Dendritic Cell Vaccine for Recurrent High-Grade Gliomas in Pediatric and Adult Subjects: Clinical Trial Protocol

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BACKGROUND: Although there have been significant advances in understanding the basic pathogenesis of glioblastoma multiforme, the median survival of patients has changed little in the past 25 years. Recent studies have suggested that immune modulation through dendritic cell (DC) vaccines may stimulate the immune system against tumor antigens and potentially increase survival.

OBJECTIVE: To determine whether the use of adjuvant vaccination with autologous DCs (matured in situ after being loaded with tumor cell lysate derived from autologous refractory gliomas) is safe, feasible, and beneficial for adult and pediatric patients with recurrent high-grade gliomas.

METHODS: The study design is a single-center, nonrandomized, open phase I clinical trial. A total of 20 patients with malignant gliomas will be enrolled preoperatively over 2 years. Patients will be given adjuvant vaccination with autologous DCs loaded with tumor lysate after maximal safe surgical resection.

EXPECTED OUTCOMES: Using topical imiquimod before vaccination, it is anticipated that the immune response in vaccinated patients and potentially Overall survival will be greater than that demonstrated in the literature. We anticipate that there will be minimal side effects (minor dermatitis) associated with this treatment.

DISCUSSION: In the current trial, we assess immune response, safety, and survival using a novel vaccine protocol developed in Belgium that seems to markedly increase survival of certain subjects. Nevertheless, larger randomized clinical studies need to be performed to evaluate fully the efficacy of this therapy for both recurrent and newly diagnosed glioblastoma.

KEY WORDS: Clinical trial, Dendritic cell vaccine, Malignant gliomas

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Glioblastoma multiforme (GBM) is the most common brain tumor in adults, occurring in approximately 39% of central nervous system neoplasms. Patients typically present in their fifth decade with a history of headaches or seizures.[1,2] Treatment typically includes extensive surgical resection coupled with cytotoxic chemotherapy and ionizing radiation. However, this combination has limited efficacy and increased toxicity. Although there have been significant advances in understanding the basic pathogenesis of GBM (grade IV), the median survival of patients has changed little in the past 25 years, with a 5-year survival rate of only 13.4%.[3,4] Although current treatment remains universal for all GBM patients, recent evidence has demonstrated marked heterogeneity in the molecular profiles of these tumors.[5,6] Therefore, recent studies have suggested that immune modulation through vaccines may serve a role in enhancing specificity for individual tumors and potentially increasing survival.

Dendritic cell (DC) vaccines are intended to train the body's immune system to elicit an antitumoral cellular response. To elicit an immune response, the lysate of the patient's tumor (obtained from surgery) is exposed to autologous DCs, which are then injected in the patient. In many cases, CD4/CD8-mediated cellular immune response is activated to help target infiltrating

ABBREVIATIONS: DC, dendritic cell; **GBM,** glioblastoma multiforme; **PGE2,** prostaglandin E_2

tumor cells. Several phase I/II trials or case reports have been published to date of patients with malignant gliomas treated using slightly different variants of DC vaccination. The results of these studies have suggested that autologous DC vaccines for newly diagnosed/recurrent malignant gliomas are safe and feasible and may contribute to greater overall survival.[7-14] In some reports, the median overall survival is increased by 20% after administration of an autologous DC vaccine for patients with GBM.[9] Nevertheless, in some cases, clinical immune response is not elicited; therefore, an alternative method to enhance clinical immune response may be indicated. A plausible concern in many DC protocols is the use of prostaglandin E_2 (PGE2) in the maturation cocktail to generate DCs. Nair et al[15] have shown in murine systems that PGE2 can be eliminated and imiquimod, a Toll-like receptor 7 and 8 agonist, used to mature the DCs in situ.

Recent studies by a Belgian group (HGG-IMMUNO) demonstrated improved immune responses by eliminating PGE2 in this fashion from the maturation cocktail and by using topical imiquimod before vaccine administration. In a pilot study involving 12 patients, this method has demonstrated increased quantitative immunological responses in recurrent malignant gliomas (grades III and IV). Specifically, 33% of the patients who received a gross macroscopic resection were disease free at 5-year follow-up. This group has an ongoing clinical trials program in Belgium, including protocols using the approach in newly diagnosed GBM patients in conjunction with standard chemotherapy and radiation therapy. Although there are initial data on the efficacy of this approach in Belgium, the safety of this protocol using a systematic protocol has yet to be confirmed in the United States. We will conduct a pilot phase I trial in the United States to assess the safety and feasibility of treating recurrent high-grade gliomas with a DC vaccine with pretreatment with imiquimod (NCT01808820).

