JAMA Oncology | Original Investigation

Association of Autologous Tumor Lysate-Loaded Dendritic Cell Vaccination With Extension of Survival Among Patients With Newly Diagnosed and Recurrent Glioblastoma

A Phase 3 Prospective Externally Controlled Cohort Trial

Linda M. Liau, MD, PhD; Keyoumars Ashkan, MD, FRCP, FRCS; Steven Brem, MD; Jian L. Campian, MD, PhD; John E. Trusheim, MD; Fabio M. Iwamoto, MD; David D. Tran, MD, PhD; George Ansstas, MD; Charles S. Cobbs, MD; Jason A. Heth, MD; Michael E. Salacz, MD; Stacy D'Andre, MD; Robert D. Aiken, MD; Yaron A. Moshel, MD, PhD; Joo Y. Nam, MD; Clement P. Pillainayagam, MD; Stephanie A. Wagner, MD; Kevin A. Walter, MD; Rekha Chaudary, MD; Samuel A. Goldlust, MD; Ian Y. Lee, MD; Daniela A. Bota, MD, PhD; Heinrich Elinzano, MD; Jai Grewal, MD; Kevin Lillehei, MD; Tom Mikkelsen, MD, FRCPC; Tobias Walbert, MD; Steven Abram, MD; Andrew J. Brenner, MD, PhD; Matthew G. Ewend, MD; Simon Khagi, MD; Darren S. Lovick, MD; Jana Portnow, MD; Lyndon Kim, MD; William G. Loudon, MD; Nina L. Martinez, MD; Reid C. Thompson, MD; David E. Avigan, MD; Karen L. Fink, MD, PhD; Francois J. Geoffroy, MD; Pierre Giglio, MD; Oleg Gligich, MD; Dietmar Krex, MD; Scott M. Lindhorst, MD; Jose Lutzky, MD; Hans-Jörg Meisel, MD, PhD; Minou Nadji-Ohl, MD; Lhagva Sanchin, MD; Andrew Sloan, MD; Lynne P. Taylor, MD; Julian K. Wu, MD; Erin M. Dunbar, MD; Arnold B. Etame, MD, PhD; Santosh Kesari, MD, PhD; David Mathieu, MD; David E. Piccioni, MD, PhD; David S. Baskin, MD; Michel Lacroix, MD; Sven-Axel May, MD; Pamela Z. New, MD; Timothy J. Pluard, MD; Steven A. Toms, MD; Victor Tse, MD; Scott Peak, MD; John L. Villano, MD, PhD; James D. Battiste, MD, PhD; Paul J. Mulholland, MD; Michael L. Pearlman, MD; Kevin Petrecca, MD, PhD; Michael Schulder, MD; Robert M. Prins, PhD; Alton L. Boynton, PhD; Marnix L. Bosch, PhD

IMPORTANCE Glioblastoma is the most lethal primary brain cancer. Clinical outcomes for glioblastoma remain poor, and new treatments are needed.

OBJECTIVE To investigate whether adding autologous tumor lysate-loaded dendritic cell vaccine (DCVax-L) to standard of care (SOC) extends survival among patients with glioblastoma.

DESIGN, SETTING, AND PARTICIPANTS This phase 3, prospective, externally controlled nonrandomized trial compared overall survival (OS) in patients with newly diagnosed glioblastoma (nGBM) and recurrent glioblastoma (rGBM) treated with DCVax-L plus SOC vs contemporaneous matched external control patients treated with SOC. This international, multicenter trial was conducted at 94 sites in 4 countries from August 2007 to November 2015. Data analysis was conducted from October 2020 to September 2021.

INTERVENTIONS The active treatment was DCVax-L plus SOC temozolomide. The nGBM external control patients received SOC temozolomide and placebo; the rGBM external controls received approved rGBM therapies.

MAIN OUTCOMES AND MEASURES The primary and secondary end points compared overall survival (OS) in nGBM and rGBM, respectively, with contemporaneous matched external control populations from the control groups of other formal randomized clinical trials.

RESULTS A total of 331 patients were enrolled in the trial, with 232 randomized to the DCVax-L group and 99 to the placebo group. Median OS (mOS) for the 232 patients with nGBM receiving DCVax-L was 19.3 (95% CI, 17.5-21.3) months from randomization (22.4 months from surgery) vs 16.5 (95% CI, 16.0-17.5) months from randomization in control patients (HR = 0.80; 98% CI, 0.00-0.94; P = .002). Survival at 48 months from randomization was 15.7% vs 9.9%, and at 60 months, it was 13.0% vs 5.7%. For 64 patients with rGBM receiving DCVax-L, mOS was 13.2 (95% CI, 9.7-16.8) months from relapse vs 7.8 (95% CI, 7.2-8.2) months among control patients (HR, 0.58; 98% CI, 0.00-0.76; P < .001). Survival at 24 and 30 months after recurrence was 20.7% vs 9.6% and 11.1% vs 5.1%, respectively. Survival was improved in patients with nGBM with methylated MGMT receiving DCVax-L compared with external control patients (HR, 0.74; 98% CI, 0.55-1.00; P = .03).

CONCLUSIONS AND RELEVANCE In this study, adding DCVax-L to SOC resulted in clinically meaningful and statistically significant extension of survival for patients with both nGBM and rGBM compared with contemporaneous, matched external controls who received SOC alone.

TRIAL REGISTRATION ClinicalTrials.gov Identifier: NCT00045968

JAMA Oncol. doi:10.1001/jamaoncol.2022.5370
Published online November 17, 2022.

Multimedia

Supplemental content

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Marnix L. Bosch, PhD, Northwest Biotherapeutics, Inc, 4800 Montgomery Ln, Bethesda, MD 20814 (marnix@nwbio.com).

Glioblastoma is a highly lethal brain cancer, with a nearly 100% recurrence rate and dismal patient survival. Standard of care (SOC) for newly diagnosed glioblastoma (nGBM) includes surgery, radiotherapy, and chemotherapy. Following initial surgery, tumors typically recur in 6 to 8 months,[1] median overall survival (mOS) is 15 to 17 months, and 5-year survival is generally less than 5%.[2] For recurrent glioblastoma (rGBM), there is no established SOC.[3] Among more than 400 clinical trials since 2005, with more than 32 000 patients, testing diverse treatment modalities,[4] only 1 phase 3 trial in nGBM and no phase 3 trials in rGBM have demonstrated a survival benefit.[5]

We report the overall survival (OS) and safety outcomes of a phase 3 nonrandomized controlled trial testing an autologous tumor lysate-loaded dendritic cell vaccine (DCVax-L) combined with SOC for treatment of glioblastoma. Dendritic cells present tumor antigens to the immune system, prime T cells, and mobilize antitumor responses.[6,7]

Many trials, especially for incurable diseases, incorporate a crossover design for feasibility and/or ethical reasons. A crossover was considered necessary when our study began in 2007 to make patient enrollment and retention feasible when novel immunotherapies were not yet generally viewed as promising for cancer. The crossover was also important to justify the placebo group for patients undergoing a leukapheresis—an invasive procedure necessary for blinding and for manufacturing vaccine but offering no benefit to patients in the placebo group if they could not receive their autologous vaccine.

