A Phase I/II Clinical Trial of Autologous CMV-Specific Cytotoxic T Cells for GBM Patients

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Protocol Body

1.0 Objectives

1.1 Phase I: Dose Escalation in Recurrent glioblastoma

1.1.1 Primary Objective: To determine the Maximum Feasible Dose (MFD) or Maximum tolerated Dose (MTD) and safety of CMV-specific T cells in combination with dose-dense temozolomide in patients with recurrent glioblastoma.

1.1.2 Exploratory Objective: To determine the persistence and expansion of adoptively-infused CMV-specific T cells by multiparameter flow cytometry in serially-sampled peripheral blood.

1.2 Phase II (Dose Expansion): Recurrent glioblastoma undergoing resection

1.2.1 Primary:

1.2.1.1 To evaluate the immunological effects in resected glioblastoma after intravenous administered cytomegalovirus (CMV)-stimulated adoptive T cells in patients with recurrent glioblastoma.

1.2.1.2 To correlate 6-month PFS rate (PFS6) with objective clearance of CMV antigens as measured by IHC and by *ex vivo* T-cell-specific effector responses using intracellular cytokine profiling.

1.2.2 Secondary:

1.2.2.1 Time to progression, overall survival (OS) as well as immunological reactivity and safety.

1.2.3 Exploratory:

1.2.3.1 To identify imaging characteristics such as MRI textural analysis associated with immunological changes in tumor following treatment with CMV-stimulated adoptive T cells.

1.2.3.2 To ascertain if adoptive transfer of CMV-specific T cells leads to the expansion of T cells with specificity to other glioblastoma antigens (i.e. epitope spreading) by performing longitudinally monitoring of antigen-specific T cell frequency with ELISPOT.

1.3 Phase II (Dose Expansion): Newly diagnosed glioblastoma

1.3.1 Primary: Overall survival (OS)

1.3.2 Secondary:

1.3.2.1 Safety and tolerability of dose-dense temozolomide in combination with intravenous administered CMV-stimulated adoptive T cells in patients receiving adjuvant therapy after completing external beam radiotherapy with concurrent temozolomide for newly diagnosed glioblastoma

1.3.2.2 Overall objective response rate (ORR), Median duration of response, PFS6.

1.3.3 Exploratory: To determine the persistence and expansion of adoptively-infused CMV-specific T cells by multiparameter flow cytometry in serially-sampled peripheral blood.

2.0 Background and Rationale

2.1 CMV: The pathogen and the immune system

CMV is a B-herpes virus that is very common in humans and leads to asymptomatic infection followed by viral persistence and latency. A recent analysis in the United States revealed an overall 50% seroprevalence among adults [1], but rates in some populations are even higher. For example, approximately 90% of Mexican-Americans in the United States are seropositive by age 50, as are 88% of stem cell transplantation patients in Italy and 96% of individuals in southern Brazil [2-4]. Despite its high worldwide prevalence, CMV infections are generally not apparent, except in newborns and immunocompromised individuals, for whom they can cause life-threatening disease affecting many organ systems.

Following initial infection, a complex set of host responses conspires to limit CMV replication. Multiple defense systems sense the foreign nature of the virus very early after contact. The specific pathogen-associated molecular patterns likely include virion glycoproteins and the viral genome itself [5, 6]. Among the earliest responses are elaboration of interferon and cytokines that help establish an anti-viral state [7].

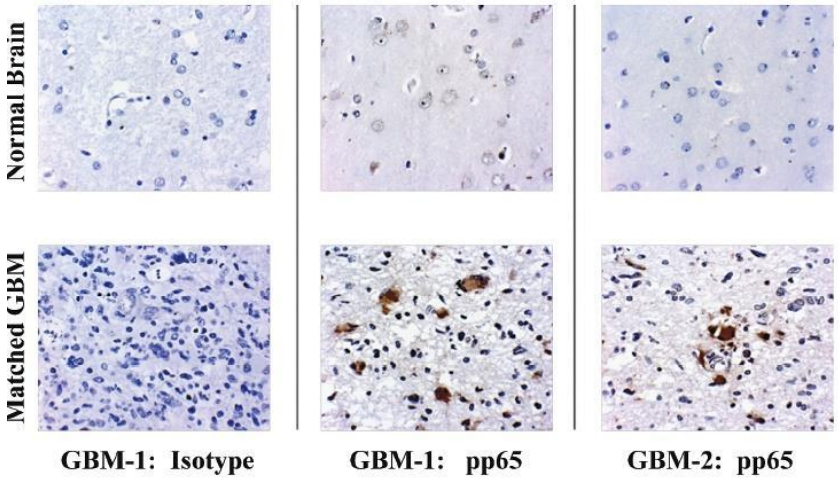
In seropositive humans, a strikingly high fraction (10% or more) of circulating T lymphocytes target CMV [8]. Use of tetramers has shown that pp65 is recognized by a high fraction of T cells, but many other gene products are also recognized [8, 9]. Moreover, the fraction of CMV-specific T cells tends to increase with age, which supports the hypothesis that CMV contributes to immune system exhaustion and dysfunction associated with aging [10].

2.2 CMV and glioblastoma

Dr. Cobbs was the first to discover that CMV is associated with GBM and that CMV gene products are expressed in >95% of GBM cases [11]. The presence of CMV in malignant gliomas has since been confirmed by several other groups [12-14].

Table 1. Summary of HCMV detection in GBM specimens

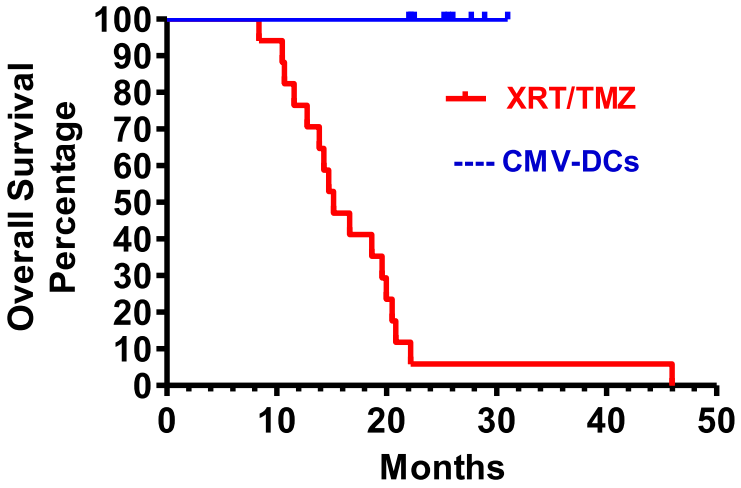
HCMV	GBM Tissue Specimen	Primary GBM Cultures
IE1 IHC	42/45 (93%)[a]	4/4 (100%)
pp65 IHC	30/33 (91%)[a]	12/12 (100%)
HCMV DNA ISH	16/16 (selected cases)	not tested
gB PCR	21/34 (61.7%)[b]	13/17 (70.6%)
IE1 PCR	8/34 (24%)[b]	9/17 (53%)



From Mitchell, et al., Neuro-Oncology, 2010
HCMV, human cytomegalovirus
IE1, immediate-early protein 1
IHC, immunohistochemistry
ISH, in situ hybridization
gB, soluble envelope glycoprotein B
pp65, tegument protein

Malignant gliomas have been postulated to arise from transformed neural progenitor cells, and these cells are permissive to CMV infection [15] resulting in abnormal differentiation [16] or inhibition of differentiation into normal astrocytes [17]. Platelet-derived growth factor-alpha receptor (PDGFR-α) is expressed on neural progenitor/stem cells [18], is required for CMV infection, and causes subsequent phosphoinositide-3-kinase (PI3K) signaling as a result of binding its envelope protein gB [19]. Blockade of PDGFR-α or its function with small molecule inhibitors of the kinase activity inhibits CMV internalization and gene expression [20]. Our group at MD Anderson has previously shown that CMV enhances glioblastoma mediated immunosuppression through the activation of the signal transducer and activator of transcription STAT3 [21] and others have shown that the CMV viral antigen pp65 plays a role in immune evasion by preventing expression of antigens by MHC molecules, inhibiting NK cell toxicity, and degrading the HLA-DR alpha chain [20, 22]. Although the role of CMV in the etiology of glioblastoma is still debatable, the association of CMV antigens with glioblastoma is well established [23].

Previously, glioblastoma patients treated with an autologous tumor lysate-pulsed dendritic cell immunotherapy were shown to have a robust CMV-specific CD8+ T-cell response to the pp65 CMV immune dominant epitope [24].The most compelling rationale for the use CMV targeted immunotherapy is based on a recently published clinical trial conducted at Duke University Medical Center. In a phase II clinical trial of newly diagnosed glioblastoma patients treated with autologous dendritic cells pulsed with CMV mRNA pp65 there was unprecedented progression free survival and overall survival (Mitchell DA, et al, Nature 2015) [35].



However, dendritic cell approaches entail complex and expensive processing. As such we would propose to use CMV-specific T cell immune therapeutics especially given the ubiquity of expression of CMV antigens in glioblastoma (>90%). Thus, we propose to test the therapeutic efficacy of adoptively-transferred T cells pulsed with CMV peptides in glioblastoma patients.

2.3. Adoptive therapy with CMV specific CTLs

Use of donor-derived antigen specific T cells is routinely used for immune reconstitution in transplant patients and has been studied in phase I and II trials. Riddell et al. was the first to establish the proof of concept for CMV cytotoxic T lymphocytes (CTLs) as adoptive therapy in 1994 [25, 26]. Since then many trials administering CMV CTL either used pre-emptively or prophylactically have provided encouraging results. There are currently two trials of CMV-specific T-cell adoptive therapy for the treatment of patients with CMV infection post transplant at our center (M.D. Anderson protocols 2013-0620 and 2013-0657).

To be suitable for clinical applications, the cells used for adoptive T-cell transfer must be virus-specific T cells generated by *in vitro* induction and expansion from a small number of precursor cells, over a short period of culture, under highly reproducible conditions, and in accordance with good manufacturing practice (GMP). CMV-specific memory T cells are

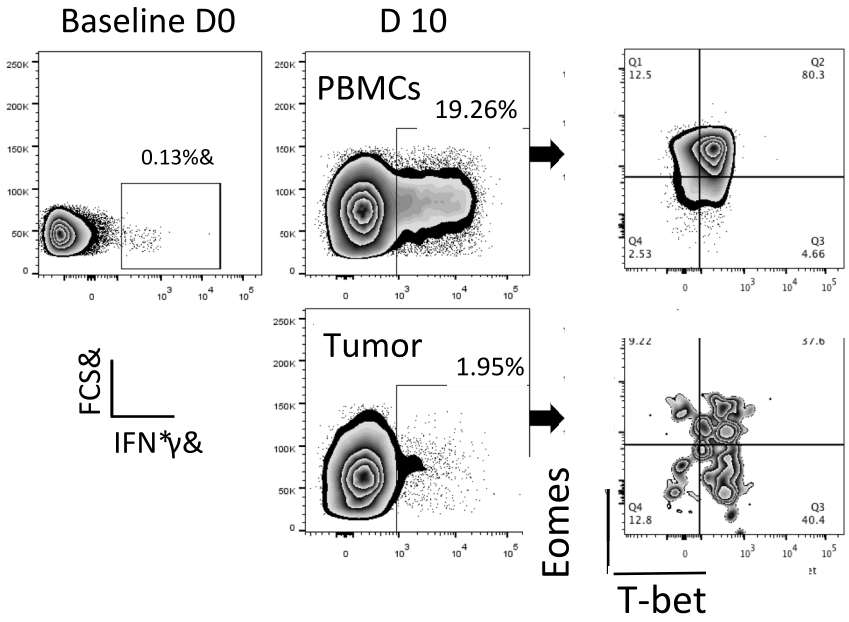
present at high frequencies in the blood of healthy CMV-seropositive donors. Typically, they represent 0.5% to 4% of the CD8+ T-cell pool and 0.05% to 1.6% of the CD4+ T helper (Th) cell pool [27]. Most protocols for the generation of virus-specific T cells use peptide-loaded monocyte-derived dendritic cells (DCs), artificial antigen-presenting cells (aAPCs), or CMV-infected immature dendritic cells as stimulator cells. However, these protocols are difficult to standardize and often laborious to adapt to GMP conditions.

2.4 Rapid Generation of CMV-Specific T cells from GBM patients

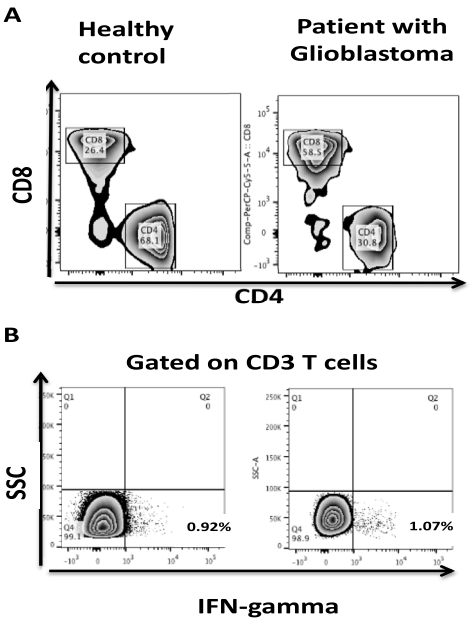
The CTL therapies described employed methods of T cell production that required prolonged periods of activation and expansion and also require specialized GMP facilities and significant regulatory support. This reduces the practicality of adoptive immunotherapy, since CTL lines must be made long in advance of disease, and few centers have the facilities or infrastructure required for this type of cell processing. A recently reported clinical trial using tetramer selection of CMV peptide-specific T cells isolated directly from peripheral blood avoided both ex vivo expansion and live viral antigens. Although exclusively CD8+ T cells were infused, they expanded by several logs after infusion into patients following allogeneic stem cell transplantation, clearing infection in 8/9 cases [28]. Again this approach is limited by the availability of tetramers for uncommon HLA types and lack of class II tetramers. In addition production of clinical-grade tetramers for multiple HLA types for use under GMP may limit wider application of this strategy. Capture of T cells that secrete cytokines in response to stimulation with viral antigens also allows rapid T cell selection as demonstrated for adenovirus specific T cells [29]. This strategy has the advantage that knowledge of epitopes is not required and there is no HLA restriction. Unfortunately it is inefficient, producing small numbers of selected cells, impeding the product characterization essential to later phase clinical study.

We have developed a GMP-compliant strategy to rapidly generate a single preparation of polyclonal (CD4+ and CD8+) T cells against CMV from the peripheral blood of patients with GBM. CMV-specific T cells can be rapidly produced (10 days) by a single stimulation of donor peripheral blood mononuclear cells (PBMCs) with a peptide mixture spanning the CMVpp65 antigen in the presence of the potent pro-survival cytokines interleukin-2 (IL-2), IL4 and IL7. We have shown the feasibility of expanding CMV-specific CTLs from the peripheral blood of MD Anderson immune suppressed glioblastoma patients - even following temozolomide therapy. These expanded CMV-specific T cells are highly functional and produce effector cytokines in response to stimulation with CMV antigen, express normal levels of eomes and T-bet and upregulate expression of CXCR3, a chemokine receptor important for T cell homing to the central nervous system

CMV-specific CD8+ and CD4+ T cells will be generated by our rapid expansion technology in MDACC GMP laboratories. Our current protocol yields a mean 277-fold expansion of CMV-specific CD8+ and CD4+ T cells. Thus, using this approach, the GMP cell manufacturing facility at MD Anderson has been able to reliably expand CMV-specific CD8+ and CD4+ T cells from the leukapheresis product of patients with GBM to meet the total cell number requirements for the clinical protocol (5-10 $X10^8$ T cells).



