A Phase II Clinical Trial Evaluating Autologous Dendritic Cells Pulsed with Tumor Lysate Antigen +/- Toll-like Receptor Agonists for the Treatment of Malignant Glioma

Version 9.0
May 21, 2015

Study Agent(s): Autologous Dendritic Cells (DC) Derived from PBMC Cultured with GM-CSF (Leukine®, Genzyme or Miltenyi Biotec) and IL-4 (CellGenix or Miltenyi Biotec); Resiquimod cream (3M Pharmaceuticals, Inc.); poly ICLC (Hiltonol®, Oncovir, Inc.).

UCLA IRB Number: 10-000202
IND Number: 11053

Sponsor: **Robert M. Prins, Ph.D.**
UCLA Dept. of Neurosurgery
Address: 10833 LeConte Ave, CHS 74-145, PO Box 956901
Los Angeles, CA 90095
Phone: 310-825-4207
Fax: 310-206-2093

Principal Investigator Agreement:

I the undersigned, have reviewed this protocol and I agree to conduct this protocol in accordance with Good Clinical Practice, the ethical principals set forth in the Declaration of Helsinki and the U.S. Code of Federal Regulations governing the protection of human subjects (21 CFR 50), Financial Disclosure of interests in Sponsor (21 CFR 54), Institutional Review Boards (21 CFR 56) and the obligations of clinical investigators (21 CFR 312).

Robert M. Prins

Signature:_____ Date 05/21/2015

Print name: Robert M. Prins, Ph.D.

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

Protocol Synopsis

Protocol Title: A Phase II Clinical Trial Evaluating Autologous Dendritic Cells Pulsed with Tumor Lysate Antigen +/- Toll-like Receptor Agonists for the Treatment of Malignant Glioma

Protocol No.: 105212015, v9.0

Study Agent: Autologous DC pulsedwith patient tumor lysate +/- 0.2% resiquimod or 20 μg/kg poly ICLC (Hiltonol®)).

Indication: Treatment of patients with newly diagnosed or recurrent glioma of WHO Grade III or IV (anaplastic astrocytoma, anaplastic astro-oligodendroglioma, or glioblastoma).

Objectives:
Primary: The primary objective of this study is to determine the best combination of autologous tumor lysate-pulsed DC vaccine, with or without adjuvant toll-like receptor (TLR) agonists.

Secondary: The secondary objectives are to monitor time to tumor progression (TTP) and overall survival (OS) induced by tumor lysate-pulsed DC vaccination in these patients.

Study Design:
The study is designed as a randomized Phase II clinical trial. The trial will enroll approximately 60 total patients with malignant glioma into the trial. Approximately 20 patients per group will be randomized into one of three experimental treatment cohorts. Cohort #1 will receive autologous tumor lysate-pulsed DC vaccination together with a placebo cream or intramuscular injection of saline. Cohort #2 will receive autologous tumor lysate-pulsed DC vaccination together with adjuvant 0.2% resiquimod (TLR7 agonist). Cohort #3 will receive autologous tumor lysate-pulsed DC vaccination together with adjuvant poly ICLC (TLR3 agonist). Patients who randomize to Cohort #1 will be treated with either a placebo cream or intramuscular injection of saline. All of these investigational treatments will be performed in addition to standard treatment.

Patients in each treatment cohort will receive up to 10 immunizations with autologous tumor lysate-pulsed DC vaccination (+/- TLR agonists) over a period of 3 years.

Safety of autologous tumor lysate-pulsed DC vaccination +/- TLR agonists will be assessed by comparing the frequency and intensity of adverse events, along with physical examination data and clinical laboratory testing results during the study period, between each treatment cohort.

Population:
Patients, 18 and older with newly diagnosed or recurrent malignant glioma of WHO III or IV, who have undergone surgery at UCLA, are eligible to enroll in the protocol. All patients must have a Karnofsky Performance Score of ≥60, 8 week life expectancy, no other prior malignancy within the last 10 years, and no active infections.

Treatment Schedule:
Two intradermal (i.d.) injections of autologous tumor lysate-pulsed DC +/- TLR agonists per treatment; each injection consists of 2.5 million DC in 0.15 ml, in the upper arm. Treatments will be given at days 0, 14, 28, and at future follow up appointments that are associated with routine clinical visits if enough tumor material is available and deemed clinically appropriate. Adjuvant 0.2% resiquimod will be given as a cream, with 1 tube given once a day on days 1, 3, and 5 post DC vaccination. Poly ICLC will be given as a one-time intramuscular injection (20 μg/kg) immediately before the DC vaccination.

Assessments:
Disease progression will be assessed every 2 months by brain MRI scans. KPS is assessed during clinic visits.

Primary and Secondary Endpoint, Hypothesis, and Analytic Method
Primary Endpoint- The most effective combination of DC vaccine components.

Hypothesis: anti-tumor immune responses will be enhanced for patients treated with adjuvant TLR agonists in the treatment cohorts #2 and #3 compared with patients treated with autologous tumor lysate-pulsed DC vaccination alone.

Analytic Method: immune monitoring.

Secondary Endpoint- time to tumor progression and overall survival.

Hypothesis: Survival will be longer for patients treated with adjuvant TLR agonists in the treatment cohorts #2 and #3 compared with patients treated with autologous tumor lysate-pulsed DC vaccination alone.

Analytic Method: Cox regression.

A Phase II Clinical Trial Evaluating Autologous Dendritic Cells Pulsed with Tumor Lysate Antigen +/- Toll-like Receptor Agonists for the Treatment of Malignant Glioma

STUDY PROTOCOL

Table of Contents

1.0 INTRODUCTION

The main purpose of this study is to evaluate the most effective immunotherapy vaccine components in patients with malignant glioma. Our previous phase I study (IRB #03-04-053) already confirmed that this vaccine procedure is **safe** in patients with malignant brain tumors, and with an indication of extended survival in several patients. However, the previous trial design did not allow us to test which formulation of the vaccine was the most effective. This phase II study will attempt to dissect out which components are most effective together. Dendritic cells (DC) (cells which "present" or "show" cell identifiers to the immune system) isolated from the subject's own blood will be treated with tumor-cell lysate isolated from tumor tissue taken from the same subject during surgery. This pulsing (combining) of antigen-presenting and tumor lysate will be done to try to stimulate the immune system to recognize and destroy the patient's intracranial brain tumor. These pulsed DCs will then be injected back into the patient intradermally as a vaccine. We will also utilize adjuvant resiquimod or poly ICLC in some treatment cohorts. It is thought that the host immune system might be taught to "recognize" the malignant brain tumor cells as "foreign" to the body by effectively presenting unique tumor antigens to the host immune cells (T-cells) *in vivo*.

2.0 SPECIFIC AIMS / OBJECTIVES

The primary objectives of the study are:

2.1 To determine the most effective DC vaccine combination, based on formulations tested in our Phase I clinical trial (03-04-053).

Secondary objectives of this study are:

2.2 To monitor time to tumor progression (TTP) and overall survival (OS), in brain tumor patients injected with tumor lysate-pulsed dendritic cells.

3.0 BACKGROUND AND RATIONALE

3.1 Review of Brain Cancer

It was estimated that there were 17,200 new cases of brain and other nervous system cancers accounting for 13,100 deaths in the United States in the year 2001 (1, 2). Survival rates for brain tumors differ greatly by location, behavior, histologic type, and age. Nonetheless, 5-year survival rates for the most malignant primary brain tumor, glioblastomamultiforme, is less than 2% (2).

In an effort to improve the outcome of patients with brain cancer, there have been attempts to give adjunctive therapies consisting of radiation with or without chemotherapy. Thus far, research over 3 decades has failed to provide any definitive evidence of improved outcome (overall survival, disease free survival) in patients. Clearly, different modalities other than chemotherapy and/or radiation therapy must be explored in the adjuvant setting.

3.2 Tumor-Specific Immunotherapy

One alternative to conventional cancer treatments is utilizing the immune system to target and eliminate tumor cells. The potential therapeutic benefit of eliciting an anti-tumor immune response in cancer patients was first suggested over a decade ago when the direct association between immunosuppression and increased incidence of melanoma was initially observed. The original tumor vaccines consisted of irradiated, allogeneic melanoma cells. Some patients

achieved prolonged survival, although the high serum IgM titers produced in response to cell membrane antigens likely decreased the response rate. In a Phase II report, Morton *et al.*treated 136 patients with a vaccine consisting of three melanoma lines demonstrating over-expression of melanoma-associated antigens. In several patients, overall survival increased which correlated with positive skin tests against the immunogen(3).

New strategies are designed to increase tumor vaccine immunogenicity, resulting in enhanced, specific T cell responses. Some procedures involve genetically altered tumor vaccines; that is, introducing genes coding for cytokines, co-stimulatory molecules, or components of the major histocompatibility complex (MHC) (4).

Conversely, other approaches utilize altered autologous antigen-presenting cells (APC) to present tumor-associated antigens (5-8). New and exciting strategies in anticancer therapeutics are emerging since several of the molecular mechanisms involved in immune recognition were elucidated. Researchers now understand some of the crucial portions of primary and secondary signaling pathways that are activated when T and B lymphocytes produce an anti-tumor immune response (8, 9). T-cell recognition of antigen requires the formation of a tri-molecular complex comprised of: 1) the major histocompatibility complex (MHC); 2) the T cell receptor (TCR); and 3) a short segment of intracellularly-processed protein associated with the MHC. Antigen presentation of cell-surface peptides to T cells can occur in association with either MHC class I or II molecules; the former associated with $CD8^+$ T cell responses [usually cytolytic T lymphocytes (CTL)], and the latter associated with $CD4^+$ T cell responses [usually helper T cells (T_H)]. After tumor-specific proteins are proteolytically cleaved into fragments of 8-12 amino acids in length, peptides are presented on the cell surface in association with MHC class I, and the complex is recognized by the TCR of naïve T cells (8, 9). Most tumors do not express MHC class II. As a result, it is generally accepted that the enhancement of $CD8^+$-mediated immune responses is of paramount importance in anti-cancer immunotherapeutics.

Significant progress is evident in the discovery and characterization of tumor-associated antigens (TAA), beginning with the report of the first melanoma antigen (MAGE) in 1991 (10). Intensive research is underway into the use of TAAs as potential targets of immune-based cancer treatment. They can be classified into four general groups:

- "Cancer/testis" antigens, including the MAGE gene family (10-12), whose expression in normal tissues is limited to testis and whose genes have been mapped to the X chromosome;

- Antigens derived from viruses such as Human Papilloma Virus (13, 14) and Epstein-Barr Virus (15);

- Differentiation antigens such as PSA and prostate-specific membrane antigen (PSMA) (16, 17) and Melan-A/MART-1, TRP-2 and/or gp100(7, 18-28);

- Antigens existing in a modified or mutated state in tumors as compared to normal tissue, such as ras(29-31) and p53 (32, 33).

Favorable results continue to be reported as compared with standard treatments such as chemotherapy and radiotherapy, and so hold promise for decreasing patient mortality.

3.3 Dendritic Cell-Based Immunotherapy

Dendritic cells (DC) are the professional antigen-presenting cells of the body. Activation of the immune response by dendritic cells results in the potential recruiting of all effector mechanisms of the immune system, such as CTL and antibodies, as well as natural killer cells. In addition, DC are able to induce a *de novo* response to antigens and to induce long-lasting immune memory. None of these distinct advantages occur with passive immunotherapy, and they make active immunotherapy a preferred method to induce anti-tumor immunity. This advantage prompted a large number of clinical trials that employ DC-based immunotherapy against various cancers (5, 6, 34-64).

The following conclusions can be drawn from the DC clinical trial data currently in the literature. First, DC therapy appears safe and well–tolerated. To date, no serious side effects have been reported with any of these trials. Second, many of these trials report induction of cellular immunity, humoral immunity, or both, against vaccine components. Most importantly, these trials often report that DC immunotherapy may significantly impact disease-free and overall survival. The true benefit of these therapies needs to be assessed in double-blind, placebo controlled trials, some of which are underway.

DC-based immunotherapy can accommodate many different forms of antigen. DC have been used with materials based on autologous tumor cells, synthetic peptides, tumor-eluted peptides, killed tumor cells, tumor cell lysates, and recombinant proteins. The advantage of using such materials is that this approach is applicable against cancers in which a true tumor-specific or tumor-associated antigen is unknown, such as in malignant gliomas.

3.4 Immunotherapy for Brain Cancer

An emerging strategy in the treatment of various neoplasms involves the stimulation of an immune response against the malignant cells. Among the new treatments currently being investigated for malignant cancers, immunotherapy is theoretically very appealing, because it offers the potential for high tumor-specific toxicity. However, there is concern about the applicability of immunologically based therapies to central nervous system (CNS) tumors. One explanation for this is that the CNS is “immunologically privileged” -- that it is devoid of lymphoid reactivity and normal immune surveillance (65, 66). While this may be true in healthy brain tissue, many recent studies have documented that T-lymphocytes and major histocompatability (MHC) antigens are easily detectable in the CNS during illness and disease, (i.e., multiple sclerosis, encephalitis, tumor) (67, 68). Furthermore, is has been observed by several different laboratories that effective anti-CNS tumor immune responses can be generated through the use of cytokine-modified tumor cell vaccines (58, 69-91).Therefore, the implication that the immune system can mediate interactions with CNS disease, (such as brain tumors) allow for excellent opportunities to investigate possible immunologic modes of therapy.

3.5 Pre-clinical data

3.5.1 Prolonged survival of rats with intracranial 9L gliomas by treatment with tumor antigen-pulsed dendritic cells.

The survival of tumor-bearing rats injected subcutaneously with syngeneic bone marrow-derived DC pulsed (co-cultured) with acid-eluted tumor peptides was significantly prolonged, relative to the survival of rats receiving equivalent numbers of DC pulsed with control (normal rat astrocyte) peptides, un-pulsed DC, or control media alone ($P<0.05$). Seven of twelve (58%) of the animals treated with tumor antigen-pulsed DC were alive at 31 days. In contrast, 0/6

(0%) of the animals treated with unpulsed DC, 0/10 (0%) of the rats treated with control peptide-pulsed DC, and 0/10 (0%) of the animals treated with control media survived past 31 days. Rats treated with control media, *un*pulsed DC, or control peptide-pulsed DC had median survivals of 16, 17, and 22 days, respectively. In contrast, rats in the 9L antigen-pulsed DC-treated group had a significantly longer median survival of 35 days (P = 0.027). When the experiment was carried out to 60 days, 3/12 (25%) of the 9L antigen-pulsed DC-treated rats were still surviving (36).

3.5.2 Histologic characterization of intracranial tumors treated with antigen-pulsed DC revealed no evidence of toxicity

The brains from each of the animals in the different treatment groups were removed shortly after death for histopathological and immunohistochemical examination. The tumors ranged in size from 125 mm^3 to 1000 mm^3. In specimens from all groups, the brain parenchyma outside the immediate peri-tumoral regions appeared histologically normal, with no appreciable inflammatory infiltration or demyelination. There was no evidence of experimental allergic encephalitis (EAE) induced by the DC-based vaccine therapy, both in the animals that died early and in the long-term survivors.

Analysis of intracranial tumor volumes in all rats that died within 21 days post-implantation was performed. Seven of the animals that received control media, 4 of those given unpulsed DC, 4 of those given normal peptide-pulsed DC, and 3 treated with the 9L antigen-pulsed DC were dead by three weeks. In these animals that died early, there was no significant difference in the mean tumor size at death among the four experimental groups *(data not shown).* This further supports the observation that the DC-treated animals that died early probably died of their intracranial tumor load and not of any unusual immune inflammation compared to the control animals. The brains of the three animals treated with 9L antigen-pulsed DC that survived past 60 days revealed no evidence of tumor, suggesting eradication of established tumors in these long-term survivors.

