A Phase I Study of Autologous Dendritic Cell Vaccine Pulsed with Allogeneic Stem-like Cell Line Lysate in Patients with Newly Diagnosed or Recurrent Glioblastoma

Jethro L. Hu[1,2], Oluwaseun A. Omofoye[2], Jeremy D. Rudnick[1], Sungjin Kim[1], Mourad Tighiouart[1], Surasak Phuphanich[1], Hongqiang Wang[2], Mia Mazer[2], Toni Ganaway[2], Ray M. Chu[2], Chirag G. Patil[2], Keith L. Black[2], Stephen L. Shiao[3], Rongfu Wang[4,5], and John S. Yu[2]

ABSTRACT

Purpose: Glioblastoma (GBM) is a heterogeneous malignancy with multiple subpopulations of cancer cells present within any tumor. We present the results of a phase I clinical trial using an autologous dendritic cell (DC) vaccine pulsed with lysate derived from a GBM stem-like cell line.

Patients and Methods: Patients with newly diagnosed and recurrent GBM were enrolled as separate cohorts. Eligibility criteria included a qualifying surgical resection or minimal tumor size, ≤ 4-mg dexamethasone daily dose, and Karnofsky score ≥70. Vaccine treatment consisted of two phases: an induction phase with vaccine given weekly for 4 weeks, and a maintenance phase with vaccines administered every 8 weeks until depletion of supply or disease progression. Patients with newly diagnosed GBM also received standard-of-care radiation and temozolomide. The primary objective for this open-label, single-institution trial was to assess the safety and tolerability of the autologous DC vaccine.

Results: For the 11 patients with newly diagnosed GBM, median progression-free survival (PFS) was 8.75 months, and median overall survival was 20.36 months. For the 25 patients with recurrent GBM, median PFS was 3.23 months, 6-month PFS was 24%, and median survival was 11.97 months. A subset of patients developed a cytotoxic T-cell response as determined by an IFNγ ELISpot assay.

Conclusions: In this trial, treatment of newly diagnosed and recurrent GBM with autologous DC vaccine pulsed with lysate derived from an allogeneic stem-like cell line was safe and well tolerated. Clinical outcomes add to the body of evidence suggesting that immunotherapy plays a role in the treatment of GBM.

Introduction

Over the past several years, outcomes for patients with many aggressive cancers have improved considerably, due in part to advancements in areas such as targeted therapy and immunotherapy. Glioblastoma (GBM) stands as a notable exception, where therapeutic advances have been relatively incremental. Standard treatment for newly diagnosed GBM consists of involved-field radiation therapy (IFRT) and concurrent temozolomide chemotherapy followed by adjuvant temozolomide cycles (1). Tumor-treating fields may be added following radiotherapy (2). Median overall survival (OS) in the clinical trial setting with these treatments is within an approximate range of 15 to 20 months, compared with 12 months with radiation alone (1, 2). Treatment options at recurrence include the antiangiogenic therapy bevacizumab, but improvements in median OS with this treatment are modest (3–5).

Dendritic cell (DC) vaccines are a promising method of generating a therapeutic antitumor immune response. DCs are professional antigen-presenting cells and are capable of initiating CTL function. DCs derived *in vitro* from peripheral blood mononuclear cells (PBMC) can be primed against tumor antigens in culture, and upon subsequent vaccination in tumor-bearing hosts, have the ability to elicit antitumor immunity. We have used DC-based vaccination protocols in six investigator-initiated trials and have demonstrated the safety and bioactivity of this approach in patients with malignant brain tumors (6–11). We previously reported that therapeutic vaccination with autologous tumor peptide-pulsed DCs is sufficient to enhance peripheral tumor-reactive CTL activity and $CD8^+$ T-cell infiltration into tumors *in situ* in patients with newly diagnosed GBM (6, 7). Furthermore, we have shown that patients who developed a postvaccination peripheral cytotoxic T-cell response survived longer than those patients who did not develop a response (8, 9).

A defining challenge of GBM is its heterogeneous nature, with multiple subpopulations of cancer cells present within any given tumor, including the presence of a subpopulation of tumor cells which exhibit stem-like behavior (12–14). These GBM stem-like cells are resistant to radiotherapy and traditional chemotherapy (15, 16). We have demonstrated that patients with tumors with high expression of stem-like markers have not responded as robustly to treatment with DC vaccine therapy compared with patients with tumors that lack expression of glioma stem-like cell markers (17).

[1]Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, California. [2]Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, California. [3]Department of Radiation Oncology, Cedars-Sinai Medical Center, Los Angeles, California. [4]Department of Pediatrics, Children's Hospital Los Angeles, Los Angeles, California. [5]Department of Medicine, Norris Comprehensive Cancer Center, The Keck School of Medicine at the University of Southern California, Los Angeles, California.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: John S. Yu, Department of Neurosurgery, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Advanced Health Sciences Pavilion A6600, Los Angeles, CA 90048. Phone: 310-423-9522; Fax: 310-967-8591; E-mail: John.Yu@cshs.org

Clin Cancer Res 2022;28:689–96

doi: 10.1158/1078-0432.CCR-21-2867

Translational Relevance

This open-label, single-institution, phase I trial evaluated an autologous dendritic cell vaccine pulsed with an allogeneic stem-like cell lysate in patients with newly diagnosed and recurrent glioblastoma (GBM). Treatment was safe and well tolerated. Median progression-free survival (PFS) was 8.75 months and median overall survival (OS) was 20.26 months for patients with newly diagnosed GBM. For patients with recurrent GBM, median PFS was 3.22 months, 6-month PFS was 24%, and median survival was 11.97 months. Although this phase I trial was not powered to assess efficacy, PFS and OS compare favorably with historical controls, and a subset of patients developed a cytotoxic T-cell response as determined by an IFNγ ELISpot assay. This trial adds to the body of evidence suggesting that immunotherapy may play a role in the treatment of GBM.

