlative laboratory manual.

Magnetic resonance (MR) images will be obtained after completing XRT/TMZ for baseline measurements and to assess progression. Patients will then be imaged at visits prior to receiving vaccines #5, #6, and #7. The RANO 2.0 criteria will be used for assessment of response and pseudoprogression following treatment [62].

9.1.1 Safety evaluations

Treatment safety will be monitored separately according to guidelines described in Section 15.5.2, and using the definitions of unacceptable and DLT provided in Section 9.1.2.

9.1.2 Toxicity Definitions

Toxicities will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5 criteria.

9.1.2.1 Definition of Unacceptable Toxicity

Any ≥ Grade 3 AEs will be considered as unacceptable toxicities if they are possibly, probably, or definitely attributable to the protocol treatment regimen, and they occur within 30 days of vaccination. Specifically, any ≥ Grade 3 organ toxicity (cardiac, renal, hepatic), including CRS-related toxicities such as hypotension and tachycardia, of any duration will be considered an unacceptable toxicity. A Grade 3 neurologic toxicity will only be declared an unacceptable toxicity if not reversible within 48 hours. Grade 3 flu-like symptoms, fever, and chills/rigors are not considered unacceptable if the duration is less than 72 hours.

Exceptions based on the disease population are as follows, with or without treatment:

- Seizures: Due to the nature of the disease under investigation in this protocol, patients may have preexisting seizures or be susceptible to new seizures as a result of the underlying disease process. Although seizures may be defined as grade 3 or 4 toxicities under NCI CTCAE, and will be reported as such in this protocol, seizures will not be considered a DLT if, in the opinion of the PI, they

have not increased in frequency or can be attributed to another recognized cause of increasing seizure frequency such as sub-therapeutic anticonvulsant levels or biopsy-proven tumor progression.

- New neurologic deficits: Due to the nature of the disease under investigation in this protocol, patients may develop new neurologic deficits as a result of tumor invasion or inflammation. A new neurologic deficit or worsening of a known neurologic deficit, which resolves (i.e. returns to baseline) within 3 weeks after initiation of medical therapy (e.g. corticosteroids) will not be considered an unacceptable toxicity.
- Thromboembolism: Due to the high incidence of deep vein thrombosis (DVT) in this patient population, patients may have undiagnosed preexisting DVTs or be susceptible to the development of DVTs due to the underlying disease process. Although DVT may be defined as a grade 3 or 4 toxicity under NCI CTCAE, and will be reported as such in this protocol, DVT will not be considered unacceptable in this protocol.
- Syndrome of inappropriate antidiuretic hormone (SIADH): Due to the high incidence of SIADH in this patient population, patients may be susceptible to the development of SIADH due to the underlying disease process. Although SIADH may be defined as grade 3 toxicity under NCI CTCAE, and will be reported as such in this protocol, SIADH will not be considered an unacceptable toxicity in this protocol unless it is refractory to medical management.
- Muscle weakness and weight gain: Due to the high incidence of generalized muscle weakness (i.e. steroid myopathy) and weight gain in patients taking steroids in this patient population, patients may be susceptible to the development of generalized muscle weakness or weight gain, which is due to steroids alone. Although generalized muscle weakness may be defined as grade 3 or grade 4 toxicity and weight gain ≥ 20% may be defined as grade 3 toxicity under NCI CTCAE, and will be reported as such in this protocol, generalized muscle weakness or weight gain will not be considered an unacceptable toxicity in this protocol if the patient has required steroids greater than the physiologic doses in the interval between the immunization and the development of the toxicity.
- Tumor progression: Due to the nature of the disease under investigation in this protocol, patients may have an increase in preexisting neurologic deficits or have an onset of new neurologic deficits due to tumor progression. Although such neurologic deficits may be defined as AEs under NCI CTCAE, these clinical changes could be an expected phenomenon in this disease due to tumor growth. As a result, neurologic deficits will not be considered unacceptable toxicities if unequivocal tumor progression can be documented radiographically or histologically.

Unacceptable ≥ Grade 3 Toxicity

Any ≥ Grade 3 organ toxicity (cardiac, renal, hepatic), including CRS-related toxicities such as hypotension and tachycardia, of any duration that is attributable to vaccination will be considered an unacceptable toxicity. Please see Section

9.1.4 for guidelines on management of immune response, including CRS. Patients will be monitored for 30 days after each vaccine. As vaccines #1 through #5 are close in proximity (priming phase of vaccination), patients will be monitored from vaccine #1 until 30 days after vaccine #5.

Patients will also be monitored for 30 days after vaccine 6 and vaccine 7 (booster phase of vaccination). Toxicities must occur within this 30-day period to be considered an unacceptable toxicity attributable to vaccination.

9.1.2.2 Definition of Dose Limiting Toxicity (DLT)

As defined in Section 9.1.2.1, a DLT is as an unacceptable toxicity that occurs during the DLT observation period that extends between vaccine #1 and 30 days after vaccine #5.

Patients receiving less than 5 vaccines without experiencing a DLT will be replaced in the decision-making concerning dose escalation.

9.1.3 Dose Modification

In the event of DLT at the initial dose level of 300 µg/peptide/dose (see Section 15.5.1 for rules for dose escalation), the dose of the P30-EPS vaccine dose will be de-escalated to 200 µg/peptide/dose. See Section 15.5.1.2 for possible actions if 200 µg/peptide/dose is deemed unsafe. No dose modifications are allowed within an individual patient.

9.1.4 Safety Considerations

Management of Toxicities

If a Grade 3 NCI CTCAE (Version 5) or greater toxicity is seen that is not attributable to a concomitant medication, co-morbid event, or radiographically or clinically documented disease progression, the next study treatment for that patient will be withheld for up to 2 months or until the toxicity improves to a Grade 2 or less. However, planned procedures requiring hospitalization or long-term clinical decline that are clearly not related to the study vaccine will not be considered an unacceptable toxicity nor will such patients have immunizations withheld.

In an abundance of caution, the current study has implemented an extended post-vaccine monitoring period in which the patients are observed in clinic with regular vital signs monitoring.

Patients will receive their 1st vaccine in the Oncology Treatment Center (OTC) and will receive a normal saline bolus by intravenously (IV) prior to vaccine administration. The IV saline lock used for the bolus will remain in place for all patients during the post-vaccine monitoring period for the purpose of quick administration of additional fluids and/or medication should either be necessary. Supportive medications for post-vaccine reactions may include the following per institutional guidelines:

- Sodium chloride NS bolus 20 mL/kg
- Benadryl 1 mg/kg IV push

- Solu-medrol 2 mg/kg IV push

Patients on study who experience a significant post-vaccine reaction at any point (i.e., ≥ Grade 2 toxicity thought to be possibly, probably, or definitely related to study vaccine), but who are not removed from study, will receive a normal saline bolus by IV prior to vaccine administration for all subsequent vaccines and the IV saline lock will remain in place as a precautionary measure, in case supportive medications are needed. Patients on study who have never experienced a post-vaccine reaction or only experienced a Grade 1 toxicity with earlier vaccines will not be required to have an IV saline lock placed prior to administration of subsequent vaccines. The treating provider may opt to proceed with the IV saline lock though if a patient experienced a mild post-vaccine reaction with previous vaccines.

We believe that the vaccine reactions in PERFORMANCE, a study that used a different vaccine platform with a longer pp65 peptide, were likely indicative of vaccine potency. In that study, patients who had a demonstrable Grade 3 reaction also had measurable cellular responses to the PEP-CMV vaccines, which may be indicative of clinical responses. In patients with AEs, the pp65-specific T cell activation may be inducing a temporary elevation of pro-inflammatory cytokines resembling CRS.

CRS is associated with elevated circulating levels of cytokines including IL-6 and IFNγ. Commonly referred to as an infusion reaction, it results from the release of cytokines from immune effector cells. When cytokines are released into the circulation, systemic symptoms such as fever, nausea, chills, hypotension, tachycardia, asthenia, headache, rash, scratchy throat, and dyspnea can result. In most patients, the symptoms are mild to moderate in severity and are managed easily. However, some patients may experience severe, life-threatening reactions that result from massive release of cytokines. Massive cytokine release is an oncologic emergency, and special precautions must be taken to prevent life-threatening complications. A CRS Management Plan based upon the ASTCT Consensus Grading [63] guidelines is provided below in Table 1 for the mitigation of symptoms of CRS. Please note that ASTCT Consensus Grading guidelines refer to the CTCAE v.5 description of constitutional symptoms for its CRS term.

Table 1. CRS Management Plan Based Upon the ASTCT Consensus Grading

Grade of Toxicity	ASTCT Definition [63]	Management Plan
1	Fever ≥ 38°C, with or without constitutional symptoms, not attributable to another cause	Symptomatic management only
2	Fever ≥ 38°C with hypotension not requiring vasopressors and/or hypoxia requiring the use of	Hypotension: Clearly establish a baseline blood pressure; if hypotension develops, give fluids.

Grade of Toxicity	ASTCT Definition [63]	Management Plan
	oxygen delivered by low-flow nasal cannula (≤6 L/minute) or blow-by	Other reactions: Patients with grade 2 toxicity will be individually tailored, depending on the patient age and medical co-morbidity, and therefore clinical judgement will be crucial prior to use of immunosuppression for grade 2 toxicity. Patients with grade 2 toxicity will be monitored in the OTC with very close cardiac monitoring.
3	Fever ≥ 38°C with hypotension requiring 1 vasopressor with or without vasopressin and/or hypoxia requiring high-flow nasal cannula (>6 L/minute), facemask, nonrebreather mask, or venturi mask not attributable to another cause	If grade 3 toxicity develops where fluid resuscitation and 1 low dose vasopressor are not sufficient to reverse the hypotension, the patient will be transferred and monitored closely in the emergency department. All patients with grade 3 toxicity will receive immunosuppressive agents including corticosteroids, such as 2mg/kg Solu-medrol. In severe cases, Tocilizumab, antihuman IL-6R mAb, will be administered. This drug is stocked in the Duke pharmacy.
4	Fever ≥ 38°C with hypotension requiring multiple vasopressors (excluding vasopressin) and/or hypoxia requiring positive pressure (e.g., CPAP, bilevel positive airway pressure, intubation, mechanical ventilation) not attributable to another cause	All patients with immediate, life-threatening toxicity will be treated with full support, including mechanical ventilation, immunosuppressive agents, Tocilizumab to prevent inflammatory cascade, and close comprehensive organ monitoring.

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

Please refer to Section 13.1 for details on AE assessments and reporting.

9.1.5 Steroid Use

Steroid use should be minimized to prevent potential dampening of the immune system in response to peptide vaccination. If patients experience symptoms requiring corticosteroid use > 4 mg per day of dexamethasone (or equivalent), it will be recommended that patients initiate bevacizumab (7.5mg/kg IV every 3 weeks) instead of increasing the steroid dose, if possible. Bevacizumab may be given by their local oncologist.

9.1.6 Missed Doses

To ensure that repetitive doses of P30-EPS vaccines will be given to patients in a timely manner, vaccines will be given with a ± 1,2, or 7 day window depending upon which vaccine it is (see Section 9.1), unless unacceptable toxicities require discontinuation of therapy or disease progression is diagnosed prior to completion of planned therapy. At the discretion of the study PI, patients who miss a vaccine administration for whatever reason will have their appointment re-scheduled to administer the vaccine as soon as possible. In these instances, a deviation will be recorded on the deviation log and the reportability will be assessed by the PI. For continued non-compliance with the scheduled vaccine appointments, the subject may be removed from the trial at the discretion of the study PI.

9.1.7 Concomitant Medications

Concomitant medications will be managed by the treating physician and recorded at each study visit by the study team starting with the CMV screening visit.

Corticosteroids should be used at the lowest dose [less than 0.1 mg/Kg/day (<4 mg of dexamethasone / day)] to control symptoms of edema and mass effect, and discontinued, if possible. Use of corticosteroids should be recorded in the electronic database.