Immunohistochemistry analysis of five animals from each group documented an increased peri-lesional and intratumoral infiltration of $CD8^+$ T-cells, (and CD4+ T-cells and macrophage cells to a lesser extent), in the group treated with antigen-pulsed DC compared to controls. Tumors from all five animals analyzed in the antigen-pulsed DC-treated group were associated with moderate to heavy T-cell infiltration, whereas no intratumoral T-cell infiltration was observed in any of the similarly stained brains examined from the control group (36).

3.5.3 Induction of tumor-specific cytotoxic T-lymphocytes (CTL) by treatment with tumor antigen-pulsed DC.

To determine whether the prolonged survival seen in our antigen-pulsed DC-treated rats was actually due to the induction of tumor-specific CTL immune responses *in vivo*, standard *in vitro* europium cytotoxicity assays were performed using splenocytes harvested from animals in each of the experimental groups. Cytotoxic activity was tested against irradiated 9L tumor cells. Because the animals in our survival study expired on different days, five separate cytotoxicity assays were performed. Three out of five of the cytotoxicity assays showed a trend that animals immunized with peptide-pulsed DC were capable of inducing 9L-specific CTL responses that were statistically significant compared with

animals immunized with control media. Two of the assays that we performed revealed no significantly increased CTL response in the treated versus control animals (36).

3.5.4 Toll-like receptors (TLR) and DC Activation

Poor response rates in vaccine trials have led to the development of various strategies for enhancing the immune response. Recent efforts have focused on the family of Toll-like receptors (TLR). TLR are the immune system's pattern recognition receptors (92-94). TLR signaling results in the activation of APC and leads to T-cell priming and adaptive immune responses. The central player in this process is the DC. The character and intensity of DC-T cell interactions are radically altered by TLR activation. TLR activity regulates the signals that DC deliver to T cells in order to promote clonal expansion and differentiation into effector cells. Distinct DC subtypes express a unique set of TLR molecules. Differential expression of TLR helps tune the specificity of the immune response.

The selective expression of different TLR in specific DC subsets precludes widespread activation of DC by TLR agonists. TLR-3 recognizes double-stranded RNA encoded by viruses (95), as well as polyriboinosinic:polyribocytidylicacid poly(I:C), a synthetic analog of dsRNA(95, 96). TLR-3 is predominantly expressed by macrophages, plasmacytoid DC and myeloid DC (96, 97), but also by microglia cells, the resident macrophages in the brain (98, 99). However, it has also been shown that astrocytes (100-102) and malignant gliomas(103) can express and respond to TLR-3 activation. Activation of TLR-3 in these cells triggers the activation of NFκb signaling, the release of pro-inflammatory cytokines and promotes the differentiation of immature T cells into Th1 cells (94). The potent stimulatory effects of TLR-3 activation on the immune response have made it an attractive pharmaceutical target.

Imiquimod, an imidazoquinoline, is a TLR-7 agonist that was approved by the U.S. Food and Drug Administration for treating external genital and perianal warts (104). More recently, newer TLR-7 agonists (e.g., ***Resiquimod/R-848***) have been developed in gel formulations that have better penetration through healthy skin (105). A variety of studies using imiquimod and resiquimod for benign and malignant lesions have demonstrated a great deal of promise (106-112). The observed antiviral and anti-tumor activity of imidazoquinolines, such as resiquimod and imiquimod, stem from their ability to recruit activated immune cells to the site of the lesion, to up regulate antigen presentation molecules in the lesion, to down regulate immunosuppressive molecules in the lesion microenvironment, and to promote directly the apoptosis of tumor cells (108, 112-114). These charactreristics led some to hypothesize that imiquimod or resiquimod might enhance tumor immunity after vaccination. Imiquimod increases the response rate and the number of tumor-reactive $CD8^+$ T cells following vaccination with melanoma peptide and adjuvant Flt3 ligand (111). Similarly, the newer TLR-7 agonist, resiquimod, also enhances T cell responses and TH1-type cytokine secretion by DC (106, 107, 110). Monocyte-derived immature DC injected into imiquimod-pre-treated sites acquired lymph node migratory capacity comparable to *ex vivo* matured DC (115). These data suggest that imiquimod and resiquimodare potent immunostimulatory molecules that might improve the immune response to DC vaccination. Therefore, one of

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

the secondary objectives added to this study is to examine the efficacy safety/toxicity of resiquimod 0.2% topical cream as an adjuvant in DC vaccination for malignant glioma in patients who experience tumor recurrence and then receive repeat DC vaccinations.

The ability of poly IC to activate DC (116, 117) and promote T cell responsiveness (103, 117-119) has stimulated interest in its ability to be utilized as an adjuvant. In fact, recent work has demonstrated the ability of poly ICLC to synergize with glioma-specific peptide immunotherapy in pre-clinical models of glioma(103), thus highlighting its ability to act as an adjuvant. Poly IC has also been tested as a single therapeutic agent in patients with advanced malignancies (120, 121). However, serum proteases in humans were found to rapidly degrade the active components. More recently, the use of polyriboinosic-polyribocytidylic acid stabilized by poly-lysine (poly-ICLC) was found to have much greater stability in humans. In fact, poly-ICLC has been utilized as a single agent therapeutic for advanced renal carcinoma, relapsed lymphoma (74) and malignant glioma patients (122), demonstrating its safety. More recently, clinical trials sponsored by the NCI-funded CNS Consortium (New Approaches to Brain Tumor Therapy, NABTT) have similarly confirmed the safety and toxicity for the combination treatment of poly ICLC and low-dose temozolomide for newly diagnosed glioblastoma patients. More data from these clinical trials are forthcoming. These data suggest that poly ICLC is a potent immunostimulatory molecule that might improve the immune response to DC vaccination. Therefore, one of the secondary objectives added to this study is to examine the efficacy safety/toxicity of poly ICLC (*Hiltonol*, 20 μg/kg i.m.) as an adjuvant in DC vaccination for malignant glioma in patients who receive repeat DC vaccinations.

3.6 Clinical Data

Two Phase I clinical trials, **"Autologous Dendritic Cells Derived from Peripheral Blood Mononuclear Cells Cultured with GM-CSF and IL-4, then Pulsed with Autologous Glioblastoma Tumor Peptides" (BB-IND #8434; UCLA IRB #99-04-019)** and **"Phase I Dose Escalation Study of Autologous Tumor Lysate-pulsed Dendritic Cell Immunotherapy for Malignant Gliomas" (FDA BB-IND #11053; UCLA IRB #03-04-053)** have been carried out at UCLA, Division of Neurosurgery, under the direction of Drs.' Linda Liau and Robert Prins. The results of our first Phase I trial have previously been published (62). The updated results of our second Phase I safety trial are summarized below.

The primary purpose of the Phase I study carried out at UCLA was:

- to determine whether Dose Limiting Toxicity (DLT) was reached in a dose escalation scheme using intradermal (i.d.) injections of autologous DC harvested from peripheral blood precursors and pulsed with tumor lysate in patients with malignant gliomas.

The secondary objectives were to monitor:

- tumor progression, and
- cellular immune responses in brain tumor patients injected with these antigen-pulsed DC.

Patient demographics are summarized in Table I.

3.6.1 Phase I Study results to date:

TABLE I. Study Subjects

Patient ID #	Age	Gender	Race	On-Study Date	Date Completed Study Injections
1-708	39	Male	Caucasian	7/7/03	2/16/05
2-261	39	Male	Hispanic	9/24/03	11/5/03
4-908	34	Male	Caucasian	10/29/03	12/17/03
5-343	40	Female	Caucasian	2/4/04	3/24/04
6-815	54	Male	Caucasian	3/3/04	4/28/04
7-799	26	Male	Caucasian	3/10/04	8/4/04
9-875	37	Male	Caucasian	5/26/04	7/14/04
10-956	58	Female	Caucasian	8/11/04	9/16/04*
11-289	70	Female	Caucasian	9/22/04	12/1/04
12-801	50	Male	Caucasian	11/10/04	12/29/04
13-209	59	Male	Caucasian	12/1/04	1/29/04
15-532	64	Male	Caucasian	4/29/05	6/10/05
16-331	66	Male	Caucasian	6/08/05	8/3/05
17-232	43	Male	Caucasian	4/10/06	5/24/06
18-748	61	Male	Caucasian	4/19/06	6/16/06
19-539	45	Female	Caucasian	4/19/06	6/7/06
20-179	59	Male	Caucasian	8/15/06	07/24/07
21-828	59	Male	Caucasian	8/16/06	10/04/06
25-062	63	Female	Asian	1/14/2008	3/27/08
26-863	41	Female	Caucasian	1/25/2008	04/08/08
27-934	34	Male	Caucasian	4/13/2008	7/01/08
28-290	38	Male	Caucasian	6/10/2008	8/13/08
31-418	49	Female	Caucasian	11/13/08	4/08/09
32-204	54	Male	Caucasian	12/16/08	4/02/09
33-296	74	Female	Caucasian	2/2/09	5/28/09
34-730	53	Male	Caucasian	3/3/09	5/27/09

Table II. Characterization of Patients with Respect to Tumor Pathology, Other Therapy, and Adverse Events Following Treatment

Patient ID #	Tumor Pathology*	Pre-Vaccine Therapy**	Post-Vaccine Therapy	Adverse Events***
1-708	GBM	Temodar	Temodar, Accutane, Celebrex, Re-operation, SRS	Seizure, fatigue, nausea/vomiting, diarrhea
2-261	GBM	Temodar	Temodar, Accutane	Fatigue, arthralgia, low-grade fever, myalgia
4-908	GBM	Temodar, Accutane	Temodar, Accutane	Lymphadenopathy, injection site reaction, low-grade fever, seizure
5-343	GBM	Temodar, Accutane		Dizziness/vertigo
6-815	Recurrent GBM	Temodar, Accutane, CCNU	Carboplatin	Allergic rhinitis, anorexia, motor weakness on left side†
7-799	GBM	Temodar, Accutane, CCNU		Anosmia, arthralgia, itching at injection site, seizure
9-875	Recurrent grade 3 Oligoastrocytoma	Temodar	Temodar, Celebrex	Sensory seizures (left hand)
10-956	Recurrent GBM	Temodar		Headaches, anxiety, depression, nausea/vomiting, speech aphasia, cognitive disturbances††
11-289	GBM	Temodar		
12-801	GBM	Temodar		Dermatitis, rash, focal motor seizures, tinnitus
13-209	GBM	Temodar		Anorexia, abdominal pain, low back pain
15-532	GBM	Temodar		
16-331	GBM	Temodar		Fatigue, left shoulder pain, arthralgia, Anorexia/weight loss
17-232	GBM	Temodar	Temodar	Sensory seizure of right upper extremity
18-748	Recurrent GBM	Temodar, Thalidomide, Accutane, Newcastle virus		
19-539	GBM	Temodar		
20-179	Grade 3 Oligoastrocytoma	Temodar		
21-828	GBM	Temodar		Decreased appetite/anorexia, Fatigue, nausea, mild decrease in facial senation on the right (neuropathy sensory), muscular weakness, decreased left facial

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

Patient ID #	Tumor Pathology*	Pre-Vaccine Therapy**	Post-Vaccine Therapy	Adverse Events***
				sensation, tremor, allergic reaction to ritalin
25-062	GBM	Temodar	Temodar	Hypersensitivity to smell, L sided facial shingles
26-863	Recurrent GBM	CPT-11, Avastin		
27-934	Recurrent GBM	Tarceva, Temodar, ANG, CCNU, Celebrex, tamoxifen,	CCNU, Celebrex, tamoxifen,	Seizure,
28-290	Grade 3 Oligoastrocytoma	Temodar		Visual seizure, fever, nausea, headache, vertigo, diarrhea, hypersensitivity to smell and tape, decreased visual processing, unsteady gait, seizures, fatigue, urinary retention, decreased proprioception upper extremities, decreased R peripheral vision, chills
31-418	Grade 3 Oligodendroglioma	PCV, tamoxifen, thalidomide		Nausea, dysphasia, dizziness, R sided numbness/tingling, memory difficulties, soreness to R arm injection site, headache, expressive aphasia, sore throat,
32-204	GBM	Temodar, Gliadel	Avastin, irinotecan	L foot pain, subgleal hematoma, L foot metatarsal stress fx, ALT elevation
33-296	Recurrent GBM	Temodar	Temodar	L hemianopsia, double vision, foreign body sensation OU, R eye retinal detachment, short-term memory deficits, periods of confusion, constipation, L sided weakness
34-730	Recurrent GBM	Temodar		Abdominal rash, post-operative hematoma, upper lip ulcers, rash to RUE, R chest wall, face, R hemiparesis, aphasia, urinary retention,

*GBM=glioblastomamultiforme;
**Pre-vaccination therapy refers to additional treatments besides surgery and standard external beam radiation therapy (up to 60 Gy); Temodar = temozolamide; Accutane = isotretinoin; CCNU = lomustine; and SRS = stereotactic radiosurgery.
***All adverse experiences related to protocol were of mild severity (NCI grades I & II). Adverse events that were of higher severity were determined to be not related to protocol and likely due to tumor progression.
†Due to tumor progression.
††Symptoms resolved with steroids.

Table III. Summary of Clinical Data: Patients with GBM

Patient ID #	Diagnosis	DC Dose x 10^6	Time to Tumor Progression (months)	Survival (months)	Current Survival Status
[a]1-708	GBM	1	12.9	33.8	Dead
2-261	GBM	1	59.37	94.0	Dead
4-908	GBM	1	No progression to date	>117.8	Alive
5-343	GBM	5	9.17	18.0	Dead
6-815	Recurrent GBM	5	8.17	11.7	Dead
7-799	GBM	5	70.13	81.4	Dead
9-875	Anaplastic Oligoastrocytoma	5	83.1	>104.8	Alive
10-956	Recurrent GBM	10	17.97	29.93	Dead
11-289	GBM	10	13.0	23.0	Dead
12-801	GBM	10	13.9	36.33	Dead
13-209	GBM	10	49.43	52.63	Dead
15-532	GBM	10	5.8	13.6	Dead
16-331	GBM	10	26.4	37.7	Dead
17-232	GBM	5	75.6	75.6	Dead
18-748	Recurrent GBM	1	18.53	18.57	Dead
19-539	GBM	5	7.63	34.97	Dead
20-179	Anaplastic Oligoastrocytoma	1	No progression to date	>49.83	Alive
21-828	GBM	1	7.9	10.3	Dead
25-062	GBM	1	No progression to date	>30.23	Alive
26-863	Recurrent GBM	1	9.83	10.93	Dead
27-934	Recurrent GBM	1	No progression to date	>67.0	Alive
28-290	Grade 3 Oligoastrocytoma	5	33.63	42.83	Dead
31-418	AO (recurrent)	5	No progression to date	>59.23	Alive
32-204	Recurrent GBM	5	22.87	>22.87	Alive
33-296	GBM	10	10.23	17.07	Dead
34-730	Recurrent GBM	10	13.27	16.23	Dead

[a]Patient #1-708 was a newly diagnosed GBM patient who completed treatment with three injections of 1 x 10^6 DC (Lot #1-708A). He subsequently has had tumor recurrence and received a repeated treatment course with 1 injection of 5 x 10^6 DC (Lot#1-708B) and then 3 booster injections of 5 x 10^6 DC (Lot #1-708C).

[b]Patient #10-956 was a recurrent GBM patient who only received one vaccination with 1x10^6 DC due to subsequent steroid use.