Feasibility of expanding functional CMV-specific CD8+ and CD8+ T cells from the peripheral blood of GBM patients (top panel) and from the tumor (bottom panel); expanded CMV-specific T cells express t-bet and eomesodermin.



2.5 Autologous CMV-Specific T cells in recurrent Glioblastoma

In a recently published study, the safety and potential clinical efficacy of autologous CMV specific T-cell therapy as consolidative treatment for recurrent GBM was found to be safe, with possible clinical benefit. In a total of 19 patients with recurrent GBM, CMV-specific CD8+ T cells were successfully expanded ex-vivo with interleukin (IL)-2 from 13 patients, 11 of whom received up to four T cell infusions. The overall survival ranged from 133-2428 days with a median overall survival of 403 days [30]. This is almost double the median survival for recurrent GBM patients and suggests a potential long-term clinical benefit requiring further exploration to optimize the efficacy of autologous CMV specific T-cell therapy. Although some patients showed a small increase in virus-specific T cells in the peripheral blood (PB) after the first few infusions, this effect was transient. This study provided an important proof-of-principle platform for a formal assessment of adoptive T-cell immunotherapy in GBM. However, a number of limitations in the study design may have contributed to the transient responses: (a) only CMV-specific CD8+ T cells were infused, without the concurrent transfer of CD4+ T-helper cells, with the latter population being crucial for the maintenance of tumor antigen specific CD8+ T cell populations; (b) T cells were expanded in the presence of IL-2, which is likely to also support the expansion of Foxp3 positive regulatory T cells (Tregs), leading to further inhibition of the expansion, cytotoxicity and persistence of adoptively-infused CMV-specific CD8+ T cells in vivo; (c) the patients included in this study did not receive uniform chemotherapy prior to T cell therapy to provide the benefit of in vivo clonotypic expansion; and (d) no tissue correlates were included to evaluate the the direct immunological effects after intravenous administration of CMV-stimulated T cells. Some patients had received no treatment at all while others received treatment with agents such as Avastin, Etoposide, Carboplatin, and Lomustine [30].

We propose that the efficacy of adoptively infused CMV-specific T cells would be increased by pretreatment with a chemotherapeutic regimen which can achieve deep lymphodepletion, such as dose-dense temozolomide. In addition to instituting a uniform lymphodepleting preparative chemotherapy regimen prior to T cell therapy, we plan to infuse both CMV-specific CD4+ and CD8+ T cells and to examine the tumor microenvironment immunobiology to assess the correlation between CMV-specific T cell frequencies following adoptive immunotherapy and clinical outcome.

2.6 Rationale for dose-dense temozolomide

We have previously demonstrated that dose-dense temozolomide renders superior immunological responses [31]. A phase II, multi-center trial was undertaken to assess the immunogenicity of an experimental EGFRvIII-targeted peptide vaccine in patients with GBM undergoing treatment with serial cycles of standard-dose (STD) (200 mg/m^2/5d) or dose-intensified (DI) TMZ (100 mg/m^2/21d). All patients receiving STD TMZ exhibited at least a transient grade 2 lymphopenia, whereas all patients receiving DI TMZ exhibited a sustained grade 3 lymphopenia (<500 cells/μL). CD3$^+$ T-cell (p=0·005) and B-cell (p=0·004) counts were reduced significantly only in the DI cohort. EGFRvIII-specific immune responses developed in all patients treated with either regimen, but the DI TMZ regimen produced humoral (p=0·037) and delayed-type hypersensitivity (DTH) responses (p=0·036) of much greater magnitude. Although a large scale clinical trial did not

demonstrate a therapeutic benefit of DI TMZ relative to STD [32], the immune modulatory properties justify is use in this particular context.

2.7 Rationale for testing in a surgical cohort with recurrent GBM

Our approach of studying the intratumoral immune response in the context of immunotherapy clinical trials diverges from the prior state of the art. Well known to immunotherapy clinical investigators is the fact that the markers measuring the immune responses in the blood do not correlate with patient outcomes and clinical responses. This is probably secondary to the lack of accounting for the influence of immune suppression present within the tumor microenvironment. We believe that analysis of immune responses from the tumor will be more likely to yield relevant biological information that will guide therapy and correlate with clinical responses since it accounts for the influence of tumor-mediated immune suppression at the effector location as opposed to merely sampling peripheral blood immune responses.

2.8 Rationale for inclusion of exploratory textural MRI

As described above, our studies focus on immune responses within the tumor itself. Ultimately we would like use non-invasive imaging to derive this information. A second key issue for immunotherapy clinical trials is resolving the problem of distinguishing tumor progression from therapeutic immune response as it pertains to clinical trial endpoints. CNS tumor inflammatory imaging would also be useful for stratifying baseline "immune reactivity", monitoring the therapeutic levels of tumor inflammation, and refining schedule and dose. We have been developing MRI inflammatory textural analysis and found that the inflammation status can be measured using Gene Set Enrichment Analysis. Volumetric and heterogeneity features are extracted from T1-post contrast MR and FLAIR images. These features are used to build a classifier capable of discriminating inflammation status. Based on six separate gene sets associated with inflammation, we found that image-derived features are capable of accurately predicting inflammation status in glioblastomas. This is being secondarily validated with a retrospective set of glioblastomas with variable inflammatory infiltration as determined by immunohistochemistry. As such, as an exploratory endpoint, we will be testing if the textural MRI imaging obtained within 48 hours of surgery, correlates with the histological and immune functional data obtained directly from the tumor.

2. 9 Brief Summary of Overall Trial Design

The clinical trial study will include **3 groups of patients**. The **first group (Phase I, Dose Escalation in recurrent GBM)** will consist of cohorts of patients with recurrent GBM to determine the maximum feasible dose (MFD) or maximum tolerated dose (MTD) of CMV T cells combined with dose-dense temozolomide, following a 3+3 design. The **second group (Phase II, Dose Expansion in recurrent GBM undergoing resection)** will consist of patients with recurrent GBM who will receive one therapeutic administration of CMV T cells, followed by tumor resection and up to 3 additional cycles of CMV T cells and dose-dense temozolomide. This group will allow us to: 1) ascertain whether the CMV-specific T cells are trafficking to the tumor microenvironment; 2) evaluate if the immune suppression in the tumor is impacting the functional outcome of our CMV T cells as

reflected by effector responses (IFN, IL-2, and TNF-α, perforin, granzyme B); and 3) determine if there is preferential clearance of viral antigens such as CMV over other tumor-associated antigens during immune therapeutic approaches. The **third group (Phase II, Dose Expansion in newly diagnosed GBM)** will consist of a group of patients with newly diagnosed GBM who will receive therapeutic administration of CMV T cells in combination with cycles of dose-dense temozolomide as adjuvant therapy after chemoradiation, to obtain preliminary data on efficacy as measured by overall survival.

3.0 Drug Information and Preparation of CMV-specific T-cells

3.1 Temozolomide

3.1.1 Temozolomide Agent Information

3.1.1.1 Formulation

Other Names: - methazolastone; Commercial name is Temodar. Temozolomide is supplied in white opaque, preservative-free, two-piece, hard gelatin capsules of the following p.o. dosage strengths: 5 mg, 20 mg, 100 mg, 140 mg, 180 mg, and 250 mg. Dose is rounded to the nearest 5 mg based on body surface area. Each capsule contains drug substance in combination with lactose, anhydrous NF, colloidal silicon dioxide NF, sodium starch glycolate NF, tartaric acid NF, and stearic acid NF. The capsule shells contain gelatin NF, titanium dioxide USP, and sodium lauryl sulfate NF.

3.1.1.2 Mode of Action

Alkylating agent of imidazotetrazinone class.

3.1.1.3 Storage and Stability

The capsules are packaged in amber glass bottles and should be stored at 25 °C. Temperature excursions between 15 and 30 °C are permissible. Refer to the commercially labeled bottles for expiration dating.

3.1.1.4 Pharmacokinetics

Temozolomide is rapidly and completely absorbed after oral administration; peak plasma concentrations occur in 1 hour. Food reduces the rate and extent of temozolomide absorption. Mean peak plasma concentration and AUC decreased by 32% and 9%, respectively, and T_{max} increased 2-fold (from 1.1 to 2.25 hours) when temozolomide was administered after a modified high-fat breakfast.

Temozolomide is rapidly eliminated with a mean elimination half-life of 1.8 hours and exhibits linear kinetics over the therapeutic dosing range. Temozolomide has a mean apparent volume of distribution of 0.4 L/kg

(%CV=13%). It is weakly bound to human plasma proteins; the mean percent bound of drug-related total radioactivity is 15%.

3.1.1.5 Metabolism and Elimination

Temozolomide is spontaneously hydrolyzed at physiologic pH to the active species, 3-methyl-(triazen-1-yl) imidazole-4-carboxamide (MTIC) and to temozolomide acid metabolite. MTIC is further hydrolyzed to 5-amino-imidazole-4-carboxamide (AIC), which is known to be an intermediate in purine and nucleic acid biosynthesis and to methylhydrazine, which is believed to be the active alkylating species. Cytochrome P450 enzymes play only a minor role in the metabolism of temozolomide and MTIC. Relative to the AUC of temozolomide, the exposure to MTIC and ACI is 2.4% and 23%, respectively. About 38% of the administered temozolomide total radioactive dose is recovered over 7 days; 37.7% in urine and 0.8% in feces. The majority of the recovery of radioactivity in urine is as unchanged temozolomide (5.6%), AIC (12%), temozolomide acid metabolite (2.3%), and unidentified polar metabolites(s) (17%). Overall clearance of temozolomide is about 5.5 L/hr/m^2.

3.1.1.6 Special Populations

3.1.1.6.1 *Creatinine Clearance*

Population pharmacokinetic analysis indicates that creatinine clearance over the range of 36-130 mL/min/m^2 has no effect on the clearance of temozolomide after oral administration. The pharmacokinetics of temozolomide has not been studied in patients with severely impaired renal function (CLcr < 36 mL/min/m^2).

Caution should be exercised when temozolomide is administered to patients with severe renal impairment. Temozolomide has not been studied in patients on dialysis.

3.1.1.6.2 *Hepatically Impaired Patients*

In a pharmacokinetic study, the pharmacokinetics of temozolomide in patients with mild to moderate hepatic impairment (Child's-Pugh Class I-II) were similar to those observed in patients with normal hepatic function. Caution should be exercised when temozolomide is administered to patients with severe hepatic impairment.

3.1.1.6.3 *Gender*

Population pharmacokinetic analysis indicates that women have an approximately 5% lower clearance (adjusted for body surface area) for temozolomide than men. Women have higher incidences of grade 4 neutropenia and thrombocytopenia in the first cycle of therapy than men.

3.1.1.6.4 *Age*

Population pharmacokinetic analysis indicates that age (range 19-78 years) has no influence on the pharmacokinetics of temozolomide. In the anaplastic astrocytoma study population, patients 70 years of age or older had a higher incidence of grade 4 neutropenia and grade 4 thrombocytopenia in the first cycle of therapy than patients less than 70 years of age. In the entire safety database, however, there did not appear to be a higher incidence in patients 70 years of age or older.

3.1.1.7 Drug-Drug Interactions

In a multiple dose study, administration of temozolomide with ranitidine did not change the C_{max} or AUC values for temozolomide or MTIC. Population analysis indicates that administration of valproic acid decreases the clearance of temozolomide by about 5%. The clinical implication of this effect is not known. Population analysis failed to demonstrate any influence of co-administered dexamethasone, prochlorperazine, phenytoin, carbamazepine, ondansetron, H_2-receptor antagonists, or phenobarbital on the clearance of orally administered temozolomide.

3.1.1.8 Adverse Events

Hematologic: Thrombocytopenia, leukopenia, myelodysplastic syndrome.

Gastrointestinal: Nausea, vomiting, anorexia.

Hepatic: Elevated liver enzymes (reversible).

Skin: Rash.

Neurologic: Convulsions, weakness on one side of the body, abnormal coordination, paralysis.

Other: Constipation, diarrhea, stomatitis, fatigue, decreased performance status, headache Please refer to the temozolomide package insert for a comprehensive list of adverse events.

3.1.1.9 Special Handling and Precautions

Capsules should not be opened or damaged. Rigorous precautions should be taken to avoid capsule contents having contact with skin or mucous membranes. Capsule contents may be irritating to skin and eyes. Mutagenic and prolonged exposure may cause serious health effects (outside of prescribed dosage in this trial).

Temozolomide is potentially mutagenic and should be handled with appropriate precautions by both staff and patients. Capsules should not be opened. If capsules are accidentally opened or damaged, rigorous precautions should be taken with the capsule contents to avoid inhalation or contact with the skin or mucous membranes. Procedures for proper handling and disposal of anticancer drugs should be considered**.**

3.1.1.10 Contraindications

Temozolomide is contraindicated in patients who have a history of a hypersensitivity reaction to any of its components or to DTIC.

3.1.1.11 Pregnancy Category D

Temozolomide may cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies in pregnant women. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. **Women of childbearing potential should be advised to avoid becoming pregnant during therapy with temozolomide.**

Treatment of a man with temozolomide may increase the risk of birth defects if he causes a woman to become pregnant while he is taking temozolomide. Men treated with temozolomide may have difficulty causing a woman to become pregnant after their treatment is completed. Men receiving temozolomide should be directed to use effective contraception while they are being treated. There is insufficient data to know what the risk of subsequent problems with fertility will be. Similarly, women who are treated with temozolomide may have difficulty becoming pregnant in the future and may at be at increased risk of having children with birth defects. There is insufficient evidence to determine what the risk of these complications will be.

3.1.1.12 Supply

Temozolomide is commercially available and it will be supplied through the patients' insurance provider.

3.2 Autologous CMV-specific T cells

3.2.1 Preparation of CMV-specific T-cells

To prepare CMV-specific CD8+ and CD4+ T cells from the peripheral blood of patients with GBM in compliance with GMP requirements, we will use a validated expansion protocol. Patients with GBM who are enrolled in this study will undergo leukapheresis 3 weeks before the infusion of CMV-specific T cells (treatment day 0; see **Trial Diagrams, 6.1.1, 6.2.1 and 6.3.1**). CMV-specific T cells will be generated by our rapid expansion technology in M.D. Anderson's GMP laboratories as outlined in section **2.4** above, cryopreserved into vials of 5 x10^6, 1 x10^7, 5 x10^7 and 1 x10^8 cells which will then be thawed and administered on day 22 ± 2 working days (expected nadir for lymphopenia) of cycles of dose-dense temozolomide.