9.1.8 Study Drug Blinding

Not applicable

9.1.9 Randomization

Not applicable

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

Vaccines will include the EphA2$_{(883-891)}$ (TLADFDPRV) [27], pp65$_{(495-503)}$ (NLVPMVATV) [64], and survivin$_{(95-104)}$ (ELTLGEFLKL) peptides. Each of these peptides will be individually linked with a furin cleavage site (RVKR) as used in our preclinical data to the P30 sequence (FNNFTVSFWLRVPKVSASHLE) and mixed with 20 μg/kg of carboxymethylcellulose, poly I:C, and poly-L-lysine double-stranded RNA (poly-ICLC / Hiltonol®). Peptides will be produced by BaChem, (Torrence, CA), which routinely produces peptides of this length with 95% purity under GMP conditions. Peptides will be pooled for administration. Initially, 300 μg of each peptide in 10% DMSO in water will be given at the same time in all groups in a vaccine schedule that has been shown to successfully induce vaccine-specific T cell responses in patients with melanoma [65] [Priming Phase: days 1, 4, 8, 15, and 22, and Booster Phase: days 84 and 140] (see Treatment Schema in Section 6). Dose escalation to 400 μg/peptide/dose is planned. In the event that 300 μg/peptide/dose is deemed unsafe, the dose will be de-escalated to 200 μg/peptide/dose (dose level minus one). If further dose reduction is needed, the pp65 peptide will be removed from the vaccine combination following FDA approval (See Section 15.5.1.2).

9.3 Rationale for Correlative Studies

Immunological assessments will be used to measure the endpoints of the study. The peripheral blood samples collected in this study are used for assessing the impact of P30 linked EphA2, CMV pp65, and survivin peptides (detailed in Section 12.6.5 Immunological Evaluations). The exploratory biomarkers outlined in Section 15.7.2 will be assessed in archival tumor tissue, as well as any additional tumor tissue that may be obtained from future surgeries from patients throughout the course of the study. DNA and RNA sequencing will be performed on the archival tissue to describe any association between clinical findings including survival and PFS, and specific signatures that have been reported in the literature to correlate with clinical outcomes in immunotherapy trials in GBM. We expect patients with better clinical outcomes such as longer OS and PFS compared with similar historical controls, to have distinct immunological characteristics that we may define from the exploratory analyses. The sample size in this trial will not have statistical power to draw meaningful conclusions about these features, but the findings are likely to be hypothesis generating.

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

Once the patient receives vaccine #1, that patient will be considered “on study” per the Institutional Review Board (IRB). Different definitions for “on study,” “on treatment,” “off treatment,” and “off study” may be required in the electronic research record. Rationale for taking a patient off protocol treatment will be documented.

Any patient who receives at least one vaccine will be included in the efficacy analysis. The details about what patients will be evaluable for various aspects of toxicity monitoring and summarization can be found in Section 15.5. The details about what constitutes an “evaluable subject” for secondary endpoints are provided in the statistical analysis plan (Section 15.6) included in this protocol.

Once all data has been collected, analyzed, and completely evaluated on all subjects as defined in the statistical analysis plan and subjects have completed follow up, expired, withdrawn, or been lost to follow up, the study will be ended.

9.5 Early Study Termination

This study can be terminated at any time for any reason by the PI/ IND sponsor. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 12.5.2**.** Section 12.5 describes procedures and processes for prematurely withdrawn patients.

10 STUDY DRUG

10.1 P30-EPS Vaccines

10.1.1 Names, Classification, and Mechanism of Action

The tumor-associated antigen (P30-EPS) vaccines will be made up of three peptides: EphA2 linked to P30 peptide, pp65 linked to P30 peptide, and survivin linked to P30 peptide. Each of these peptides will be individually linked with a furin cleavage site (RVKR) as used in our preclinical data to the P30 sequence (FNNFTVSFWLRVPKVSASHLE) and mixed with carboxymethylcellulose, poly I:C, and poly-L-lysine double-stranded RNA (poly-ICLC/ Hiltonol®). Peptides will be produced by BaChem (Torrence, CA), which routinely produces peptides of this length under GMP conditions.

P30-EPS vaccines work by "training" the immune system to recognize the TAAs commonly expressed by tumor cells. The first sequence of P30-EPS vaccines (vaccines 1-5) prime the immune system to attack the tumor directly. The second sequence (vaccines 6 and 7) serve as a booster to promote lasting memory immune responses.

10.1.2 Packaging and Labeling

The EphA2P30, pp65P30, and SurvivinP30 drug substances are manufactured under GMP conditions by BaChem. The P30-EPS vaccines will consist of all three drug substances suspended at a total net peptide concentration of 2.52 mg/mL (net peptide concentration of 0.84 mg/mL each peptide) in 10% DMSO in water and will be mixed with Hiltonol® 20 µg/kg. The volume injected i.m. will depend on the dose level (240-480 µL P30-EPS vaccine + Hiltonol® 20 µg/kg), not to exceed a total i.m. volume of 5 mL to be injected in the thigh muscle.

The P30-EPS peptides are solubilized at a total net peptide concentration 2.52 mg/mL and mixed 1:1:1 to achieve a final net peptide concentration of 0.84 mg/mL for each peptide. The dose levels are 200µg/peptide/dose, 300µg/peptide/dose, and 400µg/peptide/dose, so the total peptide in each vaccine ranges from 0.6-1.2 mg/injection. The P30-EPS drug product will be provided to the Duke Investigational Chemotherapy Service (ICS) in sterile, frozen, single use vials, which will be thawed and loaded into a syringe by the ICS prior to vaccination.

Final vaccines will be labeled on syringes as such:

- Name
- MRN
- DoB
- Drug: P30-EPS
- Lot #
- Expiration for room temperature storage
- Expiration for refrigerated storage
- Caution New Drug Limited By Federal Law To Investigational Use

Mixing:
To prepare the final vaccine, the P30-EPS drug product will be mixed with Hiltonol® (20 µg/kg) in sterile saline. Hiltonol® will be provided by Oncovir.

The P30-EPS drug product is supplied as a liquid frozen at or below 70˚C at 0.9 mL per vial with a net peptide concentration of 2.52 mg/mL (0.84 mg/mL each peptide). Each vial will be thawed at room temperature and be fully thawed within 0.5 to 3 minutes with no remaining visible frozen drug or particulate. The resultant solution of P30-EPS peptides is a clear, colorless solution.

The P30-EPS drug product is mixed with Hiltonol® (20 µg/kg). Based on the dose to be given, withdraw the volume of P30-EPS drug product (see Table 2) in a latex, silicon oil and rubber tip-free syringe. In a second syringe, withdraw 20 µg/kg of Hiltonol® solution. Connect the two syringes via a double female luer lock.

The mixing process is done in one quick step. First, transfer all material into one syringe, and then transfer back very slowly into the other syringe WITHOUT CREATING BUBBLES. The final volume should be between 0.8 and 5 mL.

Disconnect the syringe from the connector. Twist a sterile needle on the syringe and the homogeneous solution of P30-EPS drug product + Hiltonol® is ready for patient vaccination. One syringe volume is administered i.m. to the patient thigh muscle.

Table 2. P30-EPS Peptide Solution Volumes per Dose Level

Dose Level	P30-EPS Individual Net Peptide Concentration	Volume of P30-EPS Drug Product
200 µg/peptide	0.84 mg/mL	0.24 mL
300 µg/peptide	0.84 mg/mL	0.36 mL
400 µg/peptide	0.84 mg/mL	0.48 mL

10.1.3 Supply, Receipt, and Storage
Lyophilized EphA2, pp65, and survivin peptides linked with universal epitope P30 will be supplied by BaChem to Duke's Investigational Drug Services (IDS) Compounding Pharmacy, where they will be reconstituted, placed into sterile vials, and frozen before being transferred to and stored at the Duke ICS. P30-EPS vaccines should remain frozen at or below minus 70°C for long-term storage. Final vaccine (i.e., peptides mixed with Hiltonol®) should be administered within 6 hours of preparation if stored refrigerated (2-8° C) and within 3 hours of preparation if stored at room temperature.

10.1.4 Dispensing and Preparation
Patients will receive the P30-EPS vaccines from ICS directly to the clinic or OTC under the supervision of the research nurse, or designee. The patient's name, Study ID, DoB, and Duke history number will be double-verified prior to vaccine administration, as is standard Duke procedure. The P30-EPS peptide and Hiltonol®

solution will be mixed in the ICS (see Section 10.1.2), transported to the clinic, and vaccinations will be given intramuscularly in the thigh. With each injection, the side of the body will be alternated in order to maximize systemic effect and minimize potential for site reactions.

Following administration of a P30-EPS vaccine, the first 6 patients (first 3 CMV seropositive and the first 3 CMV seronegative) on study will be retained for observation. These patients will be retained for 4 hours after the first 3 vaccines administered during the Priming Phase for observation, including regular vital signs monitoring every 30 minutes for a total of 8 sets of vital signs. If a patient experiences no vaccine injection reactions following the first three vaccines (Refer to Section 9.1.4 for background information on injection reactions experienced in a related adult study), the observation time following the subsequent vaccine injections may be decreased to two hours with a total of 4 sets of vital signs and eventually to 30 minutes with one set of vital signs at the discretion of the treating sub-investigator. If a patient experiences a post-vaccine Grade 3 or higher unacceptable reaction at any point, the monitoring period will revert to 4 hours until such time as he/she has 2 consecutive vaccines with no reaction. It will not be considered a deviation if the vitals are not captured exactly at the 30-minute time mark. As long as the patient is monitored for the protocol-specified amount of time, and as long as the correct number of sets of vitals have been collected for that time period, the patient will be considered sufficiently monitored. The PI or sub-investigator may extend monitoring periods at any given visit if they feel it is clinically indicated for the patient's safety.

If the first 6 patients do not experience Grade 3 or higher unacceptable toxicity, the 4 hours monitoring will only be required after the first vaccine for subsequent patients. After the first 6 patients have been enrolled, the study team will review the data and decide if 4-hour monitoring should be continued.

As of 4/24/2024, the first 6 patients on this study have been treated. In the second set of 6 patients, that are currently being monitored for DLTs, patients received Zofran (up to 8 mg) (or similar anti-emetic) and Tylenol (up to 650 mg maximum) prior to every vaccine. These patients also received a normal saline bolus infusion by IV prior to administration of vaccine #1 only; see Section 9.1.4 for further details. Patients were monitored for 2 hours post-vaccine for all vaccines and vitals were checked every 30 minutes.

10.1.5 Compliance and Accountability

The P30-EPS vaccines and Hiltonol® will be stored in Duke ICS. The products will be signed out and distributed according to ICS Standard Operating Procedures (SOPs). Study personnel are trained to use safe medication practices to reduce the risk of medication errors and AEs. Investigational drugs are stored separately from other drugs in an area of limited access and in accordance with special storage requirements. They are clearly labeled with the identity of the study drug and other control numbers.

Drug accountability records will be maintained for all clinical trial supplies. All empty and partially used clinical trial supplies will be destroyed in accordance with institutional guidelines. ICS will maintain detailed documentation of the receipt and/or destruction of the study agent. All materials coming in contact with the study agent including the syringe and needles will be disposed of as biological waste in the treatment room.

10.1.6 Disposal and Destruction

Unused study drugs will be destroyed per institutional guidelines. Used sharps will be disposed in biohazard sharps container and incinerated for final disposal.

10.2 Hiltonol®

10.2.1 Names, Classification, and Mechanism of Action

Carboxymethylcellulose, poly (I:C), and poly-L-lysine double-stranded RNA (poly-ICLC) (Hiltonol®) will be administered 1) in conjunction with P30-EPS peptides on Days 1, 4, 8, 15, and 22 of the Priming Phase, 2) in conjunction with P30-EPS peptides on Days 84 and 140 of the Booster Phase, and 3) will be given alone (at home) on Days 98, 112, and 126 of the Booster Phase (i.e., every 2 weeks between vaccines 6 and 7). Hiltonol® will be provided by Oncovir.

10.2.2 Packaging and Labeling

Hiltonol® injections will consist of 20 µg/kg of carboxymethylcellulose, poly (I:C), and poly-ICLC. Hiltonol® will be provided to the ICS by Oncovir in single-dose vials containing 1 mL of 1.8 mg/mL opalescent white suspension. Each milliliter of Poly-ICLC for injection contains 1.8 mg/mL poly-IC, 1.5 mg/mL poly-L-lysine, and 5 mg/mL sodium carboxymethylcellulose in 0.9% sodium chloride solution and adjusted to pH 6-8 with sodium hydroxide. Hiltonol® is withdrawn from the vial under sterile conditions and is to be administered intramuscularly as supplied.