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

The following table documents our available data regarding the stability and sterility of the glioblastoma tumor lysate throughout the duration of each patient's enrollment in the clinical trial:

Table IV. Lot Release Data for Tumor Lysate

Patient ID #	Lot Identifier	Date of Test	Sterility	Protein Concentration (µg/mL)	Amount peptide per pulsing (µg)
[a]1-708A	1-708ATL-1	7/22/03	-	967	100
	1-708ATL-2	8/5/03	-	732	100
	1-708ATL-3	8/19/03	-	404	46
2-261	2-261TL-1	10/7/03	-	370	66.6
	2-261TL-2	10/21/03	-	387	65.8
	2-261TL-3	11/4/03	-	379	62.5
4-908	4-908TL-1	11/18/03	-	589	115
	4-908TL-2	12/2/03	-	633	134
	4-908TL-3	12/16/03	-	627	130
5-343	5-343TL-1	2/23/04	-	278	203
	5-343TL-2	3/9/04	-	294	51
	5-343TL-3	3/23/04	-	250	39.3
6-815	6-815TL-1	3/30/04	-	263	100
	6-815TL-2	4/13/04	-	235	100
	6-815TL-3	4/28/04	-	222	100
7-799	7-799TL-1	7/6/04	-	551	100
	7-799TL-2	7/20/04	-	669	100
	7-799TL-3	8/3/04	-	649	100
1-708B	1-708BTL-1	5/25/04	-	719	65
9-875	9-875TL-1	6/15/04	-	425	100
	9-875TL-2	6/29/04	-	723	100
	9-875TL-3	7/13/04	-	620	100
[b]10-956	10-956TL-1	9/15/04	-	747	100
11-289	11-289TL-1	11/2/04	-	291	100
	11-289TL-2	11/17/04	-	378	100
	11-289TL-3	11/30/04	-	340	100
12-801	12-801TL-1	12/2/04	-	357	100
	12-801TL-2	12/13/04	-	564	100
	12-801TL-3	12/28/04	-	618	100
13-209	13-209TL-1	12/21/04	-	367	100

Patient ID #	Lot Identifier	Date of Test	Sterility	Protein Concentration (μg/mL)	Amount peptide per pulsing (μg)
	13-209TL-1	1/4/05	-	397	100
	13-209TL-1	1/20/05	-	359	100
1-708C	1-708CTL-1	1/18/05	-	571	100
	1-708CTL-2	2/1/05	-	823	100
	1-708CTL-3	2/15/05	-	801	100
15-532	15-532TL-1	5/17/05	-	2221	100
	15-532TL-2	5/31/05	-	5702	100
	15-532TL-3	6/9/05	-	627	100
16-331	16-331TL-1	7/5/05	-	278	100
	16-331TL-2	7/19/05	-	183	100
	16-331TL-3	8/2/05	-	433	100
17-232	17-232TL-1	4/27/06	-	7643	100
	17-232TL-2	5/9/06	-	210	100
	17-232TL-3	5/23/06	-	674	100
18-748	18-748TL-1	5/18/06	-	14224	100
	18-748TL-2	6/1/06	-	9984	100
	18-748TL-3	6/15/06	-	9947	100
19-539	19-539TL-1	5/11/06	-	984	100
	19-539TL-2	5/25/06	-	467	100
	19-539TL-3	6/6/06	-	241	100
20-179	20-179TL-1	6/26/07	-	11316	100
	20-179TL-2	7/10/07	-	11226	100
	20-179TL-3	7/24/07	-	11200	100
21-828	21-828TL-1	9/7/06	-	4523	100
	21-828TL-2	9/19/06	-	3777	100
	21-828TL-3	10/3/06	-	7283	100
25-062	25-062TL-1	2/28/08	-	1842	100
	25-062TL-2	3/12/08	-	2036	100
	25-062TL-3	3/26/08	-	2569	100
26-863	26-863TL-1	3/10/08	-	183	100
	26-863TL-2	3/24/08	-	205	100
	26-863TL-3	4/7/08	-	235	100
27-934	27-934TL-1	6/4/08	-	634	100
	27-934TL-2	6/18/08	-	586	100
	27-934TL-3	6/30/08	-	479	100

Patient ID #	Lot Identifier	Date of Test	Sterility	Protein Concentration (μg/mL)	Amount peptide per pulsing (μg)
28-290	28-290TL-1	7/15/08	-	895	100
	28-290TL-2	7/29/08	-	891	100
	28-290TL-2	8/12/08	-	815	100
31-418	31-418TL-1	3/9/09	-	4729	100
	31-418TL-2	3/24/09	-	4322	100
	31-418TL-3	4/7/09	-	3279	100
32-204	32-204TL-1	3/4/09	-	311	100
32-204	32-204TL-2	3/18/09	-	344	100
	32-204TL-3	4/1/09	-	249/225*	100
33-296	33-296TL-1	4/30/09	-	486	100
	33-296TL-2	5/13/09	-	376	100
	33-296TL-3	5/27/09	-	389	100
34-730	34-730TL-1	4/29/09	-	1375	100
	34-730TL-1	5/11/09	-	1161	100
	34-730TL-1	5/26/09	-	1269	100

[a]Patient #1-708 was a newly diagnosed GBM patient who completed treatment with three injections of 1 x 10^6 DC (Lot #1-708A). He subsequently has had tumor recurrence and received a repeated treatment course with 1 injection of 5 x 10^6 DC (Patient #1-708B), as well as three booster vaccinations with 5x10^6 DC (Lot #1-708C).
[b]Patient #10-956 was a newly diagnosed GBM patient who only received one vaccination with 1x10^6 DC due to subsequent steroid use.

Table V. Lot Release Data of Autologous Dendritic Cells

Patient No.	Lot Identifier	Dose Level (# cells)	% Viable Cells	FACScan Analysis (% large cells HLA-DR$^+$)	FACScan Analysis (% large cells CD14$^+$)	Sterility	Myco-plasma (PCR/Cx)	Endotoxin Level[a] (EU/mL)
[b]1-708A	1-708ADC-1	1 x 10^6	88	90.77	4	-	-	0.22
	1-708ADC-2	1 x 10^6	90	93.18	4	-	-	0.19
	1-708ADC-3	1 x 10^6	81	91.71	2	-	-	0.35
2-261	2-261DC-1	1 x 10^6	80	95	10	-	-	0.37
	2-261DC-2	1 x 10^6	88	90	13	-	-	0.258
	2-261DC-3	1 x 10^6	86	97	11	-	-	0.195
4-908	4-908DC-1	1 x 10^6	90	98	15	-	-	0.06
	4-908DC-2	1 x 10^6	88	99	28	-	-	0.101
	4-908DC-3	1 x 10^6	87	99	29	-	-	0.082
5-343	5-343DC-1	5 x 10^6	91	99	29	-	-	0.186
	5-343DC-2	5 x 10^6	85	99	15	-	-	0.56

Patient No.	Lot Identifier	Dose Level (# cells)	% Viable Cells	FACScan Analysis (% large cells HLA-DR$^+$)	FACScan Analysis (% large cells CD14$^+$)	Sterility	Myco-plasma (PCR/Cx)	Endotoxin Level[a] (EU/mL)
	5-343DC-2	5 x 10^6	88	98	20	-	-	0.236
6-815	6-815DC-1	5 x 10^6	94	95	24	-	-	0.282
	6-815DC-2	5 x 10^6	93	95	19	-	-	0.231
	6-815DC-3	5 x 10^6	88	89	22	-	-	0.218
7-799	7-799DC-1	5 x 10^6	81	95	20	-	-	1.1
	7-799DC-2	5 x 10^6	86	94	17	-	-	0.339
	7-799DC-3	5 x 10^6	81	96	19	-	-	0.362
1-708B	1-708BDC-1	5 x 10^6	69	92	9	-	-	2.36
9-875	9-875DC-1	5 x 10^6	78	85	7	-	-	0.288
	9-875DC-2	5 x 10^6	57	83	14	-	-	0.43
	9-875DC-3	5 x 10^6	88	90	6	-	-	0.349
[c]10-956	10-956DC-1	10 x 10^6	86	98	45	-	-	1.1
11-289	11-289DC-1	10 x 10^6	87	96	42	-	-	0.744
	11-289DC-2	10 x 10^6	92	92	44	-	-	0.97
	11-289DC-3	10 x 10^6	92	95	44	-	-	0.246
12-801	12-801DC-1	10 x 10^6	88	97	19	-	-	1.37
	12-801DC-2	10 x 10^6	84	98	22	-	-	2.57
	12-801DC-3	10 x 10^6	89	99	24	-	-	3.77
13-209	13-209DC-1	10 x 10^6	94	97	39	-	-	3.8
	13-209DC-2	10 x 10^6	94	93	38	-	-	0.21
	13-209DC-3	10 x 10^6	91	95	32	-	-	0.24
1-708C	1-708CDC-1	5 x 10^6	84	97	10	-	-	<0.5
	1-708CDC-2	5 x 10^6	79	99	9	-	-	<0.6
	1-708CDC-3	5 x 10^6	91	98	7	-	-	<1.0
15-532	15-532DC-1	10 x 10^6	85	92	44	-	-	<2.5
	15-532DC-2	10 x 10^6	92	95	41	-	-	<4.38
	15-532DC-3	10 x 10^6	90	96	47	-	-	<3.5
16-331	16-331DC-1	10 x 10^6	84	91	9	-	-	<0.25
	16-331DC-2	10 x 10^6	89	93	10	-	-	<0.25
	16-331DC-3	10 x 10^6	89	98	7	-	-	<4.0
17-232	17-232DC-1	5 x 10^6	84	99	13	-	-	<0.45
	17-232DC-2	5 x 10^6	86	99	14	-	-	<0.3
	17-232DC-3	5 x 10^6	81	99	13	-	-	0.0535
18-748	18-748DC-1	1 x 10^6	75	90	5	-	-	1.908
	18-748DC-2	1 x 10^6	72	94	5	-	-	1.636
	18-748DC-3	1 x 10^6	71	88	14	-	-	0.0282

Patient No.	Lot Identifier	Dose Level (# cells)	% Viable Cells	FACScan Analysis (% large cells HLA-DR$^+$)	FACScan Analysis (% large cells CD14$^+$)	Sterility	Myco-plasma (PCR/Cx)	Endotoxin Level[a] (EU/mL)
19-539	19-539DC-1	5 x 10^6	88	98	33	-	-	0.7684
	19-539DC-2	5 x 10^6	76	98	25	-	-	1.435
	19-539DC-3	5 x 10^6	73	96	29	-	-	0.0163
20-179	20-129DC-1	1 x 10^6	82	97	21	-	-	0.0719
	20-129DC-2	1 x 10^6	87	97	19	-	-	<0.05
	20-129DC-3	1 x 10^6	87	95	30	-	-	<0.05
21-828	21-828DC-1	1 x 10^6	71	97	9	-	-	.0649
	21-828DC-2	1 x 10^6	87	97	10	-	-	0.1121
	21-828DC-3	1 x 10^6	78	98	25	-	-	<0.05
25-062	25-062DC-1	1 x 10^6	80	98	33	-	-	<0.05
	25-062DC-2	1 x 10^6	89	98	31	-	-	<0.05
	25-062DC-3	1 x 10^6	86	99	30	-	-	<0.05
26-863	26-863DC-1	1 x 10^6	71	90	41	-	-	0.0601
	26-863DC-2	1 x 10^6	92	96	39	-	-	0.0683
	26-863DC-3	1 x 10^6	85	95	33	-	-	<0.05
27-934	27-934DC-1	1 x 10^6	82	97	29	-	-	<0.05
	27-934DC-2	1 x 10^6	75	94	36	-	-	0.0908
	27-934DC-3	1 x 10^6	72	95	28	-	-	<0.05
Patient No.	Lot Identifier	Dose Level (# cells)	% Viable Cells	FACScan Analysis (%large cells HLA-DR$^+$/CD14$^-$)	FACScan Analysis (%large cells HLA-DR$^+$/CD86$^+$)	Sterility	Mycoplasma PCR/Cx)	Endotoxin Level3 (EU/mL)
28-290	28-290DC-1	5 x 10^6	87	50	85	-	-	<0.05
	28-290DC-2	5 x 10^6	89	51	95	-	-	<0.05
	28-290DC-3	5 x 10^6	86	39	95	-	-	<0.05
31-418	31-418DC-1	5 x 10^6	82	82	95	-	-	<0.05
	31-418DC-2	5 x 10^6	80	83	94	-	-	<0.05
	31-418DC-3	5 x 10^6	73	72	97	-	-	0.0604
32-204	32-204DC-1	5 x 10^6	88	76	96	-	-	<0.05
	32-204DC-2	5 x 10^6	74	74	98	-	-	<0.05
	32-204DC-3	5 x 10^6	88	66	96	-	-	<0.05
33-296	33-296DC-1	10 x 10^6	75	70	94	-	-	<0.05
	33-296DC-2	10 x 10^6	81	n.a.	95		-	<0.05
	33-296DC-3	10 x 10^6	92	n.a.	95		-	<0.05
34-730	34-730DC-1	10 x 10^6	84	62	97	-	-	<0.05
	34-730DC-1	10 x 10^6	84	n.a.	96	-	-	<0.05
	34-730DC-1	10 x 10^6	91	n.a.	97		-	<0.05

[a]Upper limit of acceptability for endotoxin level is 5 EU/mL.

[b]Patient #1-708 was a newly diagnosed GBM patient who completed treatment with three injections of 1 x 10^6 DC (Lot #1-708A). He subsequently has had tumor recurrence and received a repeated treatment course with 1 injection of 5 x 10^6 DC (Patient #1-708B), as well as three booster vaccinations with 5x10^6 DC (Lot #1-708C).
[c]Patient #10-956 was a newly diagnosed GBM patient who only received one vaccination with 1x10^6 DC.

In the past year (2006-2007), **no** samples have tested positive for infection on sterility, mycoplasma, or endotoxin tests.

Table VI. Summary of Immune Response Data

Patient #	Tumor Pathology*	DC Dose (# cells/injection)	HLA Sub-type	Increase in Ag-specific CD8+ T Cells after vaccination*	% ↑/↓ $Foxp3^+$Treg Cells[§]	T cell Presence in Original Tumor	Survival (months)
1-708	GBM	1 x 10^6	HLA-A03, A68	ND	-47%	++	33.8
2-261	GBM	1 x 10^6	HLA-A201, A68	+ (Gp100, TRP-2, Survivin)	ND	++	>61.1
4-908	GBM	1 x 10^6	HLA-A201	+ (CMV, Gp100, TRP-2)	-	+++	>63.6
5-343	GBM	5 x 10^6	HLA-A201, A11	-	+25.64%	-	18.0
6-815	Recurrent GBM	5 x 10^6	HLA-A24, A11	ND	+6.1%	ND	11.7
7-799	GBM	5 x 10^6	HLA-A0404, A03 HLA-DR4, DR17	ND	ND	++	>68.3
9-875	Rec Gr. 3 Oligoastrocytoma	5 x 10^6	HLA-A24, A32 HLA-DR1, DR12	ND	+/-	ND	>50.6
10-956	Recurrent GBM	10 x 10^6	HLA-A201, A03 HLA-DR11, DR12	+ (Her-2)	-80.0%	ND	13.1
11-289	GBM	10 x 10^6	HLA-A201, A01 HLA-DR17, DR11	-	ND	+	23.0
12-801	GBM	10 x 10^6	HLA-A29, A69 HLA-DR7, DR15	ND	-70%	+++	36.33
13-209	GBM	10 x 10^6	HLA-A201, A63, HLA-DR4, DR13	+ (Gp100,TRP-2)	ND	++	>47.5
15-532	GBM	10 x 10^6	HLA-A03 HLA-DRB1*0404	ND	ND	++	13.6
16-331	GBM	10 x 10^6	HLA-A*0201 HLA-DRB1*0401	+ (Gp100, TRP-2, Her-2)	ND	+++	37.7
17-232	GBM	5 x 10^6					
18-748	Recurrent GBM	1 x 10^6					
19-539	GBM	5 x 10^6					
20-179	Anaplastic Oligoastrocytoma	1 x 10^6					
21-828	GBM	1 x 10^6	HLA-*A03, *A24				

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

25-062	GBM	1 x 10^6	HLA-*A0203, *A11, *BW6				
26-863	Recurrent GBM	1 x 10^6	HLA-*A26, *A29				
27-934	Recurrent GBM	1 x 10^6	HLA-*A0201, *A03				
28-290	Grade 3 Oligoastrocytoma	5 x 10^6	HLA-*A11, *A25				
31-418	AO (recurrent)	5 x 10^6					
32-204	Recurrent GBM	5 x 10^6					
33-296	GBM	10 x 10^6					
34-730	Recurrent GBM	10 x 10^6					

*(+) score reflects > 150% increase pre:post DC vaccination
§Treg percentage from d28 or d42 post DC vaccination.

