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A Phase I Study

ATTAC-P: A Phase 1 Trial of CMV RNA-Pulsed Dendritic Cells with Tetanus-Diphtheria Toxoid Vaccine in Pediatric Patients and Young Adults with WHO Grade IV Glioma, Recurrent Malignant Glioma, or Recurrent Medulloblastoma

DUKE CANCER INSTITUTE

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Amended version 1:	7/27/2018	Modifications requested by CPC in Section 13.1; clarifications to inclusion criteria in Section 10.1
Amended version 1.1:	8/6/2018	Change in diagnosis for newly diagnosed population from GBM to WHO Gr.IV glioma in Sections 4.1, 4.3, 5, 7, 8.1, 10.1, 11.7.1, and 14.4.2; revisions made to the observation period for unacceptable toxicity in Sections 4.6, 8.1.7, 14.1, and 14.4.2.1; added language regarding risk to benefit ratio and patient assent in Sections 6.3, 15.3, and 15.4.
Amended version 2:	11/16/2018	Clarify schema in Section 5; include description of bridge chemotherapy for recurrent patients after leukapheresis while their CMV-DCs are being generated (see Sections 4.3 and 8.1); Remove restriction of only up to 2 patients with prior craniospinal radiation as the 1st patient met this description and was able to generate enough PBMCs for at least 3 vaccines with one leukapheresis procedure (Sections 4.6, 14.4.1, 14.6); revisions to inclusion and exclusion criteria in Section 10; revisions to schedule of events in

Amended version 3:	4/12/2019	*Table 1* in Sections 11 and 11.4; corrections made to Table 2; new Section 8.1.3; change study pause for safety evaluation from after the 3rd patient to after the 4th patient (Sections 4.6 and 14.4.2.2). Increase eligible age for patients with WHO grade III or IV glioma (Sections 4.1, 7, 8.1, 10.1, 14.4.2); grade IV patients without measurable/progressive disease can be included on recurrent arm (Sections 4.1, 4.3, 5, 7, 8.1, 10.1); allow 2nd leukapheresis, if applicable, to be obtained at additional times during the study (Sections 4.3, 8.1, 8.1.1, 11.5, 14.4); if patients PBMCs generate only 1-2 vaccines, they may remain on study and receive all vaccines generated (Sections 4.3, 4.6, 8.1, 8.1.1, 9, 11.5, 11.7.1, 14.6); add exclusion criterion for steroid dose (Section 10.2); patients who are receiving bevacizumab may continue to do so (10.1, 10.2); window of time for obtaining blood for immune monitoring altered (Sections 8.1, 11 [Table 1], 11.4); bridge therapy option for all patients, not just recurrent added (Sections 4.3, 8.1, 8.1.3).

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3 LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATRT	Atypical Teratoid-Rhabdoid Tumor
BTIP	Brain Tumor Immunotherapy Program
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CMV-DCs	Long-Lamp CMV RNA-pulsed Dendritic Cells (study drug)
CNS	Central Nervous System
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DC	Dendritic Cell
DCI	Duke Cancer Institute
DI	Dose Intensified
DSMB	Data and Safety Monitoring Board
DUHS	Duke University Health System
ELISPOT	Enzyme-Linked Immunospot
FDA	Food and Drug Administration
GBM	Glioblastoma
GCP	Good Clinical Practice
Gd	Gadolinium
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
GMP	Good Manufacturing Practice
ICH	International Conference on Harmonization
IFC	Imaging Flow Cytometry
IRB	Institutional Review Board
IV	Intravenously
KPS	Karnofsky Performance Status
LPS	Lansky Performance Status
MG	Malignant Glioma
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
OARC	Office of Audit, Risk, and Compliance
OS	Overall Survival
PBMCs	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PD	Progressive Disease
PFS	Progression Free Survival
PI	Principal Investigator
PJP	Pneumocystis jiroveci pneumonia
PNET	Primitive Neuro-Ectodermal Tumor
Pp65-flLAMP	Full-Length LAMP Protein
RDSP	Research Data Security Plan
RT	Radiation Therapy
SAE	Serious Adverse Event
SOC	Safety Oversight Committee
Td	Tetanus Toxoid
TILs	Tumor Infiltrating Lymphocytes
TMZ	Temozolomide
ULN	Upper Limit Normal
VDLNs	Vaccine-Site Draining Lymph Nodes

4 PROTOCOL SYNOPSIS AND RESEARCH SUMMARY

4.1 Purpose

The purpose of this study is to determine the feasibility and safety of administering CMV RNA-pulsed dendritic cells (CMV-DCs) to children and young adults up to 35 years old with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma. Evidence for efficacy will also be sought.

Primary Objectives:

1. Determine the feasibility of producing CMV-DCs from the leukapheresis product collected from children and young adults up to 35 years old with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma.
2. Determine the safety of CMV-DCs in eligible patients with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma

Exploratory Objectives:

1. Describe change from baseline in T cell response to CMV-DC vaccination as measured by ELISPOT
2. Describe the overall survival (OS) and progression-free survival (PFS) in children and young adults up to 35 years old with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma.

Hypothesis:
Treatment with CMV-DCs will be feasible and safe in children and young adults up to 35 years old with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma.

4.2 Background and Significance

The incidence rate of childhood (ages 0-14 years old) primary malignant and non-malignant brain and Central Nervous System (CNS) tumors in the United States (U.S.) is 5.54 cases per 100,000 with an estimated 3,560 new cases expected to be diagnosed in the U.S. in 2018 [1]. While significant improvements have been made in the treatment of some of these children, recurrent brain tumors are generally incurable and are routinely treated with maximal surgical resection followed by radiation therapy (RT) and concomitant chemotherapy [2]. Even with improved understanding about molecular changes in pediatric tumors, only a small number of targeted therapies have demonstrated clinical benefit. Immune therapies, with their potential for highly specific tumor killing or ability to recruit the endogenous immune system against a tumor, hold great potential and are being pursued. Whereas immune checkpoint blockade has shown some promise in children with rare hyper-mutated brain tumors, responses have not been durable in most cases and these agents have limited clinical impact for most pediatric brain tumors, due in part to the lower tumor mutational burden in most pediatric tumors [2, 3]. Given that the mutational landscape and immune environment in children vary from that of adults, pediatric-focused studies are necessary to optimize immune-based therapies for treating children and young adults.

4.3 Design and Procedure

This will be a phase 1 study evaluating CMV-DC administration with Td preconditioning and GM-CSF adjuvant in children and young adults up to 35 years old with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma. This safety study will enroll a maximum of 10 patients. Eligible patients will undergo leukapheresis to obtain autologous cells for preparation of CMV-DCs. If necessary, patients who received prior craniospinal radiation (which may cause long-term suppression of their PBMCs) may undergo a second leukapheresis procedure, in an attempt to obtain adequate PBMCs to generate at least three DC vaccines.

If the number of PBMCs collected from the leukapheresis (or two leukapheresis procedures for those with previous craniospinal radiation) is less than the number needed to make at least one vaccine, the patient will be withdrawn from the study.

Newly Diagnosed Patients: After the leukapheresis procedure is completed, patients with newly diagnosed WHO grade IV glioma will undergo standard of care radiation therapy (RT) with or without concurrent TMZ prior to dose intensified (DI) adjuvant TMZ.

Recurrent Patients or WHO IV patients without recurrence: After leukapheresis procedure(s) are completed, patients may be given "bridge" therapy while the CMV-DCs are being generated (≥ 3 weeks) if needed. This decision regarding bridge therapy will be made by the treating neuro-oncologist on a case-by-case basis depending upon the patient's clinical needs. Leukapheresis procedure(s) will ideally be completed prior to initiation of bridge therapy; however, the leukapheresis procedure(s) may be done during bridge therapy, at the discretion of the PI.

Review of Appropriateness of Continuing Protocol Participation: For all patients, eligibility criteria must be confirmed prior to proceeding with DI TMZ. Patients will not proceed with DI TMZ if they do not have sufficient PBMCs to generate CMV-DCs for at least one vaccine. If bridge therapy is provided for patients during the time the vaccines are being generated (≥ 3 weeks or recurrent patients and ≥ 4 weeks for newly diagnosed patients), the patient must still meet the inclusion and exclusion criteria regarding prior treatments described in Section 10, in order to proceed with 21-day DI TMZ. Newly diagnosed patients must complete the full course of radiation to remain eligible for the study (minimum ~54 Gy). Eligibility for newly diagnosed patients will be confirmed after they have completed the standard of care radiation therapy; patients may be removed from study at that time due to not meeting eligibility or at the discretion of the PI.

Initiation of Experimental Therapy for All Patients: For patients continuing protocol participation, a single cycle of 21-day DI TMZ will be administered. On day 22 (+3 days) of their DI TMZ cycle, patients will receive the study drugs (Td preconditioning and CMV-DC vaccine containing GM-CSF, Vaccine #1) intra-dermally; Vaccine #2 and #3 will occur at 2-week intervals (± 2 days) after Vaccine #1. Study drug administration will be divided equally between left and right inguinal regions. All further available vaccines will occur monthly, approximately every 4 weeks (± 5 days). Preconditioning with Td will occur 6-24 hours prior to Vaccine #1 and Vaccine #4+, if further vaccines are available. Patients who are ≥ 18 years of age will also receive a Td booster during screening. Patients will receive as many vaccinations as can be made from their leukapheresis procedure(s).

Feasibility will be defined based on the production of at least three CMV-DC vaccines of $2x10^7$ cells from the initial leukapheresis or initial two leukapheresis procedures for patients who have had previous craniospinal radiation (only expected in recurrent medulloblastoma patients).

4.4 Selection of Subjects

Please see Section 10.

4.5 Duration of Study

Unless an unacceptable toxicity is observed, patients will receive at least all the CMV-DC vaccines generated for them. While the patient remains on study, they will be followed with MRI and clinical evaluations every three months, at the discretion of the treating physician, as possible. Upon progression or removal/withdrawal from the study, patients who have received at least one CMV-DC vaccine will be followed periodically, if possible, for progression, future medications to treat their brain tumor, and survival.

4.6 Data Analysis and Statistical Considerations

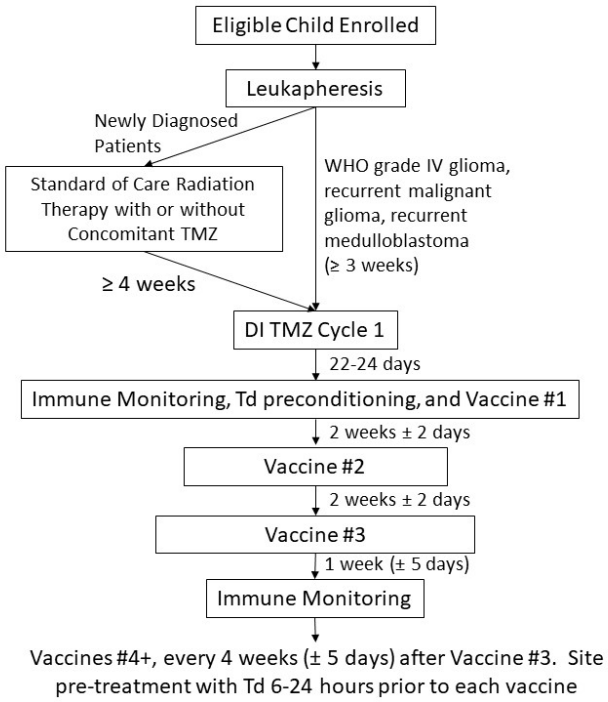
The primary objectives for this study include the assessment of feasibility for generating at least three vaccines from the pre-treatment leukapheresis of eligible patients and the safety of treating these patients with the CMV-DC vaccine.