For Vaccines 1 through 5 in the Priming Phase and Vaccines 6 and 7 in the Booster Phase, Hiltonol® will be mixed with P30-EPS peptide solution by Duke ICS. For the three home injections every two weeks apart (± 2 days), between Vaccine #6 and #7, Hiltonol® vials (along with home kits containing syringes, needles, alcohol swabs) will be provided for home dosing. Hiltonol® will be loaded into a syringe by the patient or caregiver at home prior to vaccination. If the vaccine #6 and vaccine #7 visits fall closer than 8 weeks apart due to scheduling nuances, the patient may only receive 2 Hiltonol® injections if the injections cannot be spaced apart by at least 10 days. Patients will be contacted and/or sent a reminder (phone, email, or MyChart) by a study team member prior to each injection at home to help insure that these doses are not missed. For detailed patient administration instructions, please see Appendix A in Section 18.1 for a sample Hiltonol Patient Administration Guide. Each patient's guide will indicate the appropriate volume (in mL) of Hiltonol® for self-administration based on the patient's height/weight at the start of the Booster Phase (i.e., the correct mL for a dose of 20 µg/kg). Patients will be asked to let the study team know if their weight changes significantly during the Booster Phase (e.g., a change of > 10%).

Hiltonol® will be labeled with:

- Name
- MRN
- DoB
- Drug: Hiltonol®
- Caution New Drug Limited By Federal Law To Investigational Use

10.2.3 Supply, Receipt, and Storage

Hiltonol® will be supplied by Oncovir to Duke's ICS. Hiltonol® is normally shipped or stored refrigerated at about 40ºF (2-10°C), but should NOT be frozen. Based on extended formal stability data at +5°C ± 3°C and at +25°C ± 2°C, it is justified to accept temperature excursions during transport, shipment and storage of Hiltonol® at clinical sites up to +27°C for up to 72 hours. Likewise, temperature deviations between 0°C and +2°C are acceptable and have no effect on product quality.

10.2.4 Dispensing and Preparation

Patients will be provided Hiltonol®-only injections at the Day 84 visit to be self-administered at home every 2 weeks (±2 days) until the Day 140 visit. Patients will be provided their Hiltonol®-only injections on ice and instructed to store the vials immediately in their refrigerator (not freezer) once they arrive home. Patients or patients' caregivers will be trained to administer the Hiltonol® injections by the research nurse or other qualified designee at or prior to the Day 84 visit and will be provided with educational materials to take home (please see Section 18.1 Appendix A: Hiltonol Patient Administration Guide).

10.2.5 Compliance and Accountability

Hiltonol® will be stored in Duke ICS. The products will be signed out and distributed according to ICS SOPs. Study personnel are trained to use safe medication practices to reduce the risk of medication errors and AEs. Investigational drugs are stored separately from other drugs in an area of limited access and in accordance with special storage requirements. They are clearly labeled with the identity of the study drug and other control numbers.

Drug accountability records will be maintained for all clinical trial supplies. All empty and partially used clinical trial supplies will be destroyed in accordance with institutional guidelines. ICS will maintain detailed documentation of the receipt and/or destruction of the study agent. All materials coming in contact with the study agent including the syringe and needles will be disposed of as biological waste in the treatment room.

Patients will be provided a paper diary to document the dates of Hiltonol® home administration between the Day 84 visit and Day 140 visit. Patients will be asked to return the completed diary and any unused Hiltonol® to the clinic at their Day 140 visit (please see Section 18.2 Appendix B: Hiltonol Patient Diary).

10.2.6 Disposal and Destruction

Unused study drugs will be destroyed per institutional guidelines. Patients will also be provided a biohazard sharps container. Patients will dispose of used sharps in this biohazard sharps container and will bring it back to the clinic at their Day 140 visit for final disposal.

11 SUBJECT ELIGIBILITY

11.1 Inclusion Criteria

1. Age ≥ 18 years of age
2. Newly diagnosed IDH wild type (CARIS result), MGMT promoter unmethylated (CARIS result) WHO grade 4 glioma (e.g., GBM or high grade glioma with molecular features of GBM) with definitive resection prior to enrollment and residual radiographic contrast enhancement on immediate post-surgical computed tomography (CT) or magnetic resonance imaging (MRI).
3. CMV positive or negative by Immunoglobulin G (IgG) testing.
4. Karnofsky Performance Status (KPS) of ≥ 70%.
5. Hemoglobin ≥ 9.0 g/dl, absolute neutrophil count (ANC) ≥ 1,000 cells/µl, platelets ≥ 100,000 cells/µl.
6. Serum creatinine ≤ 3 x the upper limit of normal (ULN), serum glutamic oxaloacetic transaminase (SGOT)≤ 3 times ULN
7. Bilirubin ≤ 1.5 times ULN *(Exception: Patient has known Gilbert's Syndrome or patient has suspected Gilbert's Syndrome, for which additional lab testing of direct and/or indirect bilirubin supports this diagnosis. In these instances, a total bilirubin of ≤ 3.0 x ULN is acceptable.)*
8. Signed informed consent approved by the IRB.
9. Patients must not be pregnant or breast-feeding. Patients of childbearing potential (defined as < 2 years after last menstruation or not surgically sterile) must use a highly effective contraceptive method (allowed methods of birth control, [i.e. with a failure rate of < 1% per year] are implants, injectables, combined oral contraceptives, intra-uterine device [IUD; only hormonal], sexual abstinence or vasectomized partner) during the trial and for a period of > 6 months following the last administration of trial drug(s). Patients with an intact uterus (unless amenorrhea for the last 24 months) must have a negative serum pregnancy test within 48 hours prior to first vaccination.
10. Patients that are able to produce sperm must agree to use a highly effective contraceptive method (allowed methods of birth control [i.e. with a failure rate of < 1% per year] include a partner using implants, injectables, combined oral contraceptives, IUDs [only hormonal], sexual abstinence or prior vasectomy) during the trial and for a period of > 6 months following the last administration of trial drug(s).

11.2 Exclusion Criteria

1. Patients with known potentially anaphylactic allergic reactions to gadolinium-diethylenetriaminepentaacetic acid (DTPA), or any component of the tetanus-diphtheria vaccine.

2. Patients with evidence of tumor in the brainstem, cerebellum, or spinal cord, radiological evidence of multifocal disease, or leptomeningeal disease.
3. Areas of high-grade glioma outside the original radiation field on the post XRT/TMZ MRI.
4. Patients who cannot undergo MRI.
5. Severe, active comorbidity, including any of the following:
   - Unstable angina and/or congestive heart failure requiring hospitalization;
   - Transmural myocardial infarction within the last 6 months;
   - Acute bacterial or fungal infection requiring intravenous antibiotics at the time of first vaccination;
   - Active infection requiring intravenous treatment or having an unexplained febrile illness (Tmax > 99.5°F/37.5°C)
   - Chronic obstructive pulmonary disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy;
   - Known hepatic insufficiency resulting in clinical jaundice and/or coagulation defects;
   - Known immunosuppressive disease or Human Immunodeficiency Virus (HIV) and Hepatitis C positive status;
   - Major medical illnesses or psychiatric impairments that, in the investigator's opinion, will prevent administration or completion of protocol therapy;
   - Active connective tissue disorders, such as lupus or scleroderma that, in the opinion of the treating physician, may put the patient at high risk for radiation toxicity.
6. Co-medication that may interfere with study results (e.g., immuno-suppressive agents other than corticosteroids).
7. Patients who require corticosteroid doses that cannot be reduced to a dose ≤2 mg dexamethasone per day or equivalent at the time of first vaccination. Exceptions include nasal or inhaled steroid.
8. Prior, unrelated malignancy requiring current active treatment with the exception of cervical carcinoma *in situ* and adequately treated basal cell or squamous cell carcinoma of the skin. (Treatment with tamoxifen or aromatase inhibitors or other hormonal therapy that may be indicated in prevention of prior cancer disease recurrence, are not considered current active treatment.)
9. Patients are not permitted to have had any other conventional therapeutic intervention other than surgery, steroids, and 3-6 weeks of standard of care XRT with or without concomitant TMZ prior to enrollment.
10. Patients who received previous inguinal lymph node dissection or had radiosurgery, brachytherapy, or radiolabeled monoclonal antibodies to treat a central nervous system (CNS) tumor will be excluded.
11. Current, recent (within 4 weeks of the administration of this study agent), or planned participation in an experimental drug study.
12. Known history of autoimmune disease (with the exceptions of medically-controlled hypothyroidism and Diabetes Mellitus).

12 SCREENING AND ON-STUDY TESTS AND PROCEDURES

Table 3: Schedule of Events and Interventions

Description	Screening Period		Treatment Period						
	Post-XRT/TMZ		Priming Phase					Booster phase	
Vaccine	CMV screening	Final screening	#1	#2	#3	#4	#5	#6	#7
Day		At least one week post Td booster	1	4 (±1 days)	8 (±1 days)	15 (±2 days)	22 (±2 days)	84 (±7 days)	140 (±7 days)
General Evaluations									
CMV consent	X								
Main study consent		X							
Physical Exam[1]	X	X	X	X	X	X	X	X	X
Neurologic Exam[1]	X	X	X	X	X	X	X	X	X
Performance Status[1]	X	X	X	X	X	X	X	X	X
MMSE[1]			X						
AEs			Continuous						
Laboratory Evaluations									
Tetanus immunization booster	X								
CMV screen	X								
HLA-A*0201 status[2]	X								
IDH mutation status[2]	X								
MGMT promoter methylation status[2]	X								
CBC w differential	X[3]	X[3]	X[3]	X[3]	X[3]	X	X	X	X
CMP	X[3]	X[3]	X[3]	X[3]	X[3]	X	X	X	X
Beta HCG[4]	X	X							
Whole blood for immune analysis[5]			X				X	X	X
CRS Blood Draws[6]			X					X	X
Tissue Collection (archival or on-study)[7]	Continuous								
Disease Evaluations									
MRI	X						X	X	X
Treatment									
P30-EPS Vaccine with vitals[8]			X	X	X	X	X	X	X
Hiltonol® - only injection[9]								Every 2 weeks at home (±2 days)	
Bevacizumab 7.5mg/kg IV q 3 weeks			AS NEEDED PER PHYSICIAN DISCRETION[10]						

[1] Clinical evaluations will include a general physical examination, neurologic examination, and KPS. MMSE will only be assessed once – at the vaccine #1 visit prior to vaccine administration. During screening, the physical exam, neuro exam, and KPS should be assessed to confirm eligibility and will need to be repeated if not performed within 1 week of vaccine #1 administration.
[2] Testing will be performed on surgical specimen. May be obtained prior to enrollment on study as part of standard of care evaluation.
[3] CBC w/diff and CMP needs to be drawn and resulted showing results suitable for eligibility as close as possible (within one week) prior to vaccine #1 administration. In some cases, CBC w/diff and CMP may be drawn as part of standard of care prior to the signing of main consent. The CMP will not be repeated if results meet eligibility and are within one week prior to vaccine #1; however, the CBC w/diff will need to be repeated if drawn more than 24 hours prior to vaccine #1 administration. CBC w/diff and CMP at Day 4 and/or Day 8 are not needed if they were already drawn for vaccine #1 or vaccine #2 within the same Monday through Friday "week."
[4] Beta HCG (quantitative) to be collected/resulted within 48 hours prior to vaccine #1 administration.
[5] Blood draws for immune analysis on days with vaccination should be performed before vaccine administration. Whole blood for immune analysis containing 9 yellow top ACD 8.5 mL tubes and 2 red top 10 mL tubes will be drawn prior to vaccines #1, #5, #6, and #7. For subjects who complete all study vaccines without progressing, an attempt will be made to obtain blood for immunologic monitoring 2-3 times per year at standard PRTBTC clinic visits and at progression (whichever comes first). If a patient progresses at any other vaccine visit, an attempt will be made to obtain the blood for immune analysis.
[6] Blood will be drawn at 2 hours (+15 minutes) after vaccines #1, #6, and #7 (2 10mL red top tubes) and will be sent to the designated lab for processing. Any blood draws occurring within the window of 2 hours (+15 minutes) will not be considered a deviation. Blood draws taking place outside of the window will be considered a deviation, and the reportability will be determined by the PI.
[7] We will request archival tumor tissue from patients' baseline surgical resection or prior biopsy, as well as tumor tissue from any future surgeries that patients may undergo while on-study.
[8] It will not be considered a deviation if the vitals are not captured exactly at the 30-minute time mark. As long as the patient is monitored for the protocol-specified amount of time, and as long as the correct number of sets of vitals have been collected for that time period, the patient will be considered sufficiently monitored. See Section 10.1.4 regarding the vitals schedule prior to and after each vaccine administration.
[9] Patients will be contacted and/or sent a reminder (phone, email, or MyChart) by a study team member prior to each injection at home to help ensure that these doses are not missed.
[10] Please refer to Sections 9.1 and 9.1.5.

