Journal of Clinical Neuroscience xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Clinical study

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

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ARTICLE INFO

Article history:
Received 8 September 2019
Accepted 2 March 2020
Available online xxxx

Keywords:
Glioblastoma (GBM)
Anaplastic astrocytoma (AA)
Anaplastic oligodendroglioma (AO)
Vaccine therapy
Gliadel Wafer
Overall survival (OS)
Progression-free survival (PFS)

ABSTRACT

High grade gliomas are associated with poor prognosis and high mortality. Conventional treatments and management of high grade gliomas have shown little improvement in 5-year overall survival. This phase I trial evaluated the safety, immunogenicity, and potential synergy of surgical resection with Gliadel Wafer implantation, followed by autologous tumor lysate-pulsed dendritic cell (DC) vaccine in patients with malignant glioma. Primary end points of this study were safety and surrogate markers of immunogenicity, overall survival, and progression free survival. Following surgical resection, Gliadel Wafers were placed along the resection cavity. Patients subsequently received intradermal injections of autologous tumor lysate-pulsed DC vaccines 3 times at 2 week intervals. Treatment response was evaluated clinically and through MRI at regular intervals. Twenty-eight patients received Gliadel Wafers and DC vaccination: 11 newly diagnosed (8 glioblastoma [GBM], 2 anaplastic astrocytoma [AA], and 1 anaplastic oligodendroglioma [AO]) and 17 recurrent (15 GBMs, 1 AA, and 1 AO) high grade gliomas. Immunogenicity data was collected for 20 of the 28 patients. Five of 20 patients showed elevated IFN-γ responses following vaccination. Median progression-free survival and overall survival for all GBM patients in the trial from the start of vaccination were 3.6 months and 16.9 months respectively. Comparisons between vaccine responders and non-vaccine responders were not statistically significant. Adjuvant autologous dendritic cells pulsed with tumor-lysate following resection and Gliadel Wafer placement is safe, elicits modest immunogenicity and shows similar clinical outcomes in patients who had DC vaccination in previous studies.

1. Introduction

High grade gliomas are associated with poor prognosis and high mortality. Annual incidence of high grade gliomas is about 5/100,000 [1,2]. Current standard therapy for high grade gliomas including WHO grade IV glioblastoma (GBM) is maximal resection, followed by radiation, and chemotherapy with a median survival of 14.6 months [3]. Despite advancements in treatment, gliomas typically recur locally near resection margins.

Recurrent gliomas have been treated with nitrosureas such as, BCNU[1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine)], showing safety and modest clinical efficacy [4–6]. Gliadel Wafers (biodegradable carmustine) were developed for intracranial placement along the resection cavity following maximal resection. It bypasses the blood brain barrier and decreases systemic toxicities while providing direct, prolonged, and high dose alkylating effects to residual tumor cells [7]. Gliadel Wafer treatments have shown slight improvements in overall survival in recurrent and newly diagnosed malignant gliomas [4,6].

Advancements in targeted antigen adjuvant therapies and immunotherapies can induce tumor immunogenicity. Autologous

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https://doi.org/10.1016/j.jocn.2020.03.006
0967-5868/

dendritic cell (DC) therapies pulsed with known tumor associated antigens or tumor lysate showed safety and hints at efficacy in treating cancer including glioblastoma [8–10]. Pulsing dendritic cells with patient tumor lysate offers the advantage of a unique patient regimen of glioma specific antigens. This strategy can be beneficial since high grade gliomas are typically non-homogenous, adding to the difficulty of treatment and causing eventual relapse. A prior phase II trial for GBM showed an expansion of $CD8^+$ T-cells and cytotoxic T-lymphocytes (CTL) against tumor associated antigens such as MAGE-1, gp100, and HER-2 in 4/9 patients and systemic cytotoxicity response of peripheral blood mononuclear cells (PBMCs) in 6/10 patients when dendritic cells were pulsed with tumor-lysate [9]. Anecdotally, patients who had longer OS were treated with systemic BCNU or Gliadel Wafers (intracranial BCNU). Other studies have also shown a 53% patient response rate and correlation between autologous tumor-lysed DC vaccine response and increased OS [11]. Interestingly, a correlation was seen between vaccine response followed by chemotherapy to time to progression suggesting tumor sensitization to chemotherapy. Together, these findings provided a rationale to investigate the addition of Gliadel Wafers to autologous dendritic cells loaded with tumor-lysate.