The crossover design necessitated the use of external controls to evaluate OS. Traditional (ie, within-study) randomized control comparisons were infeasible, since most placebo group patients received DCVax-L through the crossover. When randomized clinical trials (RCTs) are not feasible, use of external controls is increasingly recognized as an effective way to enable comparative analyses of outcomes.[8] There is also growing support for streamlining trials in the neuro-oncology field.[9,10]

Key Points

Question Is treatment with autologous tumor lysate-loaded dendritic cell vaccination (DCVax-L) associated with improved overall survival (OS) for patients with newly diagnosed glioblastoma (nGBM) and recurrent glioblastoma (rGBM) compared with standard of care (SOC)?

Findings In this phase 3 nonrandomized controlled trial of 331 patients, patients with nGBM receiving DCVax-L had a median OS of 19.3 months from randomization (22.4 months from surgery), while contemporaneous, matched external control patients treated with SOC had a median OS of 16.5 months from randomization; for patients with rGBM, median OS was 13.2 months from relapse in the DCVax-L group vs 7.8 months in the external control cohort. Meaningful increases in the long-term tails of the survival curves in both nGBM and rGBM were also observed.

Meaning In this study, adding DCVax-L to SOC was associated with a clinically meaningful and statistically significant improvement in median OS for patients with both nGBM and rGBM compared with matched, contemporaneous external controls.

Methods

Study Design and Oversight

This was originally a phase 3 randomized, double-blind clinical trial, with a crossover design. The trial was conducted at 94 sites in 4 countries (US, Canada, UK, and Germany). The screening and enrollment process and treatment assignment are described in **Figure 1**A and B.[11,12] The trial protocol appears in Supplement 1.

The primary end point was OS in patients with nGBM from the time of randomization (a median of 3.1 months after surgery), and the secondary end point was OS in rGBM from the time of recurrence. Each group was compared with independently selected, contemporaneous, matched external control patients as prespecified in the Statistical Analysis Plan (SAP) (Supplement 1).

The original primary end point in the 2007 study protocol was progression-free survival (PFS) determined by magnetic resonance imaging (MRI). However, while the trial was underway, the difficulty of distinguishing actual disease progression from pseudo-progression comprised of inflammation or necrosis or from vaccine-induced infiltration of immune cells was recognized.[13] Accordingly, the SAP for this study focused on OS.

On enrollment, patients were randomized 2:1 to either DCVax-L or placebo, plus SOC. Randomization was performed centrally by independent contract research organizations (CROs [Synteract, Parexel]).

Following tumor recurrence, all patients were allowed to cross over to start or continue receiving DCVax-L. The trial did not prescribe additional surgery at recurrence; the DCVax-L administered to crossover patients after recurrence was the product from the original surgery. All parties remained blinded to the treatment before crossover. Due to the crossover, the placebo group was depleted, and OS was assessed by comparison to external control populations (ECPs).

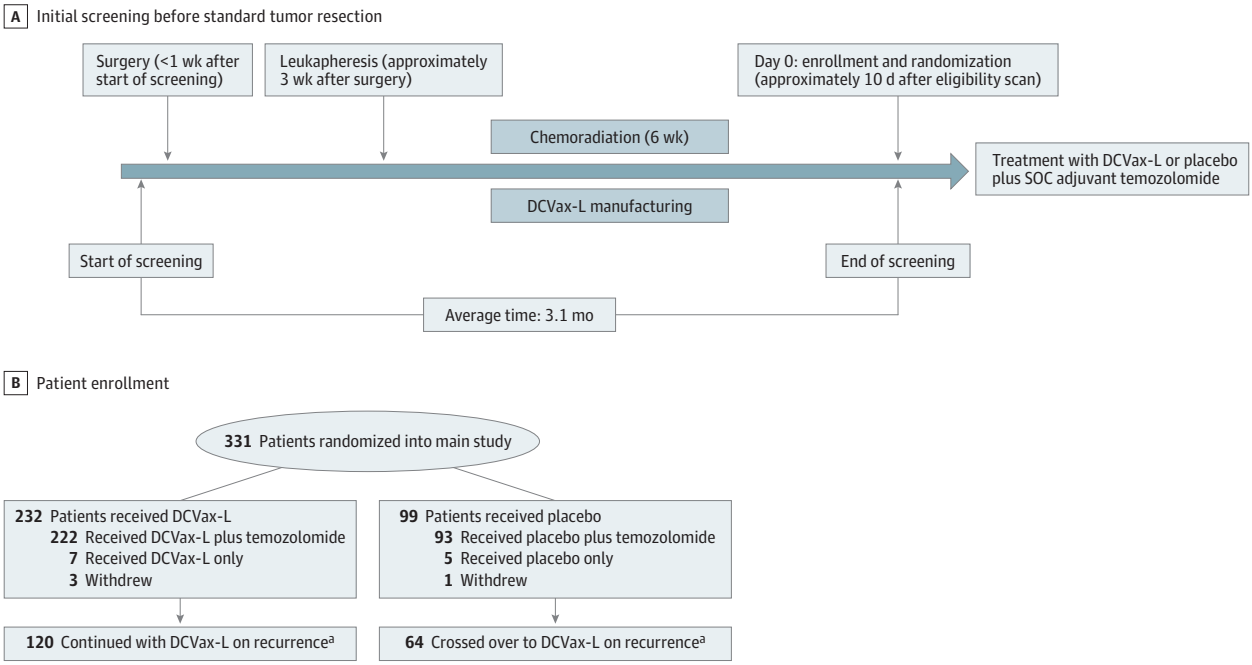
The protocol was approved by the relevant institutional review boards or ethics committees. The trial was performed in accordance with the Declaration of Helsinki.[14] The data were collected and held by independent CROs (Synteract, Parexel) and were analyzed by independent statisticians (Quantics). Patients gave informed consent for tumor collection in presurgery screening and thereafter gave consent for study participation (Figure 1A).

Patients and Study Procedures

Patients aged 18 to 70 years with nGBM (World Health Organization grade 4), Karnofsky Performance Score (KPS) of 70 or greater, life expectancy of 8 or more weeks, and adequate laboratory values were eligible for enrollment (Figure 1A). Patients centrally determined (ICON) to have radiographic evidence of early disease progression[15] following radiochemotherapy were excluded.

After initial diagnosis, all patients underwent surgery and collection of tumor tissue for manufacturing of DCVax-L. After surgery, diagnosis of glioblastoma was histologically con-

Figure 1. Study Design and Flow



A, After the surgery and centralized histological confirmation of glioblastoma, patients underwent leukapheresis to obtain their immune cells and then received 6 weeks of standard of care (SOC) radiochemotherapy, following the Stupp protocol.[11] The autologous tumor lysate-loaded dendritic cell vaccination (DCVax-L) products and placebo (peripheral blood mononuclear cells) were both manufactured for all prospective patients during the 6 weeks of radiochemotherapy, prior to enrollment. The manufacturing process for DCVax-L is described in Liau et al.[12] At the end of radiochemotherapy, patients who met the eligibility criteria were enrolled. B, Enrollment of patients into the study.

[a] (Re)started day 0, 10, and 20 and beyond DCVax-L vaccination regimen at recurrence.

firmed centrally (Quest Diagnostics; Mayo Clinic). The MGMT (O^6-methylguanine-DNA methyltransferase) gene promoter methylation status, IDH (isocitrate dehydrogenase) R132 mutation status, and postsurgery minimal (<2 cm^2) vs significant (≥2 cm^2) residual tumor were determined centrally (LabCorp; Mayo; ICON). The KPS was determined by the treating physician.