These findings suggest that targeting the subpopulation of GBM stem-like cells may be a critical element in the development of effective therapies for GBM. To evaluate this hypothesis, we performed a phase I clinical trial using an autologous DC vaccine pulsed with lysate derived from a GBM stem-like cell line.

Patients and Methods

This study was conducted at Cedars–Sinai Medical Center according to the International Council for Harmonization (ICH) guidelines. The institutional review board at Cedars–Sinai approved the study and patients were enrolled following written informed consent. This trial was registered with ClinicalTrials.gov (NCT02010606).

Eligibility criteria

Patients ≥18 years of age with either newly diagnosed (Cohort A) or recurrent (Cohort B) GBM were eligible for this phase I study. Patients in Cohort B were eligible up to third recurrence and were required to have been previously treated with IFRT and temozolomide chemotherapy. Prior bevacizumab therapy was allowed for patients in Cohort B. Patients with secondary glioblastoma (i.e., patients initially diagnosed with a lower grade glioma that subsequently transformed to GBM) were eligible for Cohort B if they had been treated with radiation and temozolomide prior to their last surgical resection. All patients underwent a qualifying surgical resection prior to enrolling in the trial (defined as having a less than 1 cm^2 residual enhancing tumor postoperatively) or had minimal tumor burden (<1 cm^2 enhancing disease) at time of enrollment. Patients with a foreseeable condition that would preclude reduction of steroids lower than dexamethasone 4 mg daily or equivalent steroid dose were not eligible for this study. Additional eligibility criteria included Karnofsky Performance Score (KPS) ≥ 70, and adequate hematologic, liver, and kidney function.

Study design

The primary objective for this open-label, single-institution phase I clinical trial was to assess safety and tolerability of the autologous DC vaccine product. Adverse events were monitored and recorded per Common Terminology Criteria for Adverse Events version 4.03 guidelines. Secondary objectives included assessment of the clinical outcome measures of progression-free survival (PFS) and OS, and evaluation of immune response by assessing cytotoxic T-cell activity *in vitro* pre-vaccination and postvaccination.

For both cohorts, vaccine treatment consisted of two phases: an induction phase with vaccine given weekly for 4 weeks, followed by a maintenance phase when vaccines were administered every 8 weeks until depletion of vaccine supply or disease progression (**Fig. 1**). This protocol was implemented on the basis of our previous data showing that T-cell responses waned after three biweekly vaccinations, and even after a fourth "booster" vaccination 6 weeks later. Patients with newly diagnosed GBM (Cohort A) were also treated with standard-of-care radiotherapy and concurrent temozolomide followed by cycles of adjuvant temozolomide. For these patients, the vaccine induction phase started 1 week following the completion of concurrent chemoradiation. For patients with recurrent GBM (Cohort B), the vaccine induction phase began as soon as the vaccine was manufactured, typically within 3 weeks of apheresis. Patients in Cohort B who were treated with bevacizumab prior to enrolling in the trial were allowed to continue receiving bevacizumab. No other disease-directed treatments were allowed to be administered while on trial.

Vaccine preparation and administration

The vaccine consisted of autologous DCs pulsed with lysate derived from an allogeneic glioblastoma stem-like cell line. The cell line from which the allogeneic lysate was derived originated from a single patient and demonstrates all properties of cancers stem cells with regard to the ability to self-renew and differentiate into multiple central nervous system cell lineages. The CSC6 cells express stem cell markers (nestin, Sox2, CD133, SSEA-1, SSEA-4, and SSEA-5) similar to that of neural stem cells and embryonic stem cells at the molecular and protein levels (Supplementary Fig. S1). The cell line was maintained in serum-free media containing basic fibroblast growth factor, EGF, and leukemia inhibitor factor in order to promote the formation of neurospheres enriched for GBM stem-like cells.

After obtaining informed consent and confirming eligibility, patients underwent apheresis to collect PBMCs required for autologous vaccine preparation. Elutra separation of apheresis product was performed into platelet, red blood cell, lymphocyte, and monocyte fractions. Fractions 4 and 5 were obtained with highly concentrated monocytes. Through FACS, $CD45^+CD14^+$high and $CD45^+CD66^+$low fraction was isolated as a source of monocytes. These monocytes were cultured in media supplemented with granulocyte–macrophage colony-stimulating factor and interleukin-4 for 4 days according to methods previously described (6–9).

On Day 4 postapheresis, cells were pulsed with lysate from the allogeneic GBM stem-like cell line described above for 20 to 24 hours. The cells were then incubated with IFNγ and lipopolysaccharide prior to being harvested and cryopreserved.