2. Methods

2.1. Study design

This is a single-center, phase I non-randomized trial. After consent was obtained, patients underwent maximal surgical resection with placement of Gliadel Wafers. Each Gliadel Wafer contains approximately 7.7 mg of carmustine. Up to 8 Gliadel Wafers were placed to cover the entire resection cavity and secured with Surgicel (oxidized cellulose). Patients with recurrent malignant glioma were screened approximately two weeks after resection (physical and neurologic evaluation, blood draw, and quality of life evaluations) with Fact-Br. One week after screening, patients underwent leukapheresis. Two weeks after leukapheresis, patients received 3 vaccines (containing up to 5×10^7 tumor lysate-pulsed dendritic cells) at 2-week intervals intradermally in the axilla. Four weeks after the last vaccination, patients underwent brain MRI and a CTL assay to determine CTL response. Other clinical and laboratory evaluations were performed routinely or as clinically indicated. Clinically stable patients were monitored with serial MRIs every 2 months. Patients were taken off trial for radiological recurrence per McDonald criteria. This study design protocol was submitted, reviewed, and approved by our IRB committee.

2.2. Patient selection

Patients 18 years or older with histologically confirmed GBM or malignant glioma including anaplastic astrocytoma (AA) and anaplastic oligodendroglioma (AO) were eligible for screening. Inclusion criteria included a Karnofsky score of at least 60%, 2 weeks from last administration of steroids prior to vaccine, and normal hematological parameters. Patients must have undergone maximal surgical resection of malignant glioma and placement of Gliadel wafers. Exclusion criteria included pregnancy; severe pulmonary, cardiac, or other systemic disease associated with an unacceptable anesthetic or operative risk; presence of an acute infection requiring active treatment; history of an autoimmune disorder or allergy to gentamicin; positive serology for hepatitis B, hepatitis C, HIV I/II, syphilis, HTLV I/II, HCV; contraindication to MRI. Patients' age, gender, tumor location, treatment received, extent of tumor resection and treatment with chemotherapy following vaccine were reported.

2.3. Autologous tumor culture

Postoperative tumor samples were processed for tissue culture by mincing them with scissors and passing them through metal meshes of decreasing pore size. The cell suspension was then plated onto tissue culture flasks and grown in DMEM/F10 (Irvine Scientific, Santa Ana, CA) plus 10% FCS (Irvine Scientific) and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA).

2.4. Preparation of autologous DCs

PBMCs were obtained fresh before vaccination using leukapheresis. A COBE Spectra Apheresis System was used to harvest the mononuclear cell layer. Leukapheresis yielded 10^{10} PBMCs. To generate autologous DCs, adherent PBMCs were cultured in complete medium for 7 days in the presence of recombinant human granulocyte macrophage colony-stimulating factor (800 units/ml; clinical grade; Immunex, Seattle, WA) and recombinant human interleukin (IL)-4 (500 units/ml; R&D Systems, Minneapolis, MN).

2.5. Pulsing of autologous DCs with autologous tumor lysate

On the day before each of the three DC vaccinations (days – 1, 13, and 27), DC cultures containing 10^7–10^8 cells were washed in RPMI 1640 with autologous patient serum supplemented with 50 μg/ml autologous tumor lysate. The DCs were incubated overnight for 18 h at 37 °C with tumor lysate on a tissue rotator to facilitate their interaction.

2.6. DC functional assay and vaccine administration

For the functional assay, DCs irradiated with 2500 rads were resuspended in RPMI 1640–10% human AB blood phenotype serum at 2×10^5 cells/ml. Allogeneic PBMCs were mixed with DCs. Phytohemagglutinin alone was added to PBMCs as a positive control. RPMI 1640 culture medium alone added to PBMCs constituted a negative control. All assays were performed in triplicate. The assay plate was incubated for 6 days in a 37 °C/5% CO_2incubator. [^{3}H] Thymidine (1 μCi/well) was added for the final 18 h of culture (i.e., on day 5). Cells were harvested with a Harvester 96-cell harvester (Tomtec, Hamden, CT), and ^{3}H counts were determined with a Microbeta 1450 Trilux liquid scintillation counter (Wallac, Gaithersburg, MD). Patients received 10^7-10^8 tumor-specific, tumor lysate-pulsed DCs s.c. in 0.5 ml of PBS in the deltoid region. Three vaccinations at 2-week intervals were administered.