Feasibility will initially be assessed separately among patients with and without prior craniospinal radiation. A maximum of 10 patients may be accrued to this study in total to achieve the number of evaluable patients.

Only patients who have one or more vaccinations generated from their leukapheresis will begin treatment with the CMV-DC vaccines. Safety is defined in terms of the proportion of patients who experience an unacceptable adverse event as defined in Section 8.1.7 after Td preconditioning, prior to initial vaccination and before the ~30 days follow-up after the 3rd vaccination, but before the 4th vaccination, if given. Safety monitoring rules that are provided in Section 14.4.2.2 combine the treatment experiences of patients with and without prior craniospinal radiation. After 4 patients have been enrolled and treated (i.e. the last patient has received Vaccine #3 and been observed for 30 days after Vaccine #3), the study will be paused for evaluation of safety before proceeding with further enrollment. A patient will be considered evaluable for the assessment of safety if the patient experiences an unacceptable adverse event before the time when Vaccine #4 would be administered, or if the patient receives three vaccines and completes the endpoint observation period that extends ~30 days after Vaccine #3 (approximately to the time that the 4th vaccine would be administered).

Survival and PFS will be described, as well as the percent change in pp65-specific T cell responses.

5 STUDY SCHEMA



6 BACKGROUND AND SIGNIFICANCE

6.1 Study Disease

The incidence rate of childhood (ages 0-14 years old) primary malignant and non-malignant brain and Central Nervous System (CNS) tumors in the United States (U.S.) is 5.54 cases per 100,000, with an estimated 3,560 new cases expected to be diagnosed in the U.S. in 2018 [1]. While significant improvements have been made in the treatment of some of these children, recurrent brain tumors are generally incurable and are routinely treated with maximal surgical resection followed by radiation therapy (RT) and concomitant chemotherapy [2]. Even with improved understanding about molecular changes in pediatric tumors, only a small number of targeted therapies have demonstrated clinical benefit. Immune therapies, with their potential for highly specific tumor killing or ability to recruit the endogenous immune system against a tumor, hold great potential and are being pursued. Whereas immune checkpoint blockade has shown some promise in children with rare hyper-mutated brain tumors, responses have not been durable in most cases and these agents have limited clinical impact for most pediatric brain tumors, due in part to the lower tumor mutational burden in most pediatric tumors [2, 3]. Given that the mutational landscape and immune environment in children vary from that of adults, pediatric-focused studies are necessary to optimize immune-based therapies for treating children and young adults.

CMV antigens have been identified in pediatric tumors including glioblastoma (GBM) and medulloblastoma, and may make excellent anti-tumor immunotherapeutic targets [4, 5]. Vaccination and adoptive T cell strategies targeting cytomegalovirus (CMV) in humans in other contexts have been found to be safe and effective. Previous studies examining adult patients with GBM have demonstrated the feasibility, safety, immunogenicity, and potential clinical efficacy of this approach [6] and Duke is currently participating in a multicenter, phase 2 study (Pro00084478) of CMV RNA-pulsed dendritic cells (DCs) in adult patients with GBM.

This study combines temozolomide (TMZ) therapy, tetanus toxoid (Td), and granulocyte macrophage-colony stimulating factor (GM-CSF) with the CMV-DC study drug to treat children and young adults with GBM, recurrent malignant glioma, or recurrent medulloblastoma. Temozolomide (TMZ) has been widely used to treat brain tumors and is routinely given to children with high grade gliomas, regardless of the fact that it is not curative and shows modest efficacy in children. Therapeutic TMZ induces lymphopenia, typically a negative effect of chemotherapy; however, repeated DC vaccination during recovery from lympho-depletion can induce potent humoral and cellular immunologic responses [7-9]. GM-CSF is a powerful adjuvant capable of stimulating macrophage function, inducing proliferation and maturation of DCs, and is able to enhance T-lymphocyte stimulatory function. It has been safely used in children with solid tumors following chemotherapy to counteract neutropenia [10, 11]. Intradermal administration of GM-CSF enhances the immunization efficacy at the site of administration in a dose-dependent fashion. Significant anti-tumor immunity has been demonstrated in preclinical murine studies in which irradiated, stably transfected tumor cell lines secreting GM-CSF have protected against subsequent tumor challenge, especially against intracerebral tumors. Finally, Td is a routinely used vaccine in the normal human population that has been shown in pilot studies to function as a potent adjuvant that may enhance DC trafficking to vaccine-site draining lymph nodes (VDLNs). Our previous studies have shown that successful DC migration to VDLNs may be a requisite for clinical activity of RNA-pulsed DCs, and administration of Td prior to vaccination may improve the effectiveness of DC vaccines in patients with GBM.

6.2 Study Agent

DCs are potent immuno-stimulatory cells that continuously sample the antigenic environment of the host and specifically activate CD4+ and CD8+ T cells and B cells [12, 13]. They are part of many networks of the immune system, and DCs represent a promising biologic entity for use as an immunotherapy. Potent immune responses and encouraging clinical results have been seen in Phase 1 and 2 human clinical trials in systemic cancers treated with DCs [14-30].

Numerous animal studies [31, 32], including many of our own [33-35], and a few clinical studies [31, 36] have demonstrated potent antitumor responses using DC-based immunotherapy against adult malignant gliomas (MGs). Specifically, CMV-targeted vaccines have been tested in adult patients at Duke in numerous IRB-approved studies, including Pro00003877 (ATTAC) and ATTAC-GM, Pro00000580 (ERADICATE), Pro00000581 (REGULATE), Pro00054740 (ELEVATE), Pro00065241 (AVERT), and Pro00084478 (ATTAC-II).

Adjuvants frequently used with vaccination include Freund's incomplete adjuvant, bacilli Calmette-Guerin, QS-21, and diphtheria toxoid. Supplemental cytokines have been used as well for the adjuvant immunological effects [37]. Granulocyte macrophage-colony stimulating factor (GM-CSF) has been commonly used, as it is commercially available and well tolerated. GM-CSF is capable of stimulating macrophage function, inducing proliferation and maturation of DCs, and is able to enhance T-lymphocyte stimulatory function. Intradermal administration of GM-CSF enhances the immunization efficacy at the site of administration in a dose dependent fashion at an optimal dose of 125 μg [38]. Significant anti-tumor immunity has been demonstrated in preclinical murine studies in which irradiated, stably transfected tumor cell lines secreting GM-CSF have protected against subsequent tumor challenge, especially against intracerebral tumors [39, 40]. Furthermore, the potency of GM-CSF has been demonstrated in a phase 1 clinical trial in melanoma patients vaccinated with irradiated autologous melanoma cells engineered to secrete GM-CSF [41]. The immunization sites were intensely infiltrated with T lymphocytes, DCs, macrophages, and eosinophils in 100% of evaluable patients. Extensive tumor destruction was seen in 11 of 16 patients. Both cytotoxic T cells and antibody responses were associated with this tumor destruction. Hence, GM-CSF has an extensive track record as both a growth factor and an adjuvant, is commercially available, and has an acceptable toxicity profile. Our experience in multiple studies at Duke, including the ACTIVATE study (Pro00000580), supports the use of GM-CSF and sets the precedent for this phase 1 study.

Td is a routinely used vaccine in the normal human population that has been shown in pilot studies to act as a potent adjuvant to enhance DC trafficking to VDLNs [6]. Our previous studies have shown that successful DC migration to VDLNs may be a requisite for clinical activity of RNA-pulsed DCs and, as such, administration of Td prior to vaccination may improve the effectiveness of DC vaccines in patients.

6.2.1 Pre-clinical experience

Systemic immunization using DCs co-cultured with uncharacterized tumor homogenate [34], whole tumor RNA [35], unidentified peptides eluted from tumor cells by gentle acid washing [32], or a distinct peptide encompassing the tumor-specific EGFRvIII mutation [33] have been shown to induce humoral and cell mediated systemic immune responses and to prolong the survival of rodents with brain tumors.

In our laboratory [34], inbred VM/Dk mice received three or four weekly intraperitoneal injections of autologous bone marrow-derived DCs transiently co-cultured with tumor homogenate. The homogenate was derived from a syngeneic murine astrocytoma cell line derived from a spontaneously occurring astrocytoma in the inbred VM/Dk mouse strain. Splenocytes from mice immunized in this way were able, *in vitro*, to lyse the astrocytoma cell line that was used to generate the tumor homogenate. They were also able to lyse other astrocytoma cell lines derived from the same inbred mouse strain, but they had no effect against syngeneic fibroblasts. Similarly, these immunized mice also demonstrated a significantly increased antibody titer against the astrocytoma cell line used to generate the homogenate. In addition, mice immunized with DCs transiently co-cultured with tumor homogenate that were subsequently challenged with a lethal dose of this astrocytoma cell line intracerebrally were found to have a median survival >160% longer than those immunized with DCs cultured without tumor homogenate (P=0.016). In addition, 50% of the mice treated with the tumor homogenate-supplemented DCs survived long-term without any evidence of tumor growth and also survived a re-challenge of tumor cells indicating that a sustained anti-tumor immune response had been established. These findings are especially significant in light of the fact that the astrocytoma cell line used is known to

secrete the immunosuppressive agent transforming growth factor-β (TGF-β[1]), which is secreted by most human gliomas [42-46].

In another report from our laboratory [35], C57BL/6 mice received three weekly intraperitoneal injections of autologous bone-marrow derived DCs co-cultured with tumor homogenate or whole tumor RNA derived from the poorly immunogenic B16F10 melanoma cell line. Standard *in vitro* cytotoxicity assays again revealed that splenocytes harvested from mice immunized with DCs transiently co-cultured with either tumor-derived homogenate or whole tumor RNA were able to lyse B16F10 melanoma cells, but not unrelated tumor cells from the same major histocompatibility complex (MHC[2]) background. In this experiment, mice immunized with autologous bone-marrow derived DCs co-cultured with tumor homogenate or whole tumor RNA increased median survival by >233% (*P*=0.0006) and 48% (*P*=0.0001), respectively, relative to mice immunized with DCs co-cultured with tumor homogenate or whole tumor RNA derived from an unrelated tumor with the same MHC background. In addition, 8/13 (61.5%) in the specific homogenate group and 4/10 (40%) in the specific RNA group survived beyond the endpoint of the study without evidence of tumor. Immunization of mice with pre-existing tumors with specific tumor homogenate also demonstrated the potency of this immunization approach by increasing survival by 62.5% relative to controls. An inflammatory infiltrate composed of mononuclear cells and polymorphonuclear leukocytes was only identified in mice treated with DCs co-cultured with tumor homogenate that matched the intracerebral tumor challenge.