The final DC product was $CD40^+$high, $CD80^+$high, $CD83^+$high, $CD86^+$high, HLA-ABC^+high, HLA-DR^+high, $CCR7^+$high, and $CD14^+$low. Release criteria included $CD11c^+$high (> = 80%), $CD83^+$high (> = 80%), and $CD11c^+CD83^+$high (> = 60%). CD11c is a widely used defining marker for DCs, and CD83 is most highly and stably expressed by mature DCs. A minimum of four vaccines was manufactured for each patient. Vaccine consisting of 1×10^7 DCs was administered intradermally in the axillary region.

Response assessments

MRI scans were performed every 8 weeks, beginning on Day 22 of the induction phase, coinciding with the administration of the fourth and final vaccine of the induction phase. For patients

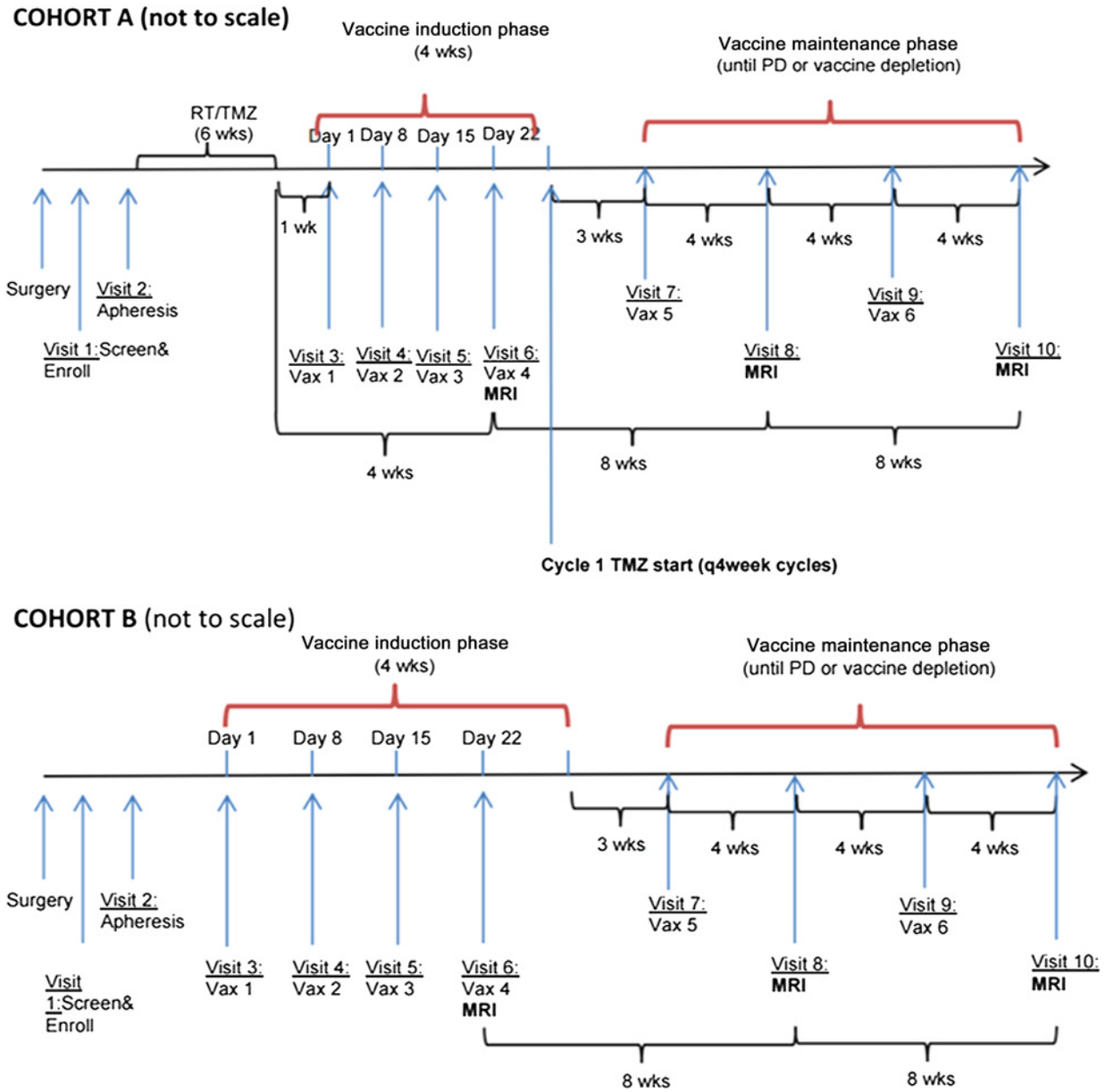


Figure 1.
Treatment and vaccine administration schedule. RT, radiotherapy; TMZ, temozolomide.

in Cohort A, this timepoint occurred 4 weeks following completion of concurrent chemoradiation. Responses were assessed using response assessment in neuro-oncology criteria, and patients were allowed to continue on trial until disease progression was noted (18).

Immune response was assessed through the measurement of IFNγ induction by patient-derived PBMCs *in vitro*. The response was compared prevaccination and postvaccination after stimulation with the allogeneic glioblastoma stem-like cell lysate. IFNγ levels were quantified using the ELISpot assay as previously described (19). A postvaccine induction of IFNγ > 1.5 times the response seen in PBMCs acquired prevaccine is evidence of a tumor-specific cytotoxic T-cell response (9).