2.7. Quantitative real-time PCR

Gene expression was measured with the use of the Icycler System (Bio-Rad) as described previously [12]. Primers and TaqMan probes (Qiagen) were designed to span exon-intron junctions to prevent amplification of genomic DNA and also to produce amplicons of fewer than 150 bp to enhance the efficiency of PCR amplification. DNA standards were generated by PCR amplification of gene products and purification, whereas quantification was determined by spectrophotometry (absorbance at 260 nm). The number of copies was calculated by dividing the total sample weight by the molecular weight of each gene amplicon. Real-time PCRs of cDNA specimens and DNA standards were conducted in 25 μl with 1 × TaqMan Master Mix (Perkin-Elmer Corp). Primers were used at 400–600 nM, and probes were used at 160 nM. Standard thermal cycler parameters were used and standard curves were generated for both IFN-γ and CD8α. PCR efficiency was assessed and was between 90% and 100%. Linear regression analysis of all standard curves demonstrated a coefficient of determination (R2) of 0.99

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or higher. Standard curve extrapolation of each copy number was performed for both IFN-γ and CD8α. Normalization of sample data was done by dividing the number of copies of IFN transcripts by the number of copies of CD8α transcripts, representing the relevant cell population.

Data were adjusted for CD8 mRNA copies on the basic immunological assumption that stimulation with a HLA class I-restricted epitope defines CD8 + T cells as the only relevant population. We calculated the ratio of IFN-γ mRNA (corrected for CD8 mRNA) obtained from PBMCs stimulated with autologous tumor lysate to that obtained from PBMCs stimulated without tumor lysate. The cutoff value for tumor-specific IFN-γ was derived by analyzing the IFN-γ:CD8 ratios in PBMCs obtained from all patients post-vaccination versus pre-vaccination. A cutoff value of 1.5 is standard for evidence of vaccine-related tumor-specific cytotoxic response [12].

2.8. Statistical methods

Statistics were reported for 28 patients. The Kaplan-Meier estimation method was used to obtain median survival times and survival probabilities Individual progression free and overall survival times were calculated from surgery and vaccine. The survival time from vaccine was calculated as the time of first vaccine to the time of death. Patients still alive were considered as censored in the survival analysis. A p-value less than or equal to 0.05 was considered to be statistically significant. All statistical analyses were conducted in SAS 9.2 (SAS Institute, Cary, NC, USA).

3. Results

A total of 35 patients were consented between March 2007 and November 2009 with 21 (60%) being female and 14 male (40%) with 30 Caucasian Non-Hispanic, 1 Hispanic, 1 Asian, and 3 African-Americans. Twenty-eight (80%) of the 35 consented received the DC vaccine. Of the 7 patients excluded, one patient failed the screen, while the remaining 6 received surgery without vaccine due to rapid tumor progression. Twenty-eight patients (10 males and 18 females) were enrolled with a median age of 55.5 years (range: 25–72 years) (Table 1). Patients were verified histologically with 17 recurrent (15 GBM, 1 AA, and 1 AO) and 11 newly diagnosed (8 GBM, 2 AA, and 1 AO) high grade gliomas. Twenty four high-grade glioma patients completed the trial receiving all 3 vaccines (20 GBMs: 8 newly diagnosed and 12 recurrent). The median KPS was 90, ranging between 60 and 100.

3.1. Summary of systemic toxicities

No severe adverse events (SAE) were reported in this study. Adverse events of grade I fatigue, mild aphasia, dizziness, dry mouth, speech difficulties, cough, and poor appetite were reported. Transient swelling and erythema of the injection site was commonly noted. One case of Grade II macular rash was reported.

3.2. Immunogenicity

Immunogenicity data was collected for 20 of the 28 patients (8 newly diagnosed and 12 recurrent GBMs). The remaining 8 patients either did not complete all 3 rounds of DC vaccinations or were non-GBM (AA/AO). Twenty-five percent of patients (5/20) were considered responders (≥1.5 fold increase in reference gene normalized IFN-γ post vaccination). Of the responders, 80% (4/5) were recurrent GBM patients (Table 2). Endogenous responders were defined as patients that had ≥1.5 fold increase in reference gene normalized IFN-γ pre-vaccination that were not responders. There was no observable association between immunogenicity response and clinical response.

3.3. Clinical outcomes

Patient specific factors, tumor location, extent of surgical resection, immune response, as well as overall survival (OS) and progression free survival (PFS) from vaccine is provided in Table 2. Patients that were still alive and have not yet progressed are denoted with asterisks. GBM patients had a median OS and PFS from the start of vaccination of 16.9 and 3.6 months, respectively (Table 3, Fig. 1ab). Twelve month PFS and OS were 21.4% and 60.7% respectively. Newly diagnosed GBM patients had a median PFS of 4.8 months and OS of 27.7 months (12 month PFS = 25%; 12 month OS = 87.5%) while the recurrent GBM patients had a median PFS of 1.9 months and OS of 10.9 months (12 month PFS = 13.3%; 12 month OS = 40%). Four patients were discontinued prior to receiving 3 doses of the DC vaccine. Patient 1 and 9 were discontinued from the trial after the first vaccine due to disease progression; patient 14 and 24 were discontinued from the trial after the second vaccine. Three patients (8, 22, and 26) have had prolonged survival and two patients (8 and 22) have not progressed (Table 2).