In a recent report from another laboratory [32], the survival of tumor-bearing rats injected subcutaneously with autologous bone marrow-derive DCs co-cultured with peptides eluted from tumor cells with a gentle acid wash was significantly prolonged compared to tumor-bearing rats receiving equivalent numbers of DCs co-cultured with peptides acid-eluted from normal astrocytes (*P*< 0.05). Median survivals in these groups were 35 and 22 days, respectively. In addition, three of the twelve rats (25%) treated with DCs co-cultured with acid-eluted tumor peptides remained alive at the end of the experiment. In addition, immuno-histochemical analysis of five animals from each group in this experiment documented an increased peri-tumoral and intratumoral infiltration of CD8+T cells, and to a lesser extent CD4+ T cells and macrophages, in the group treated with DCs co-cultured with peptides acid-eluted from tumor cells when compared to controls.

6.2.2 Clinical experience

We have compared the ability to generate DCs from adult patients with malignant brain tumors and patients undergoing craniotomy for non-tumor related procedures using a simple DC generation method described previously [47] to generate human DCs by culturing peripheral blood mononuclear cells (PBMCs[3]) in media supplemented with granulocyte-macrophage colony stimulating factor (GM-CSF[4]) and interleukin-4 (IL-4[5]). The phenotype of DCs from both tumor and normal populations were identical and were characterized as being highly positive for HLA-ABC and HLA-DR, the co-stimulatory molecules CD80 and CD86, and the DC/monocyte marker CD11c, but negative for the monocyte marker CD14. The cells were negative for the B and natural killer (NK[6]) cell lineage markers, CD19 and CD56, respectively, which is consistent with published DC phenotypes.

The number of clinical trials using DC immunotherapy in adult patients with MGs has been on the rise. A handful of these adult studies are summarized below. In the published study by Yu *et al.*[31], patients received biweekly intradermal injections of peripheral blood derived DCs pulsed with uncharacterized peptides eluted from the surface of autologous glioma cells by gentle acid washings. All patients were required to complete a course of RT and were off steroids at the time of immunization. Toxicity was minimal and included only mild fever and lymphadenopathy. There was no clinical or radiographic evidence of

[1] TGF-β, transforming growth factor-β
[2] MHC, major histocompatibility complex
[3] PBMCs, peripheral blood mononuclear cells
[4] GM-CSF, granulocyte-macrophage colony stimulating factor
[5] IL-4, interleukin-4
[6] NK, natural killer

autoimmune encephalomyelitis in any patient and no serious adverse events occurred. The immunization resulted in enhanced cytotoxic T lymphocyte (CTL[7]) activity in 4/7 patients and both cytotoxic and memory T-cells were found to have infiltrated the patient's tumors whom underwent reoperation after immunization. Although this study was performed in a selected population of patients, the median survival of 455 days in the treated group compared very favorably with an institutional control group where median survival was only 257 days. Unfortunately, no clinical responses were seen and any antigen-specific immune response could not be characterized because the immunizing antigens were not characterized.

In another Phase 1/2 trial, tumor lysate pulsed DCs were given safely to ten patients who received immunizations every 3 weeks for a minimum of one and a maximum of 10 immunizations. There were only two minor clinical responses seen. Of 5 patients evaluated by enzyme-linked immunospot (ELISPOT[8]) before and after vaccination, T-cells reactive against tumor lysate-pulsed DCs were increased in two patients [48].

In a more recent study, De Vleeschouwer *et al.* [49] investigated the safety and clinical response in both adults and children > 3 years of age with recurrent MG to DCs pulsed with tumor lysate. Fifty-six patients underwent a surgical resection followed by intradermal injection of DCs on one of three different treatment schedules: DCs at week 1 and 3 and then every 4 weeks, 5 DC vaccinations every 2 weeks and then every 4 weeks, or 4 weekly DC vaccinations with intradermal boosts of autologous tumor lysate. No serious adverse events were seen and the authors concluded that the DC regimen was safe in both children and adult patients. Univariable analysis indicated that younger age and total resection were better prognostic factors for this DC treatment regimen.

In addition to the aforementioned studies, a handful of other studies have been performed with DCs in the pediatric population. One study by Ardon *et. al.* [50] demonstrated the clinical feasibility of using DCs loaded with autologous tumor cell lysate in children with recurrent malignant glioma. In this study, 45 children were divided up into groups that received identical vaccination schedules to those listed in the De Vleeschouwer study described above with one additional group that had the DCs grown with different enhancing molecules. Each DC vaccine was given intra-dermally at a median dose of $2.8x10^6$ cells. Overall, the study determined that DC vaccination was safe and feasible in children with high grade glioma, medulloblastoma, primitive neuro-ectodermal tumor (PNET), ependymoma, and atypical teratoid-rhabdoid tumor (ATRT). Additionally, this DC vaccination method resulted in a subgroup of long-term survivors with an OS over 24 months. Another study using tumor lysate-loaded DCs in children with WHO grade III and IV glioma demonstrated that DC treatment was tolerable and feasible in pediatric patients, albeit with some limitations [51]. Three children (one with recurrent GBM, one with newly diagnosed GBM, and one with anaplastic astrocytoma) received 2-4 DC vaccinations at $1x10^6$ cells per injection. In summary, there is experience in administering DC vaccines to children with brain tumors and studies confirm that there are no major toxicities associated with the treatment.

Our research team has extensive experience with DC vaccines in adults including, most recently, a clinical trial of 11 patients with newly diagnosed glioblastoma who were given upfront DI TMZ, followed by administration of CMV-targeted DC vaccines ($2x10^7$ cells) administered bilaterally in the groin [52]. The primary objective of this study was to evaluate the safety and feasibility of vaccinating newly diagnosed patients with CMV-targeted DCs mixed with GM-CSF after DI TMZ. No patients experienced DC-related AEs during the study; however, there was one reaction to GM-CSF after vaccine 8, which was alleviated by removing the GM-CSF from the DC vaccine mixture. Another study performed at our center involved CMV-targeted DC vaccine administration ($2x10^7$ cells) following Td preconditioning [6]. Patients given Td had increased migration of DCs to VDLNs and showed a significant increase in both progression-free survival (PFS) and overall survival (OS) as compared to patients who were preconditioned with mature DCs. Additional studies we have performed include EGFRvIII-targeted DC vaccine studies in GBM [53, 54]. And, importantly, we have also administered DC

[7] CTL, cytotoxic T lymphocyte
[8] ELISPOT, enzyme-linked immunospot

vaccines to children with recurrent medulloblastoma in the RE-Match study (Pro00018020), giving our research team extensive experience with DC vaccines.

6.3 Study Purpose/Rationale

While significant improvements have been made in the treatment of children with these diagnoses, the outcome for those with recurrent brain tumors remains grim. Immune therapies, with their potential for highly specific tumor killing and/or ability to recruit the endogenous immune system against a tumor, hold great potential and are being pursued. Whereas immune checkpoint blockade has shown some promise in children with rare hyper-mutated brain tumors, responses have not been durable in most cases and these agents have limited clinical impact for most pediatric brain tumors, due in part to the lower tumor mutational burden (TMB) in most pediatric tumors [55]. Given that the mutational landscape and immune environment in children vary from that of adults, pediatric-focused studies are necessary to optimize immune-based therapies for treating children and young adults.

One such immune-based therapy being studied in children is the use of primed, autologous dendritic cell vaccines. As mentioned above in Section 6.2.2, a handful of studies have been performed with DCs in the pediatric population [50, 51]. These previous studies have used autologous tumor lysate to prime the autologous DCs; however, this requires that the patient undergo surgery to obtain a tumor sample for priming. To avoid that potentially unnecessary surgery, specifically for those with recurrent tumor, our study proposes the use of an antigen that is highly expressed on brain tumors, Cytomegalovirus (CMV). CMV antigens have been identified in pediatric tumors including glioblastoma (GBM) and medulloblastoma, and may make excellent anti-tumor immunotherapeutic targets [4, 5]. Vaccination and adoptive T cell strategies targeting CMV in humans in other contexts have been found to be safe and effective. Previous studies examining adult patients with GBM have demonstrated the feasibility, safety, immunogenicity, and potential clinical efficacy of this approach [6] and Duke is currently participating in a multicenter, phase 2 study (Pro00084478) of CMV RNA-pulsed dendritic cells (DCs) in adult patients with GBM.

We have demonstrated in murine models that DCs loaded with tumor-specific antigens in the form of peptides or RNA can induce potent and specific humoral and cell-mediated immune responses that are effective against murine intracerebral (i.c.) tumors, including a syngeneic murine astrocytoma, without inducing autoimmunity [33, 34, 56]. Our previous clinical experience has also shown that DC vaccines, in combination with standard of care radiation therapy and chemotherapy, are capable of generating potent, tumor-specific immune responses and clinical radiographic responses in patients with malignant gliomas. We and others have also shown that antigens derived from CMV are contained within malignant gliomas and may serve as potent and specific immunotherapy targets. Vaccination and adoptive T cell strategies targeting CMV in humans in other contexts, including the targeting of lesions within the CNS, have been found to be safe and effective. We have also shown that DCs generated from patients with GBM and loaded with pp65-LAMP mRNA are capable of generating CD4+ and CD8+ T cells that produce IFN-γ and kill malignant astrocytes infected with CMV in an antigen-specific fashion. We have found that tumor infiltrating lymphocytes (TILs) isolated from these patients are significantly enriched for T cells that specifically recognize CMV antigens, suggesting that this response may be important in the biology of these tumors. We have recently demonstrated that fusion of the immuno-dominant antigen CMV pp65 to full-length LAMP targeting protein enhances activation and expansion of CMV-specific T cells *in vitro*. Based on previous clinical trials and our experience in DC administration in adults with brain tumors, we are proposing a CMV-DC vaccine study in children and young adults that incorporates DI TMZ, Td preconditioning, and a GM-CSF adjuvant.

TMZ is used as a standard of care chemotherapy for patients with high grade glioma. Therapeutic TMZ induces a profound lymphopenia that may inhibit anti-tumor vaccination; however, we have shown in an animal model, that TMZ-induced lympho-depletion actually enhances the induction of antigen-specific immune responses after active and adoptive immunotherapy. This suggests that DI TMZ may enhance the DC vaccination immune response, and therefore this tactic will be applied to this study.