Statistical analysis

Stopping rules for safety and efficacy concerns were defined prior to trial initiation and were not met at any point. The Kaplan–Meier method was used to estimate the probability of survival for each cohort, and the log-rank test was used to assess survival distribution. Exploratory analyses were performed to examine associations between vaccine response, age, race/ethnicity, or O^6-Methylguanine-DNA Methyltransferase (*MGMT*) promoter methylation status and outcomes using a Cox proportional hazards model (20). The proportional hazards assumption was assessed with scaled Schoenfeld residuals (21). Analyses were performed using R package version 4.0.3 with two-sided tests and a significance level at 0.05.

Table 1. Patient characteristics ($N = 36$).

Characteristics	Newly diagnosed GBM ($N = 11$)	Recurrent GBM ($N = 25$)
Age at consent (years), mean (± SD)	55.9 (±18.2)	52.3 (±14.2)
KPS at consent, median (±SD)	85 (±6.6)	80 (±9.0)
Gender		
Female	3 (27.3)	11 (44)
Male	8 (72.7)	14 (56)
Race/Ethnicity		
Non-Hispanic White	10 (90.9)	4 (30.8)
Other	1 (9.1)	9 (69.2)
MGMT promoter methylation status		
Methylated	4 (100)	4 (30.8)
Unmethylated	0 (0)	9 (69.2)
IDH1 R132H status		
Negative	10 (90.9)	19 (76)
Positive	1 (9.1)	3 (12)
Unknown	0 (0)	3 (12)
Corticosteroid use at consent		
Yes	2 (18.2)	10 (40)
No	9 (81.8)	15 (60)

Results

From December 2013 to February 2018, 43 patients signed informed consent to participate in the trial. Five patients failed screening, and 2 patients voluntarily withdrew prior to receiving treatment. Thus, 36 patients received at least one vaccine and were eligible for analysis, 11 with newly diagnosed GBM and 25 with recurrent GBM. Mean age at consent was 55.9 years for patients with newly diagnosed GBM and 52.3 years for patients with recurrent GBM. The median KPS at enrollment was 85 for patients with newly diagnosed GBM, and 80 for patients with recurrent GBM (**Table 1**).

All 11 patients with newly diagnosed GBM and 23 of the 25 patients with recurrent GBM underwent gross total or near–gross total resection within 1 month of enrolling in the trial. Tumor *MGMT* promoter methylation status was assessed for 16 patients and was found to be methylated in 7 patients and unmethylated in 9 patients. IHC staining for *IDH R132H* was performed on tumors for 32 patients and found to be positive in 4 tumors and negative in 28 tumors.

In total, 209 vaccines were administered across 36 patients. Twenty-eight patients received all 4 vaccines in the 4-week-long induction period. The median number of vaccines administered was 5, ranging from 1 to 15. Overall, treatment was very well tolerated (**Table 2**). The most common adverse events (AE) at least possibly related to the vaccine were grade 1 cutaneous injection-site reactions (27 events in 18 patients) and fever (23 events in 8 patients; 20 events were classified as grade 1, 2 events as grade 2, and 1 event was not able to be graded due to missing information). There were 11 instances of grade 1 flu-like symptoms and 3 instances of grade 1 myalgia following vaccine administration. There were no higher-grade instances of these symptoms noted. No serious adverse events (SAE) possibly related to vaccine administration or treatment were observed. Two SAEs occurred immediately following leukapheresis for 1 patient: grade 2 bleeding from the catheter site and transient grade 1 thrombocytopenia.