12.1 Screening Examination

If patients are considered to be a potential candidate prior to XRT, the study may be mentioned to the patient, however, initial screening examination will take place at a Duke PRTBTC clinic visit after standard of care XRT/TMZ. If the patient wishes to pursue the study after XRT/TMZ, they will sign the CMV screening consent and then have blood drawn for CMV IgG testing per the Duke clinical lab requirements. The patient will also receive a tetanus booster after signing the screening consent.

All patients will need IDH and MGMT promoter methylation confirmed for eligibility purposes. These tests will need to be performed via standard of care practices, and the results will need to be available before the patient can commence study treatment. HLA status will be extracted from the electronic medical records. All HLA types are eligible in this study that focuses on safety and feasibility. However, we may restrict accrual to patients with a positive HLA-A*0201 status in subsequent future trials.

During collection of tumor sample at initial resection at Duke University, primary tissue will be banked and stored for assays, and slides will be sent for IDH-1/2 mutation analysis. For subsequent partitioning of pathology slides, tissue will be stored in formalin-fixed blocks. If the initial resection was not performed at Duke, all previously collected tissue specimens will be reviewed by Duke pathologists. MGMT gene promoter methylation and IDH mutation status will be determined using validated testing from CARIS. Even if MGMT gene promoter methylation status has already been determined outside of Duke, the test needs to be repeated by CARIS for determining eligibility. For patients where tumor resection was performed outside of Duke, tumor tissue is requested from the external hospitals as part of standard practice and will be sent directly to CARIS for MGMT gene promoter methylation. If the results of testing by CARIS are indeterminate, the results will be considered methylated and the patient will not be eligible for the study. We will also request archival tumor tissue from patients' baseline surgical resection or prior biopsy, as well as tumor tissue from future surgeries, but only in the case that the patients may undergo other clinically indicated biopsy or tumor resection while participating in this study. Further details on tissue processing are available in the ETAPA correlative laboratory manual. Available tumor tissue will be analyzed by correlative analyses as outlined in Section 9.3.

All patients will undergo a final screening visit after XRT/TMZ (at least one week post tetanus booster), in order to confirm eligibility and start vaccinations. Final screening visit tests will consist of a physical and neurological exam, performance status testing, CMV testing (if not already known), CBC with differential, CMP, and Beta HCG quantitative (if applicable). The post XRT/TMZ standard of care MRI will be used to confirm eligibility, unless a new scan is requested by the treating physician.

Once the final screening visit is complete and the patient is confirmed eligible, the study vaccinations should commence that day or the following day. If study vaccines do not start within one week of the final screening visit, the CMP and clinic visit (physical/neuro exam and KPS) will need to be repeated. The CBC w/diff needs to be

collected within 24 hours prior to vaccine #1. Mini-Mental Status Examination (MMSE) and blood for immune monitoring will also be drawn (9 yellow 8.5 mL ACD tubes and 2 red 10 mL tubes) once the patient is deemed eligible and just prior to vaccine #1.

12.1.1 MGMT Promoter Methylation

The unmethylated MGMT gene promoter status will have been determined prior to enrollment on trial via standard of care testing of the tissue obtained at the time of original surgery for tumor diagnosis using Caris Life Sciences sequencing technology, as per the PRTBTC standard of care for MGMT gene promoter status determination. No additional specimen will be obtained for the protocol for MGMT promoter status testing. MGMT testing will be obtained from the surgical specimen obtained at the time of standard of care resection for diagnosis of suspected GBM patients. In a published study in 2016, 43% of GBM tumors profiled (a mixture of newly diagnosed and recurrent tumors) were positive for MGMT methylation in testing performed by Caris Life Sciences [15]. FDA has determined this use of the companion diagnostic testing does not meet the definition of a significant risk device under § 812.3(m) of the investigational device exemptions (IDE) regulation (21 CFR 812).

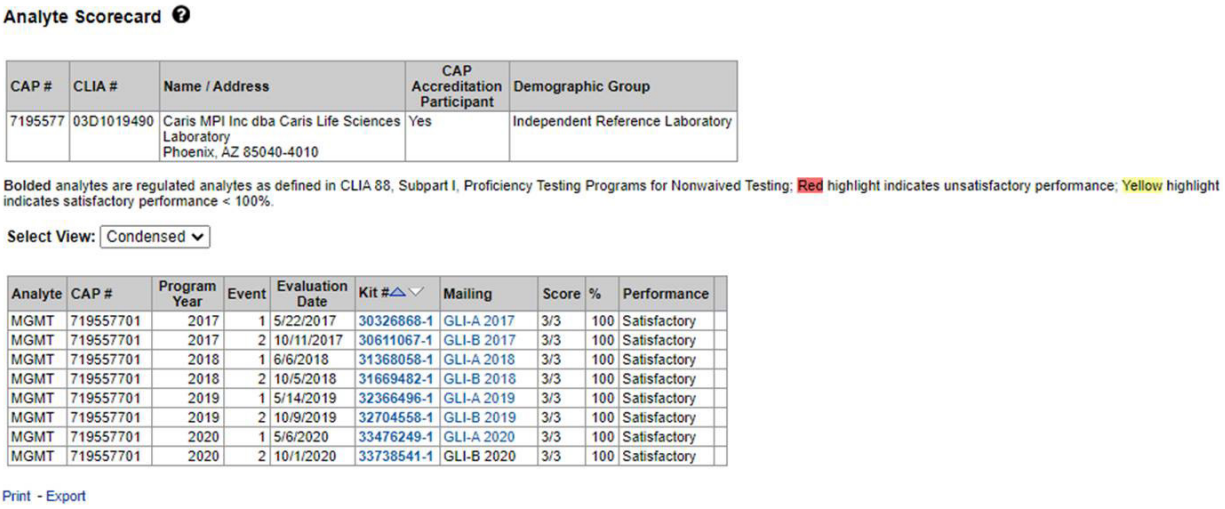
Caris Life Sciences performs MGMT-methylation (MGMT-ME) gene testing for GBM patients using pyrosequencing to detect methylation levels. Pyrosequencing was shown to be the best predictor of overall survival and PFS in GBM patients [66]. MGMT-ME status is believed to be a useful predictor of GBM patient response to TMZ, as well as offer helpful prognostic insights.

MGMT promoter methylation is evaluated by pyrosequencing. DNA extraction from paraffin-embedded tumor samples is performed for subsequent pyrosequencer-based analysis of 5 CpG sites (CpGs 74-78). All DNA samples undergo a bisulfite treatment and are polymerase chain reaction (PCR) amplified with primers specific for exon 1 of MGMT (GRCh37/hgl9 – chr10: 131,265,448- 131,265,560). Methylation status of PCR amplified products is determined using the PyroMark system. Samples with ≥ 7% and < 9% methylation are considered to be equivocal or gray zone results. If this occurs, the results are provided to the physician. If there is more tissue available from the resected tissue obtained at the time of original surgery for tumor diagnosis, the additional tissue is obtained for repeat testing. Patients with equivocal or gray zone results from the MGMT testing will not be eligible for clinical trials that specify MGMT promotor methylation status per their inclusion/exclusion criteria.

The MGMT Pyro Kit by Qiagen is intended for quantitative measurements of methylation in 4 CpG sites in exon 1 of the human MGMT gene (genomic sequence on chromosome 10 from 131,265,519 to 131,265,537: CGACGCCCGCAGGTCCTCG). Bisulfite converted genomic DNA is amplified by PCR and sequenced through the defined region in the forward direction. Sequences surrounding the defined positions serve as normalization and reference peaks for quantification and quality assessment of the analysis. The product consists of PCR primer mix and sequencing primer, 2 vials of each. The primers are delivered in

solution. Each vial contains 24 µL of primer or primer mix. The kit contains primers and reagents for amplification of the genes, plus buffers, primers, and reagents for quantitative methylation detection in real time using Pyrosequencing® technology on the PyroMark® Q24 system.

Description of the instrument used by Caris Life Sciences for detection changes in specified variable positions in DNA prepared from biological samples during pyro sequencing for methylation analysis of the MGMT promoter: Qiagen PyroMark Q96 MD instrument (Qiagen, cat# Q96MD); PyroMark CpG software, version 1.0.11.14. Caris Life Sciences has provided a performance matrix of their CAP scorecard for the MGMT promoter methylation test (Figure 5).



Analyte Scorecard

CAP #	CLIA #	Name / Address	CAP Accreditation Participant	Demographic Group
7195577	03D1019490	Caris MPI Inc dba Caris Life Sciences Laboratory Phoenix, AZ 85040-4010	Yes	Independent Reference Laboratory

Bolded analytes are regulated analytes as defined in CLIA 88, Subpart I, Proficiency Testing Programs for Nonwaived Testing; Red highlight indicates unsatisfactory performance; Yellow highlight indicates satisfactory performance < 100%.

Select View: Condensed

Analyte	CAP #	Program Year	Event	Evaluation Date	Kit #	Mailing	Score	%	Performance
MGMT	719557701	2017	1	5/22/2017	30326868-1	GLI-A 2017	3/3	100	Satisfactory
MGMT	719557701	2017	2	10/11/2017	30611067-1	GLI-B 2017	3/3	100	Satisfactory
MGMT	719557701	2018	1	6/6/2018	31368058-1	GLI-A 2018	3/3	100	Satisfactory
MGMT	719557701	2018	2	10/5/2018	31669482-1	GLI-B 2018	3/3	100	Satisfactory
MGMT	719557701	2019	1	5/14/2019	32366496-1	GLI-A 2019	3/3	100	Satisfactory
MGMT	719557701	2019	2	10/9/2019	32704558-1	GLI-B 2019	3/3	100	Satisfactory
MGMT	719557701	2020	1	5/6/2020	33476249-1	GLI-A 2020	3/3	100	Satisfactory
MGMT	719557701	2020	2	10/1/2020	33738541-1	GLI-B 2020	3/3	100	Satisfactory

Print - Export

Figure 5: Screenshot of Caris Life Sciences CAP scorecard specific to MGMT pyro sequencing testing.

In the validation study comparing the Caris Life Sciences MGMT results with an orthogonal test, they had 83% agreement with the outside lab. In the precision test, the intra-assay, inter-assay and inter-lot results generated 100% concordance across all duplicates.

12.2 Treatment Period

12.2.1 Priming Phase

Day 1

- Physical and neurological exam with KPS (if screening visit more than 1 week ago)
- MMSE (only performed once prior to vaccine #1 administration)
- CBC with differential (within 24 hours of vaccine #1) and CMP (within 1 week of vaccine #1)
- Beta HCG quantitative (if last result was drawn more than 48 hours prior to vaccine #1)
- Blood for immune monitoring
  - 9 yellow top 8.5 mL ACD tubes and 2 red top 10 mL tubes prior to vaccine administration.
- Vaccine #1 with vitals pre and post vaccine (see Section 10.1.4 for details)
- Blood for CRS

- 2 red top 10 mL tubes drawn 2 hours (+ 15 minutes) post vaccine administration.

Day 4 (±1 day)

- Physical and neurological exam with KPS
- CBC with differential and CMP (These labs are not needed if they were already drawn for vaccine #1 within the same Monday through Friday "week").
- Vaccine #2 with vitals pre and post vaccine (see Section 10.1.4 for details)

Day 8 (±1 day)

- Physical and neurological exam with KPS
- CBC with differential and CMP (These labs are not needed if they were already drawn for vaccine #2 within the same Monday through Friday "week")
- Vaccine #3 with vitals pre and post vaccine (see Section 10.1.4 for details)

Day 15 (±2 days)

- Physical and neurological exam with KPS
- CBC with differential and CMP
- Vaccine #4 with vitals pre and post vaccine (see Section 10.1.4 for details)

Day 22 (±2 days)

- MRI
- Physical and neurological exam with KPS
- CBC with differential and CMP
- Blood for immune monitoring
  - 9 yellow top 8.5 mL ACD tubes and 2 red top 10 mL tubes prior to vaccine administration.
- Vaccine #5 with vitals pre and post vaccine (see Section 10.1.4 for details)

12.2.2 Booster Phase

Day 84 (±7 days)

- MRI
- Physical and neurological exam with KPS
- CBC with differential and CMP
- Blood for immune monitoring
  - 9 yellow top 8.5 mL ACD tubes and 2 red top 10 mL tubes prior to vaccine administration.
  - 2 red top 10 mL tubes drawn 2 hours post vaccine administration.
- Vaccine #6 with vitals pre and post vaccine (see Section 10.1.4 for details)
- Blood for CRS
  - 2 red top 10 mL tubes drawn 2 hours (+ 15 minutes) post vaccine administration.
- Hiltonol® - only injections will be provided to patient for self-administration every 2 weeks (± 2 days) until Day 140 visit[9]

Day 140 (±7 days)

- MRI
- Physical and neurological exam with KPS
- CBC with differential and CMP
- Blood for immune monitoring
  - 9 yellow top 8.5 mL ACD tubes and 2 red top 10 mL tubes prior to vaccine administration.
  - 2 red top 10 mL tubes drawn 2 hours post vaccine administration.
- Vaccine #7 with vitals pre and post vaccine (see Section 10.1.4 for details)
- Blood for CRS
  - 2 red top 10 mL tubes drawn 2 hours (+ 15 minutes) post vaccine administration.