3.2.2 Adverse Events

The toxicity of autologous CMV CTLs is lower than the toxicity of allogenic T cells and CAR T cells. GBM patients have previously been dosed with autologous CMV-specific T cells every 4 weeks. The toxicity profile was excellent. There was only a single case of grade 3 seizure activity likely related to the underlying disease (glioma). Other events including infrequent grade 1 headaches, fatigue, visual hallucination, pyrexia, and high blood pressure and grade 2 alterations in liver function tests, lymphopenia, seizure and anxiety. The attribution scores were all unlikely related to the CVM CTLs. (Schuessler, et al. Autologous T cell Therapy for CVM as a Consolidative Treatment for Recurrent Glioblastoma, *Cancer Research*, 74(13), 2014). There has not been reported cytokine release syndrome or autoimmunity in this aforementioned clinical trial or in the experience of dendritic cells targeted to CMV antigens in these patients (Mitchell, et al., Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients, Nature, Mar 19:519(7543):366-9, 2015).
Additionally, we have used allogenic CMV CTLs in 6 patients with refractory CMV infection in the post stem cell transplant setting without significant adverse events (ongoing trial at MD Anderson Cancer Center). Furthermore, we have used tri-virus-specific banked third-party T cells (targeting CMV, EBV and adenovirus) to treat 50 patients with severe, refractory viral infections in the post stem cell transplant setting in a multicenter clinical trial (Leen et al, Blood 2013) with no associated infusion-related AEs. De novo graft versus host disease (GVHD) only occurred in two patients (which did not represent an increased risk of expected GVHD in this patient setting). Since we are proposing autologous CTLs, GVHD responses would not be anticipated.

4.0 Eligibility Criteria

4.1 Summary of Entry criteria for Phase I (Dose Escalation in Recurrent glioblastoma patients)

Patients must have pathology confirmed WHO grade 4 astrocytoma (glioblastoma or gliosarcoma) with any prior number of recurrences, have received prior radiation and/or temozolomide therapy and no prior therapy with bevacizumab. Patients must be at least 3 months (12 weeks) from receiving conformal radiation.

A baseline brain MRI must be obtained no more than 14 days (+ 3 working days) prior to study enrollment. The patient must either be on no steroids or a stable dose of dexamethasone no greater than 2 mg a day for at least 5 days prior to entrance onto the study. Patients having undergone recent surgery are eligible as long as they are at least 3 weeks from resection or 1 week from stereotactic biopsy, and recovering from any operative or perioperative complications. No measurable disease post resection will be required.

Otherwise, all other inclusion and exclusion criteria specified in 4.1.1 and 4.1.2 must be fulfilled.

4.1.1 Phase I Subject Inclusion Criteria

1. Be willing and able to provide written informed consent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Have histologically confirmed World Health Organization Grade IV glioma (glioblastoma or gliosarcoma). Participants will be eligible if the original histology was low-grade glioma and a subsequent histological diagnosis of glioblastoma or variants is made.

4. Patients having undergone recent surgery are eligible as long as they are at least 3 weeks from resection or 1 week from stereotactic biopsy, and recovering from any operative or perioperative complications. No measurable disease post resection will be required.

5. Any number of prior relapses.

6. CMV seropositive.

7. Be willing to provide tissue from an archival tissue sample or newly obtained core or excisional biopsy of a tumor lesion.

8. Have a performance status of ≥ 60 on the KPS.

9. If patient is on steroids, patient must be on a stable or decreasing dose of steroids for 5 days, no more than 2 mg dexamethasone (or equivalent) total per day at the time of screening and consent. If on steroids at the time of screening, the dose will need to be tapered and discontinued at least 5 days prior to CMV T cell infusion.

10. Demonstrate adequate organ function as defined in Table 2.

11. **Table** , all screening labs should be performed within 14 days (+3 working days) of treatment initiation.

Table 2 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	

Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN
[a]Creatinine clearance should be calculated per institutional standard.	

12. Female subject of childbearing potential should have a negative serum pregnancy test within 14 days (+ 3 working days) of study enrollment.

13. Female subjects of childbearing potential should be willing to use 2 methods of birth control during the study and for 30 days after the last dose of the study drug or be surgically sterile. **Female subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.**

14. Male subjects should agree to use an adequate method of contraception during the course of the study and for 30 days after the last dose of the study drug.

4.1.2 Phase I Subject Exclusion Criteria

1. Previous treatment with bevacizumab.

2. Tumor localized primarily to the posterior fossa or spinal cord.

3. Prior interstitial brachytherapy, implanted chemotherapy, or therapeutics delivered by local injection or convection enhanced delivery. Prior treatment with Gliadel® wafers.

4. Less than 12 weeks from completing external beam radiotherapy. Patients with proven PD by resection or with new lesions outside of the radiation field should not be excluded even if they are within 12 weeks of XRT, per RANO criteria for early PD.

5. Patient currently participating in a study of an investigational agent or using an investigational device for therapeutic purposes. Concurrent use of Optune® device is not allowed.

6. CMV seronegative.

7. Patient has a known history of Human Immunodeficiency Virus (HIV) (positive HIV 1/2 antibodies); HTLV1 and/or HTLV2; active Hepatitis B (e.g., HBsAg

reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected). Patients with prior HBV vaccination (anti-HBs positive, HBsAg negative, anti-HBc negative) will NOT be excluded.

8. Patient has a diagnosis of immunodeficiency or is receiving systemic immunosuppressive therapy within 7 days of study entrance.

9. Patient has had prior chemotherapy or targeted small molecule therapy, within 2 weeks prior to study Day 1, or has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

    - Note: If subject received major surgery (other than craniotomy), they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

10. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

11. Has known gliomatous meningitis, subependymal spread, or extracranial disease.

12. Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjogren's syndrome will not be excluded from the study.

13. Has evidence of interstitial lung disease or active, non-infectious pneumonitis.

14. Has an active infection requiring systemic therapy.

15. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

16. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

17. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit.

18. Has received a live vaccine within 30 days prior to the first dose of trial treatment.

19. Contraindication for undergoing MRIs.

4.2 Summary of Entry criteria for Phase II (Dose Expansion): Recurrent glioblastoma undergoing resection

Patients must have pathology confirmed* WHO grade 4 astrocytoma (glioblastoma or gliosarcoma) at first recurrence who require reoperation for tumor progression and have received prior radiation and/or temozolomide therapy. Patients must be at least 3 months (12 weeks) from receiving conformal radiation.

A baseline brain MRI must be obtained no more than 14 days (+ 3 working days) prior to study enrollment. The patient must either be on no steroids or a stable dose of dexamethasone no greater than 2 mg a day for at least 5 days prior to entrance onto the study.

Otherwise, all other inclusion and exclusion criteria specified in 4.2.1 and 4.2.2 must be fulfilled.

*PLEASE NOTE: Participants will be also eligible for this component of the study if the original histology was lower grade glioma and there is suspected transformation to glioblastoma based on imaging findings. If the final pathology report after resection fails to confirm recurrent glioblastoma or gliosarcoma, the subject will be followed for AEs and survival, but excluded for other primary and secondary objective analysis. The subject will be replaced.

4.2.1 Phase II Subject Inclusion Criteria (Dose Expansion in Recurrent GBM undergoing resection)

In order to be eligible for participation in the recurrent glioblastoma Dose Expansion, the subject must:

1. Be willing and able to provide written informed consent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Have histologically confirmed World Health Organization Grade IV glioma (glioblastoma or gliosarcoma). Participants will also be eligible if the original histology was lower grade glioma and there is suspected transformation to glioblastoma based on imaging findings.. If the final pathology report after resection fails to confirm recurrent glioblastoma or gliosarcoma, the subject will be followed for AEs and survival, but excluded for other primary and secondary objective analysis. The subject will be replaced.

4. Be at first relapse. **Note:** Relapse is defined as progression following initial therapy (i.e., radiation, chemotherapy, or radiation+chemotherapy). If the participant had a surgical resection for relapsed disease and no antitumor

therapy instituted for up to 12 weeks, this is considered one relapse. For participants who had prior therapy for a lower grade glioma, the surgical diagnosis of glioblastoma or gliosarcoma will be considered first relapse.

5. Have measurable disease consisting of a minimal volume of 1 cm^3.

6. CMV seropositive.

7. Be willing to provide tissue from an archival tissue sample or newly obtained core or excisional biopsy of a tumor lesion.

8. Have a performance status of ≥ 60 on the KPS.

9. If patient is on steroids, patient must be on a stable or decreasing dose of steroids for 5 days, no more than 2 mg dexamethasone (or equivalent) total per day at the time of screening and consent. If on steroids at the time of screening, the dose will need to be tapered and discontinued at least 5 days prior to CMV T cell infusion.

10. Demonstrate adequate organ function as defined in Table 2.

Table , all screening labs should be performed within 14 days (+3 working days) of treatment initiation.

Table 2 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN

Activated Partial Thromboplastin Time (aPTT)	
[a]Creatinine clearance should be calculated per institutional standard.	

11. Female subject of childbearing potential should have a negative serum pregnancy test within 14 days (+ 3 working days) of study enrollment.

12. Female subjects of childbearing potential should be willing to use 2 methods of birth control during the study and for 30 days after the last dose of the study drug or be surgically sterile. **Female subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.**

13. Male subjects should agree to use an adequate method of contraception during the course of the study and for 30 days after the last dose of the study drug.

4.2.2 Phase II Subject Exclusion Criteria (Dose Expansion in Recurrent GBM undergoing resection)

The subject must be excluded from participating in the recurrent glioblastoma Dose Expansion if the subject:

1. Has been treated previously with bevacizumab.

2. Has tumor localized primarily to the posterior fossa, spinal cord, or an unresectable location.

3. Has received prior interstitial brachytherapy, implanted chemotherapy, or therapeutics delivered by local injection or convection enhanced delivery. Prior treatment with Gliadel® wafers will be excluded.

4. Is $\leq$ 12 weeks from completing external beam radiotherapy. Patients with proven PD by resection or with new lesions outside of the radiation field should not be excluded even if they are within 12 weeks of XRT, per RANO criteria for early PD.

5. Is currently participating in a study of an investigational agent or using an investigational device for therapeutic purposes. Concurrent use of Optune® device is not allowed.

6. CMV seronegative.

7. Has a known history of Human Immunodeficiency Virus (HIV) (positive HIV 1/2 antibodies); HTLV1 and/or HTLV2; active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected). Patients with prior HBV vaccination (anti-HBs positive, HBsAg negative, anti-HBc negative) will NOT be excluded.

8. Has a diagnosis of immunodeficiency or is receiving systemic immunosuppressive therapy within 7 days of study entrance.

9. Has had prior chemotherapy or targeted small molecule therapy, within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

10. Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

11. Note: If subject received major surgery (other than craniotomy), they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

12. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

13. Has known gliomatous meningitis, subependymal spread, extracranial disease, or multifocal disease.

14. Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjogren's syndrome will not be excluded from the study.

15. Has evidence of interstitial lung disease or active, non-infectious pneumonitis.

16. Has an active infection requiring systemic therapy.

17. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

18. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

19. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit.

20. Has received prior therapy with any antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways.

21. Has received a live vaccine within 30 days prior to the first dose of trial treatment.

22. Contraindication for undergoing MRIs.

23. Evidence of bleeding diathesis or use of anticoagulant medication or any medication that may increase the risk of bleeding that cannot be stopped prior to surgery.

4.3 Summary of Entry criteria for Phase II (Dose Expansion): Newly diagnosed glioblastoma.

Patients must have pathology confirmed WHO grade 4 glioma (glioblastoma or gliosarcoma) and completed external beam radiotherapy in combination with temozolomide.

A baseline brain MRI obtained no more than 14 days (+ 3 working days) prior to study enrollment on a stable dose of steroids no greater than 2 mg a day of dexamethasone for at least 5 days, is required prior to entrance of a patient onto the study. Patients must be registered on the study within 5 weeks of completion of concurrent chemoradiation.

Otherwise, all other inclusion and exclusion criteria specified in 4.3.1 and 4.3.2 must be fulfilled.

4.3.1 Phase II Subject Inclusion Criteria (Dose Expansion in Newly diagnosed GBM)

In order to be eligible for participation in Dose Expansion for newly diagnosed GBM, the subject must:

1. Be willing and able to provide written informed consent for the trial.

2. Be ≥ 18 years of age on day of signing informed consent.

3. Have histologically confirmed World Health Organization Grade IV glioma (glioblastoma or gliosarcoma).

4. Patients must have completed standard radiation therapy with concurrent TMZ within 5 weeks of enrollment and must not have evidence of progressive disease on post treatment imaging. Progression can only be defined using diagnostic imaging if there is new enhancement outside of the radiation field (beyond the high-dose region or 80% isodose line) or if there is unequivocal evidence of viable tumor on histopathologic sampling (e.g., solid tumor areas [i.e, > 70% tumor cell nuclei in areas], high or progressive increase in MIB-1 proliferation index compared with prior biopsy, or evidence for histologic progression or increased anaplasia in tumor). Note: Given the difficulty of differentiating true progression from pseudoprogression, clinical decline alone, in the absence of radiographic or histologic confirmation of progression, will not be sufficient for definition of

progressive disease in the first 12 weeks after completion of concurrent chemoradiotherapy [33].

5. CMV seropositive.

6. Be willing to provide tissue from an archival tissue sample.

7. Have a performance status of ≥ 60 on the KPS.

8. If patient is on steroids, patient must be on a stable or decreasing dose of steroids for 5 days, no more than 2 mg dexamethasone (or equivalent) total per day at the time of screening and consent. If on steroids at the time of screening, the dose will need to be tapered and discontinued at least 5 days prior to CMV T cell infusion.

9. Demonstrate adequate organ function as defined in Table 2.

10. Table 2 all screening labs should be performed within 14 days (+3 working days) of treatment initiation.

Table 2 Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**
	Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN
[a]Creatinine clearance should be calculated per institutional standard.	

11. Female subject of childbearing potential should have a negative serum pregnancy test within 14 days (+ 3 working days) of study enrollment.

12. Female subjects of childbearing potential should be willing to use 2 methods of birth control during the study and for 30 days after the last dose of the study drug or be surgically sterile. **Female subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.**

13. Male subjects should agree to use an adequate method of contraception during the course of the study and for 30 days after the last dose of the study drug.

4.3.2 Phase II Subject Exclusion Criteria (Dose Expansion in newly diagnosed GBM)

The subject must be excluded from participating in the newly diagnosed Dose Expansion if the subject:

1. Has been treated previously with bevacizumab.

2. Has received prior interstitial brachytherapy, implanted chemotherapy, or therapeutics delivered by local injection or convection enhanced delivery. Prior treatment with Gliadel® wafers will be excluded.

3. Is currently participating or has participated in any other newly diagnosed therapeutic trial before or after chemoradiation.

4. CMV seronegative.

5. Has a known history of Human Immunodeficiency Virus (HIV) (positive HIV 1/2 antibodies); HTLV1 and/or HTLV2; active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected). Patients with prior HBV vaccination (anti-HBs positive, HBsAg negative, anti-HBc negative) will NOT be excluded.

6. Has a diagnosis of immunodeficiency or is receiving systemic immunosuppressive therapy within 7 days of study entrance.

7. Has had prior chemotherapy ortargeted small molecule therapy, within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.

    a. Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.

    b. Note: If subject received major surgery (other than craniotomy), they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

8. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy.

9. Has known gliomatous meningitis, extracranial disease, or multifocal disease.

10. Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjogren's syndrome will not be excluded from the study.

11. Has evidence of interstitial lung disease or active, non-infectious pneumonitis.

12. Has an active infection requiring systemic therapy.

13. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

14. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

15. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the screening visit.

16. Has received prior therapy with any antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways.

17. Has received a live vaccine within 30 days prior to the first dose of trial treatment.

18. Contraindication for undergoing MRIs

5.0 Stratification/Descriptive Factors

5.1 Phase I: Dose Escalation in Recurrent glioblastoma

Patients with histologically proven glioblastoma or gliosarcoma (WHO grade IV astrocytoma) at any recurrence (see eligibility criteria for full details).