Td is a routinely used vaccine in the normal human population that we have shown in pilot studies may function as a potent adjuvant to enhance DC trafficking to VDLNs [6]. Our previous clinical studies at Duke have shown that successful DC migration to VDLNs may be a requisite for clinical activity of RNA-pulsed DCs and administration of Td prior to vaccination may improve the effectiveness of DC vaccines in patients with GBM. The CMV-DC vaccines with the combination of Td preconditioning and GM-CSF adjuvants should result in a favorable risk to benefit ratio that provides a safe and feasible treatment regimen for children and young adults with WHO grade IV glioma, recurrent malignant glioma, and with recurrent medulloblastoma.

7 OBJECTIVES AND ENDPOINTS

	Objective	Endpoint	Analysis
Primary	Determine the feasibility of producing CMV-DCs from the leukapheresis product collected from children and young adults up to 35 years old with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma.	Percentage of patients for whom three or more vaccines can be generated from the pre-treatment leukapheresis	See Section 14.4.1
Primary	Determine the safety of intradermal administration of CMV-DCs in children and young adults up to age 35 with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma.	Percentage of patients that experience unacceptable toxicity from CMV-DC administration	See Section 14.4.2
Exploratory	Describe changes in T cell response to CMV-DC vaccination	Percent change in pp65-specific T cell responses assessed by Imaging Flow Cytometry (IFC)	See Section 14.4.3.1
Exploratory	Describe OS and PFS	Percentage of patients alive without disease progression 6 months after initiation of DI adjuvant TMZ with CMV-DC vaccination (6-month PFS) Percentage of patients alive 1 year after initiation of DI adjuvant TMZ with CMV-DC vaccination (1-year OS)	See Section 14.4.3.2

8 INVESTIGATIONAL PLAN

8.1 Study Design

This is a phase 1 study to confirm the safety of CMV-DCs in children and young adults up to 35 years old with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma. . Eligible children or adult patients will undergo a leukapheresis (3 blood volumes) for peripheral blood mononuclear cells (PBMCs). A maximum of 10 patients with WHO grade IV glioma, recurrent malignant glioma, or recurrent medulloblastoma will be treated with CMV-DCs on this phase 1 safety study. Patients who received prior craniospinal radiation will be recruited into a separate cohort as described in Section 14.4.2. Patients who have previously undergone craniospinal radiation may have a second leukapheresis procedure.

All patients will undergo leukapheresis, as soon as possible after enrollment onto the study. After the leukapheresis procedure is completed, patients with newly diagnosed WHO grade IV glioma will undergo standard of care radiation therapy (RT) with or without concurrent TMZ prior to dose intensified (DI) adjuvant TMZ. Also, after the leukapheresis procedure is completed, patients may be given “bridge” therapy while the CMV-DCs are being generated, if needed. This decision regarding bridge therapy will be made by the treating neuro-oncologist on a case-by-case basis depending upon the patient’s clinical needs. Leukapheresis

procedure(s) will ideally be completed prior to initiation of bridge therapy; however, the leukapheresis procedure(s) may be done during bridge therapy, at the discretion of the PI.

Review of Appropriateness of Continuing Protocol Participation: For all patients, the eligibility criteria must be confirmed prior to proceeding with DI TMZ. Patients will not proceed with DI TMZ if they do not have sufficient PBMCs to generate CMV-DCs for at least 1 vaccine. If bridge therapy is provided during the time the vaccines are being generated, the patient must still meet the inclusion and exclusion criteria regarding prior treatments described in Section 10, in order to proceed with 21-day DI TMZ. Newly diagnosed patients must complete the full course of radiation to remain eligible for the study (minimum ~54 Gy). Eligibility for newly diagnosed patients will be confirmed after they have completed the standard of care radiation therapy; patients may be removed from study at that time due to not meeting eligibility or at the discretion of the PI.

Initiation of Experimental Therapy for All Patients: For patients continuing protocol participation, a single cycle of 21-day DI TMZ will be administered. Patients who received prior craniospinal radiation may undergo a repeat leukapheresis to obtain adequate numbers of PBMCs to generate at least one DC vaccine. DI TMZ treatment will be at 50-100 mg/m^2/day for 21 days. On Day 22 (+3 days) of the single cycle of DI TMZ, patients will receive preconditioning with Td (1 Lf) in the right inguinal region, 6-24 hours later they will receive their first CMV-DC vaccination (Vaccine #1) given intra-dermally, divided equally between both inguinal regions. Vaccine #2 and Vaccine #3 will occur at 2-week (±2 days) intervals following Vaccine #1.

After the first three vaccines, patients will be vaccinated approximately every 4 weeks (±5 days). Patients will continue with monthly vaccinations until they have used up all of the prepared CMV-DCs or until tumor progression (whichever comes first). All patients will undergo vaccine site preconditioning with Td (1 Lf) 6-24 hours prior to Vaccine #1, Vaccine #4, and all subsequent monthly vaccines. The Td preconditioning site will alternate between the right and left inguinal region with Td prior to Vaccine #1 given in the right inguinal region, Td prior to Vaccine #4 given in the left inguinal region, Td prior to Vaccine #5 given in the right inguinal region, and so on.

Blood for immune monitoring will be collected during the initial leukapheresis, prior to Vaccine #1, ~ 1 week (±5 days) after Vaccine #3, and ~1 week (±5 days) after the child's last vaccine. Patients will have an MRI within 2 weeks of starting DI TMZ as a baseline MRI, ~3-4 weeks after Vaccine #3 (at the Vaccine #4 visit, if possible), and then every 3 months at the discretion of the treating physician. Patients will be followed for progression and future medications to treat their brain tumor until death.

8.1.1 Removal of PBMCs by Leukapheresis

Prior to the initiation of radiation therapy in newly diagnosed WHO grade IV glioma patients or prior to DI TMZ initiation in all recurrent patients, subjects will undergo a leukapheresis procedure. The leukapheresis procedure will process three blood volumes of the patient's blood. Patients who have received prior craniospinal radiation may undergo a second leukapheresis, at the discretion of the treating physician, to generate up to three vaccines to meet the feasibility endpoint. As long as at least one vaccine is generated, the patient will remain on the study.

8.1.2 Concomitant Temozolomide and Radiation Therapy (Newly Diagnosed WHO Grade IV Glioma only)

Children or young adults with newly diagnosed WHO grade IV glioma who are enrolled onto the study will undergo standard of care radiation therapy with or without concomitant TMZ, at the discretion of their treating physician, prior to initiating the first cycle of adjuvant DI TMZ. Patients must complete the full course of radiation therapy (minimum ~54 Gy), in order to continue onto the DI TMZ portion of the study.

If TMZ is administered concomitantly with radiation therapy, its management will be done by the patient's treating physician in accordance with institutional practices.

8.1.3 Optional "Bridge" Treatment

Patients may be given "bridge" therapy after leukapheresis during the time needed to generate their CMV-DCs. This decision will be made by the treating neuro-oncologist on a case-by-case basis depending upon the patient's clinical needs. This includes the type of bridge therapy that is most appropriate for the given patient, e.g., metronomic dosed chemotherapy such as daily etoposide or cyclophosphamide. If bridge therapy is provided during the time the vaccines are being generated, the patient must still meet the inclusion and exclusion criteria regarding prior treatments described in Section 10, in order to proceed with 21-day DI TMZ. Patients receiving the Tumor Treating Fields (TTFields) device called Optune™ can initiate DI TMZ immediately after ceasing device use and do not have to wait ≥ 1 week.

8.1.4 Dose Intensified Temozolomide Therapy

All patients will receive one cycle of DI TMZ. For patients with newly diagnosed WHO grade IV glioma, this adjuvant DI TMZ cycle will begin ≥ 4 weeks after completing radiation therapy. For recurrent patients, DI TMZ will begin ≥ 3 weeks after leukapheresis. DI TMZ is administered once per day at 50-100 mg/m^2/day for 21 consecutive days of a 28-day cycle. Dose reduction of DI TMZ may occur, if necessary, at the discretion of the treating physician. The Principal Investigator may also choose to discontinue DI TMZ early due to declining blood counts.

8.1.5 Tetanus and Diphtheria Toxoid Booster and Site Preconditioning

Potentially eligible patients who are ≥ 18 years of age will receive a Td booster (5 Lf) during screening.

Subjects will undergo vaccine site preconditioning with a one-fifth dose of Td (1 Lf) intradermally 6-24 hours prior to Vaccine #1, Vaccine #4, and at all subsequent vaccines. Td preconditioning will be done in the right inguinal region for Vaccine #1, in the left inguinal region for Vaccine #4, in the right inguinal region for Vaccine #5, and continuing in alternating regions until all vaccines have been given.

8.1.6 DC Vaccination

On day 22 (+3 days) of the DI TMZ cycle, patients will receive Td preconditioning and 6-24 hours later will receive their first intradermal CMV-DC immunization (Vaccine #1). The two subsequent immunizations (Vaccine #2 and #3) will be given every 2 weeks (± 2 days) for a total of three doses. Each immunization will be divided equally between both inguinal regions with a total volume of ~200 µL per side. Vaccine target dose is 2x10^7 cells.

After vaccination, patients will be monitored for thirty minutes to one hour post-immunization for the development of any adverse effects. The immunization procedures will be supervised by a nurse or physician that has completed an Advanced Cardiac Life Support (ACLS) course. A cardiac resuscitation cart will be available in the immediate vicinity when performing these immunizations in case of severe allergic reactions. After Vaccine #3, subsequent vaccines will be done every 4 weeks (± 5 days) until the patient has completed all of their available vaccines or until tumor progression, whichever comes first. If patients cannot receive the vaccine within the aforementioned time windows due to an uncontrollable circumstance such as an adverse event, manufacturing delay, natural disaster, etc., a maximum 2-week delay in the vaccine may occur. If the patient's vaccine cannot be given within 2 weeks outside of the original window, the patient will be withdrawn from the study.

8.1.7 Definition of Unacceptable Toxicity

Toxicities will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5 criteria. The timeframe for assessment of unacceptable toxicity begins with Td preconditioning prior to the initial vaccination and ends ~30 days after Vaccine #3, at the time that Vaccine #4 may be given.

Any Grade 3 or any Grade 4 non-hematologic toxicity at least possibly related to Td preconditioning or DC vaccine, other than the exceptions listed below, any life-threatening event deemed probably or definitely related to the study drug (with the exception of the Grade 4 non-hematologic toxicities that are reversible within 2 weeks), or any treatment-related death will be considered unacceptable. In addition, any Grade 2 or higher autoimmune toxicities, particularly those affecting vital organs (e.g. cardiac, renal, CNS), will be considered an unacceptable toxicity if it occurs after Td preconditioning and before vaccine #4 is administered.