Prior to each vaccine, patients will be pre-medicated with Zofran (up to 8 mg) (or similar anti-emetic) and Tylenol (up to 650 mg maximum). If a patient experiences any post-vaccine reactions (please refer to Section 9.1.4 for a description of previous reactions), the patient also may be pre-medicated with prednisone at one or more subsequent vaccine visits, at the discretion of their provider. Vital signs will be taken before each study vaccine. All vaccines are given via the i.m. route. Please refer to Section 9.1.4 for post-vaccine reaction recommendations.

Vital signs will be taken every 30 minutes after each vaccine administration during the post-vaccine injection monitoring period. Per Section 10.1.4, it will not be considered a deviation if the vitals are not captured exactly at the 30-minute time mark. As long as the patient is monitored for the protocol-specified amount of time, and as long as the correct number of sets of vitals have been collected for that time period, the patient will be considered sufficiently monitored. The PI or sub-investigator may extend monitoring periods at any given visit if they feel it is clinically indicated for the patient's safety.

12.3 End of Treatment

If a patient progresses or once all vaccinations are completed, the treatment phase of the study will be over. Patients will then be followed for serious adverse events (SAEs) for 30-days after their final treatment intervention. Concomitant medication will not be tracked after the final treatment intervention. An attempt will be made to collect immune monitoring blood at the time of progression (for patients who completed all vaccines without prior progression). If a patient undergoes other clinically indicated resections or biopsy while still being followed, the study team may request a sample of leftover tissue for DNA and RNA analysis.

12.4 End of Study

Once all data has been collected, analyzed, and completely evaluated on all subjects as defined in the statistical analysis plan and subjects have completed follow up, expired, withdrawn, or been lost to follow up, the study will be ended. The study may also be terminated early for any reason by the PI.

12.5 Early Withdrawal of Subject(s)

12.5.1 Criteria for Early Withdrawal

Subjects may voluntarily withdraw from the study at any time. Subjects may be withdrawn prior to registration by the PI and considered screen failures; the PI may also withdraw a subject from the study at any time based on his/her discretion. Reasons for PI-initiated withdrawal may include, but are not limited to the following:

- Patients with an active infection requiring treatment or having an unexplained febrile illness (T_{max} > 99.5 F);
- Patients requiring an increase in corticosteroids doses that cannot be reduced again to a dose ≤4 mg dexamethasone per day or equivalent. Exceptions include nasal or inhaled steroid. If the patient, at the time of first vaccination, requires a dose >2 mg , the patient will be removed from the study before vaccination and replaced. Please refer to Section 9.1.5 for additional information.
- Disease progression requiring treatment beyond the radiation necrosis dose of bevacizumab;
- AEs (CTCAE version 5);
- Abnormal laboratory values (clinically significant);
- Protocol deviation;
- Administrative issues;
- Pregnancy.

12.5.2 Follow-up Requirements for Early Withdrawal

All patients who receive a vaccine will be followed for PFS and survival (please see Section 15.6.5) until death or loss to follow-up. Subjects will also be assessed and followed for SAE monitoring/safety analysis for 30 days following the last vaccine injection.

12.5.3 Replacement of Early Withdrawal(s)

Subjects who are withdrawn by the PI prior to vaccine injection will be replaced.

As described in Sections 9.1.2 and 15.5.1.1, a patient who withdraws from the study before completion of the observation period that ends 30 days after vaccine #5 for a reason unrelated to the occurrence of a DLT will be replaced for the purpose of this initial acute safety monitoring. Though replaced for the acute safety monitoring, the patient will be included in other study analyses.

12.6 Study Assessments

12.6.1 Medical History

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

- All past medical and surgical history;
- Current medications;

- Changes in physical or neurologic symptoms;
- Any AEs.

12.6.2 Physical Exam

Vital signs and physical and neurologic examinations will be assessed and recorded along with a KPS score prior to enrollment and at each visit. MMSE will be performed prior to vaccine #1 for baseline documentation assessment.

12.6.3 Radiologic Evaluations

Patients with newly diagnosed GBM will be imaged by MRI as per standard of care for eligibility and baseline measurements, and to assess progression prior to vaccine therapy; although the purpose of this study is not to detect tumor responses, any evidence of tumor response will be determined using the Duke PRTBTC SOP. Progression will be defined in accordance with RANO 2.0 criteria, or "≥25% increase in the sum of bi-perpendicular diameters of enhancing disease on two separate scans at least 4 weeks apart; or new lesions; or substantial worsened T2/FLAIR; or substantial clinical decline" [62].

12.6.4 Immunologic Evaluations

Immunological response evaluations for baseline values will be conducted on the screening blood sample. Blood work and total blood draw volumes for immunologic monitoring are described in Table 3. The immune monitoring correlative studies will be conducted by a designated Study Scientist under the direction of Dr. Kent J. Weinhold, who oversees The Duke Immune Profiling Core (DIPC). DIPC is a School of Medicine Shared Resource (SOM-SR), whose academic home is the Department of Surgery. The DIPC Mission Statement is 'To identify immunologic signatures that predict clinical outcomes'. DIPC has extensive experience in designing and optimizing highly complex phenotypic and functional flow-based panels. They have developed a total of 82 different polychromatic flow cytometry (PFC, up to 18 colors) panels to support various immune monitoring efforts. Twenty of these PFC panels include functional ICS assays; the remaining phenotypic panels measure T cell subsets [activation, maturation, regulation, proliferation, apoptosis, T-helper subsetting (Th1, Th1/Th17, Th2, Th17, Th9, Th22, Tfh), B cell subsets (naïve, unswitched memory, switched memory, transitional plasmablasts), monocytes (conventional and non-conventional), myeloid-derived suppressor cells (MDSC), natural killer cell subsets, dendritic cell subsets (pDC, mDC), innate pathways (inflammasome activation, toll-like receptors, pattern recognition receptors, pyroptosis), microparticles, and immune checkpoint markers used for targeted cancer immunotherapies.

The primary objective for measuring immune responses for this trial is to assess whether P30-EPS-specific polyfunctional T cells (pp65-, EphA2-, and Survivin-specific) are increased after priming or after booster vaccination. As an exploratory objective, we will also assess the diversity of the P30-EPS-specific memory T cell repertoire via TCR sequencing (Figure 6).

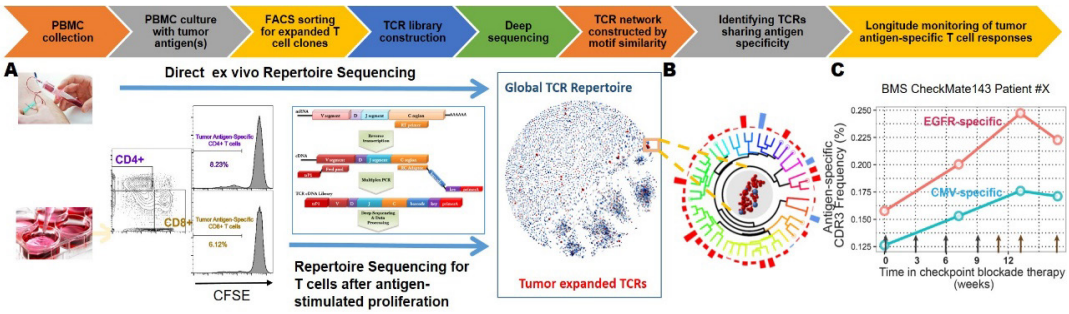


Figure 6. Experimental and computational strategies to discover tumor-antigen specific TCRs.

A) PBMCs from patients were aliquoted for direct repertoire sequencing or TCR analysis after tumor antigen stimulated-proliferation. These two sets of data were subjected to Motif Analysis, which will cluster TCR together in an unbiased manner based on their motif similarities and degrees of sharing. In this way, even with distinct TCR sequences, TCRs recognizing the tested tumor antigen from the unstimulated pool were linked to tumor antigen-specific TCRs. B) The diversity and frequency of tumor antigen-specific TCRs from a specific cluster with shared Motifs were quantified. C) With this procedure and longitude samples collected from the BMS CheckMate 143 Trial, we monitored the dynamics of T cell clonal expansion in patients with GBM with anti-PD-1 or anti-PD-1 plus anti-CTLA4 treatment. EGFR epitopes were used as a representative of tumor associated antigens; CMV pp65 epitopes represented GBM-specific but exogenous antigens.

In addition, blood will also be collected after vaccines 1, 6, and 7 as outlined in Table 3 to assess for cytokine release syndrome, which is a temporary elevation of pro-inflammatory cytokines. Serum will be separated from whole blood in a red top tube using a standard SOP and aliquoted at 0.2 mL per tube and stored at -135˚C. Blood and possibly tumor samples will also be stored for possible future research in the BTC laboratory dedicated to the analysis of the trial samples. The samples being stored are ONLY for this study. Further details are available in the ETAPA correlative laboratory manual.

12.6.5 Correlative Assessments

Tumor tissue analysis for clinical pathologic diagnosis and select biomarkers will be performed and will include both pathological diagnosis and determination of IDH 1 and 2 and MGMT promoter methylation from tissue collected from patients both archivally and from any future surgeries that the patients may undergo while on study. The tissue will be used for genetic analysis, including full genome or full exome sequencing, as well as other molecular genetic testing by Caris Life Sciences.

If the subject has any other prior or subsequent biopsies or resections, these may also be requested for the same testing. In requesting tissue from outside facilities, the study team may need to provide a copy of the subject's signed consent form to the outside facility, which shows both the subject's name and Duke medical record number. This disclosure will be described in the informed consent.

Further details on tissue processing are available in the ETAPA correlative laboratory manual.

13 SAFETY MONITORING AND REPORTING

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

13.1 AEs

An AE (AE) is any untoward medical occurrence in a subject receiving study drug and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of study drug (i.e., bevacizumab or vaccine), whether or not related to use of the study drug(s).

From the time the subject receives vaccine 1 through the End of Study visit (as defined in Section 12.4), all AEs must be recorded in the subject medical record and AEs case report form.

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

Attribution of AEs will be indicated as follows:

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

13.1.1 Reporting of AEs

A summary of all AEs (not just those considered related to the study drugs) will be kept. AEs will be categorized by organ system, relationship to which treatment, grade of severity, and resolution. Weekly review by the PI during weekly Adverse Event meeting of new AEs and the collective AEs will occur with the intention of identifying any trends or patterns in toxicity. If any such trends are identified, depending on their severity and frequency, a protocol amendment will be considered.

13.2 Serious Adverse Events (SAEs)

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

- Fatal
- Life-threatening
- Constitutes a congenital anomaly or birth defect

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

13.2.1 Reporting of SAEs

All SAEs should be reported immediately to Dr. Mustafa Khasraw (Pager: 919-206-0493) or his designee (919-684-8111). Fatal or life-threatening, unexpected AEs that are related or possibly related to the research will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the sponsor. Serious, unexpected AEs that are related or possibly related to the research but are not fatal or life-threatening will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the sponsor. Any relevant additional information obtained by the sponsor that pertains to a previously submitted IND safety report must be submitted as a Follow-up IND Safety Report as soon as possible but no later than 15 calendar days after the sponsor receives the information. At the time of the annual progress report to the FDA, a summary of the overall toxicity experience will be provided.

All AEs that are considered serious, unanticipated, and related or possibly related to the research (as defined by 21 CFR 312.32[a]) will be reported to the IRB using the appropriate SAE reporting process.

- Immediately (within 24 hours) upon learning of an unanticipated study-related death, study personnel will notify the IRB via phone or email by providing a brief summary of the event. Then within 1 week (five business days), study personnel will send to the IRB a Safety Event submission in iRIS.

- For a reportable SAE, study personnel will notify the IRB within five business days of the investigator becoming aware of the event. Study personnel will send a Safety Event submission in iRIS.

- For any other problem or event requiring prompt reporting to the IRB, within ten business days of the investigator becoming aware of the event, study personnel will send to the IRB a Safety Event submission in iRIS.