STUDY GOAL AND OBJECTIVE

The objectives of this study were to determine whether the use of adjuvant vaccination with autologous DCs (matured in situ after being loaded with tumor cell lysate derived from autologous refractory gliomas) is safe, feasible, and beneficial for adult and pediatric patients with recurrent high-grade gliomas and whether the DC vaccine is capable of initiating a tumor cell response.

We hypothesize that patients who receive the in situ matured DC vaccine after macroscopic total resection will demonstrate an increased progression-free survival and overall survival compared with the survival of historical controls with minimal toxicity.

STUDY DESIGN

The study design is a single-center, nonrandomized, open interventional clinical trial.

METHODOLOGY

Subjects

Inclusion Criteria

Patients 13 to 100 years of age with recurrent high-grade glioma (WHO grade III anaplastic astrocytoma or WHO grade IV GBM) with a life expectancy longer than 3 months will be included. These patient groups can include the following subpopulations: (1) patients with high-grade glioma, histologically proved at first surgery and (2) patients with histologically proven low-grade gliomas with radiological or histological evidence of malignant transformation to a high-grade glioma.

All patients included in the study must have undergone a subtotal or gross total resection with a postoperative magnetic resonance imaging (MRI) scan that demonstrates less than 2 cm^3 of residual disease confirmed by both the surgeon and neuroradiologist. Patients must not have received chemotherapy/radiotherapy for at least 1 month before first DC vaccination and must not have received corticosteroids for at least 1 week before vaccination. A summary of the inclusion criteria is given in the Table.

Exclusion Criteria

Patients with unresectable or partially resected tumor (>2 cm^3 of residual disease) will be excluded. Patients with a poor life expectancy of less than 3 months or other active malignancies will be excluded. Patients with immunodeficiency, liver dysfunction, or uncontrollable medical problems as well as pregnant females will also be excluded. A summary of the exclusion criteria is given in the Table.

Study Description

This study is separated into 2 cohorts: an initial safety pilot cohort and an expansion cohort.

In this initial group of patients, 5 preliminary patients will be assessed for any adverse effects. In the event that no treatment-related side effects such as anaphylaxis or toxic dermatitis are present, a second cohort of patients (for a total of 20 patients) will be enrolled. A diagram of the overall study protocol is given in Figure.

Interested participants will be enrolled in the study after informed consent is obtained and under the condition that the inclusion criteria are met. At this time, preoperative MRI will be performed to assess baseline tumor burden. Patients will be scheduled for surgical resection, and postoperative MRI is required to determine the extent of resection. Immediately after resection, tumor tissue will be lysed and stored for the vaccine. Corticosteroids will be given to patients, as determined by the neurosurgeon. The patient will undergo leukapheresis to isolate peripheral blood mononuclear cells no longer than 2 weeks after the stop of corticosteroid therapy. Peripheral blood mononuclear cells are then manipulated ex vivo in a cyclic guanosine monophosphate facility to become partially matured DCs, and then autologous tumor lysate is incubated with them. This process takes approximately 9 days. Subsequently, DCs will be administered as 6 small-volume injections intradermally in

TABLE. Inclusion/Exclusion Criteria[a]

Inclusion Criteria	Exclusion Criteria
Age >13 and <100 y	Pregnancy
Recurrence of high-grade glioma (WHO grade III or IV), histologically proved at first stage of disease (radiological evidence of recurrence suffices)	Breastfeeding
Recurrence of glioma, which was grade II at initial diagnosis, but became grade III or higher at recurrence based on radiological or pathological criteria	Concomitant participation in other therapeutic trials
Total or subtotal resection of tumor mass, confirmed by neurosurgeon and postoperative MRI scan within 72 h after surgery (residual tumor ≤2 cm^3)	Virus serology positive for HIV or other documented immunodeficiency or autoimmune disease or any other active malignancies
No previous radiotherapy and/or chemotherapy (1 mo before first vaccine administration)	Unresectable tumor
No corticosteroids or salicylates (at least 1 wk before first vaccination)	Refusal to use adequate contraception for fertile patients
Life expectancy >3 mo	Serious or uncontrolled medical or psychiatric condition
Adequate organ function	
Laboratory values (absolute neutrophil count >750/L, lymphocytes >500/L, platelets >75,000/L	
Hemoglobin >9 g/dL, AST/ALT <2.5 × ULN; if liver metastases, <5 × ULN, serum creatinine <1.5 × ULN	
Total bilirubin <3 × ULN, albumin >2 g/dL	
Karnofsky score >70 or ECOG status of 0 or 1	