3.6.2 Safety

Safety

Adverse events: Twenty-five patients with malignant glioma have been enrolled in our previous Phase I clinical trial with all having sufficient on-study time to be evaluable for safety. The median age was 51 years of age (range, 26 to 70 years). To date, there have been no serious adverse events related to the study drug.

Constitutional symptoms: Nausea/vomiting, headache and fatigue, diarrhea, low-grade fever and loss of appetite were the most common symptoms that could likely be associated with the treatment. Lymph node swelling, myalgia, arthralgia, back/neck pain, depression, dehydration, hiccoughs, dizziness, cough, somnolence, dyspnea, allergic rhinitis, and pain/itching at injection site were other less common symptoms reported (see Table II).

4.0 NUMBER OF SUBJECTS & STOPPING RULES

4.1 Enrollment and Assignment of Patients

4.1.1 Definition of terms:

- ***Enrollment*** will be used to refer to the initial entry into the study after the patient has signed the informed consent documents, met the eligibility criteria and has received a study ID number.
- ***Assignment*** will be used to refer to the time of randomization to treatment cohorts. This will be following surgery, radiation therapy, and, if appropriate, chemotherapy.

4.1.2 There will be no limit on the number of subjects enrolled in the trial except that enrollment will cease when assignment to treatment cohorts has ceased or the trial is stopped for any reason. If after assignment a subject cannot undergo leukapheresis due to any reason, that subject can be replaced in the study. However, the Study Annual Report must list all subjects, including those not receiving investigational agent.

4.1.3 Assignment to cohorts will be limited to no more than twenty (20) subjects per experimental treatment cohort, for a maximum of (60) subjects total.

It is estimated that accrual will average two participants into this study per month. The total accrual is to be approximately 60 patients. The treatment cohorts, and their expected accrual, are described below:

Treatment Cohort	***Dose***	***Frequency***	***# Subjects/cohort***
A) DC Lysate+placebo	2.5x10e6 DC/arm x 2	3 Biweekly inj.+7 boosters @ 4 month intervals	20
B) DC Lysate+o.2% resiquimod	2.5x10e6 DC/arm x 2	3 Biweekly inj.+7 boosters @ 4 month intervals	20
C) DC Lysate + poly ICLC	2.5x10e6 DC/arm x 2	3 Biweekly inj.+7 boosters @ 4 month intervals	20

For each DC immunization, two separate i.d. injections of approximately 0.15 mL (150 ul) each are given via an insulin syringe with minimal dead space to deliver a total of 5 million tumor lysate antigen-loaded DC. Injections are administered in the upper arm. The first three tumor lysate-pulsed DC injections are given biweekly for a total of three injections (on days 0, 14, and 28) plus seven boosters scheduled at follow up visits if enough autologous tumor material is available and deemed clinically appropriate. If enough DC are not generated from the leukapheresis for 3 complete injections, a lower number of pulsed DC may be injected/vaccination or a decreased number of vaccinations may be completed in each patient. DC must still satisfy all of the normal requirements for lot release.

For subjects that randomize to treatment cohort **A**, DC vaccinations will be administered together with either a placebo cream or intramuscular injection of saline.

For subjects that randomize to treatment cohort **B**, DC vaccinations will be administered together with 0.2% resiquimod cream (see description in Section 6.6).

For subjects that randomize to treatment cohort **C**, DC vaccinations will be administered together with 20μg/kg poly ICLC, given as a one-time intramuscular injection (see description in Section 6.5).

4.2 **The estimated duration of the study is 3 – 5 years**. The maximum number of subjects that will be involved is 60, and the anticipated patient enrollment rate is approximately 12-16 subjects per year at UCLA.

4.3 Study Stopping Rules

4.3.1 Toxicity Stopping Rules

4.3.1.1 The following events define Dose Limiting Toxicity (DLT):

- Grade 2 allergic reaction involving generalized urticaria.
- Any grade 3 or greater allergic reaction.
- Any grade 2 or greater autoimmune reaction.
- Any grade 3 or greater hematologic or non-hematologic toxicity.
- Any Grade 3 or 4 toxicity (as defined by the National Cancer Institute Toxicity Criteria, *Appendix C*) or life-threatening event, except when clearly due to another cause.
- Any other unexpected significant neurological deficit, which in the opinion of the investigators is "probably" related to the antigen-pulsed dendritic cell therapy.

4.3.2 Futility Stopping Rules

If suitable vaccine cannot be provided to more than three subjects in a cohort, assignment will cease. The study will then be terminated due to lack of feasibility.

If adequate vaccine that meets lot release criteria cannot be made for more than three (3) subjects in a treatment cohort, the study will stop.

5.0 PATIENT ELIGIBILITY

5.1 Eligibility at Enrollment

5.1.1 Inclusion Criteria

5.1.1.1 Patients with newly diagnosed or recurrent glioma of WHO Grade III or IV {anaplastic astrocytoma (AA), anaplastic astro-oligodendroglioma (AO), or glioblastoma (GBM)} will be eligible for this protocol.

5.1.1.2 Patients must have had surgical resection at UCLA, for which a separate informed consent was signed for the collection of their tumor prior to surgery.

5.1.1.3 After surgery, a pathological diagnosis of malignant glioma (WHO Grade III or IV) will need to be established.

5.1.1.4 Patients must be 18 years or older and able to read and understand the informed consent document. Patients must sign the informed consent indicating that they are aware of the investigational nature of this study.

5.1.1.5 Patients must have a Karnofsky performance status (KPS) rating of $\geq$ 60 prior to initiating treatment. Patients may be enrolled at a KPS of < 60 if it is felt that the patient will have adequate opportunity to recover to a KPS of $\geq$ 60 by the initiation of treatment.

5.1.2 Exclusion Criteria

5.1.2.1 Subjects with an active infection.

5.1.2.2 Inability to obtain informed consent because of psychiatric or complicating medical problems.

5.1.2.3 Unstable or severe intercurrent medical or psychiatric conditions as determined by the Investigator.

5.1.2.4 Females of child-bearing potential who are pregnant or lactating or who are not using approved contraception.

5.1.2.5 History of immunodeficiency (e.g., HIV) or autoimmune disease (e.g., rheumatoid arthritis, systemic lupus erythematosus, vasculitis, polymyositis-dermatomyositis, scleroderma, multiple sclerosis, or juvenile-onset insulin-dependent diabetes) that may be exacerbated by immunotherapy.

5.1.2.6 Subjects with organ allografts.

5.1.2.7 Inability or unwillingness to return for required visits and follow-up exams.

5.1.2.8 Subjects who have an uncontrolled systemic malignancy that is not in remission.

5.2 Age, Gender, Race

5.2.1 Patients must be $\geq$ 18 years old.

5.2.2 This study was designed to include women and minorities, but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender.

5.2.3 No exclusion in this study will be based on race. Minorities will actively be recruited to participate. For non-English speaking subjects or those who are not facile in English, the consent form will be translated into the subject's fluent language by an educated medical interpreter prior to seeking enrollment. In addition to the translated consent form, a language interpreter will be used for communicating all research-related risks and injuries with these subjects.

TREATMENT PLAN

6.1 Initial Eligibility Evaluation and Enrollment

Subjects must satisfy the initial eligibility criteria and sign the patient Informed Consent documents for study **enrollment** prior to screening procedures and leukapheresis. The Study schema is outlined in *Appendix A.*

6.2 Screening and Assignment

Subjects will be assigned to treatment cohorts following surgery, radiation therapy and/or chemotherapy. Subjects will undergo screening evaluations and must satisfy the eligibility criteria prior to **assignment** to treatment cohorts. Screening evaluations, or evaluations that determine patient eligibility, will be performed prior to leukapheresis and preparation of autologous dendritic cells for injection. These screening evaluations must be reviewed prior to study treatment. The following clinical and laboratory evaluations will be performed at Screening:

6.2.1 Objective Signs and Symptoms: Vital signs (blood pressure, pulse, temperature and respirations).

6.2.2 History and Physical Examination

6.2.3 Karnofsky Performance Status *(Appendix B)*

6.2.4 Neurological Exam

6.2.5 MRI of brain with and without contrast

6.2.6 Urinalysis: Normal routine urinalysis

6.2.7 Serum Chemistries: CMP.

6.2.8 Hematology: Complete blood count (CBC), differential, platelets, and coagulation tests including PT (Prothrombin Time) and PTT (Partial Thromboplastin Time).

6.3 Randomization to Experimental Treatment Cohorts

In order for a patient to be enrolled into one of the three investigational arms of the study, they must meet all of the eligibility criteria listed above. The patients meeting these criteria at screening will be immediately randomized into a treatment cohort (assignment and randomization happen concurrently).

6.4 Leukapheresis

Prior to the first injection, eligible patients undergo leukapheresis at the UCLA Clinical Hemapheresis Unit to isolate peripheral blood mononuclear cells (PBMC). For patients without sufficient peripheral venous access for leukapheresis, a temporary apheresis catheter may be inserted. If such a catheter is necessary, patients will sign a separate informed consent form for temporary apheresis catheter placement. To prevent the development of hypocalcemia from the citrate used for leukapheresis, all patients will be given intravenous calcium during the leukapheresis procedure. Some patients may need to have a second leukapheresis, depending on the DC yield from the first leukapheresis. The leukapheresis materials will be transported under sterile conditions from the UCLA Hemapheresis Unit to the UCLA Jonsson Cancer Center cGMP suite (14^{th} floor, Factor Building) and processed as described below.

6.5 Description of Manufacturing Process

6.5.1 Preparation of Autologous Dendritic Cells (DC)

DC will be prepared from adherent, autologous peripheral blood mononuclear cells (PBMC) exposed for one week in tissue culture to granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4).

Patients will undergo a single leukapheresis to obtain adequate numbers of PBMC for DC generation. Mononuclear cells will be isolated from cells obtained from the leukapheresis product by Ficoll-Hypaque centrifugation and used immediately to generate dendritic cells. The cells will be labeled from the time of collection with a code number consisting of the sequential patient number in order of study enrollment (1-, 2-, 3-, etc.) followed by a random 3-digit number generated by a computer program to ensure proper tracking of the specimens and patient confidentiality. Approximately 6 x 10^9 PBMC will be resuspended in a solution of RPMI-1640 (Gibco-BRL) with 10% autologous serum. The cell suspension will be added to 150 cm^2 tissue culture flasks at 15 mL/flask and 2 x 10^8 cells/flask. The flasks will be incubated at 37°C and 5% carbon dioxide (CO_2) for 2 hours to allow the cells to adhere to the flask.

After the 2-hour incubation, the medium in the flasks will be removed. The cell layer will be washed twice with 10 mL of phosphate-buffered saline (PBS) (BioWhittaker, Walkersville, MD) to remove non-adherent cells. Culturing medium containing RPMI-1640 (Gibco-BRL), and 10% autologous serum, with 500 units/mL each of recombinant human IL-4 (CellGenix, Freiburg, Germany or MiltenyiBiotec GmbH) and recombinant human GM-CSF (Leukine®; Genzyme, Cambridge, MA or Miltenyi Biotec GmbH) will be added to each flask after washing. The flasks will be placed in the incubator for 7 to 10 days of culture.

The morphology of the cells will be observed under the microscope to ensure proper formation of DC. The DC will be cryopreserved in RPMI 1640, 20% autologous serum, and 10% DMSO until the vaccination timepoints. Aliquots will be thawed at days -1, 13, and 27 for the first, second, and third immunizations, respectively. Thawed DC will be washed x2 in sterile saline. Aliquots will be tested for sterility as outlined in Section 6.3.4, and phenotyped by FACScan flow cytometry using anti-CD86 and anti-HLA-DR antibodies.

Blood (120-180 ml) will be drawn prior to leukapheresis for autologous serum for the cell cultures. Leukapheresis will be performed at the UCLA Hemapheresis Unit (200 UCLA Medical Plaza).

To ensure that samples from different patients enrolled concurrently are adequately segregated, samples from different patients will never be processed simultaneously in the same tissue culture hood and will be kept in separate shelves in the incubator.

6.5.2 Preparation of Autologous Tumor Lysate

For isolation of autologous tumor lysate, tumor specimens will be collected from brain tumor surgeries performed at UCLA Medical Center. Tumor tissue from each patient will be taken in sterile condition from the operating room at the time of surgical resection. Remnant tumor tissue not used for clinical pathological diagnoses will either be snap frozen in liquid nitrogen and placed in a sterile specimen container, or immediately minced and put into digestion buffer using fresh tumor tissue (see below). For frozen specimens, the specimen container will then be labeled, sealed, and placed on dry ice. Specimens will be stored in a –80°C freezer directly from the operating room.

The tumor tissue sample will be transferred to a clean room cGMP facility. The tumor tissue will be immediately labeled with the unique code number generated for the patient as described in Section 6.5.1 above, and taken to a sterile tissue culture hood within the UCLA Jonsson Cancer Center cGMP suite or Cognate Bioservices, whereupon it will either be frozen at -80ºC for lysate preparation at a later time or processed immediately.

For the preparation of tumor lysate, the brain tumor tissue will be minced with a sterile scalpel, rinsed with Dulbecco's phosphate buffered saline (dPBS), and incubated with 9.6 ml of a collagenase mixture (50 mg/ml collagenase dissolved in 1.6 ml sterile water + 8 ml DMEM/F12 medium). The homogenate will be sterile filtered through a 190 μm metal sieve into a sterile specimen container to which 1.6 ml of Dornase-α (Dnase I) and 59 ml of DMEM/F12 medium will be added. This will be stirred on an orbital shaker at 37°C for 2.5 hours. The tumor suspension will then be divided equally for either primary tumor cell cultures or immediate generation of fresh tumor lysate. For primary culture, the tumor cell suspension will be aseptically transferred to T25 tissue culture flasks and expanded *in vitro*. Primary tumor cells will be cultured in media consisting of DMEM/F12 (Mediatech, Inc., Herndon, VA) that is supplemented with 10% fetal calf serum (Cambrex Biosciences, Walkersville, MD) and 50 μg/mL gentamicin (American Pharmaceutical Partners, Inc., Schaumberg, IL), as performed in our previous Phase I clinical trial (IND #8434). After limited expansion *in vitro* (<9 passages), tumor cells will be trypsinized, washed 3x in dPBS, and then subjected to the identical freeze/thaw lysis as post-surgical tumor cell suspensions. As such, tumor cell suspensions (primary cell culture or fresh post-surgical cell suspensions) will be pelleted, resuspended in DMEM/F12 medium and transferred to 2 ml cryovials labeled with the patient's UCLA I.D. number. Cryovials containing the tumor suspension are then frozen at -80ºC (in freezer for >1 hr, or on dry ice ≥ 3 min). Repeat thawing and freezing three more times for a

total of 4 freeze/thaw cycles. The resulting suspension of tumor cellular proteins will then be transferred to a sterile tube and centrifuged for 10 minutes at 2000 rpm at 4ºC. The supernatant will be collected, subsequently filtered through a sterile 0.2 μm syringe filter and stored in a sterile 1.5 ml microcentrifuge tubes at –20°C. Aliquots of this tumor lysate will then be tested for sterility and protein concentration.