13.3 Unanticipated Adverse Device Effects

Companion *in vitro* diagnostic (IVDs) for MGMT and IDH1 are used to determine eligibility on the study. An adverse device effect is any SAE on health or safety or any life-threatening problem or death caused by, or associated with, use of the companion IVDs, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (e.g., SAEs related to receiving study drugs following inaccurate test results).

Reports of unanticipated adverse device effects related to the use of companion IVDs should be submitted to the IRB and the IND as required under § 812.150(b) within 10 working days after learning of the effect. Thereafter, study personnel will submit additional reports concerning the effect as FDA requests.

13.4 Safety Oversight Committee (SOCOMM)

The Duke Cancer Institute SOCOMM is responsible for annual data and safety monitoring of Duke University Health System (DUHS) sponsor-investigator phase 1 and 2, therapeutic interventional studies that do not have an independent Data Safety Monitoring Board (DSMB). The primary focus of the SOCOMM is the review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews include, but may not be limited to, review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the Principal Investigator. The SOCOMM in concert with the Duke Cancer Institute (DCI) Monitoring Team (see Section 14.1 for Monitoring Team description) oversees the conduct of DUHS cancer-related, greater-than-minimal-risk intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, SOPs, Good Clinical Practice (GCP), and applicable regulatory requirements.

13.5 External Data and Safety Monitoring Board (DSMB)

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

The Duke Office of Scientific Integrity-Conflict of Interest Office (DOSI-COI) reviews and manages research-related conflicts of interest. The Principal Investigator and Sub-Investigators must report conflicts of interest annually and within 10 days of a change in status, and when applicable, must have a documented management plan that is developed in conjunction with the Duke Research Integrity Office (RIO) and approved by the IRB/ Institutional Ethics Committee (IEC).

14 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Monitoring

This clinical research study will be monitored both internally by the PI, and institutionally by the Duke Cancer Institute (DCI). In terms of internal review, the PI will continuously monitor and tabulate AEs. Appropriate reporting to the Duke University Medical Center IRB will be made. If an unexpected frequency of Grade 3 or 4 events occur, depending on their nature, action appropriate to the nature and

frequency of these AEs will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The PI of this study will also continuously monitor the conduct, data, and safety of this study to ensure that:

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

DCI Protocol Review and Monitoring systems (PRMS) review of this protocol begins with an initial review by the Protocol Review and Monitoring Committee (PRMC). PRMC new protocol review focuses on scientific relevance, study design, adequacy of biostatistical input, protocol prioritization, feasibility of completing the study within a reasonable time frame and risk assessment of the trial. The PI will abide by PRMC assessment of the level of risk, which will determine the intensity of subsequent DCI monitoring. PRMC also conducts annual scientific progress reviews on protocols that are open to enrollment and focus on protocol prioritization, accrual and scientific progress. These reviews are conducted at the time of IRB annual renewals and documentation of all PRMC reviews will be maintained in eIRB/iRIS systems.

A determination for the degree of monitoring conducted by the DCI monitoring team is made at the time of initial PRMC approval to commensurate with the type and level of intervention, phase, endpoints, degree of risk, size and complexity of the protocol. A formal, independent monitoring will be conducted by the DCI monitoring team according to the risk level and monitoring plan assigned by the PRMC until the study is closed to enrollment or subjects are no longer receiving study drug or other interventions that are more than minimal risk. Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns. Monitoring visits may also be initiated upon request by DUHS and DCI Leadership, PRMC, SOCOMM, a sponsor, an investigator, or the IRB.

The DCI monitoring team reviews the adequacy of informed consent, enrollment of eligible patients, implementation of protocol-specified procedures and treatment, adequacy of data collection, and appropriateness of AE monitoring and reporting. The DCI monitoring team presents final monitoring reports to the DCI SOCOMM highlighting safety concerns and unresolved issues. The SOCOMM, at a convened meeting, assigns an overall rating of satisfactory, marginal, or unsatisfactory to reflect the overall quality of data, regulatory, consent, eligibility, study conduct and AE reporting. Corrective action plans (CAPs) are developed, implemented, and evaluated as indicated. The SOCOMM will notify the sponsor-investigator and DUHS IRB when significant safety concerns are identified.

The SOCOMM in concert with DCI monitoring team conducts data and safety monitoring for DUHS sponsor-investigator phase 1 and 2, therapeutic interventional

oncology studies that do not have an independent DSMB. These reviews occur at a minimum annually and more frequently for the high risk studies. The SOCOMM safety reviews include review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The SOCOMM, at a convened meeting, assigns a rating of satisfactory when adequate accrual with lack of excessive toxicity is present.

14.2 Audits

The Duke University Office of Audit, Risk and Compliance (OARC) may conduct confidential audits to evaluate compliance with the protocol and the principles of good clinical practice (GCP). The Principal Investigator (PI) agrees to allow the OARC auditor(s) direct access to all relevant documents and to allocate his/her time and the time of the study team to the auditor(s) in order to discuss findings and any relevant issues.

OARC audits are designed to protect the rights and well-being of human research subjects. OARC audits may be routine or directed (for cause). Routine audits are selected based upon risk metrics generally geared towards high subject enrollment, studies with limited oversight or monitoring, Investigator initiated Investigational Drugs or Devices, federally-funded studies, high degree of risk (based upon AEs, type of study, or vulnerable populations), phase 1 studies, or studies that involve Medicare populations. Directed audits occur at the directive of the IRB or an authorized Institutional Official.

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

14.3 Data Management and Processing

14.3.1 Case Report Forms

The electronic CRF (eCRF) will be the primary data collection document for the study and is developed in conjunction with statistical oversight. The CRFs will be updated in a timely manner following acquisition of new source data. Only the PI, the study coordinator, the data management team, and the clinical trials manager are permitted to make entries, changes, or corrections in the eCRF.

An audit trail will be maintained automatically by the electronic CRF management system. All users of this system will complete user training, as required or appropriate per DCI requirements and other regulations.

14.3.2 Data Management Procedures and Data Verification

Access to electronic databases will be managed by the PRTBTC Data Manager.

Completeness of entered data will be checked automatically by the eCRF system and users will be alerted to the presence of data inconsistencies. Additionally, the data management team and the statistical team will cross-reference the data to verify accuracy. Missing or implausible data will be brought to the attention of the PI requiring an appropriate response (i.e., confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

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

14.3.3 Study Closure

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

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

15 STATISTICAL METHODS AND DATA ANALYSIS

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

15.1 Study Design Overview

Adults with newly diagnosed, primary GBM who are IDH wild type, have unmethylated MGMT promoter, and have undergone resection will be eligible for this study. Patients who meet eligibility criteria will be offered enrollment at the clinic visit after XRT/TMZ if they can start treatment within 8 weeks of XRT/TMZ completion (and as long as this is felt to be in the best interest of the patient by the treating physician). A maximum of 36 patients will be accrued to this study. After the initial dose escalation portion is complete, patients will be tested for CMV status however enrollment will be first come, first serve, and the slots will not be allocated based on CMV results.

All patients will receive P30-EPS vaccine (EphA2, pp65, and survivin peptides each linked to P30) suspended in 10% DMSO in water, pooled, and mixed with 20 μg/kg of Hiltonol®. All P30-EPS vaccines are given initially in a Priming Phase [on days 1, 4 (± 1 day), 8 (± 1 day), 15 (± 2 days), and 22 (± 2 days)], and then in a Booster Phase (on day 84 and day 140, both ± 7 days).

The primary objective of this trial is to evaluate the safety of P30-EPS vaccines in patients with newly diagnosed GBM, while exploring the impact of the treatment regimen on the immunologic response of the patient.

15.2 Analysis Sets

Sections 15.5, 15.6 and 15.7 provide the statistical analysis plans to address each individual objective. Included in these analyses is a description of the analysis set, or the set of patients that will be considered in the planned analyses.

15.3 Patient Demographics and Other Baseline Characteristics

The clinical and sociodemographic characteristics of all patients treated will be summarized by dose level and CMV status using descriptive statistics (e.g., means/standard deviations, percentiles, frequencies).

15.4 Treatments

A frequency distribution of the number of vaccines a patient receives will be generated for each dose level within each CMV stratum.

15.5 Primary Objective

The primary objective of this trial will be to evaluate the safety profile of a cancer vaccine comprised of the universal epitope P30 linked to EphA2, CMV pp65, and survivin (P30-EPS) peptides.

Safety will be assessed within two separate strata (patients who are CMV seropositive and patients who are CMV seronegative) given our expectation that the AE profile among CMV seropositive patients and CMV seronegative patients may differ.

Within each stratum, a standard 3+3 study design will be used to escalate or de-escalate the vaccine dose. The starting dose of the P30-EPS vaccine is 300 µg/peptide/dose, and the dose will be escalated to 400 µg/peptide/dose. In the event that 300 µg/peptide/dose is deemed unsafe, the dose will be de-escalated to 200 µg/peptide/dose. If 200 µg/peptide/dose is not considered safe, then the P30-pp65 peptide may be removed from the vaccine combination; the protocol will be amended in consultation with the FDA to administer a vaccine without P30-pp65.

Section 15.5.1 describes the initial toxicity monitoring guidelines; whereas, Section 15.5.2 describe the overall monitoring guidelines. Section 15.5.3 describes other summaries of AEs that will be generated.

15.5.1 Dose Escalation / De-Escalation Guidelines

Within each stratum (CMV seropositive and CMV seronegative), cohorts of 3 patients will be accrued to the study in a manner similar to a standard phase 1 study to assess the toxicity associated with the vaccine consisting of EphA2, pp65, and survivin P30-EPS peptides linked to P30 (P30-EPS). Three dose levels are under consideration: 200 µg/peptide/dose (dose level minus 1), 300 µg/peptide/dose (dose level 1), and 400 µg/peptide/dose (dose level 2), with the starting dose being 300 µg/peptide/dose.

Within each stratum, the first 3 patients will be treated with 300 µg/peptide/dose. The enrollment of the first 2 patients treated with 300 µg/peptide/dose will be staggered by 2 weeks. After accrual of the first cohort of 3 patients, accrual will be temporarily suspended.

15.5.1.1 General Acute Monitoring Guidelines

The following monitoring guidelines apply to both the CMV seropositive and CMV seronegative strata. The period of acute toxicity monitoring begins with vaccine #1 and lasts until 30 days after vaccine #5.

- If 0 of the first 3 treated patients within a stratum experience a DLT during the observation period beginning at vaccine #1 and ending 30 days after vaccine #5, or 1 of 6 treated patients within a stratum experiences a DLT, then patient accrual will be re-initiated. If the current dose level is 300 µg/peptide/dose, the next cohort of patients will be treated at 400 µg/peptide/dose. Otherwise, subsequent patients will be treated at the same dose level. The maximum dose at which 0 of 3, or 1 of 6 patients experience a DLT during priming will be the MTD.

- If 1 of the first 3 treated patients within a stratum experiences a DLT during the observation period beginning at vaccine #1 and ending 30 days after vaccine #5, an additional cohort of 3 patients will be treated within the same stratum at the same dose level.

- If 2 of the first 3 or 6 treated patients experience a DLT during the observation period beginning at vaccine #1 and ending 30 days after vaccine #5, the next cohort of patients within that stratum will be treated at a lower dose. Treatment modifications for the CMV seropositive stratum are described below in Section 15.5.1.2.

- The MTD is the highest dose level at which 0/3 or 1/6 patients experience a DLT.

If a patient withdraws from the study before completion of the observation period that ends 30 days after vaccine #5 for a reason unrelated to the occurrence of a DLT, the patient will be replaced for the purpose of this initial acute safety monitoring. This patient will remain a part of all other analyses, if possible.

15.5.1.2 Option to Remove P30-pp65 from vaccine

If the 200 µg/peptide/dose is determined to not be safe, consideration will be given to the removal of the P30-pp65 component of the vaccine.

Sera collected post-vaccination will be analyzed for drug hypersensitivity (histamine) or CRS (Th1 cytokines). If CRS is demonstrated, P30-pp65 will be removed from the vaccine, the data will be evaluated and the protocol will be

amended in consultation with the FDA. This is based on our experience with the PERFORMANCE trial (NCT02864368) where we found that pp65-specific memory T cells in the CMV seropositive patient stratum were likely mediating pp65 long peptide vaccine-specific toxicity. Therefore, removal of pp65-P30 will likely prevent toxicity in this patient population.

15.5.1.3 Expansion at MTD

Once the MTD is determined for CMV seropositive and CMV seronegative patients, additional patients will be treated at that dose level..

15.5.2 Overall Toxicity Monitoring Guidelines

With vaccinations potentially being administered through day 140, we will be monitoring within each stratum for the occurrence of unacceptable toxicities throughout vaccine treatment.

