original reports abstract

Phase IIa Study of SurVaxM Plus Adjuvant Temozolomide for Newly Diagnosed Glioblastoma

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PURPOSE Despite intensive treatment with surgery, radiation therapy, temozolomide (TMZ) chemotherapy, and tumor-treating fields, mortality of newly diagnosed glioblastoma (nGBM) remains very high. SurVaxM is a peptide vaccine conjugate that has been shown to activate the immune system against its target molecule survivin, which is highly expressed by glioblastoma cells. We conducted a phase IIa, open-label, multicenter trial evaluating the safety, immunologic effects, and survival of patients with nGBM receiving SurVaxM plus adjuvant TMZ following surgery and chemoradiation (ClinicalTrials.gov identifier: NCT02455557).

METHODS Sixty-four patients with resected nGBM were enrolled including 38 men and 26 women, in the age range of 20-82 years. Following craniotomy and fractionated radiation therapy with concurrent TMZ, patients received four doses of SurVaxM (500 μg once every 2 weeks) in Montanide ISA-51 plus sargramostim (granulocyte macrophage colony-stimulating factor) subcutaneously. Patients subsequently received adjuvant TMZ and maintenance SurVaxM concurrently until progression. Progression-free survival (PFS) and overall survival (OS) were reported. Immunologic responses to SurVaxM were assessed.

RESULTS SurVaxM plus TMZ was well tolerated with no serious adverse events attributable to SurVaxM. Of the 63 patients who were evaluable for outcome, 60 (95.2%) remained progression-free 6 months after diagnosis (prespecified primary end point). Median PFS was 11.4 months and median OS was 25.9 months measured from first dose of SurVaxM. SurVaxM produced survivin-specific CD8+ T cells and antibody/immunoglobulin G titers. Apparent clinical benefit of SurVaxM was observed in both methylated and unmethylated patients.

CONCLUSION SurVaxM appeared to be safe and well tolerated. The combination represents a promising therapy for nGBM. For patients with nGBM treated in this manner, PFS may be an acceptable surrogate for OS. A large randomized clinical trial of SurVaxM for nGBM is in progress.

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INTRODUCTION

Glioblastoma (GBM) has very high mortality, with median survival ranging from 14.6 to 16.0 months in large, phase III clinical trials evaluating standard radiation therapy and chemotherapy.[1,2] To date, the most effective regimen consists of radiation therapy with concurrent temozolomide (TMZ) followed by adjuvant TMZ for at least six cycles. For patients whose tumors express unmethylated O-6-methylguanine-DNA methyltransferase (MGMT) genes, TMZ provides less survival benefit than for patients with methylated MGMT.[3] Tumor-treating fields (TTF) have been reported to provide additional benefit, with a median overall survival (mOS) of 20.5 months.[2]

Survivin (BIRC5) is one of the most ubiquitous cancer-associated antigens. Although expressed during fetal development, survivin is detected infrequently in normal tissues of adult organisms.[4] Malignant gliomas express survivin at high levels, although normal glial cells do not.[5,6] Survivin expression in gliomas is associated with a poor prognosis.[6]

The survivin protein is processed by the proteasome, and survivin epitopes are presented on the tumor cell surface by major histocompatibilty complex class-I molecules. Peptides presented in this manner become recognizable by CD8+ immune effector responses. In addition to its well-described intracellular sites of action, the survivin protein appears on the cell surface of a wide variety of cancer cell types[7,8] and on tumor-derived exosomes.[8-10] Therefore, survivin is also targetable by antibody-dependent cellular cytotoxicity.[7]

Patients with cancer, including those with malignant gliomas, may exhibit cellular and humoral immune responses to survivin.[11-14] Consequently, survivin

ASSOCIATED CONTENT

Appendix

Data Sharing Statement

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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CONTEXT

Key Objective

Glioblastoma (GBM) is a devastating malignancy with poor survival times and few therapeutic options. The peptide vaccine SurVaxM stimulates immune targeting of the GBM-associated molecule survivin. This phase IIa, single-arm, multisite trial evaluated survival of patients with newly diagnosed GBM treated with SurVaxM in combination with temozolomide (TMZ) following surgery and chemoradiation.

Knowledge Generated

Across 64 enrolled patients, SurVaxM was well tolerated and was not associated with any significant adverse events. Six months after diagnosis, 95.2% (60/63) of evaluable patients remained progression-free. Measured from first SurVaxM dose, median progression-free survival was 11.4 months and median overall survival was 25.9 months. Patients generated survivin-specific immune responses, and antibody response was positively associated with survival.

Relevance *(I.K. Mellinghoff)*

Treatment with SurVaxM in combination with TMZ following chemoradiation may extend survival for patients with newly diagnosed GBM. A randomized trial is underway to determine whether adding SurVaxM to TMZ chemotherapy is better than TMZ treatment alone in these patients.*

*Relevance section written by *JCO* Associate Editor Ingo K. Mellinghoff, MD, FACP.

appears to be immunogenic to some degree. Expansion of existing immune responses to survivin epitopes could break tolerance to survivin and offer a potential vehicle for cancer immunotherapy.

The synthetic survivin vaccine conjugate SurVaxM has shown significant antitumor effects in preclinical tumor models.[15,16] A first-in-human clinical trial conducted in patients with recurrent malignant glioma following standard therapy found SurVaxM to be safe and tolerable. Seven of eight patients survived longer than 1 year following initiation of treatment.[17] Here, we report the results of a multicenter phase IIa clinical trial in which 64 patients with newly diagnosed GBM (nGBM) were treated with adjuvant TMZ plus SurVaxM following surgery and chemoradiation.

METHODS

Study Design

This multicenter, open-label, single-arm, phase IIa trial in adult patients with nGBM was approved by the institutional review boards at each of the participating hospitals (ClinicalTrials.gov identifier: NCT02455557). All participants signed an informed consent before participation and study-related tests. Patients were enrolled and treated at the following institutions: Beth Israel Deaconess Medical Center, Cleveland Clinic, Dana-Farber Cancer Institute, Massachusetts General Hospital, and Roswell Park Comprehensive Cancer Center.