6.5.3 Co-Culture of Dendritic Cells with Tumor Lysate

One day prior to each immunization, aliquots of the autologous dendritic cells will be thawed. A cell count will be obtained, viability assessed, purity evaluated, and an aliquot removed for sterility testing. The DC will then be centrifuged in preparation for loading with autologous tumor lysate.

DC pellet will be resuspended in 2 ml of tumor lysate in RPMI medium (no serum) and incubated for eighteen (18) to twenty-four (24) hours at 37 ºC and 5% CO_2. Following incubation, the cell suspension will be washed three times to remove excess lysate and the DC (at the appropriate concentration for each cohort) will be resuspended in 0.3 ml of dPBS for injection.

Quality assurance for the tumor lysate-pulsed DC will be tested by culturing 50 μl of the final product for standard bacterial and fungal pathogens prior to and after washing with sterile saline for injection into patients. An aliquot of the DC, prior to being pulsed with autologous tumor lysate, will also be tested by FACScan flow cytometry (Becton Dickinson) using anti-CD86 and anti-HLA-DR. Preparations must contain > 30% of large cells as dendritic cells (e.g., $CD86^{+}HLA\text{-}DR^{+}$) to be acceptable.

6.5.4 Biosafety Testing

6.5.4.1 Autologous Tumor Lysate Testing

The autologous tumor lysate will be prepared and pass all sterility tests:

- *Sterility (Thioglycollate Broth, Tryptic Soy Blood Agar, Inhibitory Sabouraud Agar):* The purpose of the Sterility Test is to determine the presence of one or more species of bacterial and/or fungal contaminants in the test article. This is determined by inoculating a cell suspension sample onto tryptic-soy blood agar (for aerobic bacteria), and onto inhibitory Sabouraud agar (for fungal contaminants). Those test articles that cause growth in any of the media within 14 days of inoculation are not sterile and are reported as contaminated.
  Acceptance Criteria for test article: No turbidity or growth in any media.
  No bacteria seen on Gram stain.

6.5.4.2 Dendritic Cell Testing

Dendritic cells will be cultured in RPMI-1640medium, 10% heated-inactivated autologous serum, 500 units/mL each of recombinant human IL-4 (CellGenix, Freiburg, Germany or MiltenyiBiotec) and recombinant human GM-CSF (Leukine®; Genzyme, Cambridge, MA). Autologous serum will be separated from whole clotted blood, heat inactivated to 56°C for one hour and kept at -

80°C in sterile culture tubes labeled with the patient's name and UCLA identification number. Sterility testing will be performed on the autologous serum by the UCLA Clinical Laboratory prior to use, as outlined in FDA 21 CFR 610.12. Each batch of complete medium (CM) is prepared on the day each DC culture is to be started and sterile filtered (0.22 micron, Nalgene) prior to use. CM is refrigerated during the 7-day APC culture period. After the 7-day culture period, bacterial, fungal, and mycoplasma cultures will be checked in the dendritic cells (DC) before they are cryopreserved.

- *Sterility (Thioglycollate Broth, Tryptic Soy Blood Agar, Inhibitory Sabouraud Agar) and Gram Stain:* Sterility testing as outlined in Section 6.4.4.1 above will be performed by the UCLA Clinical Laboratories.
  Acceptance criteria for test article: No turbidity or growth in any media.
  No bacteria seen on Gram stain.

- *Mycoplasma (RT-PCR and Cultures)*: 100-500 μl of autologous DC cultures (cells and supernatant) will be tested for the presence of mycoplasma using RT-PCR, as outlined in the FDA's "Points to Consider in the Characterization of Cells Lines Used to Produce Biologicals (1993)".
  Acceptance criteria for test article: No mycoplasma seen on RT-PCR

- *Viability:* Prior to cryopreservation, DC will be counted and viability assessed by trypan blue exclusion.
  Acceptance criteria for test article: > 70% viable cells,

6.5.4.3 Tumor Lysate-pulsed Dendritic Cell Testing

After pulsing with autologous tumor lysate, dendritic cells will be washed with sterile saline prior to injection into patients. Prior to and after washing, the tumor lysate-pulsed DC will be tested for sterility and endotoxin levels as outlined below:

- *Sterility (Thioglycollate Broth, Tryptic Soy Blood Agar, Inhibitory Sabouraud Agar) and Gram Stain:* Sterility testing as outlined in Section 6.4.4.1 above will be performed by the UCLA Clinical Laboratories.
  Acceptance criteria for test article: No turbidity or growth in any media.
  No bacteria seen on Gram stain.

  Endotoxin Levels (Limulus Amebocyte Lysate Quantitative Chromogenic Procedure): Endotoxin testing will be performed on the final product at the UCLA GCRC Core Lab or EndoSafe®-PTS™ System located within the UCLA GMP Suites.Ten (10 μg) of autologous tumor lysate-pulsed DC will be tested in the Limulus Amebocyte Lysate assay to quantitatively determine the gram-negative bacterial endotoxin level by the quantitative chromogenic assay. Dilutions of sample (4 log range) are mixed with LAL reagent and incubated for one hour at 37°C. Positive and negative controls, as wells as endotoxin spiked into samples for possible inhibition, will be run. An endpoint chromogenic assay (OCL-1000, Bio-Whittaker) will be used with a sensitivity of 0.01 endotoxin units Eu/ml.
  Acceptance criteria for test article: < 5 Eu/ml sample

- *Viability:* To insure that the integrity of the tumor lysate-pulsed DC is not compromised during the time needed to complete the biosafety testing, an aliquot of the final product will be assessed for viability by trypan blue exclusion just prior to injection into the patient.
  Acceptance criteria for test article: >70% viable cells in final product.

- The tumor lysate-pulsed DC (final product) will not be administered to the patient until the results of the Gram stain, endotoxin testing, and viability count are known and the acceptance criteria satisfied.

6.5.4.4 Action Plan

If a sample is positive on sterility testing after the product has been administered to the patient, the patient will be notified and monitored closely for any signs or symptoms of infection. The patient will be treated with an appropriate course of antibiotics if necessary.

6.6 Method of DC Injections

6.6.1 *Initial Vaccination Cycle*

All patients receive three intradermal (i.d.) injections at biweekly intervals. Patients will receive $2.5x10^6$ glioma tumor lysate-pulsed DC i.d. in 2 syringes, each diluted in 0.15 ml sterile saline, and in opposite arms. Depending on the available sources, tumor lysate can be used from two sources: 1) fresh, tumor-derived cells generated immediately after surgery; or 2) primary tumor cell cultures generated identically as fresh, but kept in culture for <9 passages. The injections will be given by expert medical staff. If enough DC are not generated from the leukapheresis for 3 complete injections, a lower number of pulsed DC may be injected/vaccination or a decreased number of vaccinations may be completed in each patient. DC must still satisfy all of the normal requirements for lot release.

6.6.2 *Additional Vaccination Cycles*

For those patients who do not develop any toxic side effects from the experimental treatment and have stable disease for over three months, booster injections of pulsed dendritic cells may be repeated at follow up visits if tumor material is available and deemed clinically appropriate.

The maximum number of injections will be contingent upon the availability of autologous tumor lysate (200 μg/injection) for each patient. Depending on the available sources, tumor lysate can be used from two sources: 1) fresh, tumor-derived cells generated immediately after surgery; or 2) primary tumor cell cultures generated identically as fresh, but kept in culture for <9 passages.

For patients eligible to undergo additional vaccination cycles, the procedures that may be repeated include the harvesting of tumor, screening visits, leukapheresis, injection of dendritic cells, blood draws, and all subsequent follow-up visits for another 12 months. These procedures will be performed as described above. Subjects are not required to undergo additional vaccination cycles and may withdraw participation at any time. If subjects are asked to participate in additional cycles, they will be re-consented and presented with any new information regarding the clinical trial.

6.6.3 *DC Vaccinations with placebo cream or saline*

For patients that randomize to **Cohort A** (autologous tumor lysate-pulsed DC vaccination + placebo), the following procedures will occur. Patients will receive $2.5x10^6$ glioma tumor lysate-pulsed DC i.d. in 2 syringes, each diluted in 0.15 ml sterile saline, and in opposite arms. Concurrent with DC vaccination, the subjects will be treated with either a placebo cream or intramuscular injection of saline.

6.6.4 *DC Vaccinations with Resiquimod Adjuvant*

For patients that randomize to **Cohort B** (autologous tumor lysate-pulsed DC vaccination + adjuvant 0.2% resiquimod cream), the following procedures will occur. Patients will receive $2.5x10^6$ glioma tumor lysate-pulsed DC i.d. in 2 syringes, each diluted in 0.15 ml sterile saline, and in opposite arms. Concurrent with DC vaccination, the subjects will be treated with the innate immune response modulator, resiquimod. Beginning on the day of DC vaccination (day 1), patients will be treated once with 0.2% resiquimod cream topically over the DC vaccination site. Patients will then be treated with topical resiquimod0.2% cream for an additional two applications on days 3 and 5 post vaccination. Resiquimod0.2% cream will be applied immediately prior to bedtime and left on the skin for approximately 8 hours. Before applying the cream, the treatment area will be washed with mild soap and water and allowed to dry thoroughly. The cream application surface should not exceed approximately 8 cm in diameter or roughly the size of the patient's fist. The cream should be rubbed into the treatment area until the cream is no longer visible. Patients will be instructed to contact the study physicians and their treating physician if they experience any signs or symptoms related to the resiquimod application.

6.6.5 *DC Vaccinations with Poly ICLC (Hiltonol®) Adjuvant*

For patients that randomize to **Cohort C** (autologous tumor lysate-pulsed DC vaccination + adjuvant 20 μg/kg intramuscular poly ICLC), the following procedures will occur. Patients will receive $2.5x10^6$ glioma tumor lysate-pulsed DC i.d. in 2 syringes, each diluted in 0.15 ml sterile saline, and in opposite arms. Concurrent with DC vaccination, the subjects will be treated with the innate immune response modulator, Poly ICLC (*Hiltonol*®, Oncovir, Inc.). Patients will receive *Hiltonol* at a concentration of 20 μg/kg as a one-time intramuscular injection together with the DC vaccination. Patients will be instructed to contact the study physicians and their treating physician if they experience any signs or symptoms related to the *Hiltonol* application.

None of the subjects will receive concurrent treatment with both innate immune response modifiers. Thus, there should not be any additional safety concerns about potential interactions between the two drugs.

All immunization procedures will be overseen by Dr. Linda Liau, who is a licensed physician certified in Advanced Cardiac Life Support (ACLS). A crash cart will be available in the immediate vicinity when performing these immunizations in case severe allergic reactions should occur.

7.0 PATIENT EVALUATIONS

7.1 On-Study Clinical Evaluations

Interval clinical evaluations will take place according to the Study Diagram *(Appendix A).* The following clinical evaluations are done at study visits and/or when clinically indicated, unless otherwise noted.

7.1.1 Objective Signs and Symptoms: Vital signs (blood pressure, pulse, temperature and respirations), will be done.

7.1.2 Physical Examination: Targeted physical exam will be done on Study Days.

7.1.3 Karnofsky Performance Status (*Appendix B*) will be done Study Days.

7.1.4 Serum Chemistries: Comprehensive Metabolic Profile (CMP)

7.1.5 Hematology: Complete blood count (CBC), differential platelets and coagulation tests done at screening visit: including PT (Prothrombin Time) and PTT (Partial Thromboplastin Time).

7.1.6 Urinalysis, pregnancy test, and virology testing completed at screening.

7.1.7 MRI of Brain with and without contrast, per standard of care.

7.1.7 Blood draws for immunological assays.

7.2 Interval Evaluations in the Event of Progressive Disease (PD)

In the event that patients experience progressive disease, regular interval clinical evaluations will be discontinued. Such patients will be contacted quarterly (+/- 1 month) to ascertain their survival status and medical history only. This modified follow-up for patients after PD will continue until death.

7.3 Interval Evaluations in the Event that a lower number of DC are generated

If not enough DC are generated from a patient's leukapheresis to make three complete vaccines ($15x10^6$ cells), then the vaccine schedule may be shortened accordingly. If such a change is deemed necessary, regular interval clinical evaluations will not be performed on scheduled vaccine administration days.

7.4 Immunological Studies

Methods for monitoring the immune responses to treatment will be developed and assessed during this study. The following methods will be evaluated:

7.4.1 Cellular proliferation assays

Immunological testing will be conducted on bodily samples obtained prior to and after DC vaccination. 45 to 75 cc of blood will be drawn from the patient on each test day for these studies. Patients' peripheral blood lymphocytes (PBLs) will be assayed for the specific proliferation in response to autologous tumor lysate using standard ^{3}H-thymidine incorporation. A limiting dilution precursor frequency analysis of proliferating T-cells in response to serum-free media, autologous tumor lysate, or irradiated tumor cells will also be assayed by standard ^{3}H-thymidine incorporation (T-cell proliferation assays). A comparison of pre-therapy lymphocyte functions to those at intervals after each immunization step will be made. These tests may provide evidence for the development of

immune responses following dendritic cell vaccination and may play an important role in the design of future DC-based clinical trials.

7.4.2 Mixed lymphocyte-tumor cell culture

Peripheral blood mononuclear cells (PBMC) collected before and after injection oftumor lysate-pulsed DC will be co-cultured with autologous tumor cells for 5-6 days. ^{3}H-thymidine will be added for the final 18 hours of culture to labeled proliferating cells. After the 18-hour labeling period the cells are harvested onto glass fiber filters and the amount of ^{3}H-thymidine incorporated is determined by liquid scintillation counting. Negative controls will consist of PBMC in the absence of tumor cells and positive controls will utilize allogeneic PBMC as a stimulus. A stimulation index will be calculated as the ratio of counts per minute (cpm) in the presence of tumor cells to the cpm in the absence of tumor cells. A ratio greater than or equal to 3 is considered a positive response to the autologous tumor.

7.4.3 Cell mediated cytotoxicity

A standard ^{51}Chromium-release assay will be utilized. PBMC will be stimulated *in vitro* by a 7-10 day co-culture with either tumor lysate-pulsed DCor with autologous tumor cells prior to conducting the cytotoxicity assay. Autologous tumor cells will be labeled with ^{51}Cr and mixed with *in vitro* stimulated PBMC collected before and after injection. The cells will be incubated for 4 hours at 37ºC, then the culture medium will be collected and the ^{51}Cr released will be measured by gamma counting. Controls will include ^{51}Cr-labeled K562 cells to detect NK activity and labeled DC if available. Significant cytolysis of autologous tumor cells by post-treatment PBMC would indicate effective immunization.

7.4.4 ELISPOT Assays of Cytokine Profiles

The presence of tumor-specific effector T cells in immunized patients will also be assessed by enzyme-linked immunospot (ELISPOT) assays. Briefly, PBMC's will be restimulated*in vitro* by culture with tumor lysate-pulsed DC's (responder-to-stimulator ratio of 25:1) in the presence of 30 IU/ml rhIL-2 for 48 hours. Non-adherent cells (lymphocytes) will then be collected and plated (5 x 10^4 cells/100 μl) in 96-well microtiter plates (Millipore) previously coated with purified IFN-γ, GM-CSF, IL-4 or IL-10 capture antibodies (Pharmingen) and incubated for 24 hours at 37ºC/5% CO_2. The plates will then be washed with PBS and labeled with biotinylated IFN-γ, GM-CSF, IL-4 or IL-10 detection antibodies at 4ºC overnight, followed by incubation with 100 μl/well streptavidin-alkaline phosphatase (Sigma) for 1-2 hours and nitrobluetetrazolium/5-bromo-4-chloro-3-indolylphosphate substrate buffer for 15-45 minutes. The stained spots corresponding with IFN-γ, IL-4, GM-CSF or IL-10-producing cells will be counted under a dissecting microscope. A response will be interpreted as positive when the enumerated cytokine-producing cell spots post-treatment are two times the number of stained spots seen pre-vaccination.