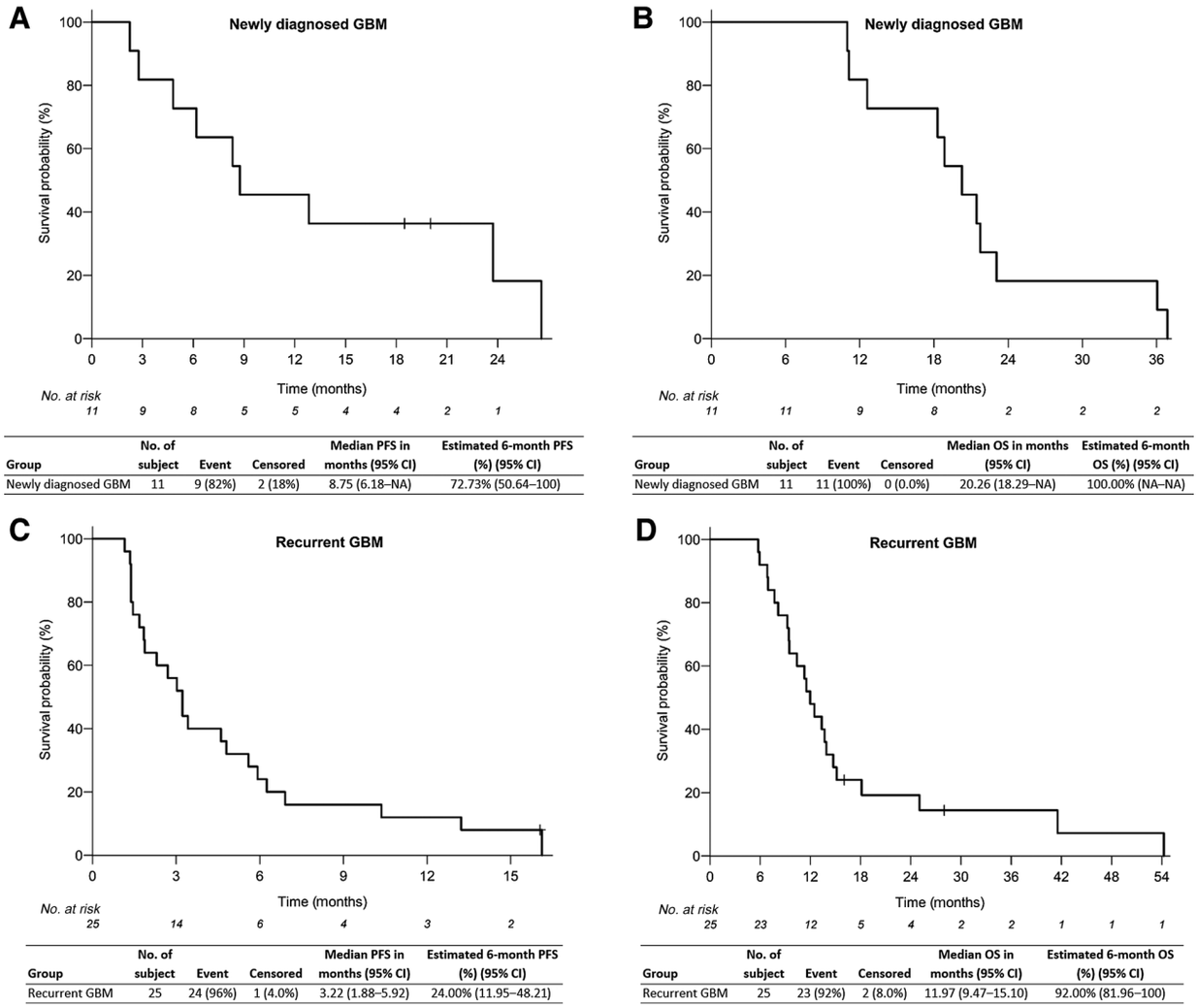
For the 11 patients with newly diagnosed GBM, median PFS was 8.75 months, and median OS was 20.26 months. For the 25 patients with recurrent GBM, median PFS was 3.22 months with a 6-month PFS of 24%. Median survival from the time of enrollment in this cohort was 11.97 months. Kaplan–Meier estimates for PFS and OS are shown in **Fig. 2**.

Immune response was assessed for 25 patients: 8 with newly diagnosed GBM and 17 with recurrent GBM (**Table 3**). For the remaining 11 patients, PBMCs were either insufficient or not collected due to early disease progression that prompted disenrollment from the trial prior to the Day 56 postvaccine collection timepoint. As described above, a ≥1.5-fold increase in IFNγ by ELISpot postvaccination was considered an immune response. By this criterion, 9 of the 25 patients qualified as immune responders—3 of 8 with newly diagnosed GBM, and 6 of 17 with recurrent GBM. In this phase I trial, exploratory univariate analysis did not reveal a statistically significant association between vaccine response (either as a binary or continuous variable) and PFS or OS. There were also no significant relationships observed between *MGMT* promoter methylation status, age at consent, or race/ethnicity with PFS or OS.

Table 2. AEs at least possibly attributable to vaccine (36 patients, total number of vaccines = 209).

Adverse event	Grade 1	Grade 2	Patients affected		Number of events
			Number	%	
Injection site reaction	27	0	18	50.0	27
Fever	20	2	7	19.4	23[a]
Flu-like symptoms	11	0	6	16.7	11
Fatigue	6	0	4	11.1	6
Bruising	3	0	1	2.8	3
Myalgia	3	0	3	8.3	3
Thrombocytopenia	2	0	2	5.6	2
Bleeding	0	1	1	2.8	1
Nasal congestion	1	0	1	2.8	1
Nausea	1	0	1	2.8	1
Productive cough	1	0	1	2.8	1
Pruritis	1	0	1	2.8	1
Total	76	3	26	72.2	80[a]

Note: One thrombocytopenia and the bleeding AE were considered SAEs. Note that there were no AEs > grade 2.
[a]One occurrence of fever was not graded due to missing information.



Group	No. of subject	Event	Censored	Median PFS in months (95% CI)	Estimated 6-month PFS (%) (95% CI)
Newly diagnosed GBM	11	9 (82%)	2 (18%)	8.75 (6.18–NA)	72.73% (50.64–100)

Group	No. of subject	Event	Censored	Median OS in months (95% CI)	Estimated 6-month OS (%) (95% CI)
Newly diagnosed GBM	11	11 (100%)	0 (0.0%)	20.26 (18.29–NA)	100.00% (NA–NA)

Group	No. of subject	Event	Censored	Median PFS in months (95% CI)	Estimated 6-month PFS (%) (95% CI)
Recurrent GBM	25	24 (96%)	1 (4.0%)	3.22 (1.88–5.92)	24.00% (11.95–48.21)

Group	No. of subject	Event	Censored	Median OS in months (95% CI)	Estimated 6-month OS (%) (95% CI)
Recurrent GBM	25	23 (92%)	2 (8.0%)	11.97 (9.47–15.10)	92.00% (81.96–100)

Figure 2.