[a]WHO, World Health Organization; MRI, magnetic resonance imaging; HIV, Human Immunodeficiency Virus; AST/ALT, aspartate aminotransferase/alanine aminotransferase; ULN, upper limit of normal; ECOG, Eastern Cooperative Oncology Group.

imiquimod-pretreated locations. A total of 4 weekly DC injections will be administered. The tumor lysate will then be given on 4 separate occasions between weeks 8 and 28 in imiquimod-pretreated areas as lysate boost alone.

Outcome Measures and Follow-up

Primary Outcome Measures

Primary outcome measures include side effect monitoring and overall, progression-free, and recurrence-free survival (radiographic or clinical evidence) for 5 years after surgery.

Secondary Outcome Measures

Secondary outcome measures include measurement of Karnofsky Performance Scores, assessment of delayed-type hypersensitivity after vaccination by induration size, immunological response by reverse transcriptase polymerase chain reaction analysis of interferon-gamma production and HLA-restricted tetramer staining of CD8 T cells.

DISCUSSION

Several studies have been conducted to evaluate the effects of autologous DC-based vaccines using tumor lysate for malignant gliomas.[8,16-18] Although current evidence of immunotherapy in glioblastoma has revealed minimal risks or side effects of treatment, immunological induction or clinical response has not been well characterized. Preliminary results seem to demonstrate that adjuvant vaccination with autologous DCs loaded with tumor lysate may increase the overall survival of patients with newly diagnosed glioblastoma by approximately 1 year.[19] Nevertheless, larger randomized clinical studies need to be performed to fully evaluate the efficacy of this therapy for both recurrent and newly diagnosed glioblastoma. In this trial, we hope to demonstrate increased immune response and safety using our vaccine protocol. Although the initial cohort may not be enough for statistical power, we will likely expand recruitment in the later phases of the clinical trial to fully assess clinical utility of autologous DC-based vaccine. We may also collaborate with the European teams assessing this methodology in future studies to increase the power of our analyses.

Trial Status

Patient recruitment has commenced and will be completed by July 2015. Data analysis will be performed continually, and final reports will be disseminated at the end of the study follow-up after 5 years.

Safety Considerations

This study will involve the treatment of up to 20 subjects to generate safety data. Because each course of treatment of vaccines takes several months, the timing of treatment for each patient is staggered so that 1 patient must receive the first 2 doses of the DCs before the next patient will receive the vaccine. This will allow adequate monitoring of any critical toxicities associated with treatment while avoiding any prolongation in study enrollment for patients with poor prognoses.

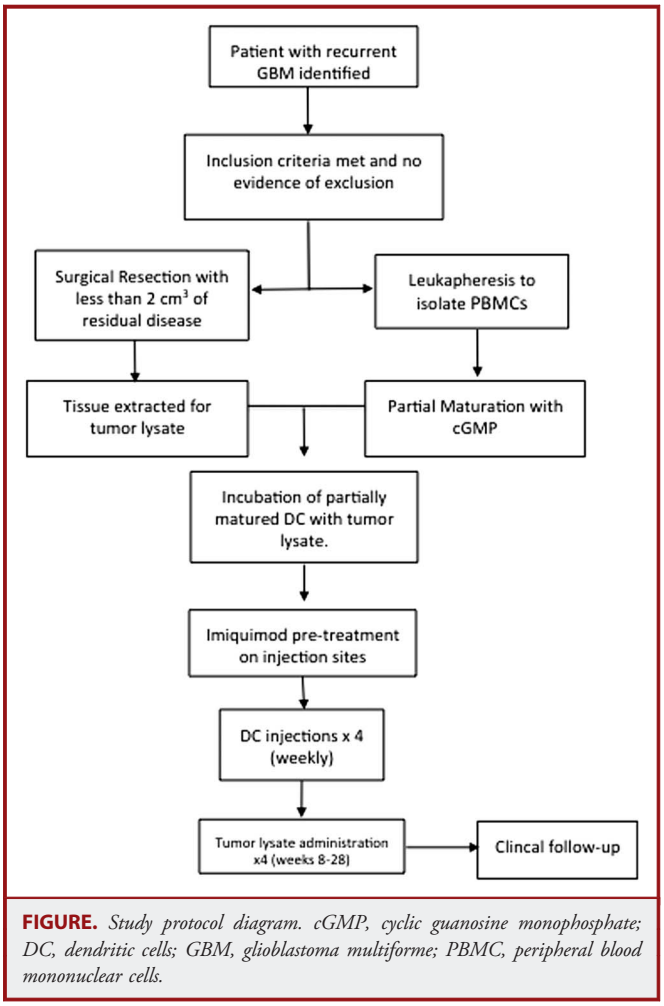

FIGURE. *Study protocol diagram. cGMP, cyclic guanosine monophosphate; DC, dendritic cells; GBM, glioblastoma multiforme; PBMC, peripheral blood mononuclear cells.*