Exceptions to these unacceptable toxicities are as follows:

- Seizures: Due to the nature of the disease under investigation in this protocol, patients may have pre-existing 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 CTC, and will be reported as such in this protocol, seizures will not be considered an unacceptable toxicity if, in the opinion of the Principal Investigator (PI) they have not increased in frequency or can be attributed to another recognized cause of increasing seizure frequency such as sub-therapeutic anti-convulsant levels or biopsy proven tumor progression.
- New or worsening neurologic deficits: Due to the nature of the disease under investigation in this protocol, patients may have an increase in pre-existing neurologic deficits or may develop new neurologic deficits as a result of tumor invasion. A new neurologic deficit, which resolves within 2 weeks after initiation of medical therapy, will not be considered an unacceptable toxicity. New neurological symptoms will not be an unacceptable toxicity if they can be ascribed to tumor progression (e.g. documented with histopathologic analyses of biopsy tissue), or they respond to treatment (e.g. oral steroids) within 2 weeks.
- Thromboembolism: While deep vein thrombosis (DVT) is not as common in children as in adults with malignant glioma, patients may have undiagnosed pre-existing DVTs or be susceptible to the development of DVTs due to the underlying disease process. Although DVT may be defined as Grade 3 or 4 toxicities under NCI CTC, and will be reported as such in this protocol, DVT will not be considered an unacceptable toxicity 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 CTC, 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 muscle weakness and weight gain in patients taking steroids in this patient population, patients may be susceptible to the development of muscle weakness or weight gain which is due to steroids alone. Although muscle weakness may be defined as Grade 3 or 4 toxicity and weight gain $\geq$ 20% may be defined as Grade 3 toxicity under NCI CTC, and will be reported as such in this protocol, muscle weakness or weight gain will not be considered an unacceptable toxicity in this protocol if the patient has required steroids greater than physiologic doses in the interval between the immunization and the development of the toxicity.

8.1.8 Safety Considerations

8.1.8.1 Allergic Reactions to DC Immunization

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

8.1.8.2 Reactions to GM-CSF

GM-CSF is routinely used to promote white blood cell recovery in patients receiving chemotherapy where it is given at much higher doses and for a longer duration than what will be given in this study. Side effects at the standard (higher) dose of GM-CSF may include mild, temporary fever, skin reactions at the injection site, fluid retention, shortness of breath, rapid or irregular heartbeat, laboratory abnormalities (possibly suggesting kidney or liver damage), and GM-CSF tolerance , which would mean it may not be effective if given in the future. These events occurred rarely, were usually mild to moderate in severity, and were usually reversible. It is unlikely that such reactions will be observed with the lower doses of GM-CSF being used in this study.

8.1.8.3 Cerebral Edema

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

8.1.8.4 Risk of Infection

The DC injections may include the risk of infection due to potential contamination of the DC product in the laboratory. This may result in localized redness, swelling, or induration at the injection site. In the most extreme situation, this may lead to systemic bacterial/fungal sepsis and possibly death. The probability of this risk is relatively low, given the small injection volume (1 mL divided between > 2 intradermal locations) and the fact that the DCs will be strictly tested for sterility prior to each injection. The risk of infection due to potential contamination of the DCs in the laboratory will be minimized by biosafety quality assurance and testing. All cell cultures will be handled under sterile conditions in a core tissue culture facility dedicated to the processing of human cells, the MPACT facility. Prior to injection into patients, DCs must pass sterility tests for aerobic, anaerobic, and fungal organisms. Following injections, patients will be monitored throughout the course of the study for any signs and symptoms of infection. If an active infection is suspected, patients will be cultured and treated with appropriate antibiotics.

8.1.8.5 Delayed Autoimmune Diseases

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

8.1.8.6 Risks of Phlebotomy

Drawing blood or inserting an intravenous catheter into an arm vein may result in bruising or swelling in the area of the insertion, bleeding at the site of the needle puncture, light

headedness, fainting and very rarely, local infection, which may be severe. These risks are reduced by the fact that the blood will be drawn by a qualified physician, nurse or phlebotomist (a professional trained to draw blood).

8.1.8.7 Risks of Leukapheresis

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

8.1.8.8 Risks of Magnetic Resonance Imaging (MRI)

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

8.1.8.9 Allergic Reactions to Contrast Agents

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

8.1.8.10 Risks of Temozolomide

TMZ has been well tolerated by both adults and children with the most common toxicity being mild myelosuppression. Other, less likely, potential toxicities include nausea and vomiting, constipation, headache, alopecia, rash, burning sensation of skin, esophagitis, pain, diarrhea, lethargy, hepatotoxicity, anorexia, fatigue and hyperglycemia. Hypersensitivity reactions have not yet been noted with TMZ. As in the case with many anti-cancer drugs, TMZ may be carcinogenic. Rats given TMZ have developed breast cancer. The significance of this finding for human is not presently known.

Based upon degree of myelosuppression experienced by the patient, dose reduction of DI TMZ may occur, if necessary, at the discretion of the treating physician. The Principal Investigator may also choose to discontinue DI TMZ early for a patient if the patient's blood counts drop too low. Vaccines will still be administered per protocol, unless otherwise contra-indicated.

8.1.8.11 Unknown Risks

The overall risk classification of this research is unknown.

8.1.9 Concomitant Medications

8.1.9.1 Steroids

Corticosteroids should be used at the lowest dose to control symptoms of edema and mass effect, and discontinued, if possible. Use of corticosteroids should be recorded in the electronic database.

8.1.9.2 Anticonvulsants

Anticonvulsants drugs should be used or continued, if indicated. Use of such anticonvulsants should be recorded in the electronic database.

8.1.9.3 Growth Factors

Routine use of growth factors outside of the protocol study drug (i.e. G-CSF, GM-CSF, and erythropoietin) is not permitted. However, therapeutic use of G-CSF in patients with serious neutropenic conditions, such as sepsis, may be used at the investigator's discretion. Use of such growth factors should be recorded in the electronic database.

8.1.9.4 Anti-emetics

The use of anti-emetics will be at the investigator's discretion. Use of anti-emetics should be recorded in the electronic database.

8.1.9.5 Proton Pump Inhibitors

The use of proton pump inhibitors (e.g. rabeprazole, omeprazole, pantoprazole, lansoprazole or esomeprazole) is allowed on this study.

8.1.9.6 Febrile Neutropenia

Febrile neutropenia should be managed according to the local institutional guidelines. Measures include laboratory testing, blood and urine cultures, and institution of broad spectrum antibiotics.

8.1.9.7 *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis

The use of medication (i.e., Bactrim) for PJP prophylaxis in patients on chronic steroids is recommended, but is at the investigator's discretion.

8.1.9.8 Neurosurgical Procedures

If a neurosurgical procedure is required for a reason other than tumor progression (i.e. the onset of hydrocephalus), these procedures should be documented, but will not constitute criteria for declaring the patient "off study."

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

The dose for this trial was selected based on the DC cell dose used in two completed clinical trials that were done in adults with newly diagnosed WHO grade IV malignant glioma (IRB# 8108 and Pro00003877). More than 24 adult patients have been treated at this dose at Duke alone. Additionally, this same dose is being used on a larger, randomized phase 2 multicenter clinical trial in adult newly diagnosed WHO grade IV malignant glioma (Pro00084478). The dose for children in this study will be the same dose that has been given safely to adults, $2x10^7$ cells/injection; details on the dosing regimen can be found in Section 11.4.

8.3 Rationale for Correlative Studies

Autologous DCs that are generated *ex vivo* and re-injected into advanced cancers, such as GBM, have shown promise but still have limited efficacy due in part to the lack of understanding of their mechanism after they are injected [6]. Given that the CMV-DC vaccine is meant to stimulate the immune system, we will test the

subject's blood for immune response. Specifically, the immune monitoring blood will be used to detect pp65 T cell responses following vaccination using IFC and possibly ELISPOT. Briefly, patient's immune cells from the blood will be stimulated with pp65 to measure IFNγ, CCL3, IL-2, and TNF-α response via IFC by intracellular cytokine staining. The pp65 protein is expressed on numerous types of brain tumor cells and therefore serves as a great target for DC vaccination. Studying the T cell response to pp65 will inform us if the DCs are stimulating an immune reaction to the pp65 protein, a significant factor for the efficacy of the CMV-DC vaccine.

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

All patients who undergo leukapheresis will be included in analyses relating to feasibility.

Patients without an unacceptable toxicity who do not receive the first three DC vaccinations at $2x10^7$ cells per dose will not be evaluable for the initial assessment of unacceptable toxicity. Any patient who receives any dose of CMV-DCs will be evaluable for efficacy of the agent.

8.5 Early Study Termination

This study can be terminated at any time for any reason by the PI-sponsor. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with Section 11.7, which describes procedures and process for prematurely withdrawn patients.

9 STUDY DRUG

Each patient will undergo at least one leukapheresis, while patients who have had previous craniospinal radiation may undergo up to two leukapheresis procedures, if necessary, to generate the three DC vaccines needed to be considered feasible. As long as at least one vaccine is generated, the patient will continue on study. Part of the contents from the leukapheresis will be used for DC generation. Each leukapheresis procedure will consist of processing three blood volumes per patient.

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

At the end of the 7-day incubation for generation of DCs, a sample of the media is taken for mycoplasma testing, the cells are then harvested and electroporated with pp65-flLAMP mRNA. The DCs are placed in a flask with AIM V media containing GM-CSF + IL-4 + TNF-α + IL-6 + IL-1β at 37^OC, 5% CO_2 for 18-20 hours for maturation. The cells are washed twice with PBS and frozen at $5x10^6$ cells/mL in 90% autologous human AB serum (Valley Biomedical, Winchester, VA 22602), 10% DMSO and 5% glucose in a controlled-rate freezer at a rate of 1^oC/minute. The DCs are then stored until needed at $–135^oC$. After freezing, an aliquot of cells is thawed for QA/QC. This testing will look at viability (>70%), endotoxin content (<5 E.U. /Kg B.W.), mycoplasma contamination (negative), and sterility testing for aerobic and anaerobic bacterial cultures ($1x10^6$ DCs) and fungal cultures ($1x10^6$ DCs).

9.1 Names, Classification, and Mechanism of Action

CMV-DCs are autologous dendritic cells derived from PBMCs loaded with RNA encoding the human CMV matrix protein pp65 as a fusion protein with the full-length LAMP protein (pp65-flLAMP) plus GM-CSF and Td vaccine as adjuvants. DCs work by activating the host immune system, specifically $CD4^+$ and $CD8^+$ T cells and B cells. Activation of these immune cells by a specific antigen, such as pp65, stimulates these immune cells to attack cells that display that antigen, such as brain tumor cells, resulting in cell killing.

9.2 Packaging and Labeling

- For CMV-DCs:
  Name

MRN
DOB
Drug: pp65DCs
Lot #: Lot 001
Caution New Drug Limited By Federal Law To Investigational Use

- For Td used in preconditioning, per Investigational Chemotherapy Services at Duke

9.3 Supply, Receipt, and Storage

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

MPACT Facility	Duke ICS Pharmacy
CMV pp65-flLAMP mRNA-pulsed DCs	Td used for preconditioning

9.4 Dispensing and Preparation

For each vaccination, cells that have passed QA/QC will be rapidly thawed at 37^{o}C, washed three times with phosphate buffered saline (PBS) and counted. The cell concentration will be adjusted to 4x10^{7} cells/mL and DCs will be re-suspended in preservative free saline and placed into a sterile tuberculin syringe with a 27 gauge needle.