Patients underwent MRI before enrollment and every 2 months thereafter. Progression was assessed centrally (ICON) on a blinded basis. Adverse events were assessed throughout the study according to National Cancer Institute Common Terminology Criteria version 3.0.[17]

Patients received either DCVax-L or placebo on days 0, 10, and 20, then in months 2, 4, and 8 and months 12, 18, 24, and 30, with monthly temozolomide as SOC. Each DCVax-L dose comprised 2.5 million DCs injected intradermally in the upper arm, alternating arms between treatment visits. The placebo was unmanipulated peripheral blood mononuclear cells.

External Control Populations

The ECPs were determined by an independent expert firm (York Health Economics Consortium) and comprised patients from the control groups from contemporaneous RCTs closely matched to the current study based on 14 criteria prespecified in the SAP (Supplement 1). These studies met the "fit for purpose" criteria outlined by Mishra-Kalyani et al.[8] We compared the treatment groups of the external trials to the ECPs to validate the methodology, applied sensitivity analyses to check for biases, and conducted a matching-adjusted indirect comparison (MAIC)[18] to adjust for imbalances in individual patient characteristics (eAppendix 1 in Supplement 2).

Statistical Analysis

Primary End Point: OS in Patients With nGBM

All statistical analyses were conducted in SAS version 9.4 (SAS Institute). The primary end point was OS from randomization to death from any cause in patients with nGBM. The 1-sided significance level was 2.5%. The O'Brien-Fleming group sequential boundary function[19] and alpha-spending function of Lan and DeMets[20] were used to adjust for sequential testing of OS. The final analysis was conducted at the 1-sided 2.409% level. OS was analyzed using log-rank test at the appropriate α level. The hazard ratio (HR) and confidence intervals were calculated using the proportional hazards model with treatment as covariate. Individual control patient survival data were reconstructed by digitizing the published Kaplan-Meier (KM) curves.[16] The algorithm to extract individual patient level data from published KM curves uses as inputs the x- and y-coordinates from digitized KM curves, the reported numbers at risk at various time points (which accounts for censored participants), and the total number of events reported. It then ap-

plies an iterative process to reconstruct the KM parameters, from which the individual patient data are obtained. Full details on the method are found in Guyot et al.[16]

Secondary End Point: OS in Patients With rGBM

For the secondary end point, OS in patients with rGBM was measured from first recurrence to death from any cause. The 1-sided significance level allocated to this end point was 2.5%. OS was analyzed using the log-rank test, and the HR and 95% CIs were calculated as described previously.

Landmark Analyses and KM Survival Curve Tails

The KM estimates of landmark survival rates at 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60 months were calculated along with Hall-Wellner 2-sided 95% CIs[21] (adjusted for multiplicity). The numbers of patients available for comparison at late time points was small, especially in the control population, resulting in relatively wide confidence intervals.

Results

Patients and Treatments

From August 2007 to November 2015, 331 patients with nGBM were enrolled (Figure 1B). The median (range) age was 56 (19-73) years, 202 participants (61.0%) were men; 7 (2.1%), Black or African American; 16 (4.8%), Hispanic or Latino; and 294 (88.8%), White. Screening and enrollment were suspended from 2008 through 2011 due to the financial crisis, resumed on a limited basis in 2012, and 303 of the 331 patients (91.5%) were enrolled during 2012 to 2015.

All patients underwent surgical resection, recovery, leukapheresis, and 6 weeks of postoperative SOC radiochemotherapy prior to enrollment (Figure 1A). The median time from surgery to randomization was 3.1 months.

Of the 331 patients, 232 were randomized to initial DCVax-L treatment and 99 to placebo. Following tumor recurrence, 64 of the 99 patients in the placebo group crossed over to receive DCVax-L, while 120 of the 232 patients who had already received DCVax-L continued to receive DCVax-L. The patients, investigators, study team, and sponsor remained blinded to the treatments before crossover.

External Controls

The ECP for the primary end point (OS in nGBM) comprised 1366 patients with nGBM treated with SOC in the control groups of 5 comparator RCTs[5,22-25] (eTable 1 in Supplement 2). The ECP for the secondary end point (OS in rGBM) comprised 640 patients with rGBM at first recurrence treated with either SOC therapies (lomustine, bevacizumab, or best supportive care) or a placebo in the control groups of 10 comparator RCTs[26-35] (eTable 1 in Supplement 2).

The patient demographic characteristics and prognostic factors of the DCVax-L cohorts were well matched with the ECPs for both the primary and secondary end points, based on the 14 criteria prespecified in the SAP (**Table 1**; eAppendix and eTable 2 in Supplement 2). The analysis of each of the 15 comparator trials, substituting our ECP for the original control groups, confirmed that the outcomes were the same as originally reported (primary end point met or not met).

In the 5 sensitivity analyses conducted to address potential known and unknown confounders in the nGBM ECP, the HR results (range, 0.77-0.82) were comparable with the HR in all 5 studies included (HR, 0.80). In the sixth sensitivity analysis, dropping 2 of the 5 comparator studies[22,23] because it was not clear whether they had excluded patients with early progression, the HR remained the same (0.80 in both). The MAIC analyses adjusted for imbalances in individual patient characteristics between the patients receiving DCVax-L and the nGBM ECPs by applying a weight to each patient in the DCVax-L cohort to result in a match with the patient characteristics of the external populations.

Survival Outcomes

OS in Patients With nGBM

The mOS for patients with nGBM assigned to the DCVax-L cohort at enrollment was 19.3 (95% CI, 17.5-21.3) months from the time of randomization (22.4 months from surgery) compared with 16.5 (95% CI, 16.0-17.5) months from randomization for the 1366-patient ECP (log-rank HR, 0.80; 95% CI, 0.00-0.94; $P = .002$) (**Figure 2**A). The data indicate a 20% relative reduction in risk of death at any point in time for patients with nGBM receiving DCVax-L, and this relative survival benefit increased over time (**Table 2**): 15.7% of patients receiving DCVax-L vs 9.9% of ECP patients were alive at 48 months after randomization, and 13.0% of DCVax-L patients vs 5.7% of ECP patients were alive at 60 months after randomization. The long-term survivors tended to have favorable prognostic characteristics, but these factors did not fully explain the survival observed (eFigure 2 in Supplement 2). The outcome of the MAIC analyses showed that after adjustment for imbalances in individual patient characteristics the difference in OS between the DCVax-L cohort and the ECP was still significant.

Six prespecified subgroup analyses were conducted (Figure 2B and eFigure 1 in Supplement 2). Patients receiving DCVax-L had HRs less than 1 in all subgroups, and the difference was statistically significant for 4 of the 6 subgroups at the 95% confidence level, and for 3 of the 6 subgroups when multiplicity correction was applied. In patients with nGBM with methylated MGMT, mOS was 30.2 (95% CI, 23.7-33.9) months from randomization (33.0 months from surgery) in 90 patients receiving DCVax-L vs 21.3 (95% CI, 18.3-25.1) months in the 199 patients in the ECP (HR, 0.74; 95% CI, 0.55-1.00, $P = .03$).

OS in Patients With rGBM

The 64 patients with rGBM who received DCVax-L after recurrence had mOS of 13.2 (95% CI, 9.7-16.8) months from relapse vs 7.8 (95% CI, 7.2-8.2) months in the ECP (HR, 0.58; 0.00-0.76; $P < .001$) (**Figure 3**). These data indicate a 42% relative reduction in risk of death at any point in time for patients with rGBM treated with DCVax-L at first recurrence, and this survival benefit continued over time (Table 2): 20.7% of the patients receiving DCVax-L vs 9.6% of the patients in the ECP were alive at 24 months after recurrence, and 11.1% vs 5.1% were alive at 30 months after recurrence.