There will be an accrual of a minimum of 15 patients and a maximum of 24 patients to the dose escalation component of the study. Dose escalation will follow a 3+3 design evaluating 4 dose levels of CMV-specific T cells to determine MFD or MTD.

5.2 Phase II (Dose Expansion): Recurrent glioblastoma undergoing resection
Patients with histologically proven glioblastoma or gliosarcoma (WHO grade IV astrocytoma) at first recurrence (see eligibility criteria for full details).

There will be an accrual of 10 evaluable patients to the surgical subset of the study, who will be treated at the MFD or MTD determined in dose escalation cohort.

5.3 Phase II (Dose Expansion): Newly diagnosed glioblastoma

Patients with histologically proven newly diagnosed glioblastoma or gliosarcoma (WHO grade IV astrocytoma) (see eligibility criteria for full details).

There will be an accrual of 20 evaluable patients to this subset of the study, who will be treated at the MFD or MTD determined in dose escalation cohort.

6.0 Treatment Plan

All patients who meet eligibility criteria must be registered with the University of Texas, MD Anderson Cancer Center (UT MD ANDERSON CANCER CENTER).

6.1 Phase I (Dose Escalation): Recurrent glioblastoma

This will be a dose escalation component in patients with recurrent glioblastoma to determine the maximum feasible dose (MFD) or maximum tolerated dose (MTD) of CMV-specific T cells in combination with dose-dense temozolomide. We will follow a 3+3 design exploring 4 dose levels of CMV-T cells: 5×10^6, 1×10^7, 5×10^7, and 1×10^8 CMV CTLs.

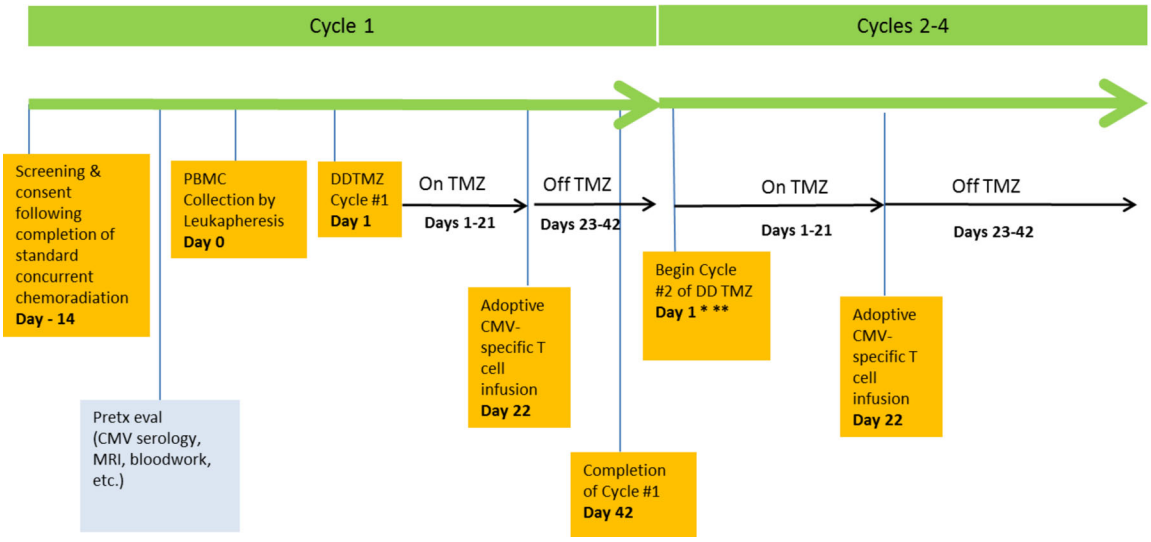
Patients will be enrolled in cohort size of 3. If two DLTs thought to be related to CMV-T cells occur at the first dose level, the MTD has been exceeded, and the trial will stop. The MTD is the dose level at which 0/6 or 1/6 patients experience a DLT with at least 2 patients experiencing DLT at the next higher dose level. A minimum of six patients must be treated at the MTD or MFD.

Number of patients with DLTs / Number of Evaluable patients	3+3 rule
0/3	Proceed with next dose escalation with three patients

1/3	Expand current dose level to 6 patients
1/6	Proceed with next dose escalation with three patients
>/=2/3 or >/=2/6	The MTD has been exceeded. Expand previous dose level to a total of 6 patients if six patients were not already treated. The MTD is defined as the dose level at which 0/6 or 1/6 patients experience a DLT with at least 2 patients experiencing DLT at the next higher dose level. A minimum of six patients must be treated at the MTD or MFD.

The DLT period will include the first cycle of therapy with dose-dense temozolomide and CMV CTLs (6 weeks). After all patients in each dose escalation cohort complete the first cycle of therapy (DLT period), all toxicities will be reviewed by the PI and sent to the IND office for review before proceeding with escalation to the next dose level of CMV-T cells. The dose escalation component will be performed in patients with recurrent glioblastoma not undergoing surgical resection, with any number of prior tumor recurrences, and otherwise fulfilling all other inclusion and exclusion criteria specified in 4.1.1 and 4.1.2. We expect to confirm a Maximum Feasible Dose (MFD) rather than an MTD in this dose escalation component. Once the MFD is confirmed (or an MTD is determined) enrollment to dose expansion arms will start. Each patient on dose escalation will receive up to 4 doses of CMV-T cells and dose-dense temozolomide, but no intrapatient dose escalation will be permitted. Cycles of dose–dense temozolomide can be continued beyond the 4th dose if patient is receiving benefit from therapy, for up to 12 cycles.

6.1.1 Trial Diagram Phase I (Dose Escalation) in Recurrent GBM



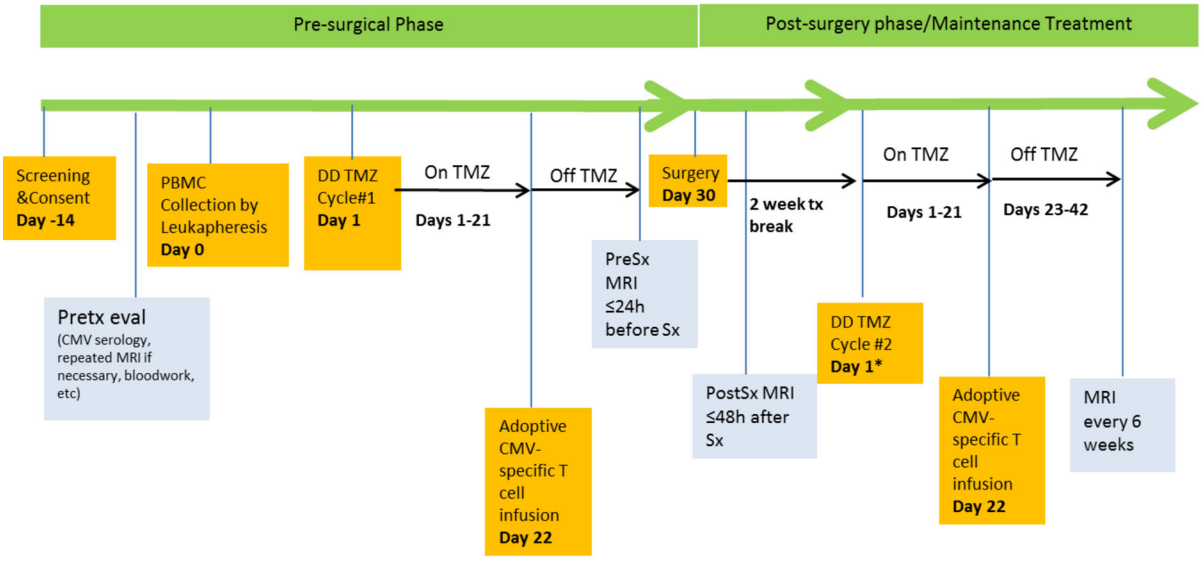
*4 cycles total of dose-dense TMZ and CMV-T cell infusion.
**Following completion of 4 cycles of dose-dense TMZ and CMV-T cell infusion, patients can receive additional 42-day cycles of dose-dense TMZ for up to 12 cycles or tumor PD.

Patients with recurrent glioblastoma or gliosarcoma who fulfil eligibility criteria (4.1.1, 4.1.2) will undergo PBMC collection on Day 0 via leukapheresis. Following leukapheresis, patients will begin treatment with 21 days of dose-dense temozolomide at a dose of 100 mg/m2/day. On day 22 (±2 working days), the patient will then undergo adoptive CMV-specific T cell transfer (at dose level of CMV-T cells assigned to the cohort). T cells will be infused into patients as per validated protocols available from the GMP and SCT laboratories. Patients will receive pretreatment with diphenhydramine and acetaminophen by mouth prior to T cell infusion to help reduce the risk of side effects. Premedication with steroids is not allowed. Vital signs (blood pressure, pulse, respiratory rate, pulse oximetry) will be monitored at baseline and 30 and 60 minutes after the T cell infusion. Patients must not receive steroids at least for 5 days prior to CMV specific T-cell infusion and 28 days post-infusion. Patients will then receive an additional 3 cycles of dose-dense temozolomide followed by CMV-specific T cell infusion, at fixed dose of CMV-specific T cells (dose assigned to the cohort). Cycles will be defined as every 42 days (first 4 cycles). After a total of 4 cycles of dose-dense temozolomide followed by CMV specific T cell infusion, participants can then continue 42-day cycles of dose-dense temozolomide alone until tumor progression, as long as there are no unacceptable toxicities (see section 6.4 and 6.5 for criteria for discontinuation of therapy based on treatment related toxicities), or until completion of a total of 12 cycles of treatment (whichever occurs first). Responses will be assessed by clinical examinations and MRI scans every 6 weeks (i.e., after each cycle).

6.2 Phase II (Dose Expansion): Recurrent glioblastoma undergoing resection

This will be an exploratory arm in patients with glioblastoma at first recurrence. Suspected transformation of a previously diagnosed lower grade glioma into glioblastoma will be considered first recurrence; this will need to be confirmed by pathology at the time of surgery for the patient to be included in analysis of primary and secondary endpoints other than safety and toxicity. If pathology does not confirm glioblastoma or gliosarcoma, the study subject will be replaced. Patients must have received prior radiation and/or temozolomide therapy. A total of 10 evaluable patients with recurrent glioblastoma or gliosarcoma who require reoperation for tumor progression will be treated with dose-dense temozolomide for lymphodepletion followed by intravenous administered CMV-stimulated adoptive T cells.

6.2.1 Trial Diagram Phase II (Dose Expansion) in Recurrent GBM undergoing resection



*Following completion of 3 cycles of dose-dense TMZ and CMV-T cell infusion in the post-surgical phase, patients can receive 42-day cycles of dose-dense TMZ alone up to 12 cycles or tumor PD

Patients with glioblastoma or gliosarcoma at first recurrence who have received prior radiation and/or temozolomide therapy and require reoperation for tumor progression will undergo PBMC collection on Day 0 via leukapheresis. Leukapheresis will be performed in the MDACC blood bank as per validated protocols. Following leukapheresis, patients will begin treatment with 21 days of dose-dense temozolomide at a dose of 100 mg/m^2/day. On day 22 (±2 working days), the patient will then undergo adoptive CMV-specific T cell transfer (first dose at MFD or MTD of CMV-specific T cells previously determined in Dose Escalation). Patients must not receive steroids for at least 5 days prior to CMV specific T-cell infusion and 28 days post-infusion. T cells will be infused into patients as per validated protocols available from the GMP and SCT laboratories. Patients will receive pretreatment with diphenhydramine and acetaminophen by mouth prior to T cell infusion to help reduce the risk of side effects. Premedication with steroids is not allowed. Vital signs (blood pressure, pulse, respiratory rate, pulse oximetry) will be monitored at baseline and 30 and 60 minutes after the T cell infusion. Surgery for resection of recurrent disease will be scheduled on day 30 (±2 working days). In anticipation of surgery, patients will be allowed to undergo platelet transfusions as necessary to keep platelets $\geq$ 50,000. Patients must be evaluated with a pre surgical MRI within 24 hours of resection and a post surgical brain MRI within 48 hours of resection. In the post-surgery phase, subsequent cycles of dose-dense temozolomide and CMV-specific T cells on Day 22 (±2 working days), will be administered for a total of 3 cycles, at the MFD or MTD determined during dose escalation component. Patients will start these subsequent cycles 2 weeks after surgery, provided that the patient has fully recovered from any perioperative complications, KPS is ≥60, and hematological and organ function values are adequate to start chemotherapy (Table 2). Cycles will be defined as every 42 days. Participants will receive a total of 3 cycles of dose-dense temozolomide followed by fixed doses of CMV-specific T cell infusion in the post-surgical

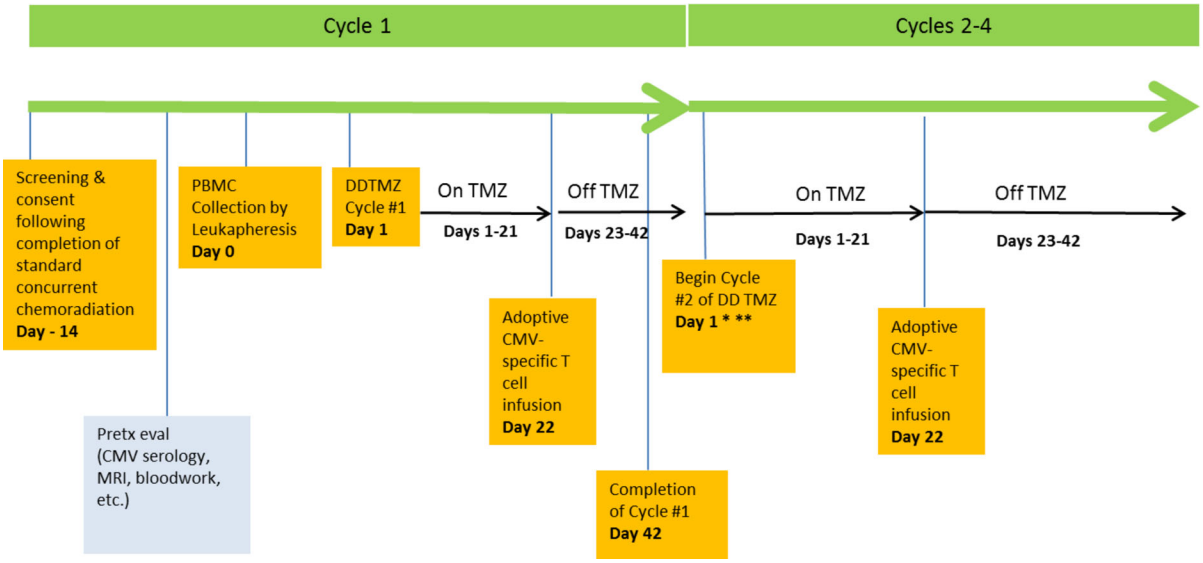
phase, after which they can remain on dose-dense temozolomide until tumor progression, as long as there are no unacceptable toxicities (see section 6.4 and 6.5 for criteria for discontinuation of therapy based on treatment related toxicities), or until completion of 12 cycles of treatment with dose-dense temozolomide (whichever occurs first). Responses will be assessed by clinical examinations and MRI scans every 6 weeks.