For all DC preparations, from the final preparation, a sample of cells will be sent for Gram stain and endotoxin testing prior to administration. DC vaccination will not be given until endotoxin testing has been passed (< 5.0 E.U./Kg) and the Gram stain has been found to be negative. An aliquot of cells will also be sent for aerobic and anaerobic bacterial cultures (1x10^{6} DCs) and fungal cultures (1x10^{6} DCs).

The CMV-DCs will be delivered from the MPACT facility directly to the clinic under the supervision of the trained research staff. Each patient vaccine will be transported to the clinic in a separate cooler.

9.5 Compliance and Accountability

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

9.6 Disposal and Destruction

All excess study drug and any item that contacts the study drug will be autoclaved and properly disposed according to institutional procedures.

10 SUBJECT ELIGIBILITY

10.1 Inclusion Criteria

1. Age requirements:
    a. ≤ 35 years for patients with grade IV glioma or recurrent WHO grade III glioma
    b. 3-35 years old for patients with recurrent medulloblastoma

2. Newly diagnosed or recurrent WHO grade IV glioma, recurrent WHO grade III glioma, or recurrent medulloblastoma (multifocal/disseminated disease is eligible, at the discretion of the PI)
3. Patients with WHO grade IV glioma who received surgery and radiation are eligible even without recurrence or progression
4. Patients must have recovered from all previous treatments, including chemotherapy, radiation therapy, surgery, and other immunotherapies, etc.
    a. If the patient was receiving bevacizumab at the time of enrollment, the treating oncologist has the discretion of administering and adjusting bevacizumab 10 mg/kg every 14 days. The rationale for continuing patients on bevacizumab is to prevent rebound cerebral edema commonly seen after stopping this agent.
5. Laboratory Studies:
    a. Platelets ≥ 100,000 cells/mm^3
    b. Creatinine ≤ 1.2 x ULN
    c. Total bilirubin, AST, ALT, alkaline phosphatase ≤ 2.5 x ULN
    d. Neutrophil count ≥ 1000 cells/mm^3
    e. Hemoglobin ≥ 9 g/dl (can be transfused)
6. Able to undergo brain MRI with and without contrast
7. Karnofsky Performance Status (KPS) ≥ 70 or Lansky Performance Status (LPS) ≥ 70
8. A signed informed consent form approved by the IRB will be required for patient enrollment into the study. Patients (if 18 years old or older) or their parent(s) or guardian(s) (if younger than 18 years old) must be able to read and understand the informed consent document and must sign the informed consent indicating that they are aware of the investigational nature of this study.
9. For females of childbearing potential, negative serum pregnancy test within 48 hours of leukapheresis
10. Females of childbearing potential must be willing to use acceptable contraceptive method to avoid pregnancy throughout the study and for at least 24 weeks after the last dose of study drug
11. Males with female partners of childbearing potential must agree to practice adequate contraceptive methods throughout the study and should avoid conceiving children for 24 weeks following the last dose of study drug
12. **Newly diagnosed WHO grade IV glioma patients only**: must be expected to complete standard of care radiation (minimum ~54 Gy)

10.2 Exclusion Criteria

1. Prior invasive malignancy (except for non-melanomatous skin cancer) unless disease free for ≥ 3 years. (For example, carcinoma in situ of the breast, oral cavity, and cervix are all permissible)
2. Disease outside of the CNS
3. HIV, Hepatitis B, or Hepatitis C seropositive
4. Known active infection requiring IV antibiotics or active immunosuppressive disease
5. Severe, active co-morbidity, defined as follows:
    a. Unstable angina and/or congestive heart failure requiring hospitalization
    b. Transmural myocardial infarction within the last 6 months
    c. Acute bacterial or fungal infection requiring intravenous antibiotics at initiation of XRT/TMZ
    d. Chronic Obstructive Pulmonary Disease exacerbation or other respiratory illness requiring hospitalization or precluding study therapy at initiation of XRT/TMZ for newly diagnosed patients or at initiation of DI TMZ for recurrent patients
    e. Hepatic insufficiency resulting in clinical jaundice and/or coagulation defects
    f. Acquired Immune Deficiency Syndrome (AIDS) based upon current CDC definition. The need to exclude patients with AIDS from this protocol is necessary because the treatments involved in this protocol may be significantly immunosuppressive
    g. Patients with autoimmune disease requiring medical management with immunosuppressant(s)
    h. Major medical illnesses or psychiatric impairments that, in the investigator's opinion, will prevent administration or completion of protocol therapy

   i. Active connective tissue disorders such as lupus or scleroderma that, in the investigator's opinion, place the patient at high risk for radiation toxicity
6. Pregnant or lactating women
7. Prior allergy to TMZ, GM-CSF, gadolinium (Gd), or Td
8. Prior history of brachial neuritis or Guillain-Barré syndrome
9. Patients treated on any other therapeutic clinical protocols within 30 days prior to study entry
10. Patients receiving > 0.1mg/kg or 4mg/day dexamethasone or equivalent
11. **For recurrent patients only**: Patients who have not recovered from the toxic effects of prior chemo- and/or radiation therapy[9]. Guidelines for this recovery period are dependent upon the specific therapeutic agent being used:
    a. Patients who have received chemotherapy or bevacizumab ≤ 4 weeks [except for nitrosourea (6 weeks) or metronomic dosed chemotherapy such as daily etoposide or cyclophosphamide (1 week)] prior to starting the study drug unless patients have recovered from the side effects of such therapy. If the patient was receiving bevacizumab at the time of enrollment, the treating oncologist has the discretion of administering and adjusting bevacizumab 10 mg/kg every 14 days.
    b. Patients who have received immunotherapy ≤ 4 weeks prior to starting the study drug unless patients have recovered from the side effects of such therapy

[9] Please note that patients who are receiving treatment with a Tumor Treating Fields (TTFields) device such as Optune™ may continue to use this device during leukapheresis and during the ≥ 3 weeks before DI TMZ if recurrent. Patients should discontinue use of Optune™ just prior to beginning DI TMZ and remain off Optune™ for the duration of time they are in the Treatment Period of the study (i.e., while they are receiving study vaccines).

11 SCREENING AND ON-STUDY TESTS AND PROCEDURES

Table 1: Schedule of Events

Description	Screening	Leukapheresis #1	At start of Adjuvant DI TMZ Cycle	Vaccine #1	Vaccine #2	Vaccine #3	Vaccine #4+[10]	Post-vaccine (follow-up period)
Week					2 weeks (± 2 days) after Vaccine #1	2 weeks (± 2 days) after Vaccine #2	4 weeks (± 5 days) after Vaccine #3	Until progression or death
Day				Day 22 (+3 days) of DI TMZ				
General Evaluations								
Physical Exam	X		X	X[11]		X	X	X
Neurologic Exam	X		X	X[11]		X	X	X
Performance Status	X		X	X[11]		X	X	X
Adverse Events		X[12]	Continuous					
Laboratory Evaluations								
CBC w/diff	X	X	X	X	X	X	X	
CMP	X	X	X	X	X	X	X	
Serum Pregnancy Test, if applicable	X	X[13]						
Whole blood for immunologic analysis		X[14]		X[14]		X[14]		X[14]
Leukapheresis		X[15]						
Disease Evaluations								
MRI	X[16]						X[16]	X[16]
Treatment								
TMZ			X[17]					
CMV-DCs				X[18]	X[18]	X[18]	X[18]	
Td booster	X[19]							
Td preconditioning				X[20]			X[20]	

[10] Subsequent vaccines occur approximately every 4 weeks (± 5 days) until all CMV-DC vaccines generated from the patient have been used.
[11] Prior to Td preconditioning
[12] AESIs related to leukapheresis only (see Section 12.1.1).
[13] Serum pregnancy test, if appropriate, within 48 hours of leukapheresis. If screening pregnancy test is within 48 hours of leukapheresis, it does not have to be repeated.
[14] Up to 76.5 mL in yellow top ACD tubes (not to exceed 3 mL/kg in a 24 hr period or 7 mL/kg in an 8 week period) and 1 red top (3 mL) prior to Vaccine #1, 1 week (± 5 days) after Vaccine #3, and 1 week (± 5 days) after the last vaccine. At leukapheresis #1, 3 yellow top tubes (25.5 mL) and 1 red top tube (3 mL),
[15] An additional leukapheresis may be done on patients who have received prior craniospinal radiation if more cells are needed.
[16] MRI must be done within 2 weeks prior to initiating the first cycle of DI TMZ as a baseline (screening MRI). For newly diagnosed patients, this baseline MRI must be done after completing XRT/TMZ and before initiating DI TMZ. For patients with recurrent medulloblastoma, a baseline MRI of the spine must also be done. If nothing is detected on the baseline MRI, then no further spinal MRIs need to occur; spinal MRIs should continue if there is spinal detection. MRI will also be done 3-5 weeks after Vaccine #3 (at Vaccine #4 visit) and then every 3 months, at the discretion of the treating physician, and at progression.
[17] 21 days of daily TMZ at a dose of 50-100 mg/m^2/day.
[18] Administer Vaccine #1 intra-dermally at day 22 (+ 3 days) of the first adjuvant TMZ cycle. Vaccines #2 and #3 will occur at 2 week intervals (± 2 days). Vaccines #4+, if available, will be given every 28 days (± 5 days). Each vaccination will be equally divided between both inguinal regions. Monitor for 30-60 minutes post-immunization for any adverse effects.
[19] For patients ≥ 18 years of age.
[20] Td preconditioning (1 Lf) 6-24 hours prior to Vaccine #1, Vaccine #4, and all subsequent vaccines.

11.1 Screening Examination

An informed consent must be signed by the patient and/or the parent(s) or legal guardian(s) before any study-specific screening procedure takes place.

Pre-treatment evaluations within 2 weeks before leukapheresis #1 to determine eligibility and as a baseline will include the following, unless otherwise indicated:

- History and physical exam, including a full neurologic assessment and KPS or Lansky Performance Status
- Laboratory Evaluations:
    - CBC with differential
    - CMP
    - Beta-HCG, if appropriate (within 48 hours of leukapheresis)
- Baseline MRI of the brain (within 2 weeks before starting adjuvant DI TMZ). *For newly diagnosed patients, this baseline MRI must be done after completing XRT/TMZ.
- Td booster (patients ≥ 18 years old only)

If a subject does not undergo leukapheresis, minimal records regarding the subject and the reason for screen failure will be retained in the study database.

11.2 Run-In Period

Not applicable.