Table 1. Baseline Demographic and Clinical Characteristics of Patients With nGBM and rGBM

Source	Patients, No.	Median age, y	Patients, %									
			Age group, y		Sex		MGMT			Residual disease		
			<65	≥65	Male	Female	Methylated	Unmethylated	Missing	Minimal	Significant	Missing
Patients with nGBM												
Gilbert et al,[23] 2013	411	NA	NA	NA	58	42	30	62	9	NA	NA	NA
Gilbert et al,[22] 2014	309	NA	NA	NA	63	37	28	69	3	NA	NA	NA
Stupp et al,[5] 2017	229	NA	80	20	69	31	34	42	25	NA	NA	NA
Weller et al,[24] 2017	374	NA	77	23	61	39	35	58	7	56	44	0
Wen et al,[25] 2019	43	NA	67	33	72	28	42	56	2	NA	NA	NA
All nGBM ECP	1366	NA	77	23	62	38	32	59	9	56	44	0
nGBM DCVax-L	232	NA	78	22	59	41	39	56	5	63	37	0
Patients with rGBM												
Cloughesy et al,[28] 2017	65	55	NA	NA	60	40	40	39	22	NA	NA	NA
Wick et al,[35] 2010	92	NA	NA	NA	61	39	NA	NA	NA	NA	NA	NA
Brandes et al,[26] 2016	40	NA	NA	NA	58	43	NA	NA	NA	NA	NA	NA
Wick et al,[34] 2017	149	60	NA	NA	61	39	25	26	50	NA	NA	NA
Narita et al,[32] 2019	30	59	NA	NA	63	37	NA	NA	NA	NA	NA	NA
Brandes et al,[27] 2019	62	59	NA	NA	73	27	19	40	40	NA	NA	NA
Taal et al,[33] 2014	46	56	NA	NA	57	43	50	44	6	NA	NA	NA
Lombardi et al,[31] 2019	60	59	NA	NA	72	28	46	54	1	NA	NA	NA
Lee et al,[30] 2020	58	58	NA	NA	62	38	NA	NA	NA	NA	NA	NA
Galanis et al,[29] 2019	38	57	NA	NA	58	42	NA	NA	NA	NA	NA	NA
All rGBM ECP	640	NA	NA	NA	63	38	33	37	31	NA	NA	NA
rGBM DCVax-L	64	56	NA	NA	66	34	44	52	5	NA	NA	NA

Abbreviations: DCVax-L, lysate-loaded dendritic cell vaccination; ECP, external control population; MGMT, O^6-methylguanine-DNA methyltransferase; NA, not available; nGBM, newly diagnosed glioblastoma; rGBM, recurrent glioblastoma.

Postprogression Treatments

The trial design did not prescribe a second surgery on recurrence, and most patients did not have a second surgery. Only 18 of the 64 patients in the placebo group (28.1%) who crossed over to start receiving DCVax-L after progression as patients with rGBM had any surgery beyond the original tumor resection when newly diagnosed. The patients who had additional surgery had shorter survival than patients who had no additional surgery (postprogression mOS of 11.8 [95% CI, 8.5-14.7] months vs 13.4 [95% CI, 7.7-19.3] months). For all crossover patients, the DCVax-L vaccines administered after progression were the products made after the original surgery. No new DCVax-L vaccines were made following any postprogression surgery.

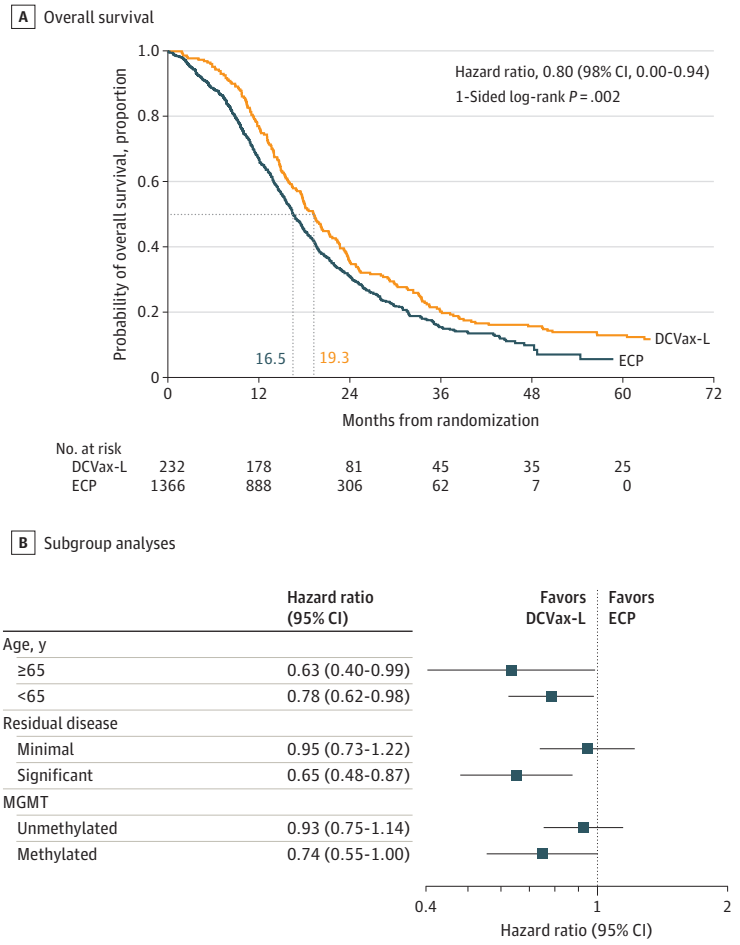
The trial design allowed additional treatments during the postrecurrence crossover period. Among the 232 patients in the DCVax-L group, 22 received bevacizumab and lomustine (9.5%), 65 (28.0%) received only bevacizumab, and 15 (6.5%) received only lomustine. Participants who received bevacizumab had shorter survival times than those who did not (16.4 [95% CI, 14.2-18.6] vs 22.1 [95% CI, 19.4-24.9] months). There was no significant survival difference between participants who received lomustine vs those who did not (18.6 [95% CI, 13.6-23.6] vs 19.3 [95% CI, 16.8-21.7] months).

Eight of the 232 patients (3.4%) receiving DCVax-L were treated with tumor-treating fields (TTF) following recurrence. Four of those 8 patients (50.0%) continued receiving DCVax-L while using the TTF device after recurrence and survived from 22.6 to more than 72.7 months from randomization. Four of the 8 patients (50.0%) stopped receiving DCVax-L while using the TTF device post-recurrence, and survived from 8.9 to 29.2 months from randomization.

Progression-Free Survival

The PFS end point became infeasible for this trial due to the challenges now well recognized in trying to distinguish true progression from pseudo-progression (including vaccine-induced immune cell infiltration).[13] There were 494 imaging time points when possible progression was observed by the independent radiologists, and 256 of these (>50%) required adjudication due to discordant interpretations. Based on these

Figure 2. Overall Survival and Subgroup Analyses for Patients with Newly Diagnosed Glioblastoma



A, Kaplan-Meier plot comparing overall survival for patients with newly diagnosed glioblastoma treated with autologous tumor lysate-loaded dendritic cell vaccination (DCVax-L) and 1366 contemporaneous matched external control participants (ECPs) treated with standard of care, derived from 5 other contemporaneous matched randomized clinical trials. B, Cox hazard ratios of overall survival in prespecified subgroups of participants receiving DCVax-L or treated with standard of care in external trials. In the age subgroup, there were 50 participants in the DCVax-L group and 45 in the ECP group aged 65 years or greater and 182 and 184, respectively in the younger than 65 years group; in the residual disease subgroup, there were 86 patients in the DCVax-L group and 163 in the ECP group with significant residual disease and 146 and 210, respectively, with minimal residual disease; in the MGMT (O^6-methylguanine-DNA methyltransferase) subgroup, there were 90 patients in the DCVax-L group and 199 in the ECP group with methylated MGMT and 131 and 349, respectively, with unmethylated MGMT. Subgroup analyses of survival, using the same parameters as the comparator publications, are presented with 95% confidence intervals to facilitate comparisons with the ECP.

assessments, the median PFS was 6.2 (95% CI, 5.7-7.4) months for patients receiving DCVax-L and 7.6 (95% CI, 5.6-10.9) months for the placebo group. This difference was not statistically significant (P = .47).