Eligibility

Patients age 18 years and older with nGBM were eligible if they had a Karnofsky performance status (KPS) $\geq$ 70 at screening, survivin expression ($\geq$ 1%) in tumor cells by immunohistochemistry, and HLA status including at least one major histocompatibilty complex class-I allele: HLA-A*02, -A*03, -A*11, or -A*24. Requirements also included adequate renal function (creatinine $\leq$ 1.8 mg/dL), absolute neutrophil count $\geq 1.5 \times 10^9$/L, platelets $\geq 100 \times 10^9$/L, hemoglobin $>$ 9.0 g/dL, total bilirubin $\leq 1.5 \times$ upper limit of normal, and ALT and AST $\leq 4.0 \times$ upper limit of normal. Patients were required to use contraceptive methods and have a negative pregnancy test. Patients with autoimmune disorders were excluded. Patients receiving any other immunotherapy, chemotherapy, investigational agent, carmustine wafers, or TTF were excluded. Patients were required to have had a brain magnetic resonance imaging (MRI) scan within 72 hours of surgical resection demonstrating $\leq$ 1 cm^3 of residual contrast enhancement or $\leq$ 100 mm^2 linear enhancement. Following standard fractionated radiation therapy with TMZ (chemoradiation), MRI scans must have shown no tumor progression; however, patients with pseudoprogression could begin treatment on Protocol (online only). Those with tumor progression at the conclusion of chemoradiation were ineligible for treatment (Appendix Fig A1B, online only). Minimal use of dexamethasone was strongly encouraged.

Treatment Regimen

Patients began treatment with SurVaxM within 28 days of completion of chemoradiation. SurVaxM (500 μg) was prepared in emulsion with Montanide ISA-51 and administered once every 2 weeks (subcutaneously). Immediately afterward, sargramostim (100 μg) was given once every 2 weeks (subcutaneously) 1-3 cm from the SurVaxM-Montanide injection site. Patients received subcutaneous vaccinations once every 2 weeks for a total of four priming

doses, and then every 12 weeks (±4 weeks) during the maintenance phase (Appendix Fig A1A). Adjuvant TMZ therapy was begun no sooner than 28 days after completion of chemoradiation and was administered once daily (orally) on days 1-5 of every 28-day cycle. Adjuvant TMZ was given for at least six cycles, or until intolerance or tumor progression occurred. Every effort was made to begin priming vaccination during the hiatus between chemoradiation and adjuvant TMZ. To the extent possible, subsequent adjuvant TMZ and vaccine cycles were aligned with trimonthly MRI scans used to determine progression. All patients received at least one priming vaccination before the start of adjuvant TMZ. Following discontinuation of TMZ, maintenance SurVaxM was continued until intolerance or disease progression.

End Points and Evaluations

The primary end point was progression-free survival at 6 months (PFS6), defined as the interval between diagnosis and first observed disease progression or death due to any cause. To permit more accurate comparisons to historical data, progression-free survival (PFS) and overall survival (OS) were also measured from the date of first dose of SurVaxM. MRI scans were performed at regular intervals, and tumor progression was assessed using RANO criteria modified to employ perfusion-weighted imaging to help distinguish pseudoprogression from tumor progression.[18] Progression was assessed by the treating investigators who were allowed to confirm progression on more than one scan before removal of patients from study. In cases of suspected pseudoprogression, patients were allowed to continue treatment and remain under observation with serial MRI scans at four-week intervals. If subsequent evaluations showed tumor progression, the date of progression was recorded as the time point at which the issue was first raised. The secondary end point of OS was defined as the interval between first vaccination with SurVaxM and death from any cause. Toxicity was evaluated and tabulated using National Cancer Institute Common Toxicity Criteria for Adverse Events. Analyses of survival in relation to MGMT, isocitrate dehydrogenase (IDH) 1, age, sex, and KPS were performed post hoc.

Statistical Analysis

The primary clinical objective was to evaluate PFS6. In addition, post hoc analyses of OS at 12 months, median PFS (mPFS), and mOS were performed in patients and the results were compared with historical controls. Patients alive and disease-free at the last study assessment were treated as censored. PFS6 was estimated using Kaplan-Meier analysis and reported with corresponding 95% CIs. An exact one-sided binomial test was used to test the primary end point that SurVaxM would increase the PFS6 fraction from 54% (historical control measured from the date of diagnosis)[1] to 70% (hypothesized experimental target) after all patients had been followed for 6 months. Estimated distributions of OS are defined as the time from the date of first dosing to death due to any cause and were obtained using the Kaplan-Meier method. For comparison of survival curves, the log-rank (Mantel-Cox) test was used. Follow-up time was determined by reverse Kaplan-Meier method. Correlation studies of PFS and OS were performed using Pearson r and two-tailed P value with standard significance considered $P < .05$ according to the method of Schemper et al.[19] Additional multivariate analyses were performed using Cox regression. Analysis was performed using GraphPad Prism version 9.3.0 for macOS, GraphPad Software, San Diego, CA.

Molecular Profiling of Patient Tumors

Tumor samples (n = 33) acquired during initial craniotomy were formalin-fixed/paraffin-embedded. RNA extraction, library preparation, and paired-end bulk RNA sequencing of samples were performed by Tempus (Chicago, IL), as described in the study by Beaubier et al.[20] Sequencing reads were aligned to the reference genome GRCh38 (GENCODE v38) using the pseudoalignment and quantification tool Salmon v1.1.0.[21] Raw transcript counts were normalized and variance-stabilizing $\log_2$-transformed using the DESeq2 Bioconductor workflow.[22] Classical, mesenchymal, and proneural signature gene sets for molecular subtyping were retrieved from the study by Wang et al.[23] Gene set variation analysis (GSVA) was applied to the variance-stabilized expression data set. Molecular subtypes were assigned to IDH1 wild-type samples on the basis of the greatest positive GSVA enrichment score reported.