11.3 Leukapheresis #1

- CBC with differential
- CMP
- Serum pregnancy test (within 48 hours of leukapheresis)
- Leukapheresis
- Blood for immune monitoring (1 red top, 3 yellow top tubes)
- AESI collection

11.4 Treatment Period

Adjuvant DI TMZ Cycle

- Physical Exam
- Neurologic Exam
- Performance Status
- CBC with differential
- CMP
- 21-day DI TMZ

Vaccine #1 (day 22 + 3 days):

- Physical Exam
- Neurologic Exam
- Performance Status
- Td preconditioning (1 Lf) 6-24 hours prior to Vaccine #1
- CBC with differential
- CMP
- Whole blood for immunologic analysis (up to 76.5 mL in 9 yellow top ACD tubes, not to exceed 3 mL/kg in a 24 hr period or 7 mL/kg in an 8 week period, and 1 red top tube)
- CMV-DCs
- AE collection

Vaccine #2 (2 weeks ± 2 days after Vaccine #1):

- CBC with differential
- CMP
- CMV-DCs
- AE collection

Vaccine #3 (2 weeks ± 2 days after Vaccine #2):

- CBC with differential
- CMP
- CMV-DCs
- Whole blood for immunologic analysis (up to 76.5 mL in 9 yellow top ACD tubes, not to exceed 3 mL/kg in a 24 hr period or 7 mL/kg in an 8 week period, and 1 red top tube) **(1 week ± 5 days after Vaccine #3)**
- AE collection

Vaccine #4+ (every 4 weeks ± 5 days after Vaccine #3):

- Physical Exam
- Neurologic Exam
- Performance Status
- CBC with differential
- CMP
- Td preconditioning (1 Lf) 6-24 hours prior to each vaccine
- CMV-DCs
- MRI at Vaccine #4 visit (~3-5 weeks after Vaccine #3) and then every 3 months, at the discretion of the treating physician
- Whole blood for immunologic analysis (up to 76.5 mL in 9 yellow top ACD tubes, not to exceed 3 mL/kg in a 24 hr period or 7 mL/kg in an 8 week period, and 1 red top tube) **(1 week ± 5 days after the last Vaccine given to the patient)**
- AE collection

11.5 Follow-up Period

Until Progression or Death:

- Physical Exam
- Neurologic Exam
- Performance Status
- MRI

Patients may not be treated with any other modality unless progressive tumor is noted or they are otherwise removed from the study. Patients will be considered off study if they do not have enough PBMCs to generate at least one DC vaccine, upon tumor progression, or upon treatment of the tumor with another modality. When subjects are considered off study, this indicates that subjects will no longer be obligated to undergo study-related tests and procedures, but the data described below will still be collected from these subjects as feasible. Patients who generate one or two vaccines after leukapheresis will have the option to receive these vaccines per the study schedule. Patients who received prior craniospinal radiation can undergo second leukapheresis during or after administration of vaccine(s). Subjects will be followed for serious adverse events (SAEs) for 30 days after coming off study. Collection of the additional data listed in the following sentence from off study subjects will be performed, if possible, but is not mandatory and will not be considered a deviation if the data cannot be obtained. Subjects' medical records will be reviewed for the remainder of their life, in order to collect data on subsequent treatments, disease progression, tumor size/volume, and survival.

11.6 End of Study

The study will be considered complete once enrollment has been met, follow-up procedures outlined in Section 11.4 have been conducted on all subjects, and data analysis is concluded. The study may also be terminated early for any reason by the PI-sponsor.

Subjects that are lost to follow-up will be documented in the patient record and in the 21 CFR Part 11 database. In the compliant database, the subject will be marked as "Patient Status Unknown," along with a corresponding explanation, if any.

11.7 Early Withdrawal of Subject(s)

11.7.1 Criteria for Early Withdrawal

Subjects may voluntarily withdraw from the study at any time. The PI may also withdraw a subject from the study at any time based on his/her discretion. Reasons for PI-initiated withdrawal may include, but is not limited to the following:

- Adverse events
- Abnormal laboratory values
- Abnormal test procedure results
- Protocol deviation
- Administrative issues
- Disease progression
- Pregnancy
- Unable to generate one CMV-DC vaccine
- A >2 week delay in vaccine administration, outside of the allowable window
- Unable to complete at least ~54 Gy of radiation (newly diagnosed WHO grade IV glioma patients only)

Subjects will be withdrawn from the study if they do not receive any CMV-DC vaccinations due to contamination or any other inadequacy in the first batch of CMV-DC vaccines from the initial leukapheresis.

11.7.2 Follow-up Requirements for Early Withdrawal

Subjects should be seen in clinic or contacted at a minimum of every 3 months for 1 year.

11.7.3 Replacement of Early Withdrawal(s)

Subjects that withdraw from the study prior to leukapheresis will not be evaluable for any objective; these subjects will be replaced. See Section 14.6 for related details.

11.8 Study Assessments

11.8.1 Medical History

Standard medical history will be obtained and documented per institutional guidelines.

11.8.2 Physical Exam

Standard physical exam and neurological assessment will be conducted and documented per institutional and Preston Robert Tisch Brain Tumor Center (PRTBTC) guidelines.

11.8.3 Radiographic Review

The modified RANO provides clinical guidelines for continuing therapy beyond suspected radiographic progression if the treating physician believes there may be a therapeutic benefit and provides criteria

for defining progression and early drug failure while also allowing for the possibility of pseudoprogression.

An update to the RANO criteria proposes strategies to establish a general framework for response assessment in neuro-oncology that is agnostic to the mechanism of action of the particular therapy (anti-angiogenic, immunotherapy, etc.) to address the challenges associated with interpretation of radiographic changes in patients with brain tumors in clinical trials.

The modified RANO proposes to use the post-radiation time point as the baseline for response evaluation and to consider only objectively defined, measurable enhancing disease in the definition of response and progression (i.e. exclusion of qualitative assessed T2/FLAIR changes).

Patients will be defined as progressive if they meet any of the following criteria:

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

11.8.4 Correlative Assessments

Whole blood will be obtained for tests of anti-tumor immune responses at leukapheresis #1, just prior to Vaccine #1, ~1 week after Vaccine #3, and ~1 week after the patient has received their final CMV-DC vaccination. Because of the large volume of the whole blood draws taken for immune monitoring, it will be essential to carefully consider the weight of each child (under the age of 18) when determining whether a blood draw may occur or how much blood may be drawn at a given time point. In the pediatric population, the maximum blood volumes are 3 mL/kg in a 24 hour period or 7 mL/kg in an 8 week period. Table 2 contains predicted blood draw volumes that are required for the planned study assessments during the indicated periods.

Table 2: Schedule of Blood Draw Volumes

	Maximum blood draw volumes [21]					
	Screening	**Leukapheresis #1**	**Adjuvant TMZ Cycle**			**Final Vaccine**
Day		**Within 2 weeks of screening**	**~22**	**~38**	**~52**	**~1 week after final Vaccine**
CBC & CMP	6 mL	6 mL	6 mL	6 mL	6 mL	
Pregnancy	2 mL	2 mL				
Whole blood immunologic analysis		28.5 mL (1 red top tube and 3 yellow top ACD tubes)	Up to 76.5 mL[22] (9 yellow top ACD tubes) + 3 mL (1 red top tube)		Up to 76.5 mL [22] (9 yellow top ACD tubes) + 3 mL (1 red top tube)	Up to 76.5 mL [22] (9 yellow top ACD tubes) + 3 mL (1 red top tube)
Total over period	**8 mL**	**36.5 mL**	**85.5 mL**	**6 mL**	**85.5 mL**	**79.5 mL**
	Max volume in 8 week period (Leukapheresis #1 and Adjuvant TMZ Cycle): 213.5 mL					

12 SAFETY MONITORING AND REPORTING

The PI is responsible for the identification and documentation of adverse events (AEs) and serious adverse events (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.

12.1 Adverse Events

An adverse event (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 CMV-DCs or Td preconditioning, whether or not related to the use of study drug(s). But laboratory value changes that require therapy or adjustment in prior therapy are considered adverse events.

From the time of the Td preconditioning prior to Vaccine #1 through the End of Study visit (as defined in Section 11.6), as well as any Special Interest AEs related to leukapheresis (as defined in Section 12.1.1), all AEs must be recorded in the subject medical record and adverse events 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:

[21] Volumes for standard lab tests are based on DUHS Clinical Labs volumes for patients age 8-15. For patients in the lower age range of this study and/or with a low body weight, lower pediatric volumes (for patients 0-8) may be used. Some volumes may be higher for patients > 15 years old.
[22] Volumes of whole blood for immunologic analysis will be reduced as necessary to not exceed 3 mL/kg in a 24 hour period or 7 mL/kg in an 8 week period.

- 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)

12.1.1 AEs of Special Interest

Adverse events will not be collected until the patient has received their first Td preconditioning prior to CMV-DC Vaccine #1, unless they are considered an Adverse Event of Special Interest related to the leukapheresis procedure. Only these special interest adverse events will be collected prior to the first Td preconditioning, all other events occurring prior to the first Td preconditioning administration will not be recorded or monitored.

Special Interest Adverse Events that may occur during the leukapheresis procedure include:

- Allergic Reaction
- Anaphylaxis
- Pre-syncope
- Syncope
- Vasovagal reaction

Special Interest Adverse Events that may occur after the procedure, but may still be related to leukapheresis include:

- Vascular Access Complications
- Venous Injury

12.1.2 Reporting of AEs

A database of all adverse events (not just those considered related to the study drug) will be maintained in 21 CFR Part 11 Compliant database. The event will be categorized by organ system, relationship to treatment, its grade of severity, and resolution. The Principal Investigator and study statistician will periodically review the collective adverse events with the intention of identifying any trends or patterns in toxicity. If any such trends are identified, depending on their severity and frequency, a protocol amendment will be considered.

12.2 Serious Adverse Events

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

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

12.2.1 Reporting of SAEs

All serious and unexpected AEs should be reported immediately to the PI or designee. Only AEs that the PI determines to be serious, unanticipated, and related or possibly/probably (i.e. more likely than not) related to the research must be reported to the IRB and the Food and Drug Administration (FDA). Fatal or life-threatening, unexpected adverse events will be reported to the FDA by telephone, facsimile, or in writing as soon as possible, but no later than 7 calendar days after first knowledge by the sponsor followed by as complete a report as possible within 8 additional calendar days. Serious, unexpected adverse events that are not fatal or life-threatening will be reported to the FDA by

telephone, facsimile, or in writing as soon as possible, but no later than 15 calendar days after first knowledge by the sponsor.

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

12.3 Safety Oversight Committee (SOC)

The Duke Cancer Institute SOC is responsible for annual data and safety monitoring of 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 SOC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews include but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The SOC in concert with the DCI Monitoring Team (see Section 13.1 for Monitoring Team description) oversees the conduct of DUHS cancer-related, sponsor-investigator 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, standing operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements.