Safety and Toxic Effects

The DCVax-L was well tolerated. Of 2151 total doses of DCVax-L administered, only 5 serious adverse events were deemed at least possibly related to the investigational treatment. There were 3 cases of intracranial edema (2 at grade 3; 1 at grade 2), 1 case of nausea (grade 3), and 1 case of lymph node infection (grade 3). There was no evidence of any auto-immune reactions or cytokine storm among patients who received DCVax-L.

Discussion

Glioblastomas are aggressive, extremely heterogeneous, immunologically "cold," and rapidly lethal. There is a pressing need for new treatments and for novel clinical trial designs to streamline their development.

This trial tested a novel fully personalized active immunotherapy. The trial also implemented an innovative design that could help accelerate advances in the field.

The survival benefit with DCVax-L vs ECP increased over time in the tails of the survival curves, with 13.0% vs 5.7% survival at 60 months in patients with nGBM and 11.1% vs 5.1% survival at 30 months after recurrence in patients with rGBM. Also of note, patients receiving DCVax-L have survived for years after completing their vaccine doses, which could be due to an effective memory immune response.[36]

Although the absolute survival was greater in patients with positive prognostic factors, the relative survival benefit of DCVax-L vs ECPs was larger in certain patients who generally fare worse with SOC, including older patients, patients with substantial residual tumor, and patients with recurrent disease. These encouraging results suggest that cancer vaccines could be relevant for a broad range of clinical settings.

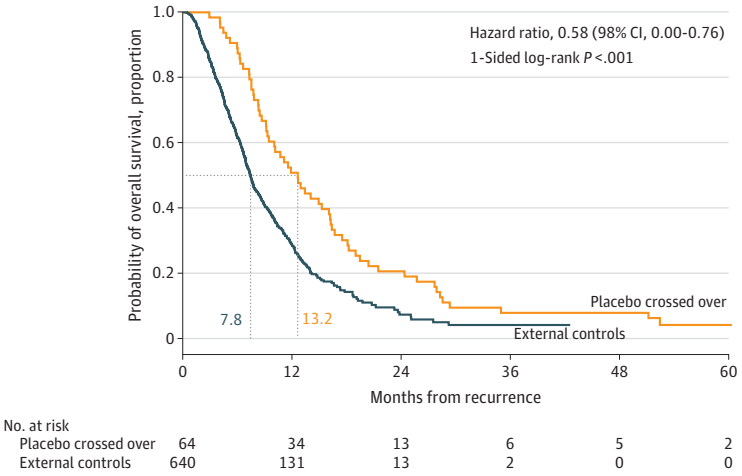
The mechanism of action of DC vaccines has been previously reported.[6,7] Using DCs as the active agent and antigen delivery method can mobilize a broader immune response (including diverse populations of T cells)[36] than with other agents.

Table 2. Landmark Survival Rates in Patients With nGBM and rGBM

Time	Patients, %		Relative rate, DCVax-L vs ECP, %
	ECP	DCVax-L group	
nGBM			
No.	1366	232	NA
Landmark survival rate			
36 mo	15.5	20.2	130
48 mo	9.9	15.7	159
60 mo	5.7	13.0	228
rGBM			
No.	640	64	
Landmark survival rate measured from date of progression			
6 mo	64.0	90.6	142
12 mo	30.8	54.1	175
18 mo	15.9	31.8	200
24 mo	9.6	20.7	215
30 mo	5.1	11.1	217

Abbreviations: DCVax-L, lysate-loaded dendritic cell vaccination; ECP, external control population; NA, not applicable; nGBM, newly diagnosed glioblastoma; rGBM, recurrent glioblastoma.

Figure 3. Overall Survival for Patients With Recurrent Glioblastoma



Kaplan-Meier plot comparing overall survival for patients with recurrent glioblastoma treated with autologous tumor lysate-loaded dendritic cell vaccination (DCVax-L; ie, patients who were randomized to the placebo group and who crossed over to begin DCVax-L following recurrence) and 640 contemporaneous, matched external controls derived from 10 other contemporaneous randomized clinical trials.

Second, using autologous rather than standardized antigens addresses the extreme heterogeneity of glioblastoma and can ensure that the treatment is targeting antigens actually present on the patient's tumor. Third, distinctively, targeting the full repertoire of antigens by using tumor lysate can prevent the patient's tumor from mutating around the targeted antigens, as has been seen when only one or a few antigens are targeted.[24,25]

Although the primary end points of this study focused on OS, exploratory analyses of immunogenicity and biomarkers of immune activation and sensitization that may correlate with therapeutic benefit are planned. We have previously shown that $CD8^+$ and $CD4^+$ T cells can traffic into glioblastomas following DC vaccination, which correlates with survival,[37,38] and we plan to confirm these prior findings with this larger phase 3 data set. Similarly, analyses of patient characteristics and baseline immune parameters (eg, tumor immune activation signatures, tumor infiltrating lymphocytes) will be correlated with outcomes but are beyond the scope of this initial report.

Treatment with DCVax-L can potentially be combined with a wide range of other treatment agents (including checkpoint inhibitors, cytokines, targeted therapies, chemotherapies, or oncolytic virus therapies).[39] The robust survival benefit in patients with MGMT methylated tumors who received DCVax-L could reflect a cooperative effect between temozolomide[40] and DCVax-L, an increase in somatic mutations associated with MGMT methylation[41] or temozolomide-induced hypermutation in MGMT methylated tumors.[42]

The benign safety profile observed with DCVax-L can enable treatment of patients vulnerable to adverse events. Furthermore, it avoids the need (and cost) for other treatments to manage side effects.

This trial highlights the feasibility and appropriateness of using independently selected, contemporaneous, matched,

and validated ECPs when a traditional RCT is not feasible.[8] This approach is highly relevant for glioblastoma, where key prognostic factors are known, patient survival remains consistently dismal, and new approaches are sorely needed to streamline and accelerate clinical trials.

Limitations

This study has limitations. Since individual patient-level data for the ECPs were not available for this trial, as is often the case, propensity score matching could not be performed, which is a potential limitation of this study. However, the MAIC analysis applied here is a powerful method to overcome the lack of such individual patient data and to enable matching of specific patient characteristics in external controls compared with patients in the investigational group. This method also has wider general applicability to provide reliable comparative evidence of benefit.[18]

Conclusions

This phase 3, nonrandomized, externally controlled trial found that the addition of DCVax-L to SOC was associated with a clinically meaningful and statistically significant extension of overall survival in both nGBM and rGBM. Treatment with DCVax-L also had an excellent safety profile and noteworthy tails of long-term survival curves.

ARTICLE INFORMATION

Accepted for Publication: August 27, 2022.