Although not specifically studied in the United States in glioma, vaccination with in situ matured DCs has been found to have an excellent safety profile in Belgium. Vaccine-related side effects such as dermatitis or eczema may be potential side effects after vaccine injection. Liver toxicity, evaluated by increased aspartate and alanine aminotransferase levels, may also occur, but are rare. Patients are regularly tested on follow-up for such side effects. Injection of autologous DCs may also theoretically stimulate autoimmune diseases; however, such an autoimmune phenomenon is relatively unlikely and has not been seen in Belgian studies. In the event that the tumor lysate is not prepared correctly, there may also be a potential for de novo tumor formation at injection sites; however, this has not been demonstrated in previous trials.

Follow-up

The patients will be followed for 5 years with 2 to 4 clinic visits per year. MRI will be performed at follow-up visits to assess disease status. Immune response will be evaluated by measuring T-cell subsets and myeloid-derived suppressor cells 1 and 3 months after completion of study treatment. In addition, clinical visits with history and physical examination will be routinely performed to assess functional status and clinical well-being. Detailed instructions are given to each patient in the event of an emergency. All patients are educated about reporting adverse events that may be related to the treatment protocol. Three years after vaccine therapy, clinical follow-up may be conducted by telephone interview.

Data Management and Statistical Analysis

Data will be collected prospectively in an electronic database collection system that is password protected and only accessed by study investigators. The data are managed and inputted by a clinical research coordinator who is directly supervised by the principal investigator. Once the data input is complete, a separate research coordinator independently verifies all clinical data to ensure accuracy and quality.

Descriptive statistics such as mean, standard deviation, and proportions and corresponding 95% confidence intervals will be calculated for immune parameters including myeloid-derived suppressor cell levels, neutrophil counts, lymphocyte counts, and lymphocyte subsets. Survival endpoints, ie, progression-free survival and overall survival, will be estimated and displaced using Kaplan-Meier product-limit approach. Median survival and 1- and 5-year survival rates will be estimated based on the Kaplan-Meier curve. The log-rank test and Cox's proportional hazard regression model will be implemented to compare survival in different subgroups and to test the effect of treatment.

Quality Assurance

The Data Safety Monitoring Committee at the University of Miami Miller School of Medicine will monitor the progress of our clinical trial in the event that there are major adverse side effects or the overall survival is markedly lower than expected. Recommendations will be directly forwarded to the principal investigator for review. Safety and feasibility will be evaluated descriptively.

Expected Study Outcome

Based on the results of other studies in Belgium, we suspect that DC vaccine with autologous tumor lysate will not be associated with major treatment-related side effects. We anticipate reports of minor dermatitis after injection of tumor lysate and autologous DC vaccine. In addition, we anticipate that patients who receive this therapy after a gross total resection of tumor will have a prolonged survival compared with patients receiving the standard treatment. Nevertheless, we cannot predict immunogenicity from this therapy; therefore, adequate follow-up with serial laboratory tests and MRI scans are necessary to assess response.

Study Duration

The study is planned to enroll patients for 8 to 12 months with a 5-year follow-up for the first phase of this clinical trial. If efficacy is established, a phase II clinical trial will be initiated.

Project Management

The study is being led by the principal investigator Dr John M. Goldberg in collaboration with the Belgian-based consortium HGG-IMMUNO. He will oversee study investigators in all aspects of study conduct and holds the Investigational New Drug application for the clinical trial (15048). Data interpretation and dissemination of results will be managed under his direct supervision.

Ethics

This study is approved by the Institutional Review Board of the University of Miami and adheres to the quality standards set by the Good Clinical Practice guidelines. This study is conducted in accordance with the Health Information Portability and Accountability Act and the guiding principles of the Declaration of Helsinki. Explicit written consent will be obtained from all patients in the study. Patients without appropriate documentation will be deemed ineligible.

Disclosures

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