7.4.5 Intracellular Cytokine Flow Cytometry

PBMC will be co-cultured with either irradiated tumor cells or tumor lysate-pulsed DC's for 5-6 days. Non-adherent cells (lymphocytes) will then be collected and restimulated for 5 hours with tumor lysate again in the presence of the protein transport inhibitor, brefeldin-A. Non-adherent PBMC will then be collected,

counted, washed in 2% FBS in dPBS (FACS Buffer), and then stained with optimal concentrations of different monoclonal antibody cocktails (CD3, CD4, CD8) for 30 minutes on ice in the dark. Cells will then be washed twice in FACS buffer and permeabilized by incubation in fixation/permeabilization buffer for 20 minutes on ice. Permeabilized cells are washed and resuspended in perm/wash buffer (BD Pharmingen) and incubated with either anti-human IFN-γ, GM-CSF, IL-4, IL-10 or an isotype-matched antibody diluted 1/50 in perm/wash for 30 min on ice. The cytokine antibodies are all conjugated with FITC. Cells will be washed twice with and resuspended in perm/wash. Four-color flow cytometry will be performed on a FACSCalibur flow cytometer with equivalent gating and voltage (UCLA Jonsson Cancer Center Flow Cytometry Core Facility).

7.4.6 Real-Time PCR Analysis of Cytokine Gene Expression

PBMC will be co-cultured either with irradiated tumor cells or pulsed with tumor lysate for 5-6 days. Non-adherent cells (lymphocytes) will then be collected and restimulated for 2 hours with irradiated tumor cells or tumor lysate. At this time, RNA will be isolated using the RNAquaous 4 PCR kit (Ambion). cDNA will be made from approximately 1-2 μg of DNA of each sample using the Omniscript RT kit (Qiagen). Quantitative assessment of cytokine (IFN-γ, TNF-α, IL-12, GM-CSF) and reference gene (CD8, CD4, β-actin) mRNA expression will be performed with a BioRad I-cycler IQ real time detection system using multiplex quantitative real time PCR. Taqman probes (Operon Technologies), labeled at the 5' end with a reporter dye (FAM, Texas Red) and at the 3' end with a quencher dye (TAMRA or Black-hole quencher), will be used to detect the real-time cleavage of sequence-specific probes from the exonuclease activity of Taq polymerase. Real time monitoring of the fluorescent emission during the exponential phase of mRNA amplification will allow definition of the threshold cycle (cycle at which fluorescence exceeds 10 times the standard deviation above the background). Cycle thresholds for cytokine and reference genes can then be compared. Fold increase of message is calculated using the formula 2^{y-x}, (where x = the PCR threshold cycle for time points other than the zero, and y = threshold cycle for time zero). Plasmid DNA standards containing sequences encoding the genes of interest (e.g., IFN-γ and CD8) will be used to quantify copy numbers of mRNA. Standard curves will be generated for each cytokine and reference genes. Normalization of message will be performed by dividing the copies of the gene of interest by the copy number of the reference gene.

7.4.7 Immunohistochemical Staining of Resected Brain Tumor Tissue

Some patients may experience disease progression following treatment with tumor lysate-pulsed DC. If surgery is indicated, the recurrent tumor will be resected and evaluated by the Division of Neuropathology at UCLA. This evaluation includes immunohistochemical staining of the tissue to detect the presence of infiltrating T cells. Successful immunization with tumor lysate-pulsed DC may result in increased infiltration of the tumor by activated, tumor-specific T cells. Frozen sections of the tumor tissue will be prepared and the sections will be stained with anti-CD3, anti-CD4 and anti-CD8 antibodies to detect T cells and T-cell subsets. Anti-CD45RA will be used to detect naïve, non-activated T cells and anti CD45RO antibody will be used to detect activated T cells. The binding of these antibodies is detected using peroxidase conjugated goat anti-mouse IgG antibodies. A peroxidase substrate is added which results in an insoluble colored

reaction product where antibody has bound. The extent and quality of the lymphocytic infiltrate will be compared with sections taken from initial surgical resection.

7.4.8 Feasibility

All of the above assays are dependent on the availability of autologous tumor cells or tumor antigen lysate in excess of that required for treatment. Excess material will not be available for all patients and it may not be available for any patients. Assays will therefore be limited based on the amount of material available.

8.0 POTENTIAL RISKS AND BENEFITS

8.1 Potential Risks

The potential risks/discomforts associated with each research procedure include the following:

ALLERGIC REACTIONS TO VACCINE. Injection of tumor lysate-pulsed dendritic cells could result in an allergic reaction, which could include redness and swelling at the injection, itching, hives, low blood pressure, difficulty breathing, or in the most extreme situation, death. In addition, if the immune system becomes overly activated, potential discomforts may include pain, redness and swelling at the injection site over the course of several days.

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

INFECTION. The DC injections may include the risk of infection due to potential contamination of the dendritic cells in the laboratory. This may result in localized redness, swelling, or induration at the injection site. In the most extreme situation, this may lead to systemic bacterial/fungal sepsis and possibly death. The probability of this risk is relatively low, given the small injection volume (1 ml intradermally) and the fact that the dendritic cells will be strictly tested for sterility prior to each injection.

DELAYED AUTOIMMUNE DISEASES. It is possible that delayed autoimmune disease(s) may develop as a result of injection with tumor lysate-pulsed DC. This means that the immune system may be stimulated to attack natural tissues in the body. It is unknown what the risk of delayed autoimmune disease is for this study.

RISKS OF PHLEBOTOMY. Drawing blood or inserting an intravenous catheter into an arm vein may result in bruising or swelling in the area of the insertion, bleeding at the

site of the needle puncture, light headedness, fainting and very rarely, local infection, which may be severe.

RISKS OF LEUKAPHERESIS. As in any donation of blood, a variety of minor reactions may occur with leukapheresis, which include fainting, dizziness, or nausea. Uncommon but serious complications may also result, which include bleeding, infection, an adverse reaction to the anticoagulant or replacement fluids, hypocalcemia, hypotension, shock, convulsions, air emboli, heart failure, or the inability to transfuse blood back into the patient.

RISKS OF MRI. The risks and/or discomforts associated with the MRI scans include anxiety from being in a tight, enclosed space (claustrophobia). In addition, the machine operates using a large and very powerful magnet. The magnetism or the machine attracts certain metals; therefore, people with these metals in them (specifically pacemakers, infusion pumps, metal aneurysm clips, metal prostheses, joints, rods or plates) will be excluded from the study.

ALLERGIC REACTIONS TO CONTRAST DYE. During the MRI, patients will be given contrast dye. This dye is given routinely to patients who get MRI scans of the brain. The dye is given by the vein and requires the placement of a catheter. The catheter placement is similar to drawing blood except that the catheter is left in the vein during the time the dye is delivered. The risks of a blood draw and insertion of a catheter are similar. There have been a few, rare cases of allergies to the dye used in the MRI.

RESIQUIMOD. The most common adverse effects associated with 0.2% resiquimod cream administration include the following: 1) injection site reactions (itching, burning, localized and temporary pain at target site, erythema at injection site); 2) headache; 3) low-grade fever; 4) flu-like symptoms.

Poly ICLC (*Hiltonol*®). The risks and/or discomforts associated with this dose of poly ICLC include the following: 1) Discomfort at injection site; 2) flu-like symptoms; 3) transient leucopenia; 4) mild and transient hepatic enzyme elevations; 4) seizure; 5) transient peritumoral edema.

8.2 Potential Benefits

Based on experience with this vaccination procedure in animals (e.g., rats and mice), in patients with other cancers (e.g., melanoma), and in our previous Phase I trials using autologous tumor lysate-pulsed antigen-presenting cells, DC-based immunotherapy may be of benefit to subjects with malignant brain tumors. Of course, because individuals respond differently to therapy, no one can know in advance if it will be helpful in a particular case. The potential benefits may include reduction and/or remission of the subject's brain cancer. Because this procedure is experimental, it cannot be guaranteed that subjects will receive any benefit as a result of participating in this research.

The information collected in this research may help scientists better understand the mechanisms involved in the immune system’s ability to fight cancer. If this understanding comes from this research, it may benefit society by leading to the development of improved treatment methods for human brain cancers in the future.

9.0 PATIENT DISCONTINUATION

Participation in this study can be discontinued for any of the following reasons listed below. An explanation will be recorded for each patient taken off treatment and the appropriate Case Report Form (CRF) is completed.

9.1 At the Patient's request

9.2 A major, Grade 4, unexpected or life-threatening toxicity requiring that treatment be discontinued

9.3 Development of clinical signs and symptoms of autoimmune disease

9.4 Generalized impairment or mental incompetence which would relinquish the patient unable to understand his/her participation in the study

9.5 If, in the Investigator's medical judgment, further participation would be injurious to the subject's health or well-being

9.6 Administrative reasons, such as subject noncompliance or a major protocol violation

9.7 Pregnancy

9.8 Injection site reactions > Grade 2 based on the modified grading *(Appendix E)*

10.0 ADVERSE EVENTS REPORTING

10.1 Adverse Event Recording

An adverse event (AE) is defined as development of any new change in signs or symptoms or abnormal lab results, and may include a single symptom or sign, a set of related symptoms or signs, or a disease while receiving the study drug. All AEs must be recorded even if a causal relationship to the study drug is unlikely.

Patients are instructed to report any AE to the investigator. On each day of evaluation, the patient is questioned in a general way regarding any new medical problems and new or changed medications. All AEs are documented in the source document and on the AE Form (located in the CRF).

The intensity of all AEs not localized to the injection site of the study drug is graded according to the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) (see *Appendix C*). AEs that are considered by the investigator to be localized or related to the injection site shall be graded according to *Appendix E*, Modified Grading of Injection Site Reaction Toxicity.

The relationship of an AE to study treatment is characterized as "not related", "possibly related", or "probably related" and is determined by the principal investigator according to the following guidelines:

- **Probably related:** a direct cause and effect relationship between the study treatment and the AE is likely;

- **Possibly related:** a cause and effect relationship between the study treatment and the AE has not been demonstrated at this time and is not probable, but is also not impossible;

- **Not related:** without question, the AE is definitely not associated with the study treatment (comment on other etiology can be made in the Comments section of the CRF).

10.2 Serious Adverse Event Reporting

10.2.1 A serious adverse event (SAE) is defined as one of the following:

- Death;
- An event that is life threatening. In the opinion of the principal investigator, the patient was at immediate risk of death due to the event as it occurred;
- An event that results in persistent or significant disability/incapacity;
- An event that requires inpatient hospitalization or prolongs hospitalization;
- An important medical event that, based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

10.2.2 The principal investigator must report any SAE to the IND at the FDA, the Jonsson Comprehensive Cancer Center (JCCC) Internal Scientific Peer Review Committee (ISPRC), and the UCLA IRB within 24 hours of the event.

10.2.3 The principal investigator will take the responsibility for reporting all AEs and any SAE to the FDA as described in *21 CFR §312.32 (IND Safety Reports)*.

10.3 Data and Safety Monitoring Plan

The JCCC Data and Safety Monitoring Board (DSMB) meets monthly to review all serious adverse event reports for trials overseen by the JCCC DSMB. All serious adverse event (SAE) reports, which have been filed since the previous meeting, are presented to the committee for review.

For all trials where the JCCC DSMB has primary oversight for SAE review, all SAEs occurring within these studies shall be reported to the JCCC DSMB in a timely manner consistent with the UCLA IRB time requirements [ten days, two days for a death] regardless of relationship, expectedness or severity. The JCCC Office of Regulatory Compliance (ORC) will review all submissions and the ORC staff will enter the information into the JCCC Clinical Trials database. Reports are generated for full JCCC DSMB review of those SAEs that have some component of relatedness to the study drug and may, at the discretion of the JCCC compliance officer, include SAE reports that may require DSMB review. For trials where the JCCC DSMB has primary DSMB review responsibility, the DSMB will request that the PI generate cumulative adverse event reports for quarterly, biannual or annual review.

The DSMB reviews each SAE report and determines whether or not protocol modifications are warranted to ensure subject safety. In this review, prior occurrences of similar toxicity with the therapy under study are taken into consideration, as well as the servity of the event and the likelihood that it was related to a study drug. The DSMB may recommend no changes to the study if the event is expected or related to other causes such as the subject's underlying condition. The DSMB may request an expert's advice of another non-Principal Investigator with national experience to support their deliberations and decisions. The JCCC DSMB has the authority to recommend to the UCLA IRB the immediate halt to the study (i.e., discontinuation of any further treatment of enrolled subjects and discontinuation of enrollment of new subjects) should there be any serious unexpected toxicity that warrants further investigation. JCCC DSMB correspondences are addressed to the Principal Investigator and copied to the UCLA IRB. Minutes of the DSMB meetings are recorded and processed into the computer file.

Monitoring, Auditing and Reporting

The NIH and NCI policy statements allow for variable monitoring and reporting plans, commensurate with the potential risks and with the size and complexity of the trial. The monitoring plan must be sufficiently rigorous and effective to ensure subject safely and to ensure protocol compliance and data validity and integrity.

Level of Risk of a Study

All interventional clinical trials undergo scientific review by the Internal Scientific Peer Review Committee (ISPRC) which requires that a Data and Safety Monitoring Plan is in place before a trial can be approved to begin. For trials overseen by the JCCC DSMB, the JCCC DSMB will determine the degree of risk of the study and will ensure that there are procedures in place to ensure the safety of the subjects that are enrolled in the trial. The intensity level of the monitoring is determined by the risk category. Some of the factors that must be considered when assigning the Level of Risk category include:

1. A biostatistical design and appropriate procedures for proper data management so that the information collected can be properly validated.

2. Appropriate Serious Adverse Event reporting procedures must be in place.

3. The study duration must be appropriate and must be based on a realistic rate of enrollment.

4. Data collection and data management must be adequate to verify and ensure subject eligibility.

Assignment of risk

Assigning risk ensures that the data and safety monitoring is based on the level of risk (low, medium, or high) to ensure that the data and safety monitoring activities are appropriate. Below are some of the criteria used to make a decision regarding the assignment of risk:

- Expected duration of the study based upon the estimated rate of enrollment.

- Type of study population (e.g., children, geriatric)

- The procedures used in the trial are commensurate with the degree of risk.

- Adequate data management systems in place and appropriate case report forms
- Proper serious adverse event reporting procedures in place
- Proper biostatistical design and data analysis procedures in place.

Level of Risk

This particular trial will be assigned a Level 1 risk category.

Level 1

Example of type of trial:

Investigator held IND of high complexity or of high toxicity potential. Examples are gene medicine, dendritic cell products from GMP suite or an experimental agent still in phase I/II development. Typically these clinical trials involve the first use of the drugs in humans, so only the investigator is knowledgeable about the test article.

1. The Compliance Officer meets with PI/Staff prior to study initiation and reviews regulatory requirements and operating system. Compliance Officer provides real time monitoring to determine eligibility prior to enrollment onto the protocol.
2. Real time QA monitoring of the subjects and data collection occurs for all subjects entered onto the trial.
3. Frequency of DSMB Summary Report is typically on a quarterly basis.
4. Comprehensive QA auditing within first year or first 10 subjects enrolled, whichever comes first. Subsequent audit frequency will be annually.

Monitoring and Auditing Activities

The compliance officer of the JCCC Office of Regulatory Compliance [ORC] will monitor the clinical records for all human subjects enrolled onto JCCC trials overseen by the JCCC DSMB. The JCCC compliance officer will perform real time review of informed consent processes and the meeting of all inclusion and exclusion criteria and screening results at study entry. Active monitoring will offer the JCCC study teams prospective information that can be used to enhance the quality of research being performed contemporaneously. Auditing is a review of historic performance of the research effort and is performed on case report forms, regulatory files and source documents to measure the quality of the research effort in a retrospective manner.

For institutional studies where there is no support for external monitoring; the DSMB serves as the primary data and safety management entity.