Published Online: November 17, 2022. doi:10.1001/jamaoncol.2022.5370

Author Affiliations: Department of Neurosurgery, University of California, Los Angeles (Liau); King's College Hospital, London, United Kingdom (Ashkan); Department of Neurosurgery, Penn Brain Tumor Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia (Brem); Division of Neurology, Washington University School of Medicine in St Louis, St Louis, Missouri (Campian); Givens Brain Tumor Center, Abbott Northwestern Hospital, Minneapolis, Minnesota (Trusheim); Columbia University Irving Medical Center, New York, New York (Iwamoto); New York-Presbyterian Hospital, New York, New York (Iwamoto); Preston A. Wells, Jr. Center for Brain Tumor Therapy, Division of Neuro-Oncology, Lillian S. Wells Department of Neurosurgery, University of Florida College of Medicine, Gainesville (Tran); Department of Neurological Surgery, Washington University School of Medicine in St Louis, St Louis, Missouri (Ansstas); Ben and Catherine Ivy Center for Advanced Brain Tumor Treatment, Swedish Medical Center, Seattle, Washington (Cobbs); Taubman Medical Center, University of Michigan, Ann Arbor (Heth); Neuro-Oncology Program, Rutgers Cancer Institute of New Jersey, New Brunswick (Salacz); Sutter Health, Sacramento, California (D'Andre); Glasser Brain Tumor Center, Atlantic Healthcare, Summit, New Jersey (Aiken, Moshel); Department of Neurological Sciences, Rush Medical College, Chicago, Illinois (Nam); Department of Neurology, The Ohio State University, Columbus (Pillainayagam); The Cancer Center of Columbus Regional Health, Columbus, Indiana (Wagner); University of Rochester, Rochester, New York (Walter); University of Cincinnati, Cincinnati, Ohio (Chaudary); John Theurer Cancer Center, Hackensack University Medical Center, Hackensack, New Jersey (Goldlust); Department of Neurosurgery, Henry Ford Health System, Detroit, Michigan (Lee, Mikkelsen, Walbert); Department of Neurology and Chao Family Comprehensive Cancer Center, University of California, Irvine (Bota); Rhode Island Hospital, Providence (Elinzano); Long Island Brain Tumor Center at NSPC, Lake Success, New York (Grewal); Department of Neurosurgery, University of Colorado Health Sciences Center, Boulder (Lillehei); Ascension St Thomas Brain and Spine Tumor Center, Howell Allen Clinic, Nashville, Tennessee (Abram); Mays Cancer Center at UT Health San Antonio, San Antonio, Texas (Brenner); Department of Neurosurgery, UNC School of Medicine and UNC Health, Chapel Hill, North Carolina (Ewend); The Geisel School of Medicine at Dartmouth, Hanover, New Hampshire (Khagi); Advent Health, Kansas City, Kansas (Lovick); Department of Medical Oncology & Therapeutics Research, City of Hope, Duarte, California (Portnow); Division of Neuro-Oncology, Icahn School of Medicine at Mount Sinai, New York, New York (Kim); Saint Joseph's Hospital, Orange, California (Loudon); Jefferson Hospital for Neurosciences, Jefferson University, Philadelphia, Pennsylvania (Martinez); Department of Neurological Surgery, Vanderbilt University Medical Center, Nashville, Tennessee (Thompson); Beth Israel Deaconess Medical Center, Harvard Medical School, Cambridge, Massachusetts (Avigan); Baylor Scott & White Neuro-Oncology Associates, Dallas, Texas (Fink); Illinois Cancer Care, Galesburg, Peoria (Geoffroy); Medical University of South Carolina Neurosciences, Charleston (Giglio); Mount Sinai Medical Center, Miami Beach, Florida (Gligich); Uniklinikum Dresden, Dresden, Germany (Krex); Hollings Cancer Center, Medical University of South Carolina, Charleston (Lindhorst); Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida (Lutzky); BG Klinikum Bergmannstrost, Halle, Germany (Meisel, Sanchin); Neurochirurgie Katharinenhospital, Klinikum der Landeshauptstadt Stuttgart, Stuttgart, Germany (Nadji-Ohl); Seidman Cancer Center, University Hospitals–Cleveland Medical Center, Cleveland, Ohio (Sloan); Department of Neurosurgery, Tufts Medical Center, Boston, Massachusetts (Taylor, Wu); Piedmont Physicians Neuro-Oncology, Piedmont Brain Tumor Center, Atlanta, Georgia (Dunbar); Department of Neuro-Oncology, Moffitt Cancer Center (Etame); Pacific Neurosciences Institute and Saint John's Cancer Institute, Santa Monica, California (Kesari); Centre de Recherche du CHUS, Université de Sherbrooke, Sherbrooke, Quebec, Canada (Mathieu); UC San Diego Moore's Cancer Center, La Jolla, California (Piccioni); Department of Neurosurgery, Houston Methodist Hospital, Houston, Texas (Baskin); Geisinger Neuroscience Institute, Danville, Pennsylvania (Lacroix); Klinik für Neurochirurgie, Chemnitz, Germany (May); Baptist Health System, San Antonio, Texas (New); Saint Luke's Cancer Institute, Kansas City, Missouri (Pluard); Departments of Neurosurgery and Medicine, The Warren Alpert Medical School of Brown University, Providence, Rhode Island (Toms); Kaiser Permanente, Redwood City, California (Tse, Peak); University of Kentucky Markey Cancer Center, Department of Medicine, Neurosurgery, and Neurology, University of Kentucky, Lexington (Villano); Oklahoma University Health Science Center, Oklahoma City (Battiste); University College London Hospitals, London, United Kingdom (Mulholland); Blue Sky Neurology/Neuro-Oncology, Englewood, California (Pearlman); Department of Neurology and Neurosurgery, Montreal Neurological Institute-Hospital, McGill University, Montreal, Quebec, Canada (Petrecca); Department of Neurosurgery, Zucker School of Medicine at Hofstra/Northwell, Uniondale, New York (Schulder); University of California, Los Angeles (Prins); Northwest Biotherapeutics, Inc, Bethesda, Maryland (Boynton, Bosch).

Author Contributions: Dr Bosch had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Liau, Ashkan, Ansstas, Walter, Prins, Boynton, Bosch.
Acquisition, analysis, or interpretation of data: Liau, Ashkan, Brem, Campian, Trusheim, Iwamoto, Tran, Ansstas, Cobbs, Heth, Salacz, D'Andre, Aiken, Moshel, Nam, Pillainayagam, Wagner, Walter, Chaudhary, Goldlust, Lee, Bota, Elinzano, Grewal, Lillehei, Mikkelsen, Walbert, Abram, Brenner, Ewend, Khagi, Lovick, Portnow, Kim, Loudon, Martinez, Thompson, Avigan, Fink, Geoffroy, Giglio, Gligich, Krex, Lindhorst, Lutzky, Meisel, Nadji-Ohl, Sanchin, Sloan, Taylor, Wu, Dunbar, Etame, Kesari, Mathieu, Piccioni, Baskin, Lacroix, May, New, Pluard, Toms, Tse, Peak, Villano, Battiste, Mulholland, Pearlman, Petrecca, Schulder, Prins, Boynton, Bosch.
Drafting of the manuscript: Liau, Ashkan, Cobbs, Elinzano, Brenner, Kim, Nadji-Ohl, Peak, Mulholland, Pearlman, Prins, Boynton, Bosch.
Critical revision of the manuscript for important intellectual content: Liau, Ashkan, Brem, Campian, Trusheim, Iwamoto, Tran, Ansstas, Heth, Salacz, D'Andre, Aiken, Moshel, Nam, Pillainayagam, Wagner, Walter, Chaudhary, Goldlust, Lee, Bota, Grewal, Lillehei, Mikkelsen, Walbert, Abram, Ewend, Khagi, Lovick, Portnow, Loudon, Martinez, Thompson, Avigan, Fink, Geoffroy, Giglio, Gligich, Krex, Lindhorst, Lutzky, Meisel, Sanchin, Sloan, Taylor, Wu, Dunbar, Etame, Kesari, Mathieu, Piccioni, Baskin, Lacroix, May, New, Pluard, Toms, Tse, Villano, Battiste, Mulholland, Petrecca, Schulder, Prins, Boynton.