T-Cell Reactivity

Cellular responses were assayed by in vitro peptide stimulation of peripheral blood mononuclear cells (PBMCs) isolated from blood obtained at the V5 time point (median = 17 weeks after first vaccination). Cells were stimulated with positive control peptide epitopes from CMV, EBV, and influenza (CEF; Mabtech, Stockholm, Sweden), or SurVaxM epitopes survivin (SVN)-1/SVN-2, for 24 hours, with protein transport inhibitor treatment at 4 hours. Cells were stained with fluor-conjugated antibodies against CD3, CD8a, CD69, HLA-DR, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ (Biolegend, San Diego, CA). Events were collected on BD Fortessa running FacsDIVA. Data were analyzed in FCSExpress (DeNovo Software, Pasadena, CA) by gating the CD3+/CD8+ population and vehicle controls for activation marker-/cytokine-positive event gating. Values were plotted in GraphPad, and compared using one-way analysis of variance.

Humoral Responses

Survivin antibody responses were measured by indirect enzyme-linked immunosorbent assay on samples collected at baseline (before first vaccination), 6-14 weeks (post-V4), 15-29 weeks (V5), 30-40 weeks (V6), and 41-55 weeks (V7). SurVaxM-coated 96-well plates were incubated with serially diluted patient serums in triplicate

followed by incubation with goat antihuman horseradish peroxidase–IgG (Bio-Rad, Hercules, CA) and TMB substrate (Biolegend). Fluorescence at 450 nm was measured and values were normalized to background. Data were fit using nonlinear regression in GraphPad and titers were determined.

RESULTS

Patient Characteristics and Study Design

Sixty-four patients were enrolled at five sites between May 28, 2015, and November 28, 2017 (Appendix Fig A1B). Sixty-three patients on study were considered evaluable for clinical efficacy, having received all four priming doses of SurVaxM. One patient received only a single priming dose of SurVaxM and was excluded from efficacy analysis but was included in the safety analysis. Patients received a median of six cycles of adjuvant TMZ. Subjects in the efficacy analysis included 38 males (60%) and 25 females (40%) ranging in age from 20 to 82 years, with a median age 60 years (Table 1). Median KPS was 90, and the most prevalent HLA haplotype combinations were HLA-A*02/A*02 (n = 19; 30.2%) and HLA-A*02/A*03 (n = 8; 12.7%). All patient tumors expressed survivin, as determined by immunohistochemistry with a median of 12% (range, 1%-40%) of cells staining positively (Table 1). No patients were excluded because of undetectable survivin expression. Following surgical resection and chemoradiation, patients received injections of SurVaxM including four priming doses at two-week intervals, followed by maintenance doses every 12 weeks (Appendix Fig A1A). The median time from initial diagnosis (surgical resection) to first vaccine dose was 3.0 months. Following chemoradiation, patients also received standard adjuvant chemotherapy with TMZ.

TABLE 1. Patient Characteristics

Characteristic	Total Patients (N = 63)
Sex, No. (%)	
Male	38 (60.0)
Female	25 (40.0)
Age, years	
Mean	56.5
Median (range)	60 (20-82)
KPS score	
Median (range)	90 (70-100)
MGMT status, No. (%)	
Unmethylated	29 (46.0)
Methylated	33 (52.0)
Unknown	1
IDH status, No. (%)	
Wt	53 (84.0)
IDH1-R32h	8 (13.0)
Unknown	2
% SVN (IHC), No. (%)	
1-4	2 (3.2)
5-9	15 (23.8)
10-19	35 (55.6)
≥ 20	12 (19.0)
Haplotype, No. (%)	
A*02/A*02	19 (30.2)
A*02/A*03	8 (12.7)
A*01/A*02	6 (9.5)
A*03/A*03	5 (7.9)
A*02/A*24	4 (6.3)
A*02/A*11	4 (6.3)
A*24/A*24	4 (6.3)
Other	13 (20.6)

Abbreviations: IDH, isocitrate dehydrogenase; IHC, immunohistochemistry; KPS, Karnofsky performance status; MGMT, O-6-methylguanine-DNA methyltransferase; SVN, survivin; Wt, wild-type.

Tumor samples from 33 of 64 patients (52.4%) were subjected to RNA sequencing. This group had similar sex (67% male and 33% female), age (median, 59 years; range, 24-76 years), and MGMT methylation status (56% methylated and 44% unmethylated) as the entire trial cohort. Aligned expression data were used to assign GBM molecular subtypes by GSVA using gene sets described by Wang et al[23] (Appendix Fig A2, online only). The sample population included 28 IDH wild-type tumors, of which 13 (46.4%) were classical, 10 (35.7%) mesenchymal, and 5 (17.9%) proneural. All molecular subtypes of GBM were therefore represented within the trial cohort.

Tumor Progression and Survival

The stated primary end point of this study was PFS6, defined as the percentage of patients without tumor progression or death from any cause 6 months after the date of diagnosis (biopsy). Six months following diagnosis, 95.2% (95% CI, 86.0 to 98.4) of evaluable patients remained progression-free. Measured from diagnosis, mPFS was 14.4 months (95% CI, 12.6 to 16.1) and mOS was 28.4 months (95% CI, 24.7 to 31.7). In addition, 69.8% (95% CI, 56.9 to 79.6) of patients remained progression-free when measured from the start of treatment (3 months after diagnosis).