There were 5 (25%) responders and 15 (75%) non-responders among the 20 GBM patients who received the DC vaccine (Table 4). Overall survival from vaccine for all GBM responders was 10.9 months (95% CI: 6.3–39.5) and PFS was 1.8 months (95% CI: 1.1–12.9) (Fig. 2). Newly diagnosed GBM patients comprised 8 patients, of which only 1 (12.5%) demonstrated vaccine response and 7 (87.5%) were non-responders. This patient had OS and PFS from vaccine of 10.5 and 1.8 months, respectively. The remaining 12 patients were recurrent GBM cases (4 [33.3%] responders and 8 [66.7%] non-responders). The OS and PFS from vaccine for recurrent GBM responders were 19.9 months (95% CI: 6.3–39.5) and 2.7 months (95% CI: 1.1–12.9).

Among 23 GBM patients, 12 (52%) had received adjuvant chemotherapy following vaccine (Table 5). Overall survival from vaccine for these patients was 19.3 months (95% CI: 5.6–39.1) vs. 10.9 months (95% CI: 8.2–39.5, p-value 0.7521) in 11 patients that did not receive chemotherapy. Of the 8 newly diagnosed GBM patients, 5 had received chemotherapy (OS: 39.1 mo, 95% CI: 13.1–43.9) vs. 3 without chemotherapy (OS: 16.4 mo, 95% CI:10.5-not yet reached, p-value 0.9842). Finally, 7/15 (46.7%) recurrent GBM patients underwent adjuvant chemotherapy following vaccine (OS: 17.4 mo, 95% CI: 3.6–21.9) vs. 8/15 (53.3%) patients without chemotherapy (OS: 10.6 mo, 95% CI: 6.3–39.5, p-value 0.6795). PFS from vaccine for all GBM patients and newly diagnosed GBM patients showed a higher trend in the adjuvant chemotherapy groups, but the recurrent GBM cohort that did not receive chemotherapy had non-statistically significantly longer PFS after vaccine compared to those that did (OS: 1.9 mo, 95% CI 1.1–7.6 vs. OS: 0.7 mo, 95% CI 0.1–6.4, p-value 0.4249).

4. Discussion

We studied the safety and clinical outcomes by combining Gliadel Wafers and DC vaccines in patients with newly diagnosed and recurrent high grade gliomas. In comparison to retrospective cohort and randomized control studies with Gliadel Wafers as well as other studies with Temozolomide, radiotherapy, and Gliadel Wafers (median OS of 20.7 months) for the treatment of newly diagnosed malignant gliomas, our study showed significantly higher median OS from surgery (32 months, 95% CI: 15.8–43.7

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Table 1
Patient characteristics and treatment.

Patient	Age	Gender	Tumor Location	Treatment	Chemotherapy after vaccine
1	66	M	L. Frontal	TMZ, Rad, Av, CPT-11	Yes*
2	56	F	L. Temporal	TMZ, Rad, Av	No
3	72	F	L. Occipital	TMZ, Rad, Av, X-knife	Yes
4	54	F	L. Frontal	TMZ, Rad	Yes
5	63	F	R. Temporal	TMZ, Rad	Yes
6	55	F	L. Parietal	TMZ, Rad, Av, CPT-11	No
7	39	F	R. Frontal	TMZ	No
8	25	F	L. Temporal	TMZ, Rad	Yes
9	41	M	L. Temporal	TMZ, Rad, BCNU, CCNU	Yes*
10	53	M	R. Parietal	TMZ, Rad, Av, CPT-11, PCV	No
11	59	F	R. Temporal	TMZ, Rad, Gliasite	Yes
12	65	M	R. Parietal	TMZ, Rad	No
13	69	F	L. Frontal	TMZ, Rad, Av	No
14	37	M	R. Temporal	TMZ, Rad, Av, CPT-11, Nexavar Etoposide	Yes*
15	37	M	L. Temporal	TMZ, Rad	Yes
16	59	F	L. Parietal	TMZ, Rad	Yes
17	47	M	R. Parietal	TMZ, Rad	No
18	30	F	R. Frontal	TMZ, Rad	No
19	63	M	R. Frontal	TMZ, Rad, Av	Yes
20	56	F	R. Frontal	TMZ, Rad, Av, CPT-11, Carboplatin	No
21	61	F	L. Parietal	TMZ, Rad	Yes
22	42	F	L. Frontal	TMZ, Rad	Yes
23	61	F	L. Temporal	TMZ, Rad	No
24	39	M	R. Frontal	TMZ, Rad, Av, CCNU, Carboplatin	Yes*
25	26	F	R. Temporal	TMZ, Rad	Yes
26	63	F	L. Parietal	TMZ, Rad	No
27	43	M	L. Temporal	TMZ, Rad, X-knife, Peptide Vaccine	Yes
28	66	F	R. Temporal	TMZ, Rad	No

Table 2
Demographics, survival/progression data, and vaccine response for 28 patients.