Statistical analysis: Liau, Bosch.
Administrative, technical, or material support: Liau, Ashkan, Brem, Iwamoto, Tran, Salacz, D'Andre, Aiken, Moshel, Nam, Wagner, Walter, Goldlust, Bota, Lillehei, Mikkelsen, Walbert, Abram, Brenner, Krex, Nadji-Ohl, Sloan, Wu, Dunbar, Baskin, Tse, Villano, Battiste, Pearlman, Petrecca, Boynton.
Supervision: Liau, Ashkan, Brem, Campian, Trusheim, Ansstas, Walter, Goldlust, Lovick, Loudon, Geoffroy, Kesari, Piccioni, Pluard, Toms, Peak, Prins, Bosch.

Conflict of Interest Disclosures: Dr Liau reported serving on the board of directors of ClearPoint Neuro outside the submitted work and having a patent pending for combinations of inhibitors with dendric cell vaccines to treat cancer. Dr Ashkan reported receiving grants from Northwest Biotherapeutics during the conduct of the study. Dr Brem reported receiving travel support from Northwest Biotherapeutics outside the submitted work. Dr Campian reported receiving grants from NeoImmue Tech and support for investigator-initiated clinical trials from Incyte, Merck, and Ipsen outside the submitted work. Dr Iwamoto reported receiving grants from Northwest Biotherapeutics and serving on the steering committee of this trial during the conduct of the study and receiving personal fees from AbbVie, Alexion, Gennao Bio, Novocure, Kiyatec, Medtronic, Merck, Guidepoint, Mimivax, Massive Bio, Tocagen, Regeneron, and Xcures outside the submitted work. Dr Tran reported receiving grants from Novocure, Moteris, Lacerta, Sarepta, Merck, Novartis, Northwest Biotherapeutics, Stemline, Celldex, Orbus, TVax, and Tocagen; receiving travel support from Novartis; and serving on the advisory board of Novocure during the conduct of the study. Dr Goldlust reported receiving institutional support from Northwest Biotherapeutics during the conduct of the study; receiving consulting fees from Boston Biomedical, Sumitomo Danippon Pharma, Cornerstone Specialty Network, Cellevolve, Daiichi Sankyo, and Novocure; serving on the speakers' bureau for Novocure and Physicians Education Resources; receiving food and drink from Novocure; and owning stock in COTA outside the submitted work. Dr Grewal reported receiving personal fees from AstraZeneca, Vivacitas Oncology, and xCures; receiving sample medication from AbbVie/Allergan; and being the founder of Genomet outside the submitted work. Dr Avigan reported serving on the advisory boards of Bristol Myer Squibb, Chugai, Merck, Kite, and Legend; receiving grants from Sanofi, and serving as a consultant for Parexel outside the submitted work. Dr Fink reported receiving funding from Northwest Biotherapeutics during the conduct of the study and receiving funding from Novocure, Denovo Biopharma, Stemline, CNS Pharmaceuticals, Servier Pharmaceuticals/Agios, and Sumitoma Pharma outside the submitted work. Dr Giglio reported receiving study support from the Medical University of South Carolina during the conduct of the study; receiving grants from Denovo Biopharma, Novocure, BioMimetix, Celgene, EORTC, the Canadian Cancer Trials Group, Institut de Recherches Internationales Servier, the Global Coalition for Adapative Research, and Prelude outside the submitted work; and having a patent pending for the epitranscriptomic analysis of glioma. Dr Lutzky reported receiving grants from Bristol Myer Squibb and serving on the advisory boards of Iovance and Castle outside the submitted work. Dr Meisel reported receiving personal fees from BG Klinikum Bergmannstrost during the conduct of the study and receiving consulting fees paid to Regenerate Life Sciences from Stayble Therapeutics and royalties from Fehling Instruments outside the submitted work. Dr Sanchin reported receiving personal fees from BG Klinikum Bergmannstrost during the conduct of the study. Dr Dunbar reported receiving speaking fees from GT Medical during the conduct of the study. Dr Pluward reported receiving grants from Northwest Biotherapeutics during the conduct of the study. Dr Mulholland reported receiving support to attend a conference from Northwest Biotherapeutics during the conduct of the study. Dr Pearlman reported receiving compensation for serving as a site principal investigator from Northwest Biotherapeutics during the conduct of the study. Dr Prins reported having patent UCLA Case No. 2015-341 pending. Drs Boynton and Bosch reported being employees of and owning shares in Northwest Biotherapeutics, Inc. Dr Boynton reported having a patent held by Northwest Biotherapeutics. Dr Bosch reporting having patent 13/492693 pending.

Funding/Support: This study was supported by Northwest Biotherapeutics, Inc.

Role of the Funder/Sponsor: Northwest Biotherapeutics contributed to the design of the study together with the principal investigators and oversaw the conduct of the study by the independent contract research organizations, which were responsible for all collection and management of the data. Northwest Biotherapeutics participated in the preparation, review, and approval of the manuscript; and in the decision to submit the manuscript for publication.

Data Sharing Statement: See Supplement 3.

Additional Information: The data from the trial were held and statistically analyzed by Quantics, which had no additional input into the manuscript or interpretation of the results. All data pertaining to this article were reviewed in full by Dr Bosch.

REFERENCES

1. Weller M, Cloughesy T, Perry JR, Wick W. Standards of care for treatment of recurrent glioblastoma—are we there yet? *Neuro Oncol*. 2013;15(1):4-27. doi:10.1093/neuonc/nos273

2. Poon MTC, Sudlow CLM, Figueroa JD, Brennan PM. Longer-term (≥ 2 years) survival in patients with glioblastoma in population-based studies pre- and post-2005: a systematic review and meta-analysis. *Sci Rep*. 2020;10(1):11622. doi:10.1038/s41598-020-68011-4

3. Alexander BM, Cloughesy TF. Adult glioblastoma. *J Clin Oncol*. 2017;35(21):2402-2409. doi:10.1200/JCO.2017.73.0119

4. Vanderbeek AM, Rahman R, Fell G, et al. The clinical trials landscape for glioblastoma: is it adequate to develop new treatments? *Neuro Oncol*. 2018;20(8):1034-1043. doi:10.1093/neuonc/noy027

5. Stupp R, Taillibert S, Kanner A, 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(23):2306-2316. doi:10.1001/jama.2017.18718

6. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice: I. morphology, quantitation, tissue distribution. *J Exp Med*. 1973;137(5):1142-1162. doi:10.1084/jem.137.5.1142

7. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. *Nat Rev Cancer*. 2012;12(4):265-277. doi:10.1038/nrc3258

8. Mishra-Kalyani PS, Amiri Kordestani L, Rivera DR, et al. External control arms in oncology: current use and future directions. *Ann Oncol*. 2022;33(4):376-383. doi:10.1016/j.annonc.2021.12.015