Analyses of the relationship of survival to MGMT status, IDH1 mutation, age, sex, and KPS were performed on a post hoc basis. The median follow-up time was 34.9 months (reverse Kaplan-Meier methodology). OS at 12 months was 87.2%, and PFS at 12 months was 47.6% (Fig 1A). At 36 months, 22.6% of patients remained progression-free, and 36-month OS was 41.4%. The median time to tumor progression (mPFS) was 11.4 months,

A

Methylation	mPFS (months)	95% CI	mOS (months)	95% CI
All patients	11.4	9.9 to 12.7	25.9	22.5 to 29.0
unMGMT	7.0	5.7 to 8.2	16.5	13.4 to 19.3
meMGMT	17.9	14.7 to 20.7	41.4	32.1 to 49.4
Methylation/IDH	**mPFS (months)**	**95% CI**	**mOS (months)**	**95% CI**
All patients/IDHwt	10.3	8.9 to 11.6	23.0	19.8 to 25.9
unMGMT/IDHwt	6.9	5.6 to 8.0	15.6	12.6 to 18.3
meMGMT/IDHwt	19.3	15.4 to 22.6	NR (> 41.4)	37.1 to 59.4 (at 41.4)
Survival	**PFS (%)**	**95% CI**	**OS (%)**	**95% CI**
All patients				
6 months	69.80	56.8 to 79.5	—	—
12 months	47.60	34.9 to 59.3	87.20	76.1 to 93.4
24 months	26.60	16.4 to 37.9	51	38.3 to 63.0
36 months	22.60	12.9 to 33.9	41.40	27.8 to 54.5

B

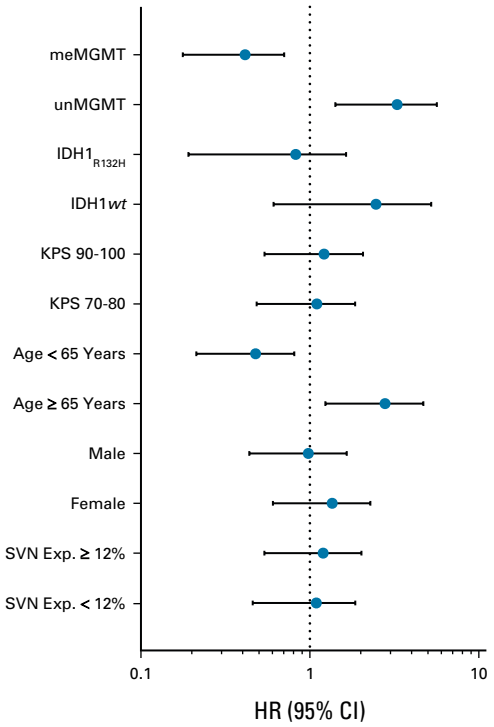

FIG 1. Survival outcomes of treated patients measured from start of SurVaxM treatment. (A) mPFS and mOS in months for patients from the start of treatment (first immunization). PFS or OS as a percentage of all evaluable patients at 6, 12, 24, and 36 months from the start of treatment. (B) Data points represent Cox HRs of OS for each subgroup. Error bars represent 95% CI of HRs. $P < .01$ for methylated MGMT patients (HR, 0.36; 95% CI, 0.18 to 0.71) and patients younger than 65 years (HR, 0.41; 95% CI, 0.21 to 0.81) Stratification by IDH1, KPS, and survivin expression did not produce significantly different HRs (post hoc analyses). HR, hazard ratio; Exp., expression; IDH, isocitrate dehydrogenase; KPS, Karnofsky performance status; meMGMT, methylated MGMT; MGMT, O-6-methylguanine-DNA methyltransferase; mOS, median overall survival; mPFS, median progression-free survival; NR, no response; OS, overall survival; PFS, progression-free survival; SVN, survivin; unMGMT, unmethylated MGMT; wt, wild-type.

and mOS was 25.9 months (Figs 1A and 2A). PFS and OS were closely associated (r = 0.79; 95% CI, 0.66 to 0.87; Fig 2B). When stratified by tumor methylation status, mPFS was 17.9 months for patients with tumors with MGMT methylation (n = 33) and 7 months for those with unmethylated MGMT (n = 29; Figs 1A and 2C). mOS was 41.4 months for patients with methylated MGMT and 16.5 months for patients with unmethylated MGMT (Figs 1A and 2D). For the eight patients whose tumors contained the IDH1 mutation R32H, mPFS was 15.5 months, and mOS was 41.4 months (Figs 1A, 2E and 2F). Analysis of age and outcome showed significant effects on mPFS and mOS. Patients younger than 65 years had an mPFS of 14.8 months and an OS of 36 months, compared with an mPFS of 6.7 months and an mOS of 15.8 months for those older than 65 years (Figs 2G and 2H).

Safety and Tolerability

There were no serious adverse events (AEs) attributed to SurVaxM, Montanide ISA-51, or sargramostim. The regimen was well tolerated with the most common AEs being Common Toxicity Criteria for Adverse Events grade 1 injection site reaction (Table 2). Two patients developed localized granulomatous panniculitis at injection sites, which was most likely caused by Montanide ISA-51. Both cases resolved with conservative therapy, including local corticosteroids. Neurologic events were largely attributable to the underlying disease state. Leukopenia and alterations in hematologic parameters were due to TMZ and were dose-related.

Cell-Based Immune Responses to Vaccination

CD8+ T-cell responses from 38 patients carrying at least one HLA-A*02 allele were measured in PBMCs in vitro following stimulation with HLA-A*02 restricted SurVaxM survivin peptides (SVN-1 and SVN-2; Fig 3). SVN-1 stimulation induced CD69 and HLA-DR expression in ≥ 1% of the CD3+/CD8+ subpopulation in 41.2% and 50% of patients, respectively (Table 3). More than half of the patient PBMCs stimulated with SVN-2, alone or in combination with SVN-1 (SVN-1/-2), showed ≥ 1% induction of CD69 or HLA-DR, whereas control CEF induced ≥ 1%