The MRI Brain obtained within 48 hours following surgical resection will be considered the baseline MRI for evaluation of treatment response (no new MRI will be obtained before cycle #2). The PFS6, median duration of response, and OS will be determined. Upon the second tumor recurrence, if recommended by the patient's treating physician, repeat biopsy and/or resected tumor will be analyzed for the persistence of CMV antigens post treatment and the peripheral blood will be sampled for CMV-specific T cell frequency and effector functions.

6.3 Phase II (Dose Expansion): Newly diagnosed glioblastoma

This will be a single arm exploratory Phase 2 clinical trial in patients with newly diagnosed glioblastoma. Patients must have completed external beam radiotherapy in combination with temozolomide within 5 weeks of accrual to this study.

6.3.1 Trial Diagram Phase II (Dose Expansion) in Newly Diagnosed GBM



*4 cycles total of dose-dense TMZ followed by adoptive CMV T cell infusion.
Following completion of 4 cycles of dose-dense TMZ and CMV T cell infusion, patients can receive **standard dose TMZ 200 mg/m^2 days 1-5 every 28 days up to 12 cycles or tumor PD.

Patients with newly diagnosed glioblastoma or gliosarcoma who have completed standard concurrent chemoradiation with low dose daily temozolomide within the previous 5 weeks will undergo PBMC collection on Day 0 via leukapheresis. Following leukapheresis, patients will begin treatment with 21 days of dose-dense temozolomide at a dose of 100 mg/m^2/day. On day 22

(±2 working days), the patient will then undergo adoptive CMV-specific T cell transfer (first dose at MFD or MTD of CMV-specific T cells previously determined in Dose Escalation). T cells will be infused into patients as per validated protocols available from the GMP and SCT laboratories. Patients will receive pretreatment with diphenhydramine and acetaminophen by mouth prior to T cell infusion to help reduce the risk of side effects. Premedication with steroids is not allowed. Vital signs (blood pressure, pulse, respiratory rate, pulse oximetry) will be monitored at baseline and 30 and 60 minutes after the T cell infusion. Patients must not receive steroids at least for 5 days prior to CMV specific T-cell infusion and 28 days post-infusion. Patients will then receive an additional 3 cycles of dose-dense temozolomide followed by CMV-specific T cell infusion, at fixed dose of CMV-specific T cells (MFD or MTD determined in dose escalation). Cycles will be defined as every 42 days (first 4 cycles). After a total of 4 cycles of dose-dense temozolomide followed by CMV specific T cell infusion, participants can then continue on standard dose temozolomide (200 mg/m^2 days 1-5 every 28 days) (i.e., 28-day cycles starting in cycle 5) until tumor progression, as long as there are no unacceptable toxicities (see section 6.4 and 6.5 for criteria for discontinuation of therapy based on treatment related toxicities), or until completion of a total of 12 cycles of treatment (whichever occurs first). Responses will be assessed by clinical examinations and MRI scans every 6 weeks during the 4 cycles of dose-dense temozolomide combined with CMV-specific T cell infusions, and every 8 weeks during treatment with standard dose temozolomide (at the beginning of each odd cycle, i.e., cycles 7, 9 and 11). The MRI Brain following chemoradiation will be considered the baseline MRI for evaluation of treatment response. The PFS6, ORR, median duration of response, and OS will be determined.

6.4 Temozolomide Administration and Dose Modifications

6.4.1. Dose-dense Temozolomide Administration (All patients).

6.4.1.1 Temozolomide will be administered orally once per day for 21 consecutive days (days 1-21) of a 42-day cycle (6 weeks). The starting dose for the first cycle will be 100 mg/m2/day.

6.4.1.2 The dose will be determined using the body surface area (BSA) calculated at the beginning of each treatment cycle. Capsules of temozolomide are available in 5, 20, 100, 180 and 250 mg. The daily dose will be rounded to the nearest 5 mg. Each daily dose should be given with the least number of capsules.

6.4.1.3 Prior to each treatment cycle with temozolomide a complete blood count (CBC) will be obtained on Day 1 or within 3 days prior to dosing. Additionally, CBC and differential will be performed weekly during Cycle 1, and then every 2 weeks after the first daily dose of TMZ during each subsequent cycle. The start of the first cycle of dose-dense temozolomide will be scheduled 1 day (+ 3 working days) after leukapheresis. The start of all subsequent cycles of dose-dense temozolomide will be scheduled every 42 days (± 3 working days) after the first daily dose of temozolomide of the preceding cycle, provided that the patient has recovered from any drug-related toxicities during the preceding cycle and there is no tumor progression. Patients in the recurrent subset cohort must have recovered from any perioperative complications by week 2 post surgery to restart dose-dense temozolomide; patients unable to restart temozolomide by week 4 post surgery will stop treatment.

6.4.1.4 Duration of treatment:
Patients will be treated with dose-dense temozolomide for up to 4 cycles along with autologous CMV specific T cells unless there is evidence of tumor progression as defined below (section 9.0) or treatment related toxicity (section 6.4.2 below). At the completion of 4 cycles:

a) Patients with recurrent GBM (Dose Escalation and Dose Expansion) may receive up to 8 additional cycles of dose-dense temozolomide (therefore, a maximum of 12 cycles) if treatment has been well tolerated and at least one of the following criteria are met:

- Serial MR studies show continued tumor response as evidenced by reduction in tumor size.
- The patient demonstrates progressive improvement in overall performance status.
- The patient demonstrates clinical improvement by improvement in neurologic function.

Both the treating physician and PI must agree that the patient is receiving sustained benefit from therapy, based on the criteria above.

b) Patients with newly diagnosed GBM (Dose Expansion) may also receive additional cycles of standard-dose temozolomide for 8 additional cycles if the above criteria are met.

6.4.1.5 Patients may keep leftover temozolomide after pill count if they wish to.

6.4.2 Dosing Modifications for Dose-Dense Temozolomide

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

Dose Level	Dose mg/m2	Remarks
-2	50	Reduction if prior AE at Dose level -1
-1	75	Reduction if prior AE at Dose level 0
0	100	Starting dose for Cycle 1

First cycle
Temozolomide will be started at a dose of 100 mg/m2/day.

Subsequent cycles
The dose of temozolomide will be determined according to (1) treatment-related non-hematologic AE during the preceding treatment cycle, as well as (2) the worst ANC and platelet counts.

Delay
On day 1 of each cycle (within the prior 3 days), ANC $\geq 1.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L and all grade 3 or 4 treatment-related non-hematologic AEs (except for alopecia, nausea and vomiting) must have resolved (to grade ≤ 1). If AEs persists, treatment should be delayed by 1

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

Dose escalation

If, during any cycle the dose of temozolomide is decreased to dose level -1 or -2, no subsequent dose escalations will be permitted.

Dose reductions

If any treatment-related non-hematologic AE observed was grade > 2 (except alopecia, nausea and vomiting) and/or if platelets < 50 x 10^9/L and/or ANC < 1 x 10^9/L, then the dose should be reduced by one dose level. Patients who require more than two dose reductions will have treatment stopped.

If any treatment-related non-hematological AE observed was grade 4 (except alopecia, nausea and vomiting) then dose-dense temozolomide treatment should be stopped.

Summary of Dose Modifications or Discontinuation During Dose-dense Temozolomide

Worst Treatment-Related Non-Hematological AE (except for alopecia, nausea and vomiting) During the Previous Cycles	
Grade	**Dose Modification**
0-2	No dose modifications for non-hematologic AEs. Dose reductions based on ANC and platelet counts are applicable.
3	Reduce by one dose level (except alopecia, nausea and vomiting).
4	Stop (except alopecia, nausea and vomiting). Dose modifications based on ANC and platelet counts are not applicable.

Worst Treatment-Related Hematologic AE During the Previous Cycle

Nadir Values (Worst AE)	**Platelets**			
		≥ 100 x 10^9/L	**50-99 x 10^9/L**	**< 50 x 10^9/L**
ANC	**≥ 1.5 x 10^9/L**	Continue same dose level	Continue same dose level	Reduce by 1 dose level
	≥1 & <1.5 x 10^9/L	Continue same dose level	Continue same dose level	Reduce by 1 dose level

	< 1 x 10^9/L	Reduce by 1 dose level	Reduce by 1 dose level	Reduce by 1 dose level

Note: CBC, differential, and platelets will be performed weekly during cycle 1. Then, every two weeks after the first daily dose of temozolomide during each cycle or at any other time if considered medically necessary by treating physician.

Hematological AE on Day 1 of Each Cycle (within 3 days before Day 1)	
AE	**Delay**
ANC< 1.5 x 10^9/L and/or Platelet count < 100 x 10^9/L	Delay up to 4 weeks until all resolved. If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on non-hematologic AEs are applicable.

Treatment-related Non-Hematologic AE (except for alopecia, nausea and vomiting) On the day 1 of Each Cycle (within the prior 3 days)	
Grade	**Delay**
3	Delay up to 4 weeks until all resolved (to grade ≤ 1). If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on ANC and platelets are applicable.

6.4.3. Dosing Modifications for Standard-dose Temozolomide (Phase II/Dose Expansion in Newly diagnosed GBM, cycles 5 to 12).

Temozolomide will be administered orally once per day for 5 consecutive days (days 1-5) of a 28-day cycle (4 weeks). The starting dose for the first cycle will be 200 mg/m2/day.

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

Dose Level	Dose mg/m2	Remarks
-2	125	Reduction if prior AE at Dose level -1
-1	150	Reduction if prior AE at Dose level 0
0	200	Starting dose for Cycle 5

Delay

On day 1 of each cycle (within the prior 3 days), ANC $\geq 1.5 \times 10^9$/L, platelet count $\geq 100 \times 10^9$/L and all grade 3 or 4 treatment-related non-hematologic AEs (except alopecia, nausea, and vomiting) must have resolved (to grade ≤ 1).

If AEs persists, treatment should be delayed by 1 week for up to 3 consecutive weeks. If, after 4 weeks of delay, all AEs have still not resolved: then any further adjuvant treatment with temozolomide should be stopped.

Dose escalation

If, during any cycle the dose of temozolomide is decreased to dose level -1 or -2, no subsequent dose escalations will be permitted.

Dose reductions

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

If any treatment-related non-hematologic AE observed was grade > 2 (except alopecia, nausea and vomiting) and/or if platelets $< 50 \times 10^9$/L and/or ANC $< 1 \times 10^9$/L, then the dose should be reduced by one dose level. For patients who would require dose reductions to a dose level < 125 mg/m2/day, temozolomide will be stopped. Also, if any of the same treatment-related non-hematologic grade 3 AE recurs (except alopecia, nausea and vomiting) after reduction for that AE, then temozolomide will be stopped.

If any treatment-related non-hematologic AE observed was grade 4 (except alopecia, nausea and vomiting) then adjuvant temozolomide treatment should be stopped.

Summary of Dose Modification or Discontinuation During Standard-dose Temozolomide

Worst Treatment-Related Non-Hematological AE (except for alopecia, nausea and vomiting) During the Previous Cycles	
Grade	**Dose Modification**

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

Nadir Values (Worst AE)	**Platelets**			
		≥ 100 x 10^9/L	**50-99 x 10^9/L**	**< 50 x 10^9/L**
ANC	**≥ 1.5 x 10^9/L**	Continue same dose level	Continue same dose level	Reduce by 1 dose level
	≥1 & <1.5 x 10^9/L	Continue same dose level	Continue same dose level	Reduce by 1 dose level
	< 1 x 10^9/L	Reduce by 1 dose level	Reduce by 1 dose level	Reduce by 1 dose level

Note: In addition to Day 1, a complete blood count must be performed 21 days (± 48 hours) after the first daily dose of each cycle of standard-dose temozolomide.

Hematological AE on Day 1 of Each Cycle (within 3 days before Day 1)	
AE	**Delay**
ANC< 1.5 x 10^9/L and/or Platelet count < 100 x 10^9/L	Delay up to 4 weeks until all resolved. If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on non-hematologic AEs are applicable.

Treatment-related Non-Hematologic AE (except for alopecia, nausea and vomiting) On the day 1 of Each Cycle (within the prior 3 days)	
Grade	**Delay**
3	Delay up to 4 weeks until all resolved (to grade ≤ 1). If unresolved after 4 weeks then stop. If resolved, dose delay/reductions based on ANC and platelets are applicable.

6.5 CMV-specific T cells

Treatment-related limiting toxicities of CMV CTLs will include infusional toxicity resulting in intubation or death.

The development of grade 3 or 4 non-hematological toxicity believed to be related to CMV CTLs will lead to discontinuation of T cell infusions. The development of hematological toxicity will be more likely related to temozolomide and will be managed as specified in sections 6.4.2 and 6.4.3 of the protocol.

7.0 Pretreatment Evaluation

7.1 Phase I (Dose Escalation) in Recurrent glioblastoma and Phase II (Dose Expansion) in Newly diagnosed glioblastoma

7.1.1 A complete history, physical and neurological examination (to include documentation of the patient's Karnofsky Performance Status) as well as baseline 3 Tesla MRI shall be performed on all patients. The scan done prior to study entry will be reviewed by the patient's treating physician. The baseline scan should be performed within 14 days (+3 working days) of registration.

7.1.2 Pre-study laboratory tests shall include CBC, differential, platelets, basic metabolic panel, serology for HIV, HBV, HCV, HTLV1, HTLV2, CMV, West Nile virus, Syphilis, Chagas Disease and serum pregnancy test for women of childbearing potential. Pre-study laboratory tests must be obtained within 14 days (+3 working days) of registration. In women of childbearing potential, a urine pregnancy test must be obtained within 72 hours of starting temozolomide (C1D1).

7.1.3 Tumor tissue from original resection will be collected and held in the Neuropathology tissue bank.

7.1.4 EKG and Chest X Ray will be obtained within 14 days (+3 working days) of registration)

7.2 Phase II (Dose Expansion): Recurrent glioblastoma undergoing resection

7.2.1 A complete history, physical and neurological examination (to include documentation of the patient's Karnofsky Performance Status) as well as baseline 3 Tesla MRI shall be performed on all patients. The scan done prior to study entry will be reviewed by the patient's treating physician. The baseline scan should be performed within 14 days (+3 working days) of registration.

7.2.2 Pre-study laboratory tests shall include CBC, differential, platelets, basic metabolic panel, coags, serology for HIV, HBV, HCV, HTLV1, HTLV2, CMV, West Nile virus, Syphilis, Chagas Disease and serum pregnancy test for women of childbearing potential. Pre-study laboratory tests must be obtained within 14 days (+3 working days) of registration. In women of childbearing potential, a urine pregnancy test must be obtained within 72 hours of starting temozolomide (C1D1).

7.2.3 Tumor tissue from original resection will be collected and held in the Neuropathology Tissue bank. Correlative studies on tumor tissue will be conducted by Dr. Amy Heimberger's lab.

7.2.4 EKG and Chest X Ray will be obtained within 14 days (+3 working days) of registration)

8.0 Evaluation during study

8.1 Phase I (Dose Escalation) in Recurrent glioblastoma and Phase II (Dose expansion) in Newly diagnosed glioblastoma

8.1.1 CBC, differential, and platelets will be performed weekly during Cycle 1 of dose-dense temozolomide, and then, every two weeks during the cycles of dose-dense temozolomide or at any other time if considered medically necessary by treating physician. Note: CBC labs at Week 3 will be obtained on the day of T cell infusion (Day 22 ±2 working days) in Cycles 2-4. Basic metabolic panel will be performed prior to (within 3 days before Day 1) and on Day 29 of each cycle of dose-dense temozolomide. During the cycles of standard-dose temozolomide, CBC, differential and platelets, and basic metabolic panel will be performed prior to each cycle (within 3 days before Day 1) of standard dose temozolomide. Serum pregnancy testing for women of childbearing potential will be obtained prior to each cycle.