Kaplan–Meier estimates for PFS and OS. **A,** Kaplan–Meier estimate of PFS in patients with newly diagnosed GBM. **B,** Kaplan–Meier estimate of OS in patients with newly diagnosed GBM. **C,** Kaplan–Meier estimate of PFS in patients with recurrent GBM. **D,** Kaplan–Meier estimate of OS in patients with recurrent GBM.

Discussion

This single-institution, phase I DC vaccine trial builds upon previous efforts by specifically targeting the GBM stem-like cell subpopulation that is resistant to standard radiation and chemotherapy (15, 16). Thirty-six patients received vaccine treatment in this study, 11 with newly diagnosed GBM and 25 with recurrent GBM. Vaccine treatment was safe and well tolerated. This trial was based on the successful results of our previous work using a single allogeneic cancer stem cell (CSC) line lysate. We have shown that CSC-pulsed DCs induce stronger cytotoxic T-cell response against 9 L tumor cells. Using a 9 L rat glioma model, we showed that vaccination with DCs loaded with neurosphere-derived antigens, but not antigens from daughter cells or conventionally cultured cells, induced CTL responses against glioma stem-like cells and significantly extended survival of animals bearing 9 L CSC tumors (17). In this phase I study, using lysate derived from an allogeneic GBM stem-like cell line to pulse autologous DCs did not result in any adverse autoimmune events. Although this trial was not powered to assess efficacy, PFS and OS compare favorably with historical controls, and a subset of patients developed a cytotoxic T-cell response as measured by an IFNγ ELISpot assay.

Recent advances in immunotherapy have transformed the treatment landscape for many cancers, but there are currently no FDA-approved immunotherapies for GBM. Thus far, outcomes using checkpoint inhibitors targeting CTLA-4, PD-1, and PD-L1 have largely been disappointing. The phase III trial CheckMate 143 randomized patients with recurrent GBM to receive the PD-1 inhibitor nivolumab or bevacizumab (22). PFS and OS in this trial were 1.5 months and 9.8 months respectively for nivolumab versus 3.5 months and 10 months for bevacizumab. CheckMate 498 randomized patients with newly diagnosed *MGMT*-unmethylated GBM to receive nivolumab with radiotherapy versus temozolomide with radiotherapy; no improvement in OS was seen in the nivolumab group. CheckMate 548 randomized patients with newly diagnosed *MGMT*-methylated GBM to receive either nivolumab versus placebo with both

Table 3. Univariate analyses, including immune response by IFNγ ELISpot assay.

	PFS					
	Newly diagnosed GBM			Recurrent GBM		
Variables	***n***	**HR (95% CI)**	***P***	***n***	**HR (95% CI)**	***P***
Vaccine response (continuous)	8	0.45 (0.14–1.46)	0.185	17	1.50 (0.95–2.36)	0.083
Vaccine response (binary)						
Responder	3	0.62 (0.12–3.25)	0.572	6	0.90 (0.33–2.50)	0.842
Nonresponder	5	1 (Reference)		11	1 (Reference)	
MGMT						
Methylated	4	NA		4	0.80 (0.21–3.02)	0.741
Unmethylated	0			9	1 (Reference)	
Age at consent (years)	11	0.98 (0.95–1.02)	0.266	25	0.98 (0.95–1.01)	0.264
Race/Ethnicity						
NH White	10	0.70 (0.08–6.03)	0.744	19	1.31 (0.51–3.35)	0.579
Other	1	1 (Reference)		6	1 (Reference)	

	OS					
	Newly diagnosed GBM			Recurrent GBM		
Variables	***n***	**HR (95% CI)**	***P***	***n***	**HR (95% CI)**	***P***
Vaccine response (continuous)	8	0.38 (0.10–1.42)	0.150	17	1.28 (0.86–1.89)	0.223
Vaccine response (binary)						
Responder	3	0.52 (0.10–2.69)	0.433	6	1.21 (0.43–3.46)	0.718
Nonresponder	5	1 (Reference)		11	1 (Reference)	
MGMT						
Methylated	4	NA		4	0.44 (0.09–2.10)	0.304
Unmethylated	0			9	1 (Reference)	
Age at consent (years)	11	0.98 (0.95–1.01)	0.235	25	0.99 (0.97–1.02)	0.603
Race/Ethnicity						
NH White	10	0.70 (0.08–6.03)	0.744	19	1.71 (0.62–4.73)	0.303
Other	1	1 (Reference)		6	1 (Reference)	

Abbreviation: NH White, non-Hispanic White

groups also receiving temozolomide and radiotherapy. This trial was designed with primary endpoints of PFS and OS. In September 2019, it was announced that no improvement in PFS was seen, while follow-up for OS is ongoing. An exploratory phase I cohort within CheckMate 143 evaluated the combination of nivolumab with the anti–CTLA-4 drug, ipilimumab (23). Limited conclusions on efficacy can be drawn from this small cohort, but treatment-related adverse events leading to treatment discontinuation were more frequent in ipilimumab-treated patients. The results of a phase I trial of the PD-1 inhibitor pembrolizumab in the neoadjuvant setting for patients with surgically resectable recurrent GBM were more encouraging (24). In this small trial of 35 patients, the cohort that received neoadjuvant pembrolizumab demonstrated an OS of 13.7 months compared with 7.5 months for patients who received pembrolizumab only postoperatively. Further investigation is needed to evaluate this approach.