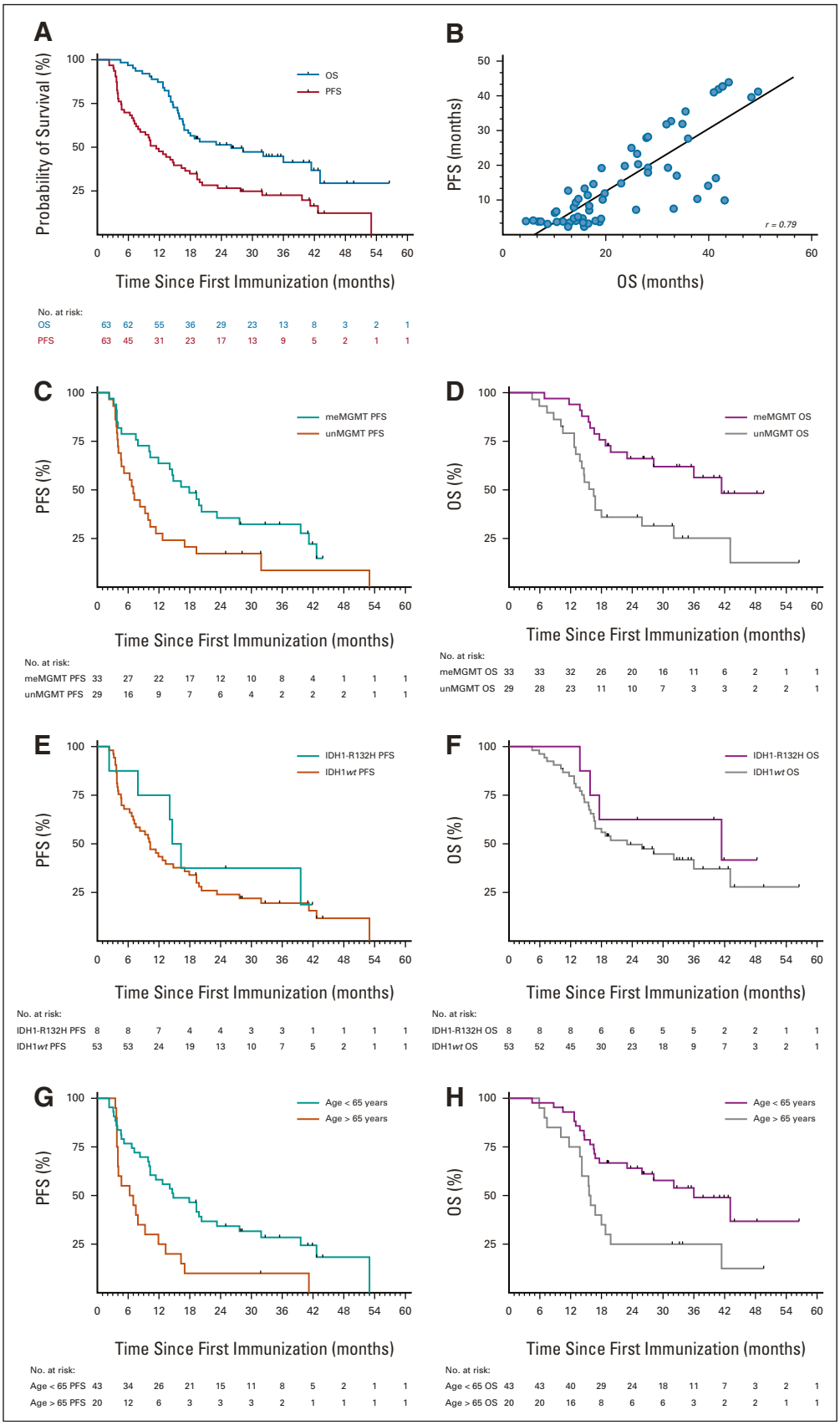

FIG 2. PFS and OS of patients treated with SurVaxM. (A) Kaplan-Meier curves of PFS and OS for all evaluable patients. (B) Correlation between OS and PFS for all patients (r = 0.79; 95% CI, 0.66 to 0.87). (continued on following page)

FIG 2. (Continued). Kaplan-Meier curves of (C) PFS and (D) OS for patients on the basis of MGMT status. Kaplan-Meier curves of (E) PFS and (F) OS for patients by IDH1 status. (G) PFS and (H) OS by age. *P* values use log-rank (Mantel-Cox). IDH, isocitrate dehydrogenase; meMGMT, methylated MGMT; MGMT, O-6-methylguanine-DNA methyltransferase; OS, overall survival; PFS, progression-free survival; unMGMT, unmethylated MGMT; wt, wild-type.

expression of these markers in only 16.2% and 27% of PBMCs, respectively. SVN-2 and SVN-1/-2 peptides induced CD69 by a significantly greater extent than CEF (Fig 3A). HLA-DR expression in CD3+/CD8+ cells was induced significantly by SVN-1, SVN-2, and SVN-1/2, but not by CEF (Fig 3B). SVN-1/2 induction of HLA-DR in CD3+/CD8+ cells was significantly greater than that of CEF.

TABLE 2. Adverse Events and Toxicities

Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4
Alopecia	1/1 (1.6%)			
Amnesia	2/2 (3.1%)			
Arthralgia	3/3 (4.7%)			
Asthenia		1/1 (1.6%)		
Back pain	1/1 (1.6%)			
Chills	1/1 (1.6%)			
Confusion			1/1 (1.6%)	
Decreased appetite	1/1 (1.6%)	1/1 (1.6%)		
Fatigue	12/12 (18.8%)	1/1 (1.6%)		
Hyperhidrosis	1/1 (1.6%)			
Hypersensitivity				
Hypertension—aggravated		1/1 (1.6%)		
Influenza-like illness	7/3 (4.7%)			
Injection site haematoma	5/4 (6.3%)			
Injection site induration	5/3 (4.7%)			
Injection site pain	12/9 (14%)			
Injection site pruritus	2/2 (3.1%)			
Injection site reaction	37/24 (37.5%)	3/3 (4.7%)		
Injection site swelling	2/2 (3.1%)			
Lymphopenia	2/2 (3.1%)	6/6 (9.4%)	1/1 (1.6%)	1/1 (1.6%)
Malaise	2/2 (3.1%)			
Myalgia	4/4 (6.3%)	1/1 (1.6%)		
Nausea	1/1 (1.6%)			
Neutrophil count decreased	2/2 (3.1%)	2/2 (3.1%)		1/1 (1.6%)
Panniculitis		2/2 (3.1%)		
Paresthesia	3/3 (4.7%)			
Platelet count decreased	2/2 (3.1%)			
Pruritus	2/2 (3.1%)	1/1 (1.6%)		
Pyrexia	2/2 (3.1%)			
Rash	2/2 (3.1%)	1/1 (1.6%)	1/1 (1.6%)	
Rash maculopapular			1/1 (1.6%)	
Skin hypertrophy	1/1 (1.6%)			
Subcutaneous nodule	3/3 (4.7%)			
Transaminases increased		1/1 (1.6%)		
Urticaria	1/1 (1.6%)	1/1 (1.6%)		
Leukopenia	4/4 (6.3%)			

NOTE. All adverse events that were definitely, probably, or possibly related to SurVaxM, Montanide ISA-51 VG, sargramostim, or temozolomide. Data represent events/affected patients (percent at risk).