Patient	Age	Diagnosis	Resection	KPS	Survived Time (months)		Time to Progression (months)		Immune Response
					From surgery	From vaccine	From surgery	From vaccine	
1	66	GBM-R	ICR	90	5.13	3.65	1.68	0.2	NA***
2	56	GBM-R	CR	90	9.67	8.15	3.39	1.87	Non-responder
3	72	GBM-N	CR	90	18.18	13.05	8.98	3.85	Non-responder
4	54	AA-N	CR	90	36.53	31	13.35	7.82	NA***
5	63	GBM-N	CR	100	43.73	39.06	30.15	25.48	Non-responder
6	55	GBM-R	PR	100	19.3	10.26	16.8	7.76	Non-responder
7	39	AO-R	ICR	80	56.45	54.31	8.09	5.95	NA***
8	25	AA-N	ICR	80	122.24*	118.72*	122.24**	118.72**	NA***
9	41	GBM-R	CR	90	23.01	21.3	1.84	0.13	NA***
10	53	GBM-R	ICR	90	40.73	39.52	4.87	3.65	Responder
11	59	GBM-R	ICR	100	18.84	17.39	2.1	0.66	Non-responder
12	65	GBM-R	ICR	80	9.9	8.61	2.76	1.48	Non-responder
13	69	GBM-N	ICR	80	15.78	10.49	7.07	1.78	Responder
14	37	GBM-R	CR	90	7.63	5.56	2.7	0.62	NA***
15	37	GBM-R	ICR	90	32.52	28.93	16.5	12.92	Responder
16	59	GBM-N	CR	90	18.21	14.6	15.09	11.47	Non-responder
17	47	GBM-R	CR	90	47.21	44.45	18.48	15.72	Non-responder
18	30	GBM-R	ICR	80	16.27	11.21	6.97	1.91	Non-responder
19	63	GBM-R	CR	60	27.48	21.9	11.97	6.38	Non-responder
20	56	GBM-N	CR	80	20.19	16.44	9.57	5.82	Non-responder
21	61	GBM-N	CR	80	47.64	43.89	7.2	3.45	Non-responder
22	42	GBM-N	CR	90	115.99*	112.54*	115.99**	112.54**	Non-responder
23	61	GBM-R	PR	90	12.36	10.92	2.53	1.08	Responder
24	39	AA-R	PR	90	13.18	11.8	1.84	0.46	NA***
25	26	AO-N	PR	90	33.3	29.85	23.41	19.96	NA***
26	63	GBM-N	CR	90	68.15*	64.64*	4.67	1.15	Non-responder
27	43	GBM-R	PR	80	13.18	9.63	6.97	3.42	Non-responder
28	66	GBM-R	ICR	100	8.25	6.28	3.72	1.74	Responder

* Patient is still alive; ** Patient has not progressed ICR: Incomplete Complete Resection.
Abbreviations: AA – Anaplastic Astrocytoma; AO – Anaplastic Oligodendroglioma; CR – Complete Resection; GBM – Glioblastoma Multiforme; KPS – Karnofsky Performance Score; PR – Partial Resection; N – Newly Diagnosed; R – Recurrent; NA – Not Available.
Immune response≥1.5 fold increase of normalized IFN-γ after vaccination.

[data not shown]) and from vaccine (27.7 months, 95% CI: 10.5–39.1) [6,13–15]. Similarly, our results in recurrent glioma treatment from surgery (median OS = 16.3, 95% CI: 8.3–23.0) had parallel overall survival as others which have shown median survival up to 14.5 months [7,13,16,17]. Overall survival and progression of patients with newly diagnosed gliomas with BCNU and autologous dendritic cells pulsed with tumor-lysate was significantly longer than those with recurrent gliomas (Fig. 1).

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Table 3
Median overall survival (OS), Progression Free survival rates, and 95% confidence intervals (CI) for GBM and Anaplastic Tumors from surgery and vaccine.