Protocol oversight, monitoring activities, and auditing activities of JCCC can be visualized in the tables found in Appendices A and B which describe the areas of monitoring and auditing for JCCC research sites, including the TORI network sites.

11.0 CRITERIA FOR ENDPOINT EVALUATIONS

11.1 Safety

Toxicity will be monitored and graded according to NCI Common Toxicity Criteria *(Appendix C*). The overall incidence of adverse events will be calculated.

11.2 Response Criteria

11.2.1 COMPLETE RESPONSE (CR): Complete disappearance of all measurable and evaluable disease. No new lesions. No evidence of non-evaluable disease. All measurable, evaluable and non-evaluable lesions and sites must be assessed using the same techniques as baseline. Responders must be on the same or decreasing doses of dexamethasone and have stable or improved neurological exams.

11.2.2 PARTIAL RESPONSE (PR): Greater than or equal to 50% decrease under baseline in the sum of products of perpendicular diameters of all measurable lesions. No progression of evaluable disease. No new lesions. All measurable and evaluable lesions and sites must be assessed using the same techniques as baseline. Responders must be on the same or decreasing doses of dexamethasone and have stable or improved neurological exams.

11.2.3 STABLE / NO RESPONSE: Does not qualify for CR, PR, or Progression. The designation of Stable/No Response requires a minimum of 12 weeks duration. All measurable and evaluable sites must be assessed using the same techniques as baseline. Responders must be on the same or decreasing doses of dexamethasone and have stable or improved neurological exams.

11.2.4 PROGRESSION: 25% increase in the sum of products of all measurable lesions over smallest sum observed (over baseline if no decrease) using the same techniques as baseline, OR clear worsening of any evaluable disease, OR appearance of any new lesion/site, OR failure to return for evaluation due to death or deteriorating condition (unless clearly unrelated to this cancer).

11.2.5 UNKNOWN: Progression has not been documented and one or more measurable or evaluable sites have not been assessed.

11.2.6 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. Only one assessment of response is necessary to call a response either a complete or partial response; however, two assessments at least 12 weeks apart are required for a description of stable disease. The response evaluation for this study will be assessed every 6-8 weeks. As an example, if a patient achieves a complete response at week 6 but shows evidence of progression at week 12, the best response will still remain a complete response. This holds true for partial response, as well. 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.

11.3 Neurological Exam

Although not used for determining response, it is useful to evaluate improvement in the neurologic exam, which should coincide with objective measurement of tumor size.

11.3.1 Normal exam

11.3.2 Improvement from on-study state (patient better)

11.3.3 Same as on-study state (patient same)

11.3.4 Worse than on-study state (patient worse)

11.4 Performance Status

Patients will be graded according to Karnofsky Performance Status (*Appendix B*)

11.5 Time To Tumor Progression (TTP)

From date of surgery to date of first observation of progressive disease measured on MRI (as defined in Section 11.2).

11.6 Survival Time

From date of surgery to date of death due to any cause.

12.0 STATISTICAL CONSIDERATIONS

12.1 Statistical Considerations for Clinical, Imaging, and Immunological Evaluation Endpoints

12.1.1 CLINICAL & IMAGING MEASURES: Although we will be monitoring patients for response, time to tumor progression (TTP), and survival, these measures for the whole group will not be statistically valid, as this experimental therapy will be given as adjuvant treatment in some and in the setting of recurrence in others. Therefore, this data will only be exploratory in nature.

12.1.2 IMMUNOLOGIC MEASURES: This study will not be powered to statistically measure these endpoints. The immunologic measures are secondary objectives of this study; and, therefore, such data will be considered exploratory in nature.

13.0 References

1. Chakrabarti I, Cockburn M, Cozen W, Wang YP, Preston-Martin S. A population-based description of glioblastoma multiforme in Los Angeles County, 1974-1999. Cancer. 2005;104:2798-806.
2. Deorah S, Lynch CF, Sibenaller ZA, Ryken TC. Trends in brain cancer incidence and survival in the United States: Surveillance, Epidemiology, and End Results Program, 1973 to 2001. Neurosurg Focus. 2006;20:E1.
3. Morton DL, Hoon DS, Nizze JA, Foshag LJ, Famatiga E, Wanek LA, et al. Polyvalent melanoma vaccine improves survival of patients with metastatic melanoma. Ann N Y Acad Sci. 1993;690:120-34.
4. Laheru DA, Pardoll DM, Jaffee EM. Genes to vaccines for immunotherapy: how the molecular biology revolution has influenced cancer immunology. Mol Cancer Ther. 2005;4:1645-52.
5. Bernhard H, Disis ML, Heimfeld S, Hand S, Gralow JR, Cheever MA. Generation of immunostimulatory dendritic cells from human CD34+ hematopoietic progenitor cells of the bone marrow and peripheral blood. Cancer Res. 1995;55:1099-104.
6. Hsu FJ, Benike C, Fagnoni F, Liles TM, Czerwinski D, Taidi B, et al. Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. Nat Med. 1996;2:52-8.
7. Mukherji B, Chakraborty NG, Yamasaki S, Okino T, Yamase H, Sporn JR, et al. Induction of antigen-specific cytolytic T cells in situ in human melanoma by immunization with synthetic peptide-pulsed autologous antigen presenting cells. Proc Natl Acad Sci U S A. 1995;92:8078-82.
8. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature. 2007;449:419-26.
9. Jondal M, Schirmbeck R, Reimann J. MHC class I-restricted CTL responses to exogenous antigens. Immunity. 1996;5:295-302.
10. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science. 1991;254:1643-7.
11. Gaugler B, Van den Eynde B, van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, et al. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J Exp Med. 1994;179:921-30.
12. Boel P, Wildmann C, Sensi ML, Brasseur R, Renauld JC, Coulie P, et al. BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity. 1995;2:167-75.
13. Ressing ME, Sette A, Brandt RM, Ruppert J, Wentworth PA, Hartman M, et al. Human CTL epitopes encoded by human papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201-binding peptides. J Immunol. 1995;154:5934-43.
14. Ressing ME, van Driel WJ, Celis E, Sette A, Brandt MP, Hartman M, et al. Occasional memory cytotoxic T-cell responses of patients with human papillomavirus type 16-positive cervical lesions against a human leukocyte antigen-A *0201-restricted E7-encoded epitope. Cancer Res. 1996;56:582-8.
15. Chen BP, DeMars R, Sondel PM. Presentation of soluble antigen to human T cells by products of multiple HLA-linked loci: analysis of antigen presentation by a panel of cloned, autologous, HLA-mutant Epstein-Barr virus-transformed lymphoblastoid cell lines. Hum Immunol. 1987;18:75-91.

16. Murphy GP, Barren RJ, Erickson SJ, Bowes VA, Wolfert RL, Bartsch G, et al. Evaluation and comparison of two new prostate carcinoma markers. Free-prostate specific antigen and prostate specific membrane antigen. Cancer. 1996;78:809-18.
17. Murphy GP, Tino WT, Holmes EH, Boynton AL, Erickson SJ, Bowes VA, et al. Measurement of prostate-specific membrane antigen in the serum with a new antibody. Prostate. 1996;28:266-71.
18. Bakker AB, Schreurs MW, de Boer AJ, Kawakami Y, Rosenberg SA, Adema GJ, et al. Melanocyte lineage-specific antigen gp100 is recognized by melanoma-derived tumor-infiltrating lymphocytes. J Exp Med. 1994;179:1005-9.
19. Coulie PG, Brichard V, Van Pel A, Wolfel T, Schneider J, Traversari C, et al. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med. 1994;180:35-42.
20. Cox AL, Skipper J, Chen Y, Henderson RA, Darrow TL, Shabanowitz J, et al. Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science. 1994;264:716-9.
21. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Rivoltini L, Topalian SL, et al. Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci U S A. 1994;91:3515-9.
22. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Sakaguchi K, Appella E, et al. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. Proc Natl Acad Sci U S A. 1994;91:6458-62.
23. Kawakami Y, Eliyahu S, Sakaguchi K, Robbins PF, Rivoltini L, Yannelli JR, et al. Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2-restricted tumor infiltrating lymphocytes. J Exp Med. 1994;180:347-52.
24. Topalian SL, Rivoltini L, Mancini M, Markus NR, Robbins PF, Kawakami Y, et al. Human CD4+ T cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. Proc Natl Acad Sci U S A. 1994;91:9461-5.
25. Kawakami Y, Eliyahu S, Jennings C, Sakaguchi K, Kang X, Southwood S, et al. Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. J Immunol. 1995;154:3961-8.
26. Van den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T. A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. J Exp Med. 1995;182:689-98.
27. Parkhurst MR, Salgaller ML, Southwood S, Robbins PF, Sette A, Rosenberg SA, et al. Improved induction of melanoma-reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A*0201-binding residues. J Immunol. 1996;157:2539-48.
28. Wang RF, Appella E, Kawakami Y, Kang X, Rosenberg SA. Identification of TRP-2 as a human tumor antigen recognized by cytotoxic T lymphocytes. J Exp Med. 1996;184:2207-16.
29. Cheever MA, Chen W, Disis ML, Takahashi M, Peace DJ. T-cell immunity to oncogenic proteins including mutated ras and chimeric bcr-abl. Ann N Y Acad Sci. 1993;690:101-12.
30. Peace DJ, Smith JW, Chen W, You SG, Cosand WL, Blake J, et al. Lysis of ras oncogene-transformed cells by specific cytotoxic T lymphocytes elicited by primary in vitro immunization with mutated ras peptide. J Exp Med. 1994;179:473-9.
31. Van Elsas A, Nijman HW, Van der Minne CE, Mourer JS, Kast WM, Melief CJ, et al. Induction and characterization of cytotoxic T-lymphocytes recognizing a mutated p21ras peptide presented by HLA-A*0201. Int J Cancer. 1995;61:389-96.
32. Yanuck M, Carbone DP, Pendleton CD, Tsukui T, Winter SF, Minna JD, et al. A mutant p53 tumor suppressor protein is a target for peptide-induced CD8+ cytotoxic T-cells. Cancer Res. 1993;53:3257-61.

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

33. Ciernik IF, Berzofsky JA, Carbone DP. Human lung cancer cells endogenously expressing mutant p53 process and present the mutant epitope and are lysed by mutant-specific cytotoxic T lymphocytes. Clin Cancer Res. 1996;2:877-82.
34. Chakraborty NG, Sporn JR, Tortora AF, Kurtzman SH, Yamase H, Ergin MT, et al. Immunization with a tumor-cell-lysate-loaded autologous-antigen-presenting-cell-based vaccine in melanoma. Cancer Immunol Immunother. 1998;47:58-64.
35. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med. 1998;4:328-32.
36. Liau LM, Black KL, Prins RM, Sykes SN, DiPatre PL, Cloughesy TF, et al. Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. J Neurosurg. 1999;90:1115-24.
37. Mackensen A, Krause T, Blum U, Uhrmeister P, Mertelsmann R, Lindemann A. Homing of intravenously and intralymphatically injected human dendritic cells generated in vitro from CD34+ hematopoietic progenitor cells. Cancer Immunol Immunother. 1999;48:118-22.
38. Morse MA, Coleman RE, Akabani G, Niehaus N, Coleman D, Lyerly HK. Migration of human dendritic cells after injection in patients with metastatic malignancies. Cancer Res. 1999;59:56-8.
39. Morse MA, Deng Y, Coleman D, Hull S, Kitrell-Fisher E, Nair S, et al. A Phase I study of active immunotherapy with carcinoembryonic antigen peptide (CAP-1)-pulsed, autologous human cultured dendritic cells in patients with metastatic malignancies expressing carcinoembryonic antigen. Clin Cancer Res. 1999;5:1331-8.
40. Murphy GP, Tjoa BA, Simmons SJ, Jarisch J, Bowes VA, Ragde H, et al. Infusion of dendritic cells pulsed with HLA-A2-specific prostate-specific membrane antigen peptides: a phase II prostate cancer vaccine trial involving patients with hormone-refractory metastatic disease. Prostate. 1999;38:73-8.
41. Schott M, Feldkamp J, Schattenberg D, Seissler J, Scherbaum WA. Dendritic cell immuno-therapy in disseminated parathyroid carcinoma. Lancet. 1999;353:1188-9.
42. Thurner B, Haendle I, Roder C, Dieckmann D, Keikavoussi P, Jonuleit H, et al. Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J Exp Med. 1999;190:1669-78.
43. Thurner B, Roder C, Dieckmann D, Heuer M, Kruse M, Glaser A, et al. Generation of large numbers of fully mature and stable dendritic cells from leukapheresis products for clinical application. J Immunol Methods. 1999;223:1-15.
44. Tjoa BA, Simmons SJ, Elgamal A, Rogers M, Ragde H, Kenny GM, et al. Follow-up evaluation of a phase II prostate cancer vaccine trial. Prostate. 1999;40:125-9.
45. Mackensen A, Herbst B, Chen JL, Kohler G, Noppen C, Herr W, et al. Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells. Int J Cancer. 2000;86:385-92.
46. Muderspach L, Wilczynski S, Roman L, Bade L, Felix J, Small LA, et al. A phase I trial of a human papillomavirus (HPV) peptide vaccine for women with high-grade cervical and vulvar intraepithelial neoplasia who are HPV 16 positive. Clin Cancer Res. 2000;6:3406-16.
47. Panelli MC, Wunderlich J, Jeffries J, Wang E, Mixon A, Rosenberg SA, et al. Phase 1 study in patients with metastatic melanoma of immunization with dendritic cells presenting epitopes derived from the melanoma-associated antigens MART-1 and gp100. J Immunother. 2000;23:487-98.
48. Geiger JD, Hutchinson RJ, Hohenkirk LF, McKenna EA, Yanik GA, Levine JE, et al. Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression. Cancer Res. 2001;61:8513-9.

49. Lau R, Wang F, Jeffery G, Marty V, Kuniyoshi J, Bade E, et al. Phase I trial of intravenous peptide-pulsed dendritic cells in patients with metastatic melanoma. J Immunother. 2001;24:66-78.
50. Shimizu K, Thomas EK, Giedlin M, Mule JJ. Enhancement of tumor lysate- and peptide-pulsed dendritic cell-based vaccines by the addition of foreign helper protein. Cancer Res. 2001;61:2618-24.
51. Thomas R, Padmanabha J, Chambers M, McFadyen S, Walpole E, Nielssen G, et al. Metastatic lesions in the joint associated with acute inflammatory arthritis after dendritic cell immunotherapy for metastatic melanoma. Melanoma Res. 2001;11:167-73.
52. Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, et al. Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. Cancer Res. 2001;61:842-7.
53. Asavaroengchai W, Kotera Y, Mule JJ. Tumor lysate-pulsed dendritic cells can elicit an effective antitumor immune response during early lymphoid recovery. Proc Natl Acad Sci U S A. 2002;99:931-6.
54. Chang AE, Redman BG, Whitfield JR, Nickoloff BJ, Braun TM, Lee PP, et al. A phase I trial of tumor lysate-pulsed dendritic cells in the treatment of advanced cancer. Clin Cancer Res. 2002;8:1021-32.
55. Holtl L, Zelle-Rieser C, Gander H, Papesh C, Ramoner R, Bartsch G, et al. Immunotherapy of metastatic renal cell carcinoma with tumor lysate-pulsed autologous dendritic cells. Clin Cancer Res. 2002;8:3369-76.
56. Butterfield LH, Ribas A, Dissette VB, Amarnani SN, Vu HT, Oseguera D, et al. Determinant spreading associated with clinical response in dendritic cell-based immunotherapy for malignant melanoma. Clin Cancer Res. 2003;9:998-1008.
57. Morse MA, Clay TM, Colling K, Hobeika A, Grabstein K, Cheever MA, et al. HER2 dendritic cell vaccines. Clin Breast Cancer. 2003;3 Suppl 4:S164-72.
58. Prins RM, Odesa SK, Liau LM. Immunotherapeutic targeting of shared melanoma-associated antigens in a murine glioma model. Cancer Res. 2003;63:8487-91.
59. Pullarkat V, Lee PP, Scotland R, Rubio V, Groshen S, Gee C, et al. A phase I trial of SD-9427 (progenipoietin) with a multipeptide vaccine for resected metastatic melanoma. Clin Cancer Res. 2003;9:1301-12.
60. Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ. Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. Cancer Res. 2004;64:4973-9.
61. Holtl L, Ramoner R, Zelle-Rieser C, Gander H, Putz T, Papesh C, et al. Allogeneic dendritic cell vaccination against metastatic renal cell carcinoma with or without cyclophosphamide. Cancer Immunol Immunother. 2005;54:663-70.
62. Liau LM, Prins RM, Kiertscher SM, Odesa SK, Kremen TJ, Giovannone AJ, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clin Cancer Res. 2005;11:5515-25.
63. Ribas A, Camacho LH, Lopez-Berestein G, Pavlov D, Bulanhagui CA, Millham R, et al. Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206. J Clin Oncol. 2005;23:8968-77.
64. Schadendorf D, Ugurel S, Schuler-Thurner B, Nestle FO, Enk A, Brocker EB, et al. Dacarbazine (DTIC) versus vaccination with autologous peptide-pulsed dendritic cells (DC) in first-line treatment of patients with metastatic melanoma: a randomized phase III trial of the DC study group of the DeCOG. Ann Oncol. 2006;17:563-70.
65. Carson MJ, Sutcliffe JG. Balancing function vs. self defense: the CNS as an active regulator of immune responses. J Neurosci Res. 1999;55:1-8.