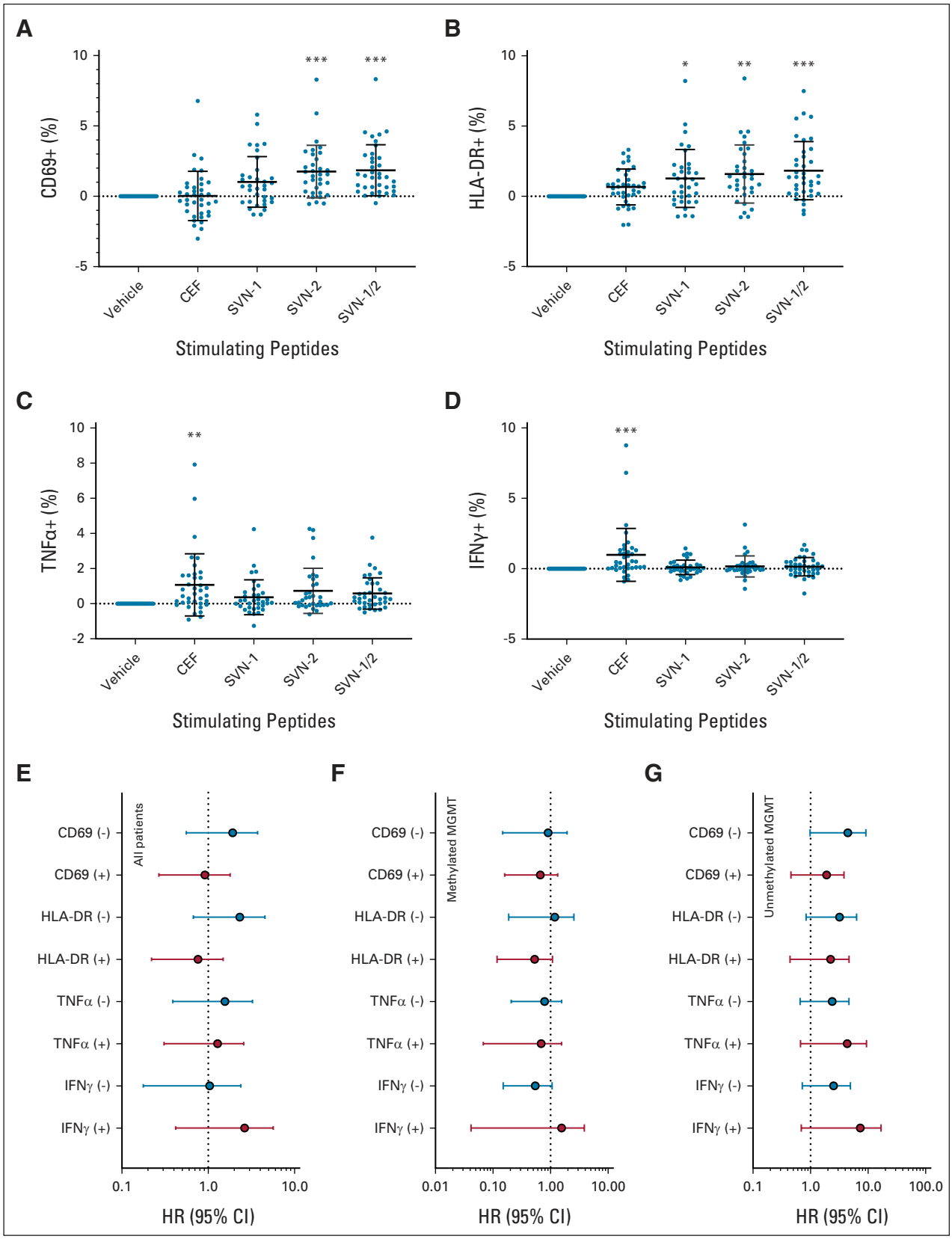

FIG 3. CD8+ T-cell responses to SurVaxM. PBMCs were harvested from blood obtained at approximately 17 weeks (V5 time point). Stimulation of patient PBMCs by peptides in vitro was followed by assessment of activation markers and cytokines. (A) CD69-, (B) HLA-DR-, (C) TNFα-, and (D) IFNγ-positive cells (gated on CD3+CD8+) following stimulation with the specified peptides. (continued on following page)

FIG 3. (Continued). *$P < .05$; **$P < .005$; ***$P < .0005$. (E-G) Plots showing expression of activation markers and cytokines expressed by CD3+CD8+ PBMCs (> 1% increase) following stimulation with SVN-1/-2, versus OS. Data points represent Cox HRs of OS for each subgroup. Error bars represent 95% CI of HRs. Stratification did not produce significantly different HR for T-cell response correlations with OS (post hoc analyses). CEF, CMV-EBV-influenza peptides; HR, hazard ratio; IFN, interferon; MGMT, O-6-methylguanine-DNA methyltransferase; OS, overall survival; PBMCs, peripheral blood mononuclear cells; SVN, survivin; TNF, tumor necrosis factor.

CEF stimulation induced TNFα and IFNγ production by CD3+/CD8+ cells significantly more than induction by SVN-1, SVN-2, and SVN-1/-2 (Figs 3C and 3D). SVN-2 induced expression of TNFα or IFNγ in 30.3% and 12.1% of patient PBMCs, respectively, and SVN-1/-2 induced expression in 27% and 10.8% of patient PBMCs, respectively (Table 3).