Tabulated below (Table 4) are the conditions under which accrual to a stratum will be temporarily suspended and data carefully reviewed to determine if accrual should be continued or terminated, and whether elements of the treatment regimen should be modified. Accrual will also be suspended whenever a death occurs that is possibly, probably, or definitely related to vaccine treatment.

Table 4: Conditions for Temporary Accrual Suspension

Number of Patients Within CMV Stratum Treated at a Particular Dose Level	Number of patients with unacceptable toxicity
0-6	≥2
7-9	≥3
10-14	≥4
≥15	≥5

15.5.3 Other AE Summaries

Ultimately, AEs will be tabulated in different ways for the manuscript summarizing the results of this study, annual reports for the SOCOMM, and the final report included within ClinicalTrials.gov. All patients who receive an initial vaccination will be included in these summaries.

- For the summary of AEs and SAEs within the ClinicalTrials.gov report, the percentage of patients who experience each type of AE regardless of attribution will be summarized.
- For the manuscript, AEs that are possibly, probably, and definitely treatment-related will be summarized. For each type of toxicity, the maximum grade experienced by each patient will be summarized with frequency distributions. Toxicity experienced with vaccines 1-5 may also be summarized separately from those experienced with vaccines 6 and 7.
- Two tabulations will be generated for review by the SOCOMM including one that includes all toxicities regardless of attribution, and another that includes

only toxicities that are possibly, probably, and definitely related to study regimen. For each of these tabulations, the maximum grade of each type of toxicity experienced by each patient will be summarized with frequency distributions.

15.6 Secondary Objectives

Secondary objectives include an assessment to assess the immunogenicity of P30-tethered EphA2, CMV pp65, and survivin, and to describe the survival and PFS of patients treated. The analyses of immunogenicity described below will focus primarily on those patients treated at the MTD.

Analyses proposed for secondary analyses assume methods appropriate for normally distributed data are appropriate. If underlying assumptions for planned analyses are violated, plans will be modified. Alternative analytic methods may also be considered to assess the robustness of inferences.

15.6.1 Secondary Objective: Tumor Antigens (pp65) After Priming Vaccination

Objective: To assess whether pp65-specific polyfunctional T cells increase after priming vaccination with P30-linked pp65 peptide

Analysis Set: Analyses will be conducted in 2 different datasets:

i. All CMV seropositive patients treated at the CMV seropositive MTD who have day 1 (pre-vaccine #1), day 22 (post-vaccine #5) and day 84 (post-vaccine #6) PBMCs collected, and

ii. All CMV seronegative patients treated at the CMV seronegative MTD who have day 1, day 22 and day 84 (post-vaccine #6) PBMCs collected.

Outcome: For analyses associated with pp65 among CMV seropositive patients, the outcome is mean fold increase in pp65-specific T cells between day 1 and 22, and the mean fold increase in pp65-specific T cells between day 1 and 84.

For analyses associated with pp65 among CMV seronegative patients, the outcome is percentage of patients who develop pp65-specific polyfunctional T cells due to vaccination between day 1 and 22, and the percent of patients who develop pp65-specific polyfunctional T cells due to vaccination between day 1 and 84.

Hypothesis: Vaccination with pp65 tethered to P30 will increase the number of pp65-specific $CD8^+$ polyfunctional T cells over baseline (pre-vaccine day 1).

Analysis Plan: Among CMV seropositive patients, a one-sample t-test will assess the mean fold increase in pp65-specific T cells between day 1 and day 22.

Among CMV seronegative patients, the proportion of patients with a pp65-specific response between day 1 and day 22 will be estimated.

Similar analyses will be conducted for the change between day 1 and day 84. Analyses for the change between day 1 and day 84 will be limited to those who receive all of the initial 5 vaccines. Additional analyses will focus on patients who receive vaccine #6 and #7 without completion of all of the 5 initial vaccines.

Power Analysis: Within the ERADICATE study (Pro00000580), the mean (SD) fold change in 3-function polyfunctionality among CMV seropositive patients is 1.93 (1.38). Assuming normality, there is 78% power to detect a similar increase in the fold change in CD8+ with at least 6 CMV seropositive patients (α=0.05).

Prior studies of pp65 mRNA-loaded DC vaccine conducted at Duke in CMV seronegative patients using this technique showed a pp65-specific T cells response rate of 2.5%. If the true response rate among CMV seronegative patients treated with the P30-EPS vaccine is similar, the expected number of responders is 0. Tabulated below is the probability of observing 1 or more responders out of 12 CMV seronegative patients as a function of the true (and unknown) response rate. The probability of observing 1 or more responders will be greater if more than 12 CMV seronegative patients are evaluable for this endpoint.

True Response Rate	Probability of Observing 1 or more Responders
2.5%	0.26
5%	0.46
10%	0.72
15%	0.86
20%	0.93
25%	0.97
30%	0.99

15.6.2 Secondary Objective: Tumor Associated Antigens (EphA2 and survivin) After Priming Vaccination

Objective: To assess whether EphA2- and survivin specific polyfunctional T cells increase after priming vaccination with P30-linked EphA2 and survivin peptides

Analysis Set: All patients treated at the MTD who have day 1, day 22 and day 84 PBMCs collected

Outcome: For analyses associated with EphA2 and survivin, the mean fold increase in EphA2- and survivin-specific T cells between day 1 and day 22 and the mean fold increase between day 1 and day 84.

Hypothesis: Vaccination with EphA2 and survivin linked to P30 will increase the number of EphA2- and survivin-specific $CD8^+$ polyfunctional T cells over baseline (pre-vaccine day 1).

Analysis Plan: A one-sample t-test will assess whether there is a significant mean fold increase in EphA2- and survivin-specific T cells between day 1 and day 22.

A similar analysis will explore the change between day 1 and day 84.

Power Analysis: Within the ERADICATE study (Pro00000580), the mean (SD) fold change in 3-function polyfunctionality among CMV seropositive patients is 1.93 (1.38). Assuming normality, there is >99% power to detect a similar increase in the fold change in EphA2-, and survivin specific T cells with 18 or more patients (α=0.05).

If the MTD among CMV seropositive patients is different from the MTD for CMV seronegative patients, then these analyses may be conducted separately within the two strata.

15.6.3 Secondary Objective: Tumor Antigens (pp65) After Booster Vaccination

Objective: To assess whether pp65-specific polyfunctional T cells increase after booster vaccination with P30-linked pp65 peptide

Analysis Set: Analyses will be conducted in 2 different datasets:

i. All CMV seropositive patients treated at the CMV seropositive MTD who have day 84 and day 140 PBMCs collected, and

ii. All CMV seronegative patients treated at the CMV seronegative MTD who have day 84 and day 140 PBMCs collected.

Outcome: For analyses associated with pp65 among CMV seropositive patients treated at the CMV seropositive MTD, the outcome is mean fold increase in pp65-specific T cells between day 84 and 140.

For analyses associated with pp65 among CMV seronegative patients treated at the CMV seronegative MTD, the outcome is percent of patients who develop pp65-specific polyfunctional T cells due to vaccination between day 84 and day 140.

Hypothesis: Booster vaccination with pp65-linked P30 will increase the number of pp65-specific $CD8^+$ polyfunctional T cells on day 140 over day 84.

Analysis Plan: Among CMV seropositive patients treated at the CMV seropositive MTD, a one-sample t-test will assess the mean fold increase in pp65-specific T cells between day 84 and day 140.

Among CMV seronegative patients treated at the CMV seronegative MTD, the proportion of patients with a pp65-specific response between day 84 and day 140 will be estimated.

Analyses described will be conducted among patients who complete all of the first 5 vaccines. Analyses will be conducted separately for patients who receive vaccine #6 and #7 without completion of the first 5 vaccines.

15.6.4 Secondary Objective: Tumor Associated Antigens (EphA2 and survivin) After Booster Vaccination

Objective: To assess whether EphA2- and survivin-specific polyfunctional T cells increase after booster vaccination with P30-linked EphA2 and survivin peptides

Analysis Set: All patients treated at the MTD who have day 84 and day 140 PBMCs collected

Outcome: For analyses associated with EphA2 and survivin, the outcome is mean fold increase in EphA2- and survivin-specific T cells between day 84 and day 140

Hypothesis: Booster vaccination with EphA2 and survivin linked to P30 will increase the number of EphA2- and survivin-specific $CD8^+$ polyfunctional T cells on day 140 over day 84.

Analysis Plan: A one-sample t-test will assess whether there is a significant mean fold increase in EphA2- and survivin-specific T cells between day 84 and day 140.

If the MTD among CMV seropositive patients is different from the MTD for CMV seronegative patients, then these analyses may be conducted separately within the two strata.

15.6.5 Secondary Objective: Survival and PFS

Objective: Describe the survival and PFS of patients who received a P30-EPS vaccine

Analysis Set: All patients who initiate P30-EPS vaccine treatment

Outcome: Survival time is the time between first P30-EPS vaccination and death, or last patient follow-up if the patient remains alive. PFS is defined as the time between first P30-EPS vaccination and initial failure (disease progression or death). PFS is censored at the last follow-up, if the patient remains alive without disease progression.

Hypothesis: Descriptive

Analysis Plan: The Kaplan-Meier estimator will describe the survival of patients treated on this protocol. Median survival will be estimated with confidence intervals, along with estimates for 12, 24, and 36-month survival.

Additionally, the Cox proportional hazards model may explore the impact of CMV status on survival and PFS outcome.

15.7 Exploratory Objectives

This study has four main exploratory objectives: (1) Assess the diversity of the P30-EPS-specific memory T cell repertoire via TCR sequencing, (2) Define the relationships between reported biomarkers of response to immunotherapy in GBM and survival, (3) Assess resected tumor for the expression of vaccine targets (i.e. EphA2, pp65, and survivin), and (4) Assess changes in the tumor immune microenvironment, mutational heterogeneity/clonality, neoantigen depletion, and their association with survival.

Additional objectives which are not explicitly stated here may also be explored. These additional analyses will be motivated by the results of analyses addressing the objectives stated a priori, as well as changes or new additional to the clinical literature.

15.7.1 Exploratory Objective: Diversity of the P30-EPS-specific memory T cell repertoire via TCR Sequencing

Objective: Assess the diversity of the P30-EPS-specific memory T cell repertoire via TCR sequencing

Analysis Set: Patients who have samples available for TCR analyses at baseline and after 5 P30-EPS vaccines

Outcome: Change in normalized entropy of P30-EPS-specific T cell clonotypes between baseline and post-vaccine 5

Hypothesis: P30-EPS vaccination will broaden the number of different T cell clones recognizing a single antigen

Analysis Plan: Patients' samples will be divided into two aliquots: one will be directly sequenced to record the overall repertoire; the other will be stimulated and expanded by the designated P30-EPS for 7 days and then sequenced. The raw sequencing reads from baseline (before vaccine #1) and an assessment after vaccine #5 will be stored in fastq files and pre-processed using the IMSEQ [67] software using reference barcode sequences from International Immunogenetics Information System [68]. TCR sequences are characterized by aligning the input to the reference V, D and J regions as well as the random regions. We will focus on the TCR beta-chain. To control alignment qualities, we will exclude reads with average quality score below 10. After the pre-processing step, the IMSEQ software will produce the clone-types of T cells. We will then derive the clonotypes and their relative frequencies. Clones which have fewer than 20 reads or 0.5% of total reads will be excluded. Alternatively, we will leverage a new method we developed to perform error-tolerance mapping. This method will not only produce the mapping results, but also assign a posterior probability to each read that measures how likely it belongs to a clone type.

With the P30-EPS-stimulated samples, we will perform Motif analysis to enrich P30-EPS-specific TCR clonotypes. All TCR clonotypes from the sequencing will be

included to generate all possible amino acid motifs with varying lengths using the R tcR package. Two separate pools of CDR3 sequences were created initially for each of these motifs from directly sequenced aliquot and P30-EPS-stimulated aliquot. To account for different sequence depths across samples, we will normalize the number of CDR3s containing each motif by the total number of CDR3s containing all motifs in each sample. The motif frequency ratios between directly sequenced aliquot and P30-EPS-stimulated aliquot will be compared. A higher ratio represents more abundant motifs in P30-EPS-stimulated aliquot compared with directly sequenced one, enabling the identification of a set of candidate motifs as P30-EPS-specific. The importance of each motif will be evaluated using both Mean Decrease Accuracy and Mean Decrease Gini in the random forest. A standard receiver operating characteristic (ROC) approach will be applied to evaluate the predictive power of each motif. These analyses will be performed using R *randomForest* [69] and *ROC* packages.