12.4 External Data and Safety Monitoring Board (DSMB)

Due to a potential conflict of interest in other PI-initiated studies at Duke University Medical Center, an external DSMB-Plus was created. The current study will be monitored by this DSMB-Plus. The external DSMB-Plus will be responsible for safeguarding the interests of trial subjects and assessing the safety of the interventions during the trial. The DSMB-Plus will provide recommendations about stopping or continuing enrollment in the trial. To contribute to enhancing the integrity of the trial, the DSMB-Plus may also formulate recommendations relating to the selection, recruitment, and retention of subjects and their management. Additional details regarding the responsibility of the DSMB-Plus and its chair may be found in the charter document.

13 QUALITY CONTROL AND QUALITY ASSURANCE

13.1 Monitoring

The Duke Cancer Institute (DCI) Monitoring Team will conduct monitoring visits to ensure subject safety and to ensure that the protocol is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, good clinical practice, and applicable regulatory requirements. As specified in the DCI Data and Safety Monitoring Plan, the DCI Monitoring Team will conduct routine monitoring after the third subject is enrolled, or annually if less than 3 subjects enroll in the first year, followed by annual monitoring of 1 – 3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk.

Additional monitoring may be prompted by findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns and may be initiated upon request of DUHS and DCI leadership, the DCI Cancer Protocol Committee, the SOC, the PI, or the IRB. All study documents must be made available upon request to the DCI Monitoring Team and other authorized regulatory authorities, including but not limited to the National Institute of Health, NCI, and the FDA. Every reasonable effort will be made to maintain confidentiality during study monitoring.

13.2 Audits

The Duke Office of Audit, Risk, & Compliance (OARC) office may conduct confidential audits to evaluate compliance with the protocol and the principles of Good Clinical Practice (GCP). The 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 OARC 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 adverse events, 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.

13.3 Data Management and Processing

13.3.1 Case Report Forms (CRFs)

The electronic CRF (eCRF) will be the primary data collection document for the study. 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.

13.3.2 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 appropriate responses (i.e. confirmation of data, correction of data, completion or confirmation that data is not available, etc.).

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

13.3.3 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

14 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.

14.1 Analysis Sets

Any patient who undergoes leukapheresis will be included in analyses of feasibility.

Any patient who experiences an unacceptable toxicity from the time of Td preconditioning until 30 days after the 3rd vaccination or any patient who remains on study until 30 days after the 3rd vaccination and has no unacceptable toxicity will be included in the primary safety analyses. For all other safety analyses, all patients who receive at least 1 vaccination will be included.

Efficacy analyses will include all patients who receive at least 1 vaccination.

Analyses of T cell response data will include all patients who received at least 3 vaccines and have assessments completed before Vaccine #1 and after Vaccine #3.

14.2 Patient Demographics and Other Baseline Characteristics

A summary of clinical and socio-demographic characteristics will be generated. Categorical descriptors will be summarized using frequency distributions; whereas, interval variables will be summarized using percentiles, as well as means and standard deviations.

14.3 Treatments

Among patients who initiate DI TMZ, a frequency distribution will be generated for the number of vaccines received by each patient.

14.4 Primary Objective

This study has two primary objectives: the assessment of feasibility of manufacturing at least three CMV-DCs from a single leukapheresis (or two leukapheresis procedures for those who have received previous craniospinal radiation) of eligible patients, and the safety of intradermal administration of CMV-DC.

14.4.1 Primary Objective #1: Feasibility

Feasibility will be defined in terms of the percentage of patients for whom three or more vaccines can be generated from the pre-treatment leukapheresis.

This study was originally designed to assess feasibility within two separate cohorts: patients **with** prior craniospinal radiation and patients **without** prior craniospinal radiation. Prior to an amendment (protocol version 2, dated 11/16/2018), up to 2 patients were to be accrued to the cohort with prior craniospinal radiation with the stipulation that the limit of 2 patients would be removed if 3 or more vaccines could be generated from the pre-treatment leukapheresis procedures in both patients. Given that the first two patients with prior craniospinal radiation had 3 or more vaccines generated, the limitation was removed in protocol version 2.

14.4.2 Primary Objective #2: Safety

The second primary objective of this study is to assess the safety of intradermal administration of CMV-DCs in in patients up to age 35 with WHO grade IV glioma, recurrent malignant glioma (WHO grade III-IV glioma), and with recurrent medulloblastoma.

14.4.2.1 Variable

The percentage of patients who experience an unacceptable adverse event as defined in Section 8.1.7 after Td preconditioning and within the 30-day window after Vaccine #3, prior to the 4th vaccination.

A patient will be considered evaluable for the primary assessment of safety if the patient experiences an unacceptable adverse event during this noted period, or if the patient receives 3 vaccines and completes the endpoint observation period of 30 days after Vaccine #3.

14.4.2.2 Adverse Event Monitoring

The experience of patients treated with CMV-DC will be continuously monitored to determine whether an unacceptable adverse event as defined by Section 8.1.7 occurs. If any patient experiences a grade 5 treatment-related adverse event, the initiation of CMV-DC treatment will be suspended until all data are carefully reviewed. The review will result in a decision as to whether treatment and accrual will continue with or without modification, or whether the study will be permanently terminated. Otherwise, the following guidelines will be used to determine whether accrual should be suspended and adverse event data reviewed:

Number of Patients Treated Who are Evaluable for Assessment of Toxicity	Criteria for Accrual Suspension
3	≥2 with unacceptable adverse event
4	≥2 with unacceptable adverse event
5	≥2 with unacceptable adverse event
6	≥3 with unacceptable adverse event
7	≥3 with unacceptable adverse event
8	≥3 with unacceptable adverse event
9	≥4 with unacceptable adverse event
10	≥4 with unacceptable adverse event

Though the study targets the treatment of 6 patients with CMV-DC who are evaluable for the assessment of adverse event per section 14.4.2.1, it is possible that up to 10 patients will be treated, depending on the number of accrued subjects that are deemed feasible and as such should be treated with CMV-DCs.

After 4 patients have been enrolled and treated (i.e. the last patient has received Vaccine #3 and been observed for 30 days after Vaccine #3), the study will be paused for evaluation of safety before proceeding with further enrollment.

14.4.2.3 Other Safety Analyses

Additional summaries of adverse events will be tabulated. In these additional summaries, all patients who receive at least one vaccination will be included. Different summaries will be generated for different purposes:

- For the summary of adverse events and serious adverse events within the ClinicalTrials.gov report, the percentage of patients who experience each type of adverse event regardless of attribution will be summarized.

- For the manuscript, adverse events 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.
- Two tabulations will be generated for review by the Safety Oversight Committee 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.

14.4.3 Exploratory Objectives

This study includes two exploratory objectives that examine T cell response and efficacy. Given the overall small sample size of this study, all exploratory analyses will be descriptive.

14.4.3.1 Exploratory Objective #1: T cell response

For each patient, the percent change in pp65-specific T cell responses assessed by polyfunctional T cell responses, including but not limited to IFNγ, CCL3, IL-2, and TNFα, between pre-chemotherapy baseline (initial leukapheresis), post-chemotherapy baseline (just prior to Vaccine #1), and the follow-up assessment 1 week (± 5 days) after Vaccine #3 and ~1 week after the administration of the final vaccine will be calculated. A one-sample Wilcoxon test or t-test will assess whether significant changes in these outcomes occurred between baseline and post-Vaccine #3.

14.4.3.2 Exploratory Objective #2: Survival and Progression-Free Survival

Survival, defined as the time between initiation of DI TMZ and death, will be censored at last follow-up for patients alive at the time of analyses. Progression-free survival (PFS), defined as the time between initiation of DI TMZ and progression, or death if the patient dies without documentation of progression. A patient that is alive without disease progression at the time of analyses will have PFS censored at the time of last follow-up. The Kaplan-Meier estimator will be used to describe the survival and PFS of the patients treated on this protocol. Estimates of 6-month, 1-year, 2-year, and 3-year OS and PFS will be generated.

14.5 Interim Analysis

No interim analyses of efficacy will be conducted. Interim analysis of safety is described in Section 14.4.2.

14.6 Sample Size Calculation

All patients accrued to the study will be treated regardless of ability to generate at least three CMV-DC vaccines from the pre-treatment leukapheresis (or 2 leukaphereses for those who have had previous craniospinal radiation).

The goal is to treat at least 6 patients with CMV-DC who are evaluable for the assessment of treatment safety. As noted in Section 14.4.2.2, the actual number that will be treated may be greater than 6 and as many as 10 patients.

Assuming we are targeting 10 patients to be treated with CMV-DC, then the probability of terminating early as a function of the true, though unknown, probability of an unacceptable adverse event is tabulated below. Included is also the probability of early study termination based upon the monitoring rules described in Section 14.4.2.2.

True Unknown Probability of Unacceptable Adverse Event	Probability of Terminating Early	Average Number of Patients Treated
0.1	0.092	9.6
0.2	0.322	8.3

0.3	0.596	6.8
0.4	0.791	5.5
0.5	0.920	4.6

15 ADMINISTRATIVE AND ETHICAL CONSIDERATIONS

15.1 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.

15.2 DUHS Institutional Review Board and DCI Cancer Protocol Committee

The protocol, informed consent form, advertising material, and additional protocol-related documents must be submitted to the DUHS IRB and DCI Cancer Protocol Committee (CPC) for review. The study may be initiated only after the PI has received written and dated approval from the CPC and IRB.

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

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

15.3 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 PI or authorized key personnel will discuss with the potential subject and/or the potential subject's parents or guardians 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 or parents/guardians who cannot read or understand English or are visually impaired. Potential subjects and/or their parents or guardians 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. Written assent is required for subjects who are aged 12 to 17 years old. Verbal assent is required for subjects who are aged 6 years old to 11 years old. In the instances where a child's assent is required, the child should be given an explanation of the proposed research procedures in a language that is appropriate to the child's age, experience, maturity, and condition.

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

15.4 Privacy, Confidentiality, and Data Storage

The PI will ensure that subject privacy and confidentiality of the subject's data will be maintained. Research Data Security Plans (RDSPs) will be approved by the appropriate institutional Site Based Research group.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his family, if desired. For all future visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private exam room. All research related interactions with the participant and their parent/guardian, if applicable, 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 dedicated 21 CFR Part 11 compliant database, which is housed in an encrypted and password-protected file on a secure network drive. Access to electronic databases will be managed by the PRTBTC Data Manager. The security and viability of the IT infrastructure will be managed by the DCI and/or Duke Medicine.

Upon completion of the study, research records will be archived and handled per DUHS HRPP policy. Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

15.5 Data and Safety Monitoring

Data and Safety Monitoring will be performed in accordance with the DCI Data and Safety Monitoring Plan.

15.6 Protocol Amendments

All protocol amendments must be initiated by the PI 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 PI must inform the IRB and all other applicable regulatory agencies of such action immediately.

15.7 Records Retention

The PI will maintain study-related records until the youngest child on study is 21 years old, or for at least six years after study completion, whichever is longer (Duke policy).

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