	Glioblastoma			Anaplastic*
	All Cases (N = 23)	Newly Diagnosed (N = 8, 34.8%)	Recurrent (N = 15, 65.2%)	AA/AO (N = 5)
Overall Survival From Vaccine				
Median OS (CI)*	16.9 (10.9–29.6)	27.7 (10.5–39.1)	10.9 (6.3–21.3)	31.0 (11.8–54.3)
Survival rates, % (CI)				
6-month	92.9 (74.3–98.2)	100%	86.7 (56.4–96.5)	100
12-month	60.7 (40.4–78.0)	87.5 (38.7–93.1)	40.0 916.5–62.8)	80.0 (20.4–97.0)
18-month	46.4 (27.7–63.3)	50.0 (15.2–77.5)	33.3 (12.5–56.4)	80.0 (20.4–97.0)
24-month	39.3 (21.7–56.5)	50.0 (15.2–77.5)	20.0 (4.9–43.9)	80.0 (20.4–97.0)
36-month	28.6 (13.5–45.6)	50.0 (15.2–77.5)	13.3 (2.2–34.6)	40.0 (6.0–75.3)
Progression Free Survival from Vaccine				
Median PFS (CI)	3.6 (1.7–6.4)	4.8 (1.2–25.5)	1.9 (0.6–3.6)	7.8 (0.5–20.0)
6-month	35.7 (18.9–53.0)	37.5 (8.7–67.4)	26.7 (8.3–49.6)	60.0 (12.6–88.2)
12-month	21.4 (8.7–37.8)	25.0 (3.7–55.8)	13.3 (2.2–34.6)	40.0 (5.2–75.3)
18-month	14.3 (4.5–29.5)	25.0 (3.7–55.8)	0	40.0 (5.2–75.3)
24-month	10.7 (2.7–25.1)	25.0 (3.7–55.8)	0	20.0 (0.8–58.2)
36-month	7.1 (1.2–20.4)	12.5 (0.7–42.2)	0	20.0 (0.8–58.2)

*AA: Anaplastic Astrocytoma, AO: Anaplastic Oligodendroglioma.

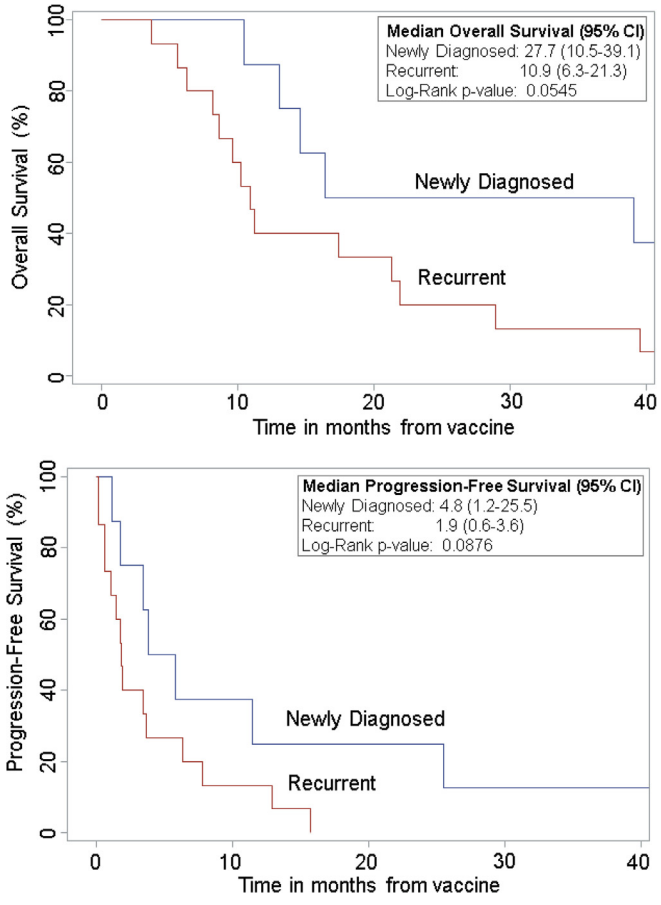

Fig. 1. ab: Kaplan Meier Curves of Overall (upper-panel) and Progression Free (lower-panel) Survival from vaccine for Newly Diagnosed and Recurrent GBM Patients.