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

66. Greenwood J, Etienne-Manneville S, Adamson P, Couraud PO. Lymphocyte migration into the central nervous system: implication of ICAM-1 signalling at the blood-brain barrier. Vascul Pharmacol. 2002;38:315-22.
67. Couraud PO. Infiltration of inflammatory cells through brain endothelium. Pathol Biol (Paris). 1998;46:176-80.
68. Prins RM, Liau LM. Immunology and immunotherapy in neurosurgical disease. Neurosurgery. 2003;53:144-52; discussion 52-3.
69. Eguchi J, Hiroishi K, Ishii S, Baba T, Matsumura T, Hiraide A, et al. Interleukin-4 gene transduced tumor cells promote a potent tumor-specific Th1-type response in cooperation with interferon-alpha transduction. Gene Ther. 2005;12:733-41.
70. Eguchi J, Kuwashima N, Hatano M, Nishimura F, Dusak JE, Storkus WJ, et al. IL-4-transfected tumor cell vaccines activate tumor-infiltrating dendritic cells and promote type-1 immunity. J Immunol. 2005;174:7194-201.
71. Ehtesham M, Samoto K, Kabos P, Acosta FL, Gutierrez MA, Black KL, et al. Treatment of intracranial glioma with in situ interferon-gamma and tumor necrosis factor-alpha gene transfer. Cancer Gene Ther. 2002;9:925-34.
72. Fecci PE, Ochiai H, Mitchell DA, Grossi PM, Sweeney AE, Archer GE, et al. Systemic CTLA-4 blockade ameliorates glioma-induced changes to the CD4+ T cell compartment without affecting regulatory T-cell function. Clin Cancer Res. 2007;13:2158-67.
73. Fecci PE, Sweeney AE, Grossi PM, Nair SK, Learn CA, Mitchell DA, et al. Systemic anti-CD25 monoclonal antibody administration safely enhances immunity in murine glioma without eliminating regulatory T cells. Clin Cancer Res. 2006;12:4294-305.
74. Giantonio BJ, Hochster H, Blum R, Wiernik PH, Hudes GR, Kirkwood J, et al. Toxicity and response evaluation of the interferon inducer poly ICLC administered at low dose in advanced renal carcinoma and relapsed or refractory lymphoma: a report of two clinical trials of the Eastern Cooperative Oncology Group. Invest New Drugs. 2001;19:89-92.
75. Graf MR, Prins RM, Hawkins WT, Merchant RE. Irradiated tumor cell vaccine for treatment of an established glioma. I. Successful treatment with combined radiotherapy and cellular vaccination. Cancer Immunol Immunother. 2002;51:179-89.
76. Graf MR, Prins RM, Merchant RE. IL-6 secretion by a rat T9 glioma clone induces a neutrophil-dependent antitumor response with resultant cellular, antiglioma immunity. J Immunol. 2001;166:121-9.
77. Graf MR, Prins RM, Poulsen GA, Merchant RE. Contrasting effects of interleukin-2 secretion by rat glioma cells contingent upon anatomical location: accelerated tumorigenesis in the central nervous system and complete rejection in the periphery. J Neuroimmunol. 2003;140:49-60.
78. Heimberger AB, Archer GE, Crotty LE, McLendon RE, Friedman AH, Friedman HS, et al. Dendritic cells pulsed with a tumor-specific peptide induce long-lasting immunity and are effective against murine intracerebral melanoma. Neurosurgery. 2002;50:158-64; discussion 64-6.
79. Heimberger AB, Crotty LE, Archer GE, Hess KR, Wikstrand CJ, Friedman AH, et al. Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors. Clin Cancer Res. 2003;9:4247-54.
80. Heimberger AB, Crotty LE, Archer GE, McLendon RE, Friedman A, Dranoff G, et al. Bone marrow-derived dendritic cells pulsed with tumor homogenate induce immunity against syngeneic intracerebral glioma. J Neuroimmunol. 2000;103:16-25.
81. Kahlon KS, Brown C, Cooper LJ, Raubitschek A, Forman SJ, Jensen MC. Specific recognition and killing of glioblastoma multiforme by interleukin 13-zetakine redirected cytolytic T cells. Cancer Res. 2004;64:9160-6.

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

82. Kjaergaard J, Wang LX, Kuriyama H, Shu S, Plautz GE. Active immunotherapy for advanced intracranial murine tumors by using dendritic cell-tumor cell fusion vaccines. J Neurosurg. 2005;103:156-64.
83. Nishimura F, Dusak JE, Eguchi J, Zhu X, Gambotto A, Storkus WJ, et al. Adoptive transfer of type 1 CTL mediates effective anti-central nervous system tumor response: critical roles of IFN-inducible protein-10. Cancer Res. 2006;66:4478-87.
84. Okada H, Attanucci J, Giezeman-Smits KM, Brissette-Storkus C, Fellows WK, Gambotto A, et al. Immunization with an antigen identified by cytokine tumor vaccine-assisted SEREX (CAS) suppressed growth of the rat 9L glioma in vivo. Cancer Res. 2001;61:2625-31.
85. Okada H, Tsugawa T, Sato H, Kuwashima N, Gambotto A, Okada K, et al. Delivery of interferon-alpha transfected dendritic cells into central nervous system tumors enhances the antitumor efficacy of peripheral peptide-based vaccines. Cancer Res. 2004;64:5830-8.
86. Prins RM, Bruhn KW, Craft N, Lin JW, Kim CH, Odesa SK, et al. Central nervous system tumor immunity generated by a recombinant listeria monocytogenes vaccine targeting tyrosinase related protein-2 and real-time imaging of intracranial tumor burden. Neurosurgery. 2006;58:169-78; discussion -78.
87. Prins RM, Craft N, Bruhn KW, Khan-Farooqi H, Koya RC, Stripecke R, et al. The TLR-7 agonist, imiquimod, enhances dendritic cell survival and promotes tumor antigen-specific T cell priming: relation to central nervous system antitumor immunity. J Immunol. 2006;176:157-64.
88. Tatsumi T, Huang J, Gooding WE, Gambotto A, Robbins PD, Vujanovic NL, et al. Intratumoral delivery of dendritic cells engineered to secrete both interleukin (IL)-12 and IL-18 effectively treats local and distant disease in association with broadly reactive Tc1-type immunity. Cancer Res. 2003;63:6378-86.
89. Wang LX, Chen BG, Plautz GE. Adoptive immunotherapy of advanced tumors with CD62 L-selectin(low) tumor-sensitized T lymphocytes following ex vivo hyperexpansion. J Immunol. 2002;169:3314-20.
90. Wang LX, Shu S, Plautz GE. Host lymphodepletion augments T cell adoptive immunotherapy through enhanced intratumoral proliferation of effector cells. Cancer Res. 2005;65:9547-54.
91. Zhang JG, Eguchi J, Kruse CA, Gomez GG, Fakhrai H, Schroter S, et al. Antigenic profiling of glioma cells to generate allogeneic vaccines or dendritic cell-based therapeutics. Clin Cancer Res. 2007;13:566-75.
92. Medzhitov R. Recognition of microorganisms and activation of the immune response. Nature. 2007;449:819-26.
93. Huang B, Zhao J, Unkeless JC, Feng ZH, Xiong H. TLR signaling by tumor and immune cells: a double-edged sword. Oncogene. 2008;27:218-24.
94. Wang RF, Miyahara Y, Wang HY. Toll-like receptors and immune regulation: implications for cancer therapy. Oncogene. 2008;27:181-9.
95. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. Nature. 2001;413:732-8.
96. Matsumoto M, Funami K, Tanabe M, Oshiumi H, Shingai M, Seto Y, et al. Subcellular localization of Toll-like receptor 3 in human dendritic cells. J Immunol. 2003;171:3154-62.
97. Seya T, Funami K, Taniguchi M, Matsumoto M. Antibodies against human Toll-like receptors (TLRs): TLR distribution and localization in human dendritic cells. J Endotoxin Res. 2005;11:369-74.
98. McKimmie CS, Fazakerley JK. In response to pathogens, glial cells dynamically and differentially regulate Toll-like receptor gene expression. J Neuroimmunol. 2005;169:116-25.
99. Olson JK, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol. 2004;173:3916-24.

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

100. Carpentier PA, Begolka WS, Olson JK, Elhofy A, Karpus WJ, Miller SD. Differential activation of astrocytes by innate and adaptive immune stimuli. Glia. 2005;49:360-74.
101. Farina C, Krumbholz M, Giese T, Hartmann G, Aloisi F, Meinl E. Preferential expression and function of Toll-like receptor 3 in human astrocytes. J Neuroimmunol. 2005;159:12-9.
102. Bsibsi M, Persoon-Deen C, Verwer RW, Meeuwsen S, Ravid R, Van Noort JM. Toll-like receptor 3 on adult human astrocytes triggers production of neuroprotective mediators. Glia. 2006;53:688-95.
103. Zhu X, Nishimura F, Sasaki K, Fujita M, Dusak JE, Eguchi J, et al. Toll like receptor-3 ligand poly-ICLC promotes the efficacy of peripheral vaccinations with tumor antigen-derived peptide epitopes in murine CNS tumor models. J Transl Med. 2007;5:10.
104. Bilu D, Sauder DN. Imiquimod: modes of action. Br J Dermatol. 2003;149 Suppl 66:5-8.
105. Tomai MA, Miller RL, Lipson KE, Kieper WC, Zarraga IE, Vasilakos JP. Resiquimod and other immune response modifiers as vaccine adjuvants. Expert Rev Vaccines. 2007;6:835-47.
106. Chang BA, Cross JL, Najar HM, Dutz JP. Topical resiquimod promotes priming of CTL to parenteral antigens. Vaccine. 2009;27:5791-9.
107. Du J, Wu Z, Ren S, Wei Y, Gao M, Randolph GJ, et al. TLR8 agonists stimulate newly recruited monocyte-derived cells into potent APCs that enhance HBsAg immunogenicity. Vaccine. 2010;28:6273-81.
108. Hesling C, D'Incan M, Mansard S, Franck F, Corbin-Duval A, Chevenet C, et al. In vivo and in situ modulation of the expression of genes involved in metastasis and angiogenesis in a patient treated with topical imiquimod for melanoma skin metastases. Br J Dermatol. 2004;150:761-7.
109. Powell AM, Russell-Jones R, Barlow RJ. Topical imiquimod immunotherapy in the management of lentigo maligna. Clin Exp Dermatol. 2004;29:15-21.
110. Rajagopal D, Paturel C, Morel Y, Uematsu S, Akira S, Diebold SS. Plasmacytoid dendritic cell-derived type I interferon is crucial for the adjuvant activity of Toll-like receptor 7 agonists. Blood. 2010;115:1949-57.
111. Shackleton M, Davis ID, Hopkins W, Jackson H, Dimopoulos N, Tai T, et al. The impact of imiquimod, a Toll-like receptor-7 ligand (TLR7L), on the immunogenicity of melanoma peptide vaccination with adjuvant Flt3 ligand. Cancer Immun. 2004;4:9.
112. Urosevic M, Maier T, Benninghoff B, Slade H, Burg G, Dummer R. Mechanisms underlying imiquimod-induced regression of basal cell carcinoma in vivo. Arch Dermatol. 2003;139:1325-32.
113. Urosevic M, Dummer R, Conrad C, Beyeler M, Laine E, Burg G, et al. Disease-independent skin recruitment and activation of plasmacytoid predendritic cells following imiquimod treatment. J Natl Cancer Inst. 2005;97:1143-53.
114. Michalopoulos P, Yawalkar N, Bronnimann M, Kappeler A, Braathen LR. Characterization of the cellular infiltrate during successful topical treatment of lentigo maligna with imiquimod. Br J Dermatol. 2004;151:903-6.
115. Nair S, McLaughlin C, Weizer A, Su Z, Boczkowski D, Dannull J, et al. Injection of immature dendritic cells into adjuvant-treated skin obviates the need for ex vivo maturation. J Immunol. 2003;171:6275-82.
116. Mattei F, Schiavoni G, Belardelli F, Tough DF. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. J Immunol. 2001;167:1179-87.
117. Montoya M, Schiavoni G, Mattei F, Gresser I, Belardelli F, Borrow P, et al. Type I interferons produced by dendritic cells promote their phenotypic and functional activation. Blood. 2002;99:3263-71.
118. Salem ML, El-Naggar SA, Kadima A, Gillanders WE, Cole DJ. The adjuvant effects of the toll-like receptor 3 ligand polyinosinic-cytidylic acid poly (I:C) on antigen-specific CD8+ T

Original submission: 05/25/2010, 7/20/2010, 8/8/2010, 5/23/11, 02/15/2012, 02/05/2013, 07/02/2013, 10/10/2014, 05/21/2015

cell responses are partially dependent on NK cells with the induction of a beneficial cytokine milieu. Vaccine. 2006;24:5119-32.
119. Trumpfheller C, Caskey M, Nchinda G, Longhi MP, Mizenina O, Huang Y, et al. The microbial mimic poly IC induces durable and protective CD4+ T cell immunity together with a dendritic cell targeted vaccine. Proc Natl Acad Sci U S A. 2008;105:2574-9.
120. Cornell CJ, Jr., Smith KA, Cornwell GG, 3rd, Burke GP, McIntyre OR. Ssytemic effects of intravenous polyriboinosinic-polyribocytidylic acid in man. J Natl Cancer Inst. 1976;57:1211-6.
121. Robinson RA, DeVita VT, Levy HB, Baron S, Hubbard SP, Levine AS. A phase I-II trial of multiple-dose polyriboinosic-polyribocytidylic acid in patieonts with leukemia or solid tumors. J Natl Cancer Inst. 1976;57:599-602.
122. Salazar AM, Levy HB, Ondra S, Kende M, Scherokman B, Brown D, et al. Long-term treatment of malignant gliomas with intramuscularly administered polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose: an open pilot study. Neurosurgery. 1996;38:1096-103; discussion 103-4.

14.0 Appendix

Appendix A	Study Schema
Appendix B	Karnofsky Performance Scale
Appendix C	NCI Common Toxicity Criteria (v3)
Appendix D	Leukapheresis Procedure Subjects' Information
Appendix E	Modified Grading of Injection Site Reaction Toxicity
Appendix F	Informed Consent for *Temporary Aphaeresis Catheter Placement*