Although T-cell activation by survivin peptides was detected (Figs 3A-3D), T-cell responses did not correlate with OS (Figs 3E-3G). PBMCs stimulated by SVN-1/-2 trended toward positive correlation with OS in methylated patients, but not in unmethylated patients.

Humoral Immune Responses

Patient serum was subjected to enzyme-linked immunosorbent assay to measure antibodies to SurVaxM. Significant increases in anti-SurVaxM titers were observed for all time points compared with baseline. Responses were maintained over time (Fig 4A). Anti-SurVaxM titers ≥ 1:10,000 were achieved in 80% (47/59) of patients, and titers > 1:100,000 were achieved in 31% (18/59) of patients. Three patients had reactive titers > 1:10,000 before vaccination. Higher anti-SurVaxM serum immunoglobulin G levels (titer > 30,000) correlated significantly with OS (Figs 4B and 4C) and showed positive trends for both methylated and unmethylated patients (Fig 4B).

DISCUSSION

SurVaxM induces antitumor immunity that targets a conserved amino acid sequence shared by several survivin isoforms that traffic from different cell compartments.[7,8,16,17] SurVaxM itself is a mimetic antigen (an altered multiepitope, long survivin peptide-keyhole limpet hemocyanin conjugate) that produces both survivin-specific T cells and antisurvivin antibodies. In preclinical models and in both the current study and one previous clinical study, SurVaxM induced both survivin-specific antibody production and cellular (CD8+ and CD4+) antitumor immunity.[15,16] Although vaccinated patients developed both CD8+ T-cell and antibody responses to SurVaxM, only the unique antibody response was associated with longer PFS and OS, suggesting that cell-surface survivin may be a significant immunotherapeutic target.

Surgical resection and chemoradiation provide disease remission during which immunogens may stimulate more effective immune responses than at later time points in the course of disease.[24] Thus, the optimal time to introduce an immunotherapeutic intervention for GBM may be during the interval following chemoradiation when disease burden has been reduced. Therefore, we chose to perform vaccinations beginning during the hiatus between chemoradiation and the start of adjuvant chemotherapy.

Toxicity of the experimental regimen was low. The most common AEs attributable to the vaccine combination were local injection site reactions, which have been described previously with peptide vaccines given in Montanide ISA-51.[25] Two patients developed local granulomatous panniculitis at injection sites, which has been reported with similar vaccine regimens.[26,27] Both patients responded well to local corticosteroid therapy, and the AEs were not regimen-limiting. Significantly, no signs of autoimmunity were encountered. Other AEs including leukopenia, thrombocytopenia, nausea, and constipation were observed to be no higher than has been reported for TMZ monotherapy.[28] Thus, the combination of SurVaxM in Montanide ISA-51 with locally administered sargramostim as an add-on to TMZ adjuvant chemotherapy was a safe and well-tolerated regimen in patients with nGBM.

TABLE 3. CD8 T-Cell Stimulation by Peptides In Vitro

T-Cell Phenotype	CEF	SVN-1	SVN-2	SVN-1/2
CD3+/CD8+/CD69+	6/37 (16.2%)	14/34 (41.2%)	20/33 (60.6%)	22/37 (59.5%)
CD3+/CD8+/HLA-DR+	10/37 (27%)	17/34 (50%)	19/33 (57.6%)	20/37 (54.1%)
CD3+/CD8+/TNFα+	14/37 (37.8%)	6/34 (17.6%)	10/33 (30.3%)	10/37 (27%)
CD3+/CD8+/IFNγ+	13/37 (35.1%)	3/34 (8.8%)	4/33 (12.1%)	4/37 (10.8%)

NOTE. Proportion of CD3+CD8+ T cells showing activation marker (CD69, HLA-DR) or cytokine (TNFα, IFNγ) induction ≥ 1% above control cells following stimulation with positive control CEF peptide, SVN-1 peptide, SVN-2 peptide, or SVN-1 plus SVN-2 peptides.

Abbreviations: CEF, CMV-EBV-influenza peptides; INF, interferon; SVN, survivin; TNF, tumor necrosis factor.