In contrast to checkpoint inhibitor immunotherapy, which "releases the brakes" of the immune system in a generalized fashion, DC vaccine therapies are designed to elicit a tumor-specific cytotoxic T-cell response. Since 1999, we have conducted 6 investigator-initiated DC vaccine trials at our institution. We previously demonstrated that DC vaccination generates an immune response that corresponds to prolonged survival (6, 7, 9, 11). OS in these studies ranged from 15.4 months from a phase I acid eluted MHC-1–associated peptide-loaded DC study of 7 newly diagnosed patients (6) to 2.5 years of recurrent GBM patients in a phase I tumor lysate-loaded phase I DC study (7). An analysis of the 17 patients with newly diagnosed GBM who participated in a phase I trial of a multiepitope-pulsed DC vaccine in conjunction with standard chemoradiation demonstrated encouraging PFS of 16.9 months and OS of 38.4 months (10). A follow-up randomized multicenter phase II trial using this approach demonstrated a nonsignificant trend towards improved OS (18.3 months vs. 16.7 months with placebo injection), and a significant improvement in the secondary outcome measure of PFS (11.2 months vs. 9.0 months; HR, 0.57; $P = 0.011$; ref. 25). OS was 23.1 months in the subgroup of patients with HLA-A2 haplotype with confirmed immune response by IFNγ assay (25). Interim results of the phase III trial, DCVax-L, in which patient-derived DCs are pulsed with autologous tumor lysate, are also encouraging, although unblinding has not yet been performed for this randomized study that allows crossover at recurrence (26). The variance of survival outcomes from small early phase trials and that of larger randomized trials warns of the hazards of putting too much weight on the clinical outcomes of early phase trials with selected patients that may be impacted by subject and investigator biases.

The clinical outcomes reported in this small phase I trial compare favorably with historical controls. CheckMate 143, as an example, reported PFS of 1.5 months and OS of 9.8 months for patients with recurrent GBM treated with nivolumab, whereas PFS and OS were 3.22 months and 11.97 months in this trial (22). The landmark phase III EORTC–NCIC trial that established temozolomide as standard of care for newly diagnosed GBM demonstrated PFS and OS of 6.9 months and 14.6 months respectively, compared with 8.75 months and 20.26 months in this trial (1). More recently, EF-14, a randomized phase III trial evaluating the addition of tumor-treating fields to standard of care for patients with newly diagnosed GBM demonstrated OS of 20.9 months (2). OS for the cohort of patients that were randomized to standard treatment without tumor-treating fields was

16 months. The patients with newly diagnosed GBM in the trial reported here did not receive tumor-treating fields following radiotherapy.

It is important to note that, as with other trials, patients who participated in this study were motivated, had good functional status, and were otherwise relatively healthy. Patients enrolled in this study also had a low steroid requirement. In addition, most patients underwent gross total or near–gross total resection. In the EORTC–NCIC trial, OS was 18.8 months for patients who had gross total resection at diagnosis (27). In the EF-14 trial, gross total resection was associated with OS of 18.5 months in patients who did not receive tumor-treating fields, and 22.6 months in those who did (2).

In previous DC vaccine trials performed at our institution which used either autologous tumor lysate, tumor-associated peptides or epitopes to pulse the DCs generated measurable immune response by IFNγ in approximately half of the patients (6–11). We used cytotoxic T-cell response measured by an IFNγ ELISpot assay as an indicator of immune activation. This decision was based on our previous research which has shown that neurosphere-pulsed DCs induce stronger cytotoxic T-cell response against 9 L tumor cells. In this trial, immune response was elicited in only 36% of assessable patients. The proportion of responder patients after DC vaccination was consistent in our previous trials (6, 7, 25). Potentially, the use of an allogeneic GBM stem-like cell line may have affected the ability of the DCs to generate an endogenous immune response. Alternatively, the IFNγ assay was performed by stimulating patient-derived PBMCs with lysate derived from the allogeneic GBM stem-like cell line; perhaps a more relevant measure of immune response would have been to assess IFNγ response to autologous tumor or autologous neurospheres. The generation of autologous neurospheres for each patient, particularly recurrent patients, may represent a technical challenge. In fact, this technical challenge was the main impetus to use an allogeneic line rather than autologous neurospheres for vaccine generation. It is also unclear exactly how well the IFNγ assay captures the immune response, as non-CNS cancer vaccine trials have not demonstrated a significant correlation between any immune metric and clinical outcome.

In summary, the results obtained from this phase I DC vaccine trial are encouraging and add to the body of evidence suggesting that immunotherapy may play a role in the treatment of GBM. Many questions remain: Which tumor antigen source elicits the strongest immune response? What is the optimal timing and dosing for vaccine therapy? Which treatments—including other immunotherapies—synergize with vaccine therapy? These questions and others need to be addressed in future studies.

Authors' Disclosures

J.D. Rudnick reports other support from Novocure outside the submitted work. J.S. Yu reports a patent for US9023338B2 Cancer stem cell antigen vaccines and methods issued and licensed to Precision Lifesciences Group. No disclosures were reported by the other authors.