Dendritic cell vaccines have shown success against GBMs when primed against total tumor-lysates. When immune response were stratified to recurrent GBM responders (n = 4) against recurrent GBM non-responders (n = 8), a survival benefit from vaccine adminstration was in slight favor of responders (median OS 19.9 months and 10.7 months; median PFS 2.70 months and 2.70 months, respectively). Comparisons were not statistically significant. Juxtaposed against our other tumor-lysed dendritic cell studies that reach response rates of up to 44%, this study's response rate was lower (25% overall and 33% for recurrent GBMs) [9]. It was originally suggested that endogenous responsiveness may proportionally reduce post-vaccine response due to a pre-existing T-cell activity, resulting in an immune mediated selection and increased glioma cancer stem cell gene expression [11,18,19]. Interestingly, a weak inverse correlation remains true in this study (Sup Fig. 1). Different from earlier studies, no relationship between post-vaccine IFN-γ levels to OS was observed. This can likely be explained by our low number of responders (it was suggested > 11 responders were required to observe this correlation). Furthermore, the relatively low post-vaccination immune response rate in this trial may have been secondary to the immunosuppressive effects of the Gliadel wafer intratumorally. Although the half-life of Gliadel is thought to be within several days, which would have disappeared long before the initiation of the first vaccination, there may have been lingering BCNU alkylating agent that may have prevented T cell proliferation and cytolysis. Although intracranial T cell suppression may not directly impact the peripheral T cell recognition of antigen as measured by the IFN-gamma response to tumor antigen, the localized T cell suppressive effect of intracranial Gliadel may have secondarily impacted T cell proliferation and recognition peripherally. An alternative explanation may be that the BCNU from the Gliadel wafer may have been distributed peripherally and impacted DC antigen presentation or T cell proliferation in the periphery. These are unconfirmed hypotheses that warrant testing in a syngeneic murine glioblastoma model.

No SAE reactions were reported thus confirming safe response to combination Gliadel and DC vaccine therapy. Quality of life (QOL) data in the form of Fact-Br collected for 13 GBM patients approximately before and 56 days after vaccination shows weak correlation to PFS (r = 0.32) and OS (r = 0.28). In addition to the changes in overall survival, 7/13 patients showed an improvement in Fact-Br score after initial vaccine therapy (data not shown). One major goal that was accomplished in this study was to demonstrate safety of a local cylotoxic chemotherapeutic effect with immunotherapy.

We were unable to match the median OS in our previous trial that evaluated recurrent glioblastoma patients treated with DC pulsed with tumor lysate (median OS 133 weeks for the study

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Table 4
Median overall survival (OS), progression-free survival (PFS), and 95% confidence intervals (CI) for GBM cohort by vaccine response status.

	All Cases (N = 20)		Newly Diagnosed (N = 8, 40%)		Recurrent (N = 12, 60%)	
	Responder	Non-responder	Responder	Non-responder	Responder	Non-responder
Cohort Size (%)	5 (25.0%)	15 (75.0%)	1 (12.5%)	7 (87.5%)	4 (33.3)	8 (66.7)
Median OS (CI)						
from surgery	15.8 (8.3–40.7)	19.3 (13.2–43.7)	15.8**	43.7 (18.2–43.7)	22.4 (8.3–40.7)	17.6 (9.7–27.5)
from vaccine	10.9 (6.3–39.5)	16.4 (9.6–39.1)	10.5**	39.1 (13.1–39.1)	19.9 (6.3–39.5)	10.7 (8.2–12.9)
Median PFS (CI)						
from surgery	4.9 (2.5–16.5)	9.0 (3.4–15.1)	7.1**	9.6 (4.7–30.1)	4.3 (2.5–16.5)	7.0 (2.1–16.8)
from vaccine	1.8 (1.1–12.9)	3.8 (1.5–7.8)	1.8**	5.8 (1.6–25.5)	2.7 (1.1–12.9)	2.7 (0.7–7.8)

All comparisons by responder status were non-significant.
** corresponds to one patient.