8.1.2 A brain MRI will be done every 6 weeks prior to cycles 2, 3 and 4. Beyond cycle 4, Phase I (Dose Escalation) patients will be evaluated with MRIs every 6 weeks; Phase II Dose Expansion-newly diagnosed patients will be evaluated with MRIs every 8 weeks. If there

is a treatment delay to start the next cycle of therapy, there will be no need to repeat another MRI prior to starting the cycle. The next scan will be obtained 6 weeks after the actual start day of the delayed cycle. All imaging studies will be done on a 3 Tesla MRI scanner. All relevant information regarding drug doses, concomitant medications, and doses, measurable lesions with measurements, tumor response, laboratory examinations, and treatment-related toxicities shall be documented in the patient's medical record and flow sheets.

8.1.3 A complete physical and neurologic exam (to include documentation of the patients Karnofsky Performance Status) will be performed at each visit on Day 1 of every cycle, including C1D1(within 3 working days before Day 1).

8.1.4 All patients who discontinue study treatment will be contacted for safety evaluations (e.g., adverse events, concomitant medications) 30 days following the last dose of study treatment. If patients refuse to return for these visits or are unable to do so, every effort should be made to contact them or a knowledgeable informant by telephone to determine their status.

8.1.5 Patients will be evaluated for adverse events at the time of each clinic visit and at least weekly during their first cycle of therapy. These adverse events will be reported to the Study Chair.

8.1.6 Adverse events for subsequent cycles will be reported at every visit with MRI. In addition all serious adverse events will be reported to the study chair.

8.1.7 A total of approximately 60 mL of blood (5 Heparin Green Top Tubes and 1 EDTA tube) for Laboratory Correlates will be collected at the time points specified in Table 6 (Section 8.3).

8.2 Phase II (Dose Expansion): Recurrent glioblastoma undergoing resection

8.2.1 CBC, differential, and platelets will be performed weekly during cycle 1 of dose dense temozolomide (presurgical). Then, every two weeks during subsequent temozolomide cycles or at any other time if considered medically necessary by treating physician. Note: CBC labs at Week 3 will be obtained on the day of T cell infusion (Day 22 ±2 working days) in cycles 2-4. Basic metabolic panel will be performed prior to (within 3 days before Day 1) and on Day 29 of each cycle of dose-dense temozolomide. Serum pregnancy testing for women of childbearing potential will be obtained prior to each cycle.

8.2.2 A brain MRI will be done within 24h of resection and within 48 hours of surgical resection, followed by MRIs every 6 weeks prior to every cycle (no MRI will be obtained before cycle #2; the postsurgical MRI obtained within 48 hours of resection will serve as the baseline) . If there is a treatment delay to start the next cycle of therapy, there will be no need to repeat another MRI prior to starting the cycle. The next scan will be obtained 6

weeks after the actual start day of the delayed cycle. All imaging studies will be done on a 3 Tesla MRI scanner. All relevant information regarding drug doses, concomitant medications, and doses, measurable lesions with measurements, laboratory examinations, and treatment-related toxicities shall be documented in the patient's medical record and flow sheets.

8.2.3 A complete physical and neurologic exam (to include documentation of the patients Karnofsky Performance Status) will be performed at each visit on Day 1 of every cycle, including C1D1 (within 3 working days before Day 1).

8.2.4

All patients who discontinue study treatment will be contacted for safety evaluations (e.g., adverse events, concomitant medications) 30 days following the last dose of study treatment. If patients refuse to return for these visits or are unable to do so, every effort should be made to contact them or a knowledgeable informant by telephone to determine their status.

8.2.5 Patients will be evaluated for adverse events at the time of each clinic visit and at least weekly during their first cycle of therapy. These adverse events will be reported to the Study Chair. Evaluation of adverse events can be performed by phone when the patient is not due for a clinic visit.

8.2.6 Adverse events for subsequent cycles will be reported at the end of each cycle. In addition all serious adverse events will be reported to the study chair.

8.2.7 A total of approximately 60 mL of blood (5 Heparin Green Top Tubes and 1 EDTA tube) for Laboratory Correlates will be collected at the time points specified in Table 4 and 5 (Section 8.3).

8.3 **Peripheral Blood and Tumor Tissue Biomarkers**

The persistence and expansion of adoptively-infused CMV-specific T cells will be determined by multiparameter flow cytometry in serial peripheral blood samples (Dose Escalation and both Dose Expansion groups) and tumor-infiltrating lymphocytes (TILs) (Dose Expansion in Recurrent GBM undergoing resection) at time points detailed in **Table 4, Table 5** and **Table 6.** These correlative studies will be performed at Dr. Katy Rezvani's and Dr. Amy Heimberger's lab.

Trafficking of CMV-specific T cells after adoptive transfer to the tumor in the CNS will be studied in the Dose Expansion-recurrent GBM group by resecting the recurrent tumor, isolating TILs, and assessing these for CMV-specificity ex vivo by peptide/multimers and by functional characterization (intracellular cytokine, cytotoxicity and transcription factors). The CMV antigen expression before (from the original resection) and after treatment (recurrent) will be determined using immunohistochemistry to identify the potential for tumor escape variants and will be compared to a matched set of GBM patients undergoing standard-of-care therapy. Correlative intratumoral immune assays, such as T effector:Treg ratios, will be performed. The persistence of

CMV-specific T cells in the peripheral blood and the frequency of effector CMV-specific T cell responses within the tumor will then be correlated with clinical outcomes.

Table 4: Phase II (Dose Expansion) in Recurrent GBM undergoing resection. Schedule of sampling for CMV-specific T cell correlative studies in peripheral blood and resected tumor tissue after first infusion of CMV T cells.

FIRST INFUSION OF CMV T CELLS (Cycle 1)	Collecting Times				
	Pre surgery (within 48h prior to surgery)	Day of Surgery (C1D30)	Day 8 Post surgery (*) (C1D38)	Day 14 Post surgery (**) (C2D1, before first dose of TMZ)	Day 30 Post surgery (C2D 16) (*)
CMV-specific T cell frequencies, phenotype & functional immunoassays	x		x	X	X
Cytokine analysis	x		x	X	X
TCR clonotyping and diversity	x				X
Tumor-infiltrating lymphocytes		x			

(*) With a margin of +/- 7 working days.
(**) "Day 14" of first infusion can be obtained with a margin of ±7 working days, as long as it's obtained BEFORE initiation of the next dose-dense temozolomide cycle.

Table 5: Phase II (Dose Expansion) in Recurrent GBM undergoing resection. Schedule of sampling for CMV-specific T cell correlative studies in peripheral blood in subsequent cycles of CMV T cells.

SUBSEQUENT INFUSIONS OF CMV T CELLS (Cycles 2, 3 and 4)	Collecting Times				
	Pre infusion C2D22 C3D22 C4D22	Day 8 Post infusion (*) C2D30 C3D30 C4D30	Day 42 (**) C3D1, C4D1, C5D1, before first dose of TMZ	Day 180# (*) C8D34	Disease progression (***)
CMV-specific T cell frequencies, phenotype & functional immunoassays	X	x	x	x	
Cytokine analysis	X	x	x		
TCR clonotyping and diversity	X		x	x	
Tumor-infiltrating lymphocytes					x

#After cycle#4 only (last administration of CMV-specific T cells)
(*) With a margin of +/- 7 working days.
(**) "Day 42" of subsequent infusions can be obtained with a margin of ±7 working days, as long as it's obtained BEFORE initiation of the next dose-dense temozolomide cycle.

(***) Tumor tissue can be collected at disease progression if patient undergoes surgical resection for clinical indications.

Table 6: Phase I (Dose Escalation) in Recurrent GBM and Phase II (Dose Expansion) in Newly diagnosed GBM. Schedule of sampling for CMV-specific T cell correlative studies in peripheral blood in all cycles of CMV T cells

ALL INFUSIONS OF CMV T CELLS (Cycles 1, 2, 3 and 4)	Collecting Times				
	Pre infusion C1D22 C2D22 C3D22 C4D22	Day 8 Post infusion (*) C1D30 C2D30 C3D30 C4D30	Day 42 (**) C2D1, C3D1, C4D1, C5D1, before first dose of TMZ	Day 180# (*) C8D34 (Phase I rGBM) or C10D20 (Phase II nGBM)	Disease progression (***)
CMV-specific T cell frequencies, phenotype & functional immunoassays	X	x	x	x	
Cytokine analysis	X	x	x		
TCR clonotyping and diversity	X		x	x	
Tumor-infiltrating lymphocytes					x

#After cycle#4 only (last administration of CMV-specific T cells)
(*) With a margin of +/- 7 working days.
(**) "Day 42" can be obtained with a margin of ±7 working days, as long as it's obtained BEFORE initiation of the next dose-dense temozolomide cycle.
(***) Tumor tissue can be collected at disease progression if patient undergoes surgical resection for clinical indications.
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9.0 Criteria for Evaluation and Endpoint Definitions

9.1. Endpoints

9.1.1 Phase I (Dose Escalation) in Recurrent glioblastoma

The primary endpoint is the determination of Maximum Feasible Dose (MFD) or Maximum tolerated Dose (MTD) and safety of CMV-specific T cells in combination with dose-dense temozolomide in patients with recurrent glioblastoma.

9.1.2 Phase II (Dose Expansion) in Recurrent glioblastoma undergoing resection

The primary efficacy endpoint will be to evaluate the immunological effects in resected glioblastoma after intravenous administered CMV-stimulated adoptive T cells in patients with recurrent glioblastoma undergoing resection for clinical indications.

9.1.3 Phase II (Dose Expansion) in Newly diagnosed glioblastoma

The primary efficacy endpoint is to determine overall survival. Secondary endpoints will include determination of the safety and tolerability of dose-dense temozolomide in combination with intravenous administered CMV-stimulated adoptive T cells, ORR, median duration of response and PFS6.

Overall Survival (OS) will be defined as the time from definitive* histological diagnosis until the time of death. Patients who are alive will be censored at the time of last contact.

*If a patient undergoes further resection after initial biopsy or partial resection, the date of this second (definitive) surgery will be considered the date of diagnosis.

Progression-free survival (PFS) will be defined as the time from study enrollment until the time of first disease progression, relapse, or death due to disease. Patients who are alive without progression or relapse will be censored at the time of last contact. The point estimate of 6-month progression-free survival (PFS6) will be analyzed.

9.2 Imaging evaluation: RANO criteria.

Traditionally, progression was determined using criteria set forth by Macdonald et al [34]. These criteria relied heavily on changes in contrast enhancement to establish whether a tumor was radiologically stable, improved, or progressing. More recently, given the recognition of the phenomena of pseudoprogression as well as widespread use of anti-angiogenic agents that rapidly affect blood vessel permeability, attempting to establish a diagnosis based on these criteria has led to increasing ambiguity. In recognition of this, the Response Assessment in Neuro-Oncology (RANO) working group has recently put forth updated criteria for establishing treatment response in neuro-oncology patients. These criteria will be used for determining radiographic outcomes for this study and are outlined below.

Note: As the therapeutic basis of immunotherapy involves the induction of an inflammatory response in the tumor micro environment, it is often challenging to properly evaluate radiological responses. The appearance of measurable clinical activity at the tumor site may take longer for immunotherapies than for cytotoxic therapies; responses to immunotherapies may occur after conventional progressive disease (tumor burden increase in MRI scans), and immune based therapies may be associated with increased edema and associated T2/FLAIR changes which may inaccurately be interpreted to represent tumor progression

For these reasons, we will apply the following modification to the RANO imaging criteria:

1. **Progressive Disease (PD) will require confirmation by resection or a second scan at least 4 weeks apart,** provided that the patient remains clinically stable or improves rapidly with a dose of steroids below 2 mg daily of dexamethasone. This category will be classified as "Unconfirmed PD". If tumor resection or a subsequent scan confirms the presence of recurrent tumor, the date of PD will be the date of the first scan that showed "Unconfirmed PD".

For those patients who are not candidates for surgical resection to confirm PD, these guidelines will be followed:

1.1 Imaging assessment should be repeated a minimum of 4 weeks later in order to confirm PD with the option of continuing treatment as described below while awaiting radiologic confirmation of progression.
1.2 If repeat imaging shows a reduction in the tumor burden compared to the initial scan suggesting PD, treatment may be continued / resumed.
1.3 If repeat imaging confirms PD, defined as a 25% increase from baseline or best response, then the date of PD will be the first date the subject met criteria for progression and the subject will be discontinued from study therapy. Subjects who have confirmed PD will discontinue study medication and enter the follow up/survival phase of the study.
1.4 In determining whether or not the tumor burden has increased or decreased, investigators should consider all target lesions as well as non-target lesions.

2. Immune based therapies are expected to be associated with inflammatory changes that may include edema. RANO expanded the previously utilized Macdonald criteria to include the development of "significantly" increased T2 or FLAIR abnormality in the definition of PD because such changes can be a major component defining radiographic progression following therapeutic use of VEGF/VEGFR-targeting therapeutics, which are known to elicit potent anti-permeability changes that limit contrast uptake. **Our study will define radiographic PD by assessment of enhancing tumor burden and will not incorporate assessment of T2 or FLAIR changes as outlined in RANO** because:

(1) there is no expectation that immunotherapy agents will falsely diminish enhancing tumor burden as has been noted with anti-angiogenic therapies; and

(2) immune based therapies may be associated with increased edema and associated T2/FLAIR changes which may inaccurately be interpreted to represent tumor progression (i.e. pseudoprogression).

In subjects who have initial evidence of radiographic PD ("Unconfirmed PD"), it is at the discretion of the treating physician whether to continue a subject on study treatment until repeat imaging is obtained a minimum of 4 weeks later. This clinical judgment decision should be based on the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. **Subjects may receive study treatment while**

waiting for confirmation of PD if they are clinically stable as defined by the following criteria:

• The subject is believed to demonstrate clinical benefit from the study regimen as determined by the treating physician;
• The subject is adequately tolerating study therapy.
• The subject remains clinically stable or improves rapidly with a dose of steroids below 2 mg of dexamethasone daily.

When feasible, subjects should not be discontinued until radiographic PD is confirmed. This allowance to continue treatment despite initial radiologic PD takes into account the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response. Subjects that are exhibiting significant neurologic decline are not required to have repeat imaging for confirmation of PD.

Evaluation of imaging response will be determined by the treating physician and/or PI and documented in the patient's chart and the protocol files.

9.3 Baseline definitions:

9.3.1 Measurable Lesions: enhancing lesions that can be measured bi-dimensionally.

9.3.2 Multicentric Lesions: For multicentric lesions that are *discrete foci of enhancement*, the approach is to measure each separately enhancing lesion that meets the inclusion criteria and then calculate the sum of the products.

Lesions that are considered multicentric (as opposed to continuous) are lesions where there is normal intervening brain tissue between the two (or more) lesions. If there is no normal brain tissue between these two (or more) lesions, they will be considered the same lesion.