Authors' Contributions

J.L. Hu: Conceptualization, resources, data curation, formal analysis, supervision, investigation, methodology, writing–original draft, project administration, writing–review and editing. **O.A. Omofoye:** Writing–review and editing. **J.D. Rudnick:** Resources, funding acquisition, investigation. **S. Kim:** Formal analysis, visualization. **M. Tighiouart:** Formal analysis, visualization. **S. Phuphanich:** Resources. **H. Wang:** Investigation. **M. Mazer:** Investigation, project administration. **T. Ganaway:** Data curation. **R.M. Chu:** Resources. **C.G. Patil:** Resources. **K.L. Black:** Conceptualization, resources, supervision, funding acquisition. **S.L. Shiao:** Formal analysis, writing–review and editing. **R. Wang:** Formal analysis, writing–review and editing. **J.S. Yu:** Conceptualization, resources, formal analysis, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing.

Acknowledgments

This study was supported by grant # NCT02010606.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 7, 2021; revised September 27, 2021; accepted November 23, 2021; published first December 2, 2021.

References

1. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005;352:987–96.
2. Stupp R, Taillibert S, Kanner A, Read W, Steinberg DM, Lhermitte B, et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. JAMA 2017;318:2306–16.
3. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 2009;27:4733–40.
4. Chinot OL, de La Motte Rouge T, Moore N, Zeaiter A, Das A, Phillips H, et al. AVAglio: phase III trial of bevacizumab plus temozolomide and radiotherapy in newly diagnosed glioblastoma multiforme. Adv Ther 2011;28:334–40.
5. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med 2014;370:699–708.
6. 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.
7. 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.
8. Wheeler CJ, Das A, Liu G, Yu JS, Black KL. Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. Clin Cancer Res 2004;10:5316–26.
9. Wheeler CJ, Black KL, Liu G, Mazer M, Zhang XX, Pepkowitz S, et al. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. Cancer Res 2008;68:5955–64.
10. Phuphanich S, Wheeler CJ, Rudnick JD, Mazer M, Wang H, Nuño MA, et al. Phase I trial of a multiepitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. Cancer Immunol Immunother 2013;62:125–35.
11. Rudnick JD, Sarmiento JM, Uy B, Nuno M, Wheeler CJ, Mazer MJ, et al. A phase I trial of surgical resection with Gliadel Wafer placement followed by vaccination with dendritic cells pulsed with tumor lysate for patients with malignant glioma. J Clin Neurosci 2020;74:187–93.
12. Qiang L, Yang Y, Ma YJ, Chen FH, Zhang LB, Liu W, et al. Isolation and characterization of cancer stem-like cells in human glioblastoma cell lines. Cancer Lett 2009;279:13–21.
13. Suvà ML, Rheinbay E, Gillespie SM, Patel AP, Wakimoto H, Rabkin SD, et al. Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. Cell 2014;157:580–94.
14. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 2003;63:5821–8.
15. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756–60.
16. Chen J, Li Y, Yu T-S, McKay RM, Burns DK, Kernie SG, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 2012;488:522–6.

17. Xu Q, Liu G, Yuan X, Xu M, Wang H, Ji J, et al. Antigen-specific T-cell response from dendritic cell vaccination using cancer stem-like cell–associated antigens. Stem Cells 2009;27:1734–40.
18. Chukwueke UN, Wen PY. Use of the response assessment in neuro-oncology (RANO) criteria in clinical trials and clinical practice. CNS Oncol 2019;8:CNS28.
19. Czerkinsky C, Andersson G, Ekre HP, Nilsson LA, Klareskog L, Ouchterlony O. Reverse ELISPOT assay for clonal analysis of cytokine production. I. Enumeration of gamma-interferon–secreting cells. J Immunol Methods 1988;110:29–36.
20. Cox DR. Regression models and life-tables. Journal of the Royal Statistical Society. Series B 1972;34:187–220.
21. Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. Biometrika 1994;81:515–26.
22. Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, et al. Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: The CheckMate 143 phase III randomized clinical trial. JAMA Oncol 2020;6:1003–10.
23. Omuro A, Vlahovic G, Lim M, Sahebjam S, Baehring J, Cloughesy T, et al. Nivolumab with or without ipilimumab in patients with recurrent glioblastoma: results from exploratory phase I cohorts of CheckMate 143. Neuro Oncol 2018;20:674–86.
24. Cloughesy TF, Mochizuki AY, Orpilla JR, Hugo W, Lee AH, Davidson TB, et al. Neoadjuvant anti–PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. Nat Med 2019;25:477–86.
25. Wen PY, Reardon DA, Armstrong TS, Phuphanich S, Aiken RD, Landolfi JC, et al. A randomized double-blind placebo-controlled phase II trial of dendritic cell vaccine ICT-107 in newly diagnosed patients with glioblastoma. Clin Cancer Res 2019;25:5799.
26. Liau LM, Ashkan K, Tran DD, Campian JL, Trusheim JE, Cobbs CS, et al. First results on survival from a large phase III clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. J Transl Med 2018;16:142.
27. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomized phase III study: 5-year analysis of the EORTC–NCIC trial. Lancet Oncol 2009;10:459–66.