After the P30-EPS-specific CDR3 motifs are identified, we will map these motifs back to each individual TCR clones within the directly sequenced PBMC samples. These TCR clones will be considered to be P30-EPS-specific. For a given clone type, we will sum up all the read posterior probabilities if they exceed 0.2, and record the sum as the weighted frequency of the clone type. To test for overall clonal expansion, we will calculate the normalized entropy of the clonotypes at each time point. A decrease in entropy implies a decrease in diversity in clonotypes, and subsequently the expansion (increase in frequency) of one or more specific clonotypes. For each patient, the change in entropy over time will be computed. A Wilcoxon signed rank test will assess whether diversity has increased with vaccination.

15.7.2 Exploratory Objective: Define the relationship between reported biomarkers of response to immunotherapy in GBM and survival

Objective: Define the relationship between reported biomarkers of response to immunotherapy in GBM and survival

Analysis Set: Any available tumor tissue from patients on-study prior to and/or after immunotherapy with P30-EPS vaccine

Outcome: Association between the following: TMB, tumor-intrinsic Erk1/2 signaling, TMEM119+ (microglia) cell density, peripheral Ki67+ CD8 T cells, and gene expression signatures of MHC-class II, IFN-γ, and cell cycle/proliferation

Hypothesis: In patients with higher TMB, increased immune activation (Ki67+ CD8 T cells, MHC-class II, IFN-γ), and lower Erk1/2 signaling and microglial density, P30-EPS vaccination is more likely to enhance the antitumor immune response.

Analysis Plan: The following biomarkers that have been reported to be predictive for response to immunotherapy in GBM, will be assessed on surgically resected tumor

tissue using the noted approaches, including quantitative immunohistochemistry (qIHC), bulk RNAseq, and whole exome sequencing (WES):

- MEK-ERK activating mutations - WES**;** Zhao et al Nat Med 2019 [70]
- High frequency of p-ERK+ tumor cells - qIHC; Arrieta et al Nat Cancer 2021 [71]
- High MHC-class II gene expression - RNA-seq; Arrieta et al Nat Cancer 2021 [71]
- IFNγ signature; Cell cycle gene signature repression - RNAseq; Cloughesy et al Nat Med 2019 [72]
- Exhaustion signatures, inhibitory receptors - RNA-seq, qIHC; Lee et al. Nat Comm 2021 [73]
- Putative neoantigen depletion - WES, RNA-seq; Gromeier et al Nat Comm. 2021; preliminary data [74]

MEK-ERK activating mutations will be queried using existing WES data. qIHC will be performed for p-ERK1/2 and TMEM119 as described by Arrieta et al *Nature Cancer 2021* [71].

To define T cell exhaustion/inhibitory receptors, we will also similarly quantify and define spatial PD-L1, CTLA4, PD1, TIGIT, and TIM3 expression in all tumor samples, using qIHC. Bulk RNA-seq data will be used to determine MHC-class II, IFNγ and cell cycle gene expression signatures, as well as differential expression of inhibitory receptors (TIGIT, TIM3, CTLA4, and PD1).

Neoantigen depletion will be predicted using the pVAC-seq pipeline. Briefly, for the pVAC-seq pipeline, neoantigens will be defined at 8-11-mer peptides from exonic nonsynonymous single nucleotide variants that bind to a patient's respective HLA class I molecule with a binding affinity score <500nM stronger than the wildtype peptide using NetMHCpan51. Neoantigen density will be expressed as ratio of observed to expected neoantigens. Expected neoantigen levels will be determined using a previously empirically derived rate of mutation that assumes no selection against neoantigen-causing mutations normalized to the silent mutation load for each patient's tumor [75]. MHC class I epitope predictors are imperfect in predicting actual MHC class I binding epitopes [76]. Thus, nonsynonymous mutation/TMB ratios will also be used to predict neoantigen depletion: lower non-synonymous mutations out of total mutations is surrogate measure of neoantigen density [77].

Each feature will be tested for its association with post- P30-EPS vaccine survival to determine if other biomarkers are superior in identifying P30-EPS vaccine responsive GBM patients. We will also test the relationships between each biomarker to determine if they identify the same patients, and to further define the nature of patients living longer after P30-EPS vaccination.

The associations between the various biomarkers will be explored with scattergrams, and correlation (Pearson or Spearman) with adjustment for multiple comparisons. For each biomarker, Receiver-operating curves will be performed to

assess the optimal threshold for differentiating short versus long-term survival; the sensitivity and specificity of each biomarker will be compared. A Cox proportional hazards model will assess the joint effect of all biomarkers on survival to discern if an integrated biomarker approach is more effective in predicting survival outcome. Correction for multiple comparisons using gene expression analyses will use the False Discovery Rate.

15.7.3 Exploratory Objective: Assess resected tumor for expression of the vaccine targets (i.e. EphA2, pp65, and survivin)

Objective: Assess resected tumor for expression of the vaccine targets (i.e. EphA2, pp65, and survivin)

Analysis Set: Any available tumor tissue from patients on-study prior to and/or after P30-EPS vaccination

Outcome: Association between the vaccine targets (EphA2, pp65, and survivin) and clinical outcomes

Hypothesis: Expression of vaccine targets will associate with survival and other exploratory endpoints

Analysis Plan: RNA-seq analysis of tumor tissue will be used to determine expression levels of vaccine targets, in transcripts per million and expression will be linked with survival and PFS after P30-EPS vaccination. For patients with available post-P30-EPS vaccination tumor tissue, pre- vs post P30-EPS vaccine expression levels of antigens will be determined to test if antigen escape/immunoediting of vaccine antigens occurs as well as if T cell inflammation post- P30-EPS vaccination associates with vaccine antigen expression.

15.7.4 Exploratory Objective: Assess changes in the tumor immune microenvironment, mutational heterogeneity/clonality, neoantigen depletion, and their association with survival.

Objective: Assess changes in the tumor immune microenvironment, mutational heterogeneity/clonality, neoantigen depletion, and their association with survival.

Analysis Set: Any available tumor tissue from patients on-study prior to and/or after P30-EPS vaccination

Outcome: Since productive immune surveillance is associated with immunoediting (e.g., depletion of immunogenic glioma cell clones), the associations between putative neoantigen depletion, mutational heterogeneity, gene expression signatures of T cell inflammation, and survival will be tested.

Hypothesis: Neoantigen depletion, reduced mutational heterogeneity, and increased T cell inflammation will be associated with improved survival in GBM patients treated with the P30-EPS vaccine.

Analysis Plan: The following assessments will be made if samples are available from patients who have baseline tumor tissue and tissue from a second surgical procedure after they have received the P30-EPS vaccine.

1. *TMB suppression and putative neoantigen*: will be determined to test if low TMB status in GBM is associated with TMB suppression and/or neoantigen depletion, or post-P30-EPS vaccination survival.
2. *Gene expression signatures:* T cell inflammatory/MHC class II gene expression signatures, and unbiased gene set enrichment analyses (Biocarta and Reactome), and long (overall survival >24 months) vs short (overall survival <24 months) survival will be performed to determine if patients with low TMB and/or longer survival after P30-EPS vaccine exhibit distinct gene expression signatures and/or changes in specific gene expression patterns in response to P30-EPS vaccine.
3. *Mutational heterogeneity/clonality:* will be predicted using subclonal deconvolution of WES for all samples using a pre-validated pipeline employing Mutect2 [78] for SNV and InDel calling, FACETS [79] for CNV calling, and PyClone-VI [80] for sub-clonal deconvolution. Cancer Cell Fractions (CCF) for each mutation within each sample will be determined and sample-mean CCFs will be tested for their relationship with post- P30-EPS vaccine survival and TMB.

To more comprehensively define the relationships between TMB and post- P30-EPS vaccine survival with the GBM tumor immune microenvironment and mutational clonality, single nuclei RNA sequencing (snRNAseq) may be performed for a subset of tumor samples. In addition, tumors from any patients surviving >24 months post-P30-EPS vaccine will also be analyzed, in order to ensure an informative comparison of post- P30-EPS vaccine survival with these features.

snRNAseq will be performed on snap frozen tissue to qualitatively define the tumor microenvironment and link tumor microenvironment features with vaccine antigen expression, mutational landscapes, and survival. snRNAseq will also be used to define exhaustion and immunosuppressive phenotypes of tumor-associated macrophages in post- P30-EPS vaccine tissue, as well as whether these cell type densities or states differ according to TMB or long vs. short survival.

These analyses are exploratory and will primarily leverage qualitative comparisons. Changes in TMB, neoantigen levels, cellular phenotypes, and mutation clonality will be qualitatively compared within patients and tested using paired t-tests. Comparisons of TMB/neoantigen depletion, gene expression, and clonality to TMB and post-P30-EPS vaccine survival will be performed using Spearman correlation, adjusted for multiple comparisons. Similarly, the change in each of these features will be correlated. SnRNAseq (tumor immune microenvironment) based analyses are largely intended to be qualitative assessments that will be tested for correlation with TMB and post- P30-EPS vaccine survival. Differential expression analysis

(DEseq2) for snRNAseq data with be adjusted for multiple comparisons using the False Discovery Rate.

15.8 Interim Analysis

Interim efficacy analyses will not be conducted. Interim assessments of toxicity is described in Section 15.5.1.

15.9 Sample Size Calculation

The primary correlative analysis in this study will assess immune outcome at Day 140. To address these analyses, we intend to treat a sufficient number of patients with vaccine at its MTD so that approximately 18 patients will provide immune outcome data at Day 140. Based upon Stupp[6], we crudely estimate that approximately 75% of treated patients will provide Day 140 data. Hence, we anticipate that we will treat 24 patients at the vaccine's MTD. We anticipate that two-thirds of these patients treated will be CMV seronegative.

Prior to the determination of the vaccine MTD, additional patients may be treated at other dose levels. We anticipate that no more than 12 more patients (6 CMV seropositive and 6 CMV seronegative) will be treated at dose levels other than the MTD. Hence, no more than 36 patients will be accrued to this study.

Patients who withdraw from study participation before initiation of vaccine treatment will not be counted towards this accrual goal. Power calculations for selected secondary study endpoints may be found in Section 15.6.1 and 15.6.2.

16 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

16.1 Regulatory and Ethical Compliance

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

16.2 DUHS Institutional Review Board and DCI Protocol Review and Monitoring Committee

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

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

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

16.3 Informed Consent

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

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

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

16.4 Study Documentation

Study documentation includes but is not limited to source documents, CRFs, monitoring logs, appointment schedules, study team correspondence with sponsors or regulatory bodies/committees, and regulatory documents that can be found in the DCI-mandated "Regulatory Binder," which includes, but is not limited to, protocols and protocol amendments, approved informed consent forms, FDA Forms 1572, CAP and Clinical Laboratory Improvement Act (CLIA) laboratory certifications, and clinical supplies receipts and distribution records.

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

involved in the clinical trial. When possible, the original record should be retained as the source document. However, a photocopy is acceptable provided that it is a clear, legible, and an exact duplication of the original document.

An eCRF will be the primary data collection document for the study. The CRFs will be updated within two weeks of acquisition of new source data. Only approved study staff are permitted to make entries, changes, or corrections in the CRF. For eCRFs, an audit trail will be maintained by the Duke IT database.

16.5 Privacy, Confidentiality, and Data Storage

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained. A Duke Research Data Lifecycle (DRDL) plan will be approved by the appropriate institutional Site Based Research group.

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

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using a Duke IT database, which is housed by the DCI. Access to electronic databases will be limited to the Principal Investigator, key personnel, statisticians, Pharmacy personnel, and the PRTBTC data manager. Data stored on portable memory devices will be de-identified. Subject data will be deleted from the portable memory device at the earliest opportunity. The security and viability of the IT infrastructure will be managed by the DCI and/or Duke Medicine.

Upon completion of the study, research records will be archived and handled per DUHS Human Research Protections Program (HRPP) policy.

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

16.6 Data and Safety Monitoring

Data and Safety Monitoring will be performed in accordance with the DCI Data and Safety Monitoring Plan (DSMP). For a more detailed description of the DSMP for this protocol, refer to Sections 13 and 14.

16.7 Protocol Amendments

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

Principal Investigator must inform the IRB and all other applicable regulatory agencies of such action immediately.

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

16.8 Records Retention

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

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

16.9 Conflict of Interest

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

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

16.10 Registration Procedure

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

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18 APPENDICES

18.1 Appendix A – Hiltonol Patient Administration Guide

Please refer to separate document.

18.2 Appendix B – Hiltonol Patient Diary

Please refer to separate document.