9.3.3 Non-Measurable Lesions: All other lesions that do not meet the criteria for measurable disease as defined, as well as all non-enhancing and other truly non-measurable lesions, are considered non-measurable.

A non-measurable lesion will include foci of enhancement that are less than the specified smallest diameter, non-enhancing lesions seen on T2-weighted or FLAIR images, hemorrhagic or predominantly cystic or necrotic lesions, and leptomeningeal tumor. Hemorrhagic lesions often have intrinsic T1-weighted hyperintensity that could be misinterpreted as enhancing tumor, and for this reason, the pre-contrast T1-weighted image must be examined to exclude baseline or interval sub-acute hemorrhage.

9.4 Response Definitions:

9.4.1 Complete response (CR). Requires *all* of the following

- Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks
- No new lesions
- Stable or improved non-enhancing (T2/FLAIR) lesions
- Patients must be off corticosteroids (or on physiologic replacement doses only)
- Stable or improved clinically

Note: Patients with non-measurable disease only cannot have a complete response; the best response possible is stable disease.

9.4.2 Partial response (PR). Requires *all* of the following:

- >50% decrease compared with baseline in the sum of products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks
- No progression of non-measurable disease
- No new lesions
- Stable or improved non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan
- The corticosteroid dose at the time of the scan evaluation should be no greater than the dose at the time of baseline scan
- Improved or stable clinically

Note: Patients with non-measurable disease only cannot have a partial response; the best response possible is stable disease.

9.4.3 Stable disease (SD). Requires all of the following:

- Does not qualify for complete response, partial response, or progression
- Stable non-enhancing (T2/FLAIR) lesions on same or lower dose of corticosteroids compared with baseline scan. In the event that the corticosteroid dose was increased for new symptoms and signs without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that this increase in corticosteroids was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

9.4.4 Progression. Defined by any of the following:

- >25% increase in sum of the products of perpendicular diameters of enhancing lesions compared with the smallest tumor measurement obtained either at baseline (if no decrease) or best response, on stable or increasing doses of corticosteroids*.
- Any new lesion
- Clear clinical deterioration not attributable to other causes apart from the tumor (e.g. seizures, medication adverse effects, complications of

therapy, cerebrovascular events, infection, and so on) or changes in corticosteroid dose
- Failure to return for evaluation as a result of death or deteriorating condition
- Clear progression of non-measurable disease

Note: All measurable and non-measurable lesions must be assessed using the same techniques as at baseline (only MRI allowed in this trial).

*Stable doses of corticosteroids include patients not on corticosteroids.

9.4.5 Best Response: This will be calculated from the sequence of objective statuses.
For patients with all disease sites assessed every evaluation period, the best response will be defined as the best objective status as measured according to Section 9.1.6. If the response does not persist at the next regular scheduled MRI, the response will still be recorded based on the prior scan, but will be designated as a non-sustained response. If the response is sustained (i.e. still present on the subsequent MRI) it will be recorded as a sustained response, lasting until the time of tumor progression. Best response is unknown if the patient does not qualify for a best response or increasing disease and if all objective status determinations before progression are unknown.

9.5 Neurological Exam: Although not used for determining response, it is useful to evaluate improvement in the neurologic exam, (as compared to the baseline assessment), that should coincide with objective measurement of tumor size.

+2	Definitely better
+1	Possibly better
0	Unchanged
-1	Possibly worse
-2	Definitely worse

9.6 Performance Status: Patients will be graded according to Karnofsky Performance Status

9.7 Time to Death: From date of registration to date of death due to any cause.

9.8 Definition of Overall Response Rate: number of patients with PR and CR maintained for a minimum of 6 weeks.

10.0 Criteria for Removal from Study

Early trial termination will be the result of the criteria specified below:

1. Poor adherence to protocol and regulatory requirements

2. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects.

10.1 Criteria for Removal from Protocol Treatment:

10.1.1 Progression of disease (as defined in Section 9.4.4).

10.1.2 Unacceptable toxicity (see section 6.3 for criteria for discontinuation of therapy based on treatment related toxicities).

10.1.3 The patient may withdraw from the study at any time for any reason.

10.1.4 Medical or psychiatric illness which in the investigator's judgment renders the patient incapable of further therapy.

10.1.5 All reasons for discontinuation of treatment must be documented in the flow sheets.

10.1.6 Patients requiring a dose of steroids above 2 mg of dexamethasone or unable to taper and discontinue dexamethasone at least 5 days prior to T-cell infusions

11.0 Statistical Considerations

This will be a single-center, phase I/II clinical trial in patients with glioblastoma. Three groups of patients (recurrent GBM, recurrent GBM undergoing resection, and newly diagnosed GBM) will be investigated. We will proceed with enrollment to Dose Escalation first in recurrent GBM patients. Once the maximum feasible dose (MFD) or maximum tolerated dose (MTD) of CMV-T cells is identified, we will proceed with enrollment to the 2 cohorts in Dose Expansion in parallel (recurrent GBM undergoing resection and newly diagnosed GBM).

Phase I (Dose Escalation): Recurrent glioblastoma

This will be a dose escalation component in patients with recurrent glioblastoma to determine the maximum feasible dose (MFD) or maximum tolerated dose (MTD) of CMV-specific T cells in combination with dose-dense temozolomide. We will follow a 3+3 design exploring 4 dose levels of CMV-T cells: 5×10^6, 1×10^7, 5×10^7, and 1×10^8 CMV CTLs.

Patients will be enrolled in cohort size of 3. If two DLTs thought to be related to CMV-T cells occur at the first dose level, the MTD has been exceeded, and the trial will stop. The MTD is the dose level at which 0/6 or 1/6 patients experience a DLT with at least 2 patients experiencing DLT at the next higher dose level. A minimum of six patients must be treated at the MTD or MFD.

Number of patients with DLTs / Number of	3+3 rule

Evaluable patients	
0/3	Proceed with next dose escalation with three patients
1/3	Expand current dose level to 6 patients
1/6	Proceed with next dose escalation with three patients
>/=2/3 or >/=2/6	The MTD has been exceeded. Expand previous dose level to a total of 6 patients if six patients were not already treated. The MTD is defined as the dose level at which 0/6 or 1/6 patients experience a DLT with at least 2 patients experiencing DLT at the next higher dose level. A minimum of six patients must be treated at the MTD or MFD.

The DLT period will include the first cycle of therapy with dose-dense temozolomide and CMV CTLs (6 weeks). After all patients in each dose escalation cohort complete the first cycle of therapy (DLT period), all toxicities will be reviewed by the PI and sent to the IND office for review before proceeding with escalation to the next dose level of CMV-T cells. The dose escalation component will be performed in patients with recurrent glioblastoma not undergoing surgical resection, with any number of prior tumor recurrences, and otherwise fulfilling all other inclusion and exclusion criteria for participation in the trial. We expect to confirm a Maximum Feasible Dose (MFD) rather than an MTD in this dose escalation component. Once the MFD is confirmed (or an MTD is determined) enrolment to dose expansion arms will start. Each patient on dose escalation will receive up to 4 doses of CMV-T cells and dose dense temozolomide, but no intrapatient dose escalation will be permitted. Cycles of dose –dense temozolomide can be continued beyond the 4th dose if patient is receiving benefit from therapy.

Phase II (Dose Expansion): Recurrent glioblastoma undergoing resection

The purpose of this analysis is to determine if the clinical trial subjects have an enhanced effector CMV-specific T cell frequency within the glioma microenvironment relative to the historical cohort of patients receiving standard-of-care treatment (on whom we are currently collecting data) as a baseline. The primary objectives of this recurrent glioblastoma cohort are to evaluate the immunological effects in resected tumor tissue and clinical response (measured by PFS6) of dose-dense temozolomide with CMV-specific adoptive T cells. To evaluate the tumor-mediated immune suppression at the effector location, we are going to measure the markers (IFN, IL-2, and TNF-α, perforin, granzyme B) for immune responses in the tumor microenvironment rather than in the peripheral blood.

We expect to enroll 10 efficacy-evaluable recurrent glioblastoma patients undergoing surgery for clinical indications. Those patients unable to proceed with surgery on day 30 will be replaced but followed for toxicity analysis only (NOT evaluable for the efficacy analysis).

Phase II (Dose Expansion): Newly diagnosed glioblastoma

We expect to enroll 20 evaluable newly diagnosed glioblastoma patients. The primary objective will be overall survival (OS), which is defined as the time from definitive histological diagnosis until the time of death (see9.1.3). Secondary objectives will include assessment of tumor response (ORR), median duration of response, progression free survival at 6 months (PFS6) and to determine the safety and tolerability of dose-dense temozolomide in combination with intravenous administered CMV-specific adoptive T cells.

Toxicity monitoring will be conducted using Bayesian continuous monitoring (Thall et al., 1996), where the toxicity evaluation endpoint is defined as treatment-related unmanageable toxicities, including grade 3 or 4 AEs that require termination of the treatment during cycle one (6 weeks). Toxicity rate of 30% or higher will be considered unacceptable. The prior probability of toxicity is assumed to follow a Beta (0.3, 0.7) distribution with one patient worth of information. The toxicity will be monitored by a cohort size of 4, starting after at least 8 patients have completed toxicity evaluation (within cycle one), the trial will be stopped if the following statement is true

$$\Pr[\text{toxicity rate} > 30\% \mid \text{data}] > 0.90,$$

which means that the trial will be stopped for toxicity if the posterior probability of the toxicity rate being greater than 30% is greater 90%. The early stopping boundaries for toxicity, shown in the format of

(The number of patients with toxicities) / (The number of patients treated),

are ≥5/8, 6/12, 8/16.

For example, if there are 5 or more patients experience Toxicities among the first 8 patients, stop the trial early due to the agent is too toxic.

Table 11.1. Operating characteristics for the stopping rules

For excessive toxicity monitoring

True Toxicity Rate	Probability Stop Early	Average sample size
0.10	0.0008	20.0
0.20	0.0247	19.8

0.30	0.1433	18.7
0.40	0.3941	16.3
0.50	0.6902	13.3

The above stopping boundaries and operating characteristics are calculated using MultcLean (v.2.1.0) design software downloaded from http://biostatistics.mdanderson.org/SoftwareDownload.

Sample Size and Power Calculation:

For Dose Expansion- Recurrent GBM group, a sample size of 10 will give us 80% power to detect a difference of any immunological effects with effective size of 0.853 using one-sided paired t-test at the significance of 0.05.

For Dose Expansion-newly diagnosed GBM group, a sample size of 20 ensures that, if the trial is not terminated early, a 90% confidence interval for 24-month OS rate will have a maximum width of 0.184.

Replacement of Study Subjects

The following patients will be replaced and continue to be followed for toxicity analysis only (NOT evaluable for efficacy analysis):

1. Phase II/Dose Expansion - Recurrent GBM group:
   - Patients unable to proceed with surgery on Day 30 (+/- 2 working days).
   - Patients with suspected transformation to GBM from a lower grade glioma in whom the final pathology report fails to confirm recurrent GBM or gliosarcoma.

2. Phase I/Dose Escalation - Recurrent GBM, Phase II/Dose Expansion – Recurrent GBM undergoing resection, and Phase II/Dose Expansion - Newly Diagnosed GBM groups: patients in whom manufacturing failure occurs, i.e. expansion of CMV-specific autologous T cells does not provide sufficient number of T cells for at least the first infusion.

3. For Phase I Dose escalation, patients who do not complete the first cycle of treatment (DLT period) for reasons other than toxicity or progression of tumor will also be replaced.

11.2 Analysis Plan

We will use descriptive statistics to summarize the immunological effects. The objective response rate and the overall toxicity rate will be summarized by frequency and 95% confidence

interval. Adverse Events will be tabulated by grade and by their relationship to the treatment. Logistic regression will be used to explore the correlation between response rates with other important factors such as absolute lymphocyte count (ALC). Kaplan-Meier curves will be generated and median survival time will be derived for PFS, TTP and OS. Cox proportional hazard regression will be employed for multivariate analysis on time-to-event outcomes with factors such as immunological reactivity.

Patients must have received at least one CMV specific T cell infusion to be included in the statistical analysis for treatment efficacy and correlative studies. All other patients will be included for evaluation of toxicity.

Toxicity summaries for cohorts of 4 will be provided to the IND Medical Monitor after 8 patients have completed one cycle of therapy.

12.0 Safety Assessments and Toxicity Monitoring

12.1 Adverse Event Monitoring

Subjects must be carefully monitored for adverse events. This monitoring includes clinical laboratory tests. Adverse events should be assessed at least weekly during their first cycle of therapy and then on a cycle basis in the subsequent cycles, and graded according to NCI-CTCAE version 4.03. Adverse event should be assessed in terms of seriousness, severity, and relationship to the study drug. All adverse events and serious adverse events will be reported to the study chair.

All subjects will be registered in CORe and data will be entered in PDMS/CORe, the electronic CRF. Designated research staff will enter the data. All investigators will record information regarding on study, course (flowsheet), offstudy, survival, and toxicity data.

All data will be monitored on an ongoing basis by the PI(s), the research study nurses, and data managers. The PI or designee will be responsible for determining attribution for all events. Institutional Review Board (IRB) will be notified of any adverse events and provided data to permit a safety review of the study treatment. The IRB may request additional meetings or safety reports as deemed necessary. The IRB may also stop the trial following a review of results from such analysis.

12.2 Adverse Event Definitions

12.2.1 Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal

laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Lab abnormalities will be considered an AE if clinically significant. Clinically significant will be defined if requiring a holding of the treatment, a dose reduction of the treatment or a new prescription is given. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the protocol therapy or protocol procedures, is also an AE.

Progression of the cancer under study is not considered an AE unless it results in hospitalization or death.

Abnormal laboratory values or test results occurring after informed consent constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, require therapy (e.g., hematologic abnormality that requires transfusion or hematological stem cell support), or require changes in study medication(s).

AEs that begin or worsen after informed consent should be recorded in the Adverse Events CRF. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF. AE monitoring should be continued for at least 30 days following the last dose of study treatment. AEs (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE.

AEs will be assessed according to the Common Terminology Criteria for Adverse

Events (CTCAE) version 4.03. If CTCAE grading does not exist for an AE, the severity of mild, moderate, severe, and life-threatening, corresponding to grades 1 - 4, will be used. CTCAE grade 5 (death) will not be used in this study; rather, information about deaths will be collected though a Death form.

12.2.2 Unexpected Adverse Event

An unexpected adverse event is any adverse drug event, the specificity or severity of which is not consistent with the current Package Insert for temozolomide. Also, reports which add significant information on specificity or severity of a known, already documented adverse event constitute unexpected adverse events. An event more specific or more severe than described in the package insert would be considered "unexpected".

12.2.3 Serious Adverse Event Reporting (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- **Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.**
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).
- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.
- **Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.**

- **Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last study treatment/intervention, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.**

- **Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.**

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure that serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

Investigator Communication with Supporting Companies:

N/A – there is no supporting company for this trial.

12.2.4 Protocol stopping criteria

These will include: 1) death on study related to therapy; and 2) a rate of treatment-related toxicity of 30% or higher – hematological or non-hematological grade 3 or 4 adverse events that require termination of the treatment.