9. Alexander BM, Ba S, Berger MS, et al; GBM AGILE Network. Adaptive Global Innovative Learning Environment for Glioblastoma: GBM AGILE. *Clin Cancer Res*. 2018;24(4):737-743. doi:10.1158/1078-0432.CCR-17-0764

10. Alexander BM, Trippa L, Gaffey S, et al. Individualized Screening Trial of Innovative Glioblastoma Therapy (INSIGhT): a bayesian adaptive platform trial to develop precision medicines for patients with glioblastoma. *JCO Precis Oncol*. Published online March 27, 2019. doi:10.1200/PO.18.00071

11. Stupp R, Mason WP, van den Bent MJ, et al; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987-996. doi:10.1056/NEJMoa043330

12. Liau LM, Ashkan K, Tran DD, et al. First results on survival from a large phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. *J Transl Med*. 2018;16(1):142. doi:10.1186/s12967-018-1507-6

13. Ellingson BM, Chung C, Pope WB, Boxerman JL, Kaufmann TJ. Pseudoprogression, radionecrosis, inflammation or true tumor progression? challenges associated with glioblastoma response assessment in an evolving therapeutic landscape. *J Neurooncol*. 2017;134(3):495-504. doi:10.1007/s11060-017-2375-2

14. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053.

15. Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG. Response criteria for phase II studies of supratentorial malignant glioma. *J Clin Oncol*. 1990;8(7):1277-1280. doi:10.1200/JCO.1990.8.7.1277

16. Guyot P, Ades AE, Ouwens MJ, Welton NJ. Enhanced secondary analysis of survival data: reconstructing the data from published Kaplan-Meier survival curves. *BMC Med Res Methodol*. 2012;12:9. doi:10.1186/1471-2288-12-9

17. Common Terminology Criteria for Adverse events v3.0 (CTCAE). August 9, 2009. Accessed October 10, 2022. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf

18. Signorovitch JE, Sikirica V, Erder MH, et al. Matching-adjusted indirect comparisons: a new tool for timely comparative effectiveness research. *Value Health*. 2012;15(6):940-947. doi:10.1016/j.jval.2012.05.004

19. O'Brien PC, Fleming TR. A multiple testing procedure for clinical trials. *Biometrics*. 1979;35(3):549-556. doi:10.2307/2530245

20. Lan KKG, DeMets DL. Discrete sequential boundaries for clinical trials. *Biometrika*. 1983;70 (3):659-663. doi:10.2307/2336502

21. Hall WJ, Wellner JA. Confidence bands for a survival curve from censored data. *Biometrika*. 1980;67(1):133-143. doi:10.1093/biomet/67.1.133

22. Gilbert MR, Dignam JJ, Armstrong TS, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med*. 2014;370 (8):699-708. doi:10.1056/NEJMoa1308573

23. Gilbert MR, Wang M, Aldape KD, et al. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. *J Clin Oncol*. 2013;31(32):4085-4091. doi:10.1200/JCO.2013.49.6968

24. Weller M, Butowski N, Tran DD, et al; ACT IV trial investigators. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol*. 2017;18(10):1373-1385. doi:10.1016/S1470-2045(17)30517-X

25. Wen PY, Reardon DA, Armstrong TS, 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(19):5799-5807. doi:10.1158/1078-0432.CCR-19-0261

26. Brandes AA, Carpentier AF, Kesari S, et al. A phase II randomized study of galunisertib monotherapy or galunisertib plus lomustine compared with lomustine monotherapy in patients with recurrent glioblastoma. *Neuro Oncol*. 2016;18 (8):1146-1156. doi:10.1093/neuonc/now009

27. Brandes AA, Gil-Gil M, Saran F, et al. A randomized phase II trial (TAMIGA) evaluating the efficacy and safety of continuous bevacizumab through multiple lines of treatment for recurrent glioblastoma. *Oncologist*. 2019;24(4):521-528. doi:10.1634/theoncologist.2018-0290

28. Cloughesy T, Finocchiaro G, Belda-Iniesta C, et al. Randomized, double-blind, placebo-controlled, multicenter phase II study of onartuzumab plus bevacizumab versus placebo plus bevacizumab in patients with recurrent glioblastoma: efficacy, safety, and hepatocyte growth factor and o^6-methylguanine-DNA methyltransferase biomarker analyses. *J Clin Oncol*. 2017;35(3):343-351. doi:10.1200/JCO.2015.64.7685

29. Galanis E, Anderson SK, Twohy EL, et al. A phase 1 and randomized, placebo-controlled phase 2 trial of bevacizumab plus dasatinib in patients with recurrent glioblastoma: Alliance/North Central Cancer Treatment Group N0872. *Cancer*. 2019;125(21):3790-3800. doi:10.1002/cncr.32340

30. Lee EQ, Zhang P, Wen PY, et al. NRG/RTOG 1122: a phase 2, double-blinded, placebo-controlled study of bevacizumab with and without trebananib in patients with recurrent glioblastoma or gliosarcoma. *Cancer*. 2020;126(12):2821-2828. doi:10.1002/cncr.32811

31. Lombardi G, De Salvo GL, Brandes AA, et al. Regorafenib compared with lomustine in patients with relapsed glioblastoma (REGOMA): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet Oncol*. 2019;20(1):110-119. doi:10.1016/S1470-2045(18)30675-2

32. Narita Y, Arakawa Y, Yamasaki F, et al. A randomized, double-blind, phase III trial of personalized peptide vaccination for recurrent glioblastoma. *Neuro Oncol*. 2019;21(3):348-359. doi:10.1093/neuonc/noy200

33. Taal W, Oosterkamp HM, Walenkamp AM, et al. Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial. *Lancet Oncol*. 2014;15(9):943-953. doi:10.1016/S1470-2045(14)70314-6

34. Wick W, Gorlia T, Bendszus M, et al. Lomustine and bevacizumab in progressive glioblastoma. *N Engl J Med*. 2017;377(20):1954-1963. doi:10.1056/NEJMoa1707358

35. Wick W, Puduvalli VK, Chamberlain MC, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. *J Clin Oncol*. 2010;28(7):1168-1174. doi:10.1200/JCO.2009.23.2595

36. Whiteside TL. Immune responses to malignancies. *J Allergy Clin Immunol*. 2010;125(2) (suppl 2):S272-S283. doi:10.1016/j.jaci.2009.09.045

37. Liau LM, Prins RM, Kiertscher SM, 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 (15):5515-5525. doi:10.1158/1078-0432.CCR-05-0464

38. Prins RM, Soto H, Konkankit V, et al. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. *Clin Cancer Res*. 2011;17(6):1603-1615. doi:10.1158/1078-0432.CCR-10-2563

39. Antonios JP, Soto H, Everson RG, et al. PD-1 blockade enhances the vaccination-induced immune response in glioma. *JCI Insight*. 2016;1(10): e87059. doi:10.1172/jci.insight.87059

40. Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*. 2005;352(10):997-1003. doi:10.1056/NEJMoa043331

41. McDonald KL, Tabone T, Nowak AK, Erber WN. Somatic mutations in glioblastoma are associated with methylguanine-DNA methyltransferase methylation. *Oncol Lett*. 2015;9(5):2063-2067. doi:10.3892/ol.2015.2980

42. van Thuijl HF, Mazor T, Johnson BE, et al. Evolution of DNA repair defects during malignant progression of low-grade gliomas after temozolomide treatment. *Acta Neuropathol*. 2015; 129(4):597-607. doi:10.1007/s00401-015-1403-6