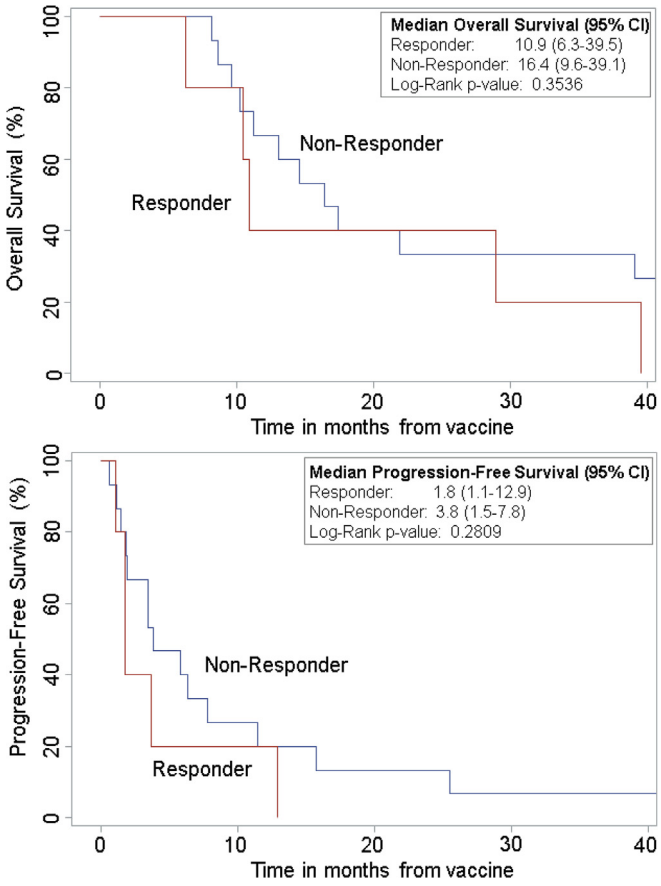

Fig. 2. ab: Kaplan Meier Curves of Overall (upper-panel) and Progression-Free (lower-panel) Survival for glioblastoma patients by vaccine response.

group [n = 8 patients] and median OS 30 weeks for the control group [n = 26 patients]) [9]. Interestingly, 5/9 (56%) of these recurrent glioblastoma patients were treated with intracranial BCNU alongside the same tumor lysed-vaccine regiment. Sixty percent (n = 6) of the prior recurrent GBM patients had a vaccine response rate. Our low number of vaccine responders and higher median patient age (55.5 years in this trial vs. 46 years in the previous trial) are likely contributors to this discrepancy.

Of note, 4 patients were discontinued due to rapid disease progression of recurrent GBMs (patients:1,9,14, and 24). This is common practice in many immunotherapy trials including the ACTIVATE. This practice would inflate the survival of patients in the recurrent GBM group who received less than 3 vaccines. In contrast to this, there were 3 newly diagnosed high grade glioma patients that were still alive at the time of this evaluation, 2 of which did not show progression. Patient 8 was an unknown responder diagnosed with AA. Patients 22 and 26 were non-responders diagnosed with GBM. Patients 22 and 26 GBM MGMT methylation status was >95% and were treated with alkylating agents, temozolomide and Gliadel Wafers. Patient 22 had a complete resection of the tumor in the frontal lobe which had high levels of EGFR (4 copies). These factors all are reported prognostic factors which may have contributed to these patients' prolonged survival [20–22]. Unfortunately, at the time of this study, MGMT was collected on only 13 patients' tumor samples and AGT data was not collected. Interestingly, patients with >95% methylated (n = 4) via IHC were all newly diagnosed GBM patients with a median OS of ~56 months.

Our study's recruitment period of 2007–2009 predates the most recent 2016 edition of the WHO Classification of Tumors of the Central Nervous System and was in a era where molecular markers that have important prognostic significance, such as IDH mutation, 1p19q co-deletion, ATRX mutation and MGMT promotor methylation status, were not routinely tested. Over the ensuing years our institution has adopted routine molecular profiling of each high grade glioma, with many cases now undergoing next generation sequencing for identification of salient genomic signatures. While the lack of molecular profiling would not have changed the overall results and conclusion of this study, it would have been interesting to stratify patients with select molecular profiles and evaluate immune response, PFS, and OS in a more granular context.

Table 5
Median overall survival (OS), progression-free survival (PFS) in months and 95% confidence intervals for GBM cohort by chemotherapy status.

	All GBM (n = 23)			Newly Diagnosed (n = 8)			Recurrent (n = 15)		
	Chemo (n = 12)	No Chemo (n = 11)	p value	Chemo (n = 5)	No Chemo (n = 3)	p value	Chemo (n = 7)	No Chemo (n = 8)	p value
OS from vaccine	19.3 (5.6–39.1)	10.9 (8.2–39.5)	0.7521	39.1 (13.1–43.9)	16.4 (10.5-NR)	0.9842	17.4 (3.6–21.9)	10.6 (6.3–39.5)	0.6795
PFS from vaccine	3.6 (0.2–12.9)	1.9 (1.2–5.8)	0.2977	11.4 (3.5–25.5)	1.8 (1.2–5.8)	0.0733	0.7 (0.1–6.4)	1.9 (1.1–7.6)	0.4249

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5. Conclusion

Adjuvant autologous dendritic cells pulsed with tumor-lysate and injected intradermally biweekly 3 times following maximal resection and Gliadel Wafer placement is safe therapeutic combination. This work showed clinical efficacy of DC vaccine and Gliadel wafers, but we observed a decreased immune response to DC vaccine compared with prior trials. To truly understand the impact of this treatment on a variety of patients, a randomized control trial study with stringent tumor characterization is needed. The modest survival figures noted in both the newly diagnosed and recurrent GBM population in this study do not support the presence of significant synergy of local chemotherapy with active immunotherapy.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jocn.2020.03.006.

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