13.0 References

1. Bate, S.L., S.C. Dollard, and M.J. Cannon, *Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988-2004.* Clin Infect Dis, 2010. **50**(11): p. 1439-47.
2. Hyde, T.B., D.S. Schmid, and M.J. Cannon, *Cytomegalovirus seroconversion rates and risk factors: implications for congenital CMV.* Rev Med Virol, 2010. **20**(5): p. 311-26.
3. Ljungman, P. and R. Brandan, *Factors influencing cytomegalovirus seropositivity in stem cell transplant patients and donors.* Haematologica, 2007. **92**(8): p. 1139-42.
4. Souza, M.A., et al., *Seroprevalence of cytomegalovirus antibodies in blood donors in southern, Brazil.* Rev Soc Bras Med Trop, 2010. **43**(4): p. 359-61.
5. DeFilippis, V.R., et al., *Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1.* J Virol, 2010. **84**(1): p. 585-98.
6. Isaacson, M.K., L.K. Juckem, and T. Compton, *Virus entry and innate immune activation.* Curr Top Microbiol Immunol, 2008. **325**: p. 85-100.
7. Marshall, E.E. and A.P. Geballe, *Multifaceted evasion of the interferon response by cytomegalovirus.* J Interferon Cytokine Res, 2009. **29**(9): p. 609-19.
8. Sylwester, A.W., et al., *Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects.* J Exp Med, 2005. **202**(5): p. 673-85.
9. Manley, T.J., et al., *Immune evasion proteins of human cytomegalovirus do not prevent a diverse CD8+ cytotoxic T-cell response in natural infection.* Blood, 2004. **104**(4): p. 1075-82.
10. Moss, P., *The emerging role of cytomegalovirus in driving immune senescence: a novel therapeutic opportunity for improving health in the elderly.* Curr Opin Immunol, 2010. **22**(4): p. 529-34.
11. Cobbs, C.S., et al., *Human cytomegalovirus infection and expression in human malignant glioma.* Cancer Res, 2002. **62**(12): p. 3347-50.
12. Cobbs, C., et al., *Human cytomegalovirus infection and expression in human malignant glioma.* Cancer Res, 2002. **62**(12): p. 3347-50.
13. Mitchell, D.A., et al., *Sensitive detection of human cytomegalovirus in tumors and peripheral blood of patients diagnosed with glioblastoma.* Neuro Oncol, 2008. **10**(1): p. 10-8.
14. Scheurer, M.E., et al., *Detection of human cytomegalovirus in different histological types of gliomas.* Acta Neuropathol, 2008. **116**(1): p. 79-86.
15. Luo, M.H., P.H. Schwartz, and E.A. Fortunato, *Neonatal neural progenitor cells and their neuronal and glial cell derivatives are fully permissive for human cytomegalovirus infection.* J Virol, 2008. **82**(20): p. 9994-10007.
16. Luo, M.H., et al., *Human cytomegalovirus disrupts both ataxia telangiectasia mutated protein (ATM)- and ATM-Rad3-related kinase-mediated DNA damage responses during lytic infection.* J Virol, 2007. **81**(4): p. 1934-50.
17. Odeberg, J., et al., *Late human cytomegalovirus (HCMV) proteins inhibit differentiation of human neural precursor cells into astrocytes.* J Neurosci Res, 2007. **85**(3): p. 583-93.
18. Jackson, E.L., et al., *PDGFR alpha-positive B cells are neural stem cells in the adult SVZ that form glioma-like growths in response to increased PDGF signaling.* Neuron, 2006. **51**(2): p. 187-99.
19. Soroceanu, L., A. Akhavan, and C.S. Cobbs, *Platelet-derived growth factor-alpha receptor activation is required for human cytomegalovirus infection.* Nature, 2008. **455**(7211): p. 391-5.
20. Kalejta, R.F., *Tegument proteins of human cytomegalovirus.* Microbiol Mol Biol Rev, 2008. **72**(2): p. 249-65, table of contents.

21. Dziurzynski, K., et al., *Glioma-associated cytomegalovirus mediates subversion of the monocyte lineage to a tumor propagating phenotype.* Clin Cancer Res, 2011. **17**(14): p. 4642-9.
22. Jenkins, C., et al., *Immunomodulatory properties of a viral homolog of human interleukin-10 expressed by human cytomegalovirus during the latent phase of infection.* J Virol, 2008. **82**(7): p. 3736-50.
23. Dziurzynski, K., et al., *Consensus on the role of human cytomegalovirus in glioblastoma.* Neuro Oncol, 2012. **14**(3): p. 246-55.
24. Prins, R.M., T.F. Cloughesy, and L.M. Liau, *Cytomegalovirus immunity after vaccination with autologous glioblastoma lysate.* N Engl J Med, 2008. **359**(5): p. 539-41.
25. Riddell, S.R., et al., *Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones.* Science, 1992. **257**(5067): p. 238-41.
26. Walter, E.A., et al., *Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor.* New England Journal of Medicine, 1995. **333**(16): p. 1038-44.
27. Cwynarski, K., et al., *Direct visualization of cytomegalovirus-specific T-cell reconstitution after allogeneic stem cell transplantation.* Blood, 2001. **97**(5): p. 1232-40.
28. Cobbold, M., et al., *Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers.* J Exp Med, 2005. **202**(3): p. 379-86.
29. Feuchtinger, T., et al., *Safe adoptive transfer of virus-specific T-cell immunity for the treatment of systemic adenovirus infection after allogeneic stem cell transplantation.* Br J Haematol, 2006. **134**(1): p. 64-76.
30. Schuessler, A., et al., *Autologous T-cell therapy for cytomegalovirus as a consolidative treatment for recurrent glioblastoma.* Cancer Res, 2014. **74**(13): p. 3466-76.
31. Sampson, J.H., et al., *Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma.* Neuro Oncol, 2011. **13**(3): p. 324-33.
32. Gilbert, M.R., et al., *Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial.* J Clin Oncol, 2013. **31**(32): p. 4085-91.
33. Wen, P.Y., et al., *Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group.* J Clin Oncol, 2010. **28**(11): p. 1963-72.
34. Macdonald, D.R., et al., *Response criteria for phase II studies of supratentorial malignant glioma.* J Clin Oncol, 1990. **8**(7): p. 1277-80.

35. Mitchell DA, Batich KA, Gunn MD et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. Nature 2015;519:366-369.

14.0 STUDY CALENDARS

Study Calendar A														
Phase I-Dose Escalation: Recurrent Glioblastoma														
Phase	**First Cycle**					**Subsequent Cycles**								
Cycle	**Screening /consent**		**C1**			**C2**			**C3 and C4**			**C5& beyond**		
Day	**Day-14**	**Day 0**	**D1**	**D2-21**	**D22**	**D1**	**D2-21**	**D22**	**D1**	**D2-D21**	**D22**	**D1**	**D2-D21**	**D22**
Informed Consent	x													
Inclusion/Exclusion Criteria	x													
Demographics and Medical History	x													
Neurological exam[10]	x		x			x			x			x		
Karnofsky Performance Status	x		x			x			x			x		
Full Physical Examination[10]	x		x			x			x			x		
Vital Signs- and Weight[7]	x		x			x			x			x		
MRI[1]	x					x			x			x		
Pregnancy Test – Serum b-HCG	x					x			x			x		
Urine Pregnancy Test[2]			x											
CBC with Differential and platelets [8]	x		x		x	x		x	x		x	x		

PT/PTT	x													
Basic metabolic panel[11]	x		x			x			x			x		
Serology[5]	x													
EKG	x													
Chest X Ray	x													
PBMC Collection by Leukapheresis		x												
DD Temozolomide[3,4]			x	x		x	x		x	x		x	x	
Adoptive CMV-specific T cell infusion[6]					x			x			x			
Correlative studies in Peripheral Blood [9]														
Correlative studies in Tumor Tissue [9]														

1. MRI every 6 weeks

2. Urine pregnancy test to be done within 72 hours of starting therapy

3. 4 cycles total of dose dense TMZ followed by adoptive T cell infusion

4. After completion of 4 cycles of dose dense TMZ and T cell infusion, patients can continue dose-dense temozolomide for up to 12 cycles or tumor PD

5. The following serology tests will be done prior to leukapheresis and after consenting (pre-study tests):
HIV-1/2 antibodies, Hepatitic C Virus Antibody, Anti HTLV I/HTLV II, Hepatitis B Antigen, Hepatitis B Core Antibody, CMV Antibody, West Nile Virus-NAT testing (WNV by Nucleic Acid Testing), Serology test for Syphilis (RPR), Chagas Disease, HIV/HCV antigen testing by Nucleic Acid Testing (NAT).

6. intravenous administered CMV-stimulated adoptive T cells, total 4 cycles. See dose levels in Section 6.1

7. Vital signs (blood pressure, pulse, respiratory rate, pulse oximetry) will be monitored at baseline and 30 and 60 minutes after the T cell infusion.

8. In addition to time points in calendar, CBC and diff will be obtained on Days 1, 8, 15, 22, 29 and 36 of Cycle 1, on Days 1, 22, and 29 of Cycles 2 – 4, and on Days 1, 15, and 29 of Cycles 5 and beyond.

9. See time points for collection in Section 8.3, Table 6

10. Within 3 working days before Day 1
11. Basic metabolic panel will be performed prior to and on Day 29 of each cycle

Study Calendar B															
Phase II-Dose Expansion: Recurrent Glioblastoma undergoing resection															
Phase	**Pre-Surgical Phase**					**Post-surgery/maintenance treatment**									
Cycle	**Screening /consent**		**C1**			**Surgery**	**C2**			**C3 and C4**			**C5 & beyond**		
Day	**Day-14**	**Day 0**	**D1**	**D2 -21**	**D22**	**D30**	**D1**	**D2 -21**	**D22**	**D1**	**D2-D21**	**D22**	**D1**	**D2-D21**	**D22**
Informed Consent	x														
Inclusion/Exclusion Criteria	x														
Demographics and Medical History	x														
Neurological exam[10]	x		x				x			x			x		
Karnofsky Performance Status	x		x				x			x			x		
Full Physical Examination[10]	x		x				x			x			x		
Vital Signs and Weight[8]	x		x				x			x			x		
MRI[1, 2]	x					x[1]				x[2]			x[2]		
Pregnancy Test – Serum b-HCG	x						x			x			x		

Urine Pregnancy Test[7]			X												
CBC with Differential and Platelet[9]	x		x		x		x		x	x		x	x		
PT/PTT	x														
Basic metabolic panel[11]	x		x				x			x			x		
Serology[5]	x														
EKG	x														
Chest X ray	x														
PBMC Collection by Leukapheresis		x													
DD Temozolomide[3]			x	x			x	x		x	x		x	x	
Adoptive CMV-specific T cell infusion[4]					x				x			x			
Correlative studies in Peripheral Blood [6]															
Correlative studies in Tumor Tissue [6]															

1. MRI: pre-surgery MRI – within 24 hours, post-surgery MRI, within 48 hours

2. MRI to be done on C3 & beyond before dosing, every 6 weeks

3. after cycle 4, patients can receive 42-day cycles of dose-dense TMZ alone up to 12 cycles or tumor PD

4. intravenous administered CMV-stimulated adoptive T cells, total 4 cycles; dose at MTD or MFD determined in Phase I

5. The following serology tests will be done prior to leukapheresis and after consenting (pre-study tests):
HIV-1/2 antibodies, Hepatitic C Virus Antibody, Anti HTLV I/HTLV II, Hepatitis B Antigen, Hepatitis B Core Antibody, CMV Antibody, West Nile Virus-NAT testing (WNV by Nucleic Acid Testing), Serology test for Syphilis (RPR), Chagas Disease, HIV/HCV antigen testing by Nucleic Acid Testing (NAT).

6. See time points for collection in Section 8.3, Tables 4 and 5

7. Urinary pregnancy test to be done within 72 hours of starting therapy

8. Vital signs (blood pressure, pulse, respiratory rate, pulse oximetry) will be monitored at baseline and 30 and 60 minutes after the T cell infusion.
9. In addition to time points in calendar, CBC and diff will be obtained on Days 1, 8, 15, 22, 29 and 36 of Cycle 1, on Days 1, 22, and 29 of Cycles 2 – 4, and on Days 1, 15, and 29 of Cycles 5 and beyond.
10. Within 3 working days before Day 1
11. Basic metabolic panel will be performed prior to and on Day 29 of each cycle

Study Calendar C														
Phase II-Dose Expansion: Newly Diagnosed Glioblastoma														
Phase	**First Cycle**					**Subsequent Cycles**								
Cycle	**Screening /consent**		**C1**			**C2**			**C3 and C4**			**C5 & beyond[5]**		
Day	**Day-14**	**Day 0**	**D1**	**D2-21**	**D22**	**D1**	**D2-21**	**D22**	**D1**	**D2-D21**	**D22**	**D1**	**D2-D21**	**D22**
Informed Consent	x													
Inclusion/Exclusion Criteria	x													
Demographics and Medical History	x													
Neurological exam[11]	x		x			x			x			x		
Karnofsky Performance Status	x		x			x			x			x		
Full Physical Examination[11]	x		x			x			x			x		
Vital Signs and Weight[8]	x		x			x			x			x		
MRI1	x					x			x			x		

Pregnancy Test – Serum b-HCG	x					x			x			x		
Urine Pregnancy Test[2]			X											
CBC with Differential and platelets [9]	x		x		x	x		x	x		x	x		
PT/PTT	x													
Basic metabolic panel[12]	x		x			x			x			x		
Serology[6]	x													
EKG	x													
Chest X Ray	x													
PBMC Collection by Leukapheresis		x												
DD Temozolomide[3,4]			x	x		x	x		x	x		x	x	
Adoptive CMV-specific T cell infusion[7]					x			x			x			
Correlative studies in Peripheral Blood[10]														
Correlative studies in Tumor Tissue [10]														

1. MRI every 6 weeks for C1 to C4. Starting cycle 5, every 8 weeks

2. Urinary pregnancy test to be done within 72 hours of starting therapy

3. 4 cycles total of dose dense TMZ followed by adoptive T cell infusion

4. After completion of 4 cycles of dose dense TMZ and T cell infusion, patients with newly diagnosed GBM can receive standard dose TMZ 200 mg/m^2 days 1-5 every 28 days up to 12 cycles or tumor PD

5. Cycle 1-4: 42 days. Cycle 5 and beyond: 28 days for standard-dose TMZ(day 1-5), for up to 12 cycles or PD

6. The following serology tests will be done prior to leukapheresis and after consenting (pre-study tests):

HIV-1/2 antibodies, Hepatitic C Virus Antibody, Anti HTLV I/HTLV II, Hepatitis B Antigen, Hepatitis B Core Antibody, CMV Antibody, West Nile Virus-NAT testing (WNV by Nucleic Acid Testing), Serology test for Syphilis (RPR), Chagas Disease, HIV/HCV antigen testing by Nucleic Acid Testing (NAT).
7. intravenous administered CMV-stimulated adoptive T cells, total 4 cycles; dose at MTD or MFD determined in Phase I
8Vital signs (blood pressure, pulse, respiratory rate, pulse oximetry) will be monitored at baseline and 30 and 60 minutes after the T cell infusion.
9. In addition to time points in calendar, CBC and diff will be obtained on Days 1, 8, 15, 22, 29 and 36 of Cycle 1, on Days 1, 22, and 29 of Cycles 2 – 4, and on Day 1 of Cycles 5 and beyond.
10. See time points for collection in Section 8.3, Table 6
11. Within 3 working days before Day 1
12. Basic metabolic panel will be performed prior to each cycle and on Day 29 of Cycles 1-4