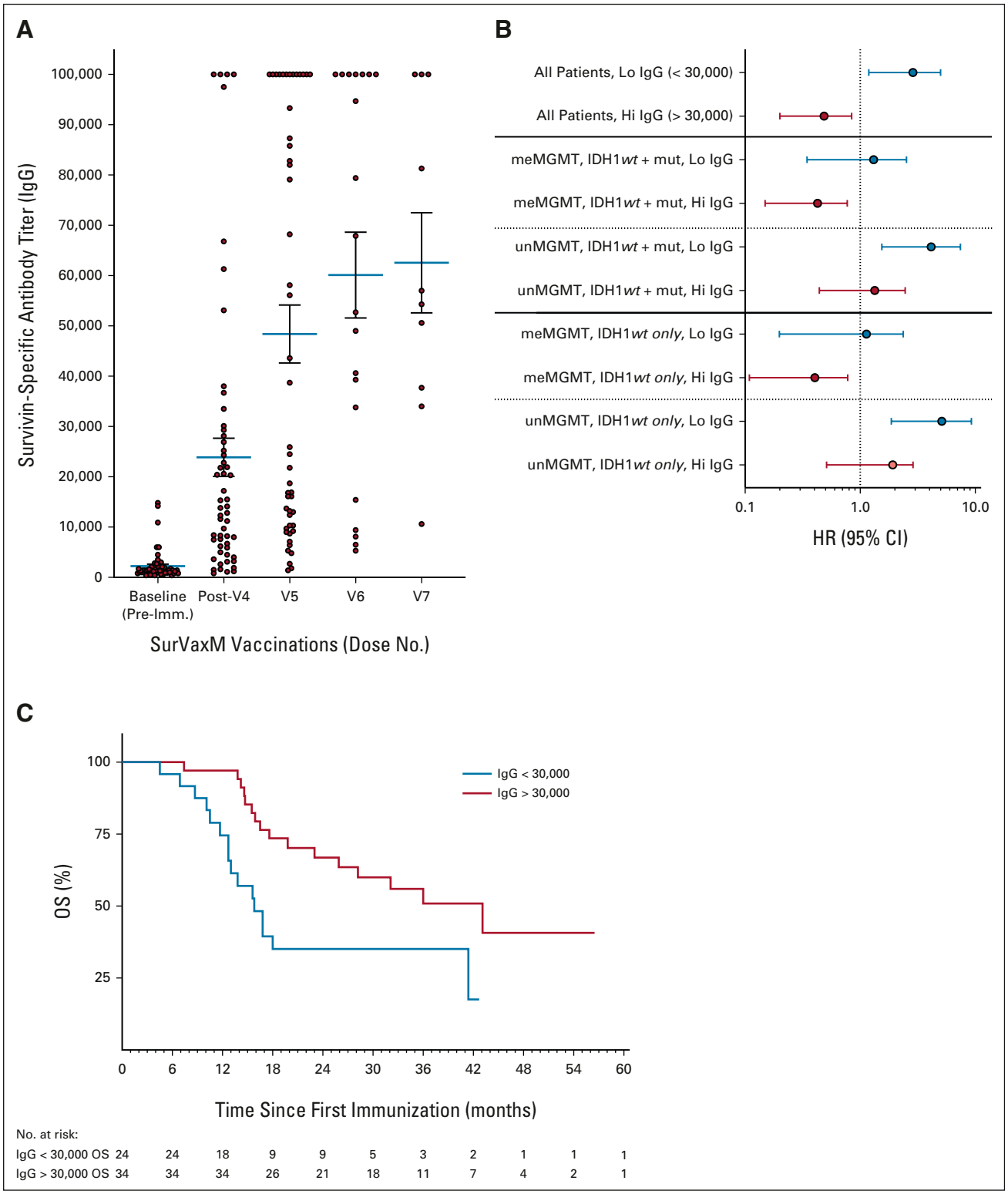

FIG 4. Humoral responses in patients treated with SurVaxM. (A) Anti–SurVaxM-specific antibody (IgG) titers in patients over time. (B) Correlation between OS and anti-SurVaxM IgG titers. Data points represent Cox HRs of OS for each subgroup. Error bars represent 95% CI of HRs. $P = .0146$ for patients with IgG responses > 30,000 in titer (HR, 0.41; 95% CI, 0.20 to 0.84). Additional stratification by IDH1 and MGMT showed trends to better HR with high (> 30,000) IgG (in red; post hoc analyses). (C) Kaplan-Meier analysis of IgG stratification. Titer > 30,000, mOS = 43.1 months; 95% CI, 12.5 to 18.7 (red). Titer < 30,000, mOS = 15.8 months; 95% CI, 33.5 to 52.3 (blue). Hi, high; HR, hazard ratio; IDH, isocitrate dehydrogenase; IgG, immunoglobulin G; Lo, low; meMGMT, methylated MGMT; MGMT, O-6-methylguanine-DNA methyltransferase; mOS, median overall survival; mut, mutation; OS, overall survival; Pre-Imm., pre-immune; unMGMT, unmethylated MGMT; wt, wild-type.

Six months following diagnosis, 95.2% of evaluable SurVaxM-treated patients remained progression-free. This was a significantly greater percentage ($P < .0001$) than the PFS6 of 54% previously reported for one notable external control group.[1] In addition, 69.8% of patients remained progression-free when measured from the start of treatment (3 months post-diagnosis), significantly greater than another external control group in which PFS6 was 37% ($P < .0001$).[2]

OS can be affected by subsequent treatments following initial tumor progression and off-study events. However, in the current study and accounting for censoring, PFS correlated strongly with OS ($r = 0.79$), suggesting that therapies subsequent to the off-study date had modest impact on OS.[29] Thus, PFS may represent an acceptable surrogate for OS as a primary end point in patients with nGBM treated with adjuvant TMZ and SurVaxM following surgical resection and chemoradiation.

In a randomized phase III multicenter trial, patients with nGBM who received 60 Gy of radiation over 6 weeks with concurrent TMZ 75 mg/m^2 once daily for 42 days, followed by adjuvant TMZ 150 mg to 200 mg/m^2 once daily for 5 consecutive days each month for 6 months, the mOS was 14.6 months.[1] In this study, patients who had surgical resection of their tumors (84%) had an mOS of 15.8 months, whereas those who had only biopsy (16%) had an mOS of 9.4 months. More recent analysis suggests that extending adjuvant TMZ treatment beyond six cycles has little impact on survival.[30]

In a recent randomized study of patients with nGBM treated with either adjuvant TMZ alone, or TMZ plus TTF, patients receiving only TMZ had an mOS of 16.0 months from random assignment. The addition of TTF to standard therapy led to an OS of 20.5 months.[2] Despite safety and apparent efficacy for nGBM, TTF is not universally adopted because of patient preference and other factors.[31] Currently, there are no data to suggest how TTF might interact with active specific vaccination immunotherapy.

MGMT methylation is another important prognostic factor in patients with nGBM. Recent aggregated data suggest that patients with methylated MGMT genes who are treated with TMZ have a mOS of 24.6 months, whereas patients with unmethylated MGMT genes have a much-reduced mOS of 14.1 months.[32] Post hoc analysis suggests improved survival in both methylation groups when receiving SurVaxM, with methylated patients reaching a mOS of 41.4 months and unmethylated patients a more modest mOS of 16.5 months.

Given the results of contemporary studies of standard-of-care therapy for nGBM, the combination of TMZ plus SurVaxM appears to be a promising adjuvant regimen for further investigation. Age, sex, performance status, extent of resection, MGMT methylation, and IDH mutation can affect outcomes in single-arm phase II studies for which the only available controls are external cohorts. Thus, to determine if the results of the current study are generalizable to patients with nGBM, a randomized placebo-controlled trial of SurVaxM plus TMZ is underway.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Phase IIa Study of SurVaxM Plus Adjuvant Temozolomide for Newly Diagnosed Glioblastoma

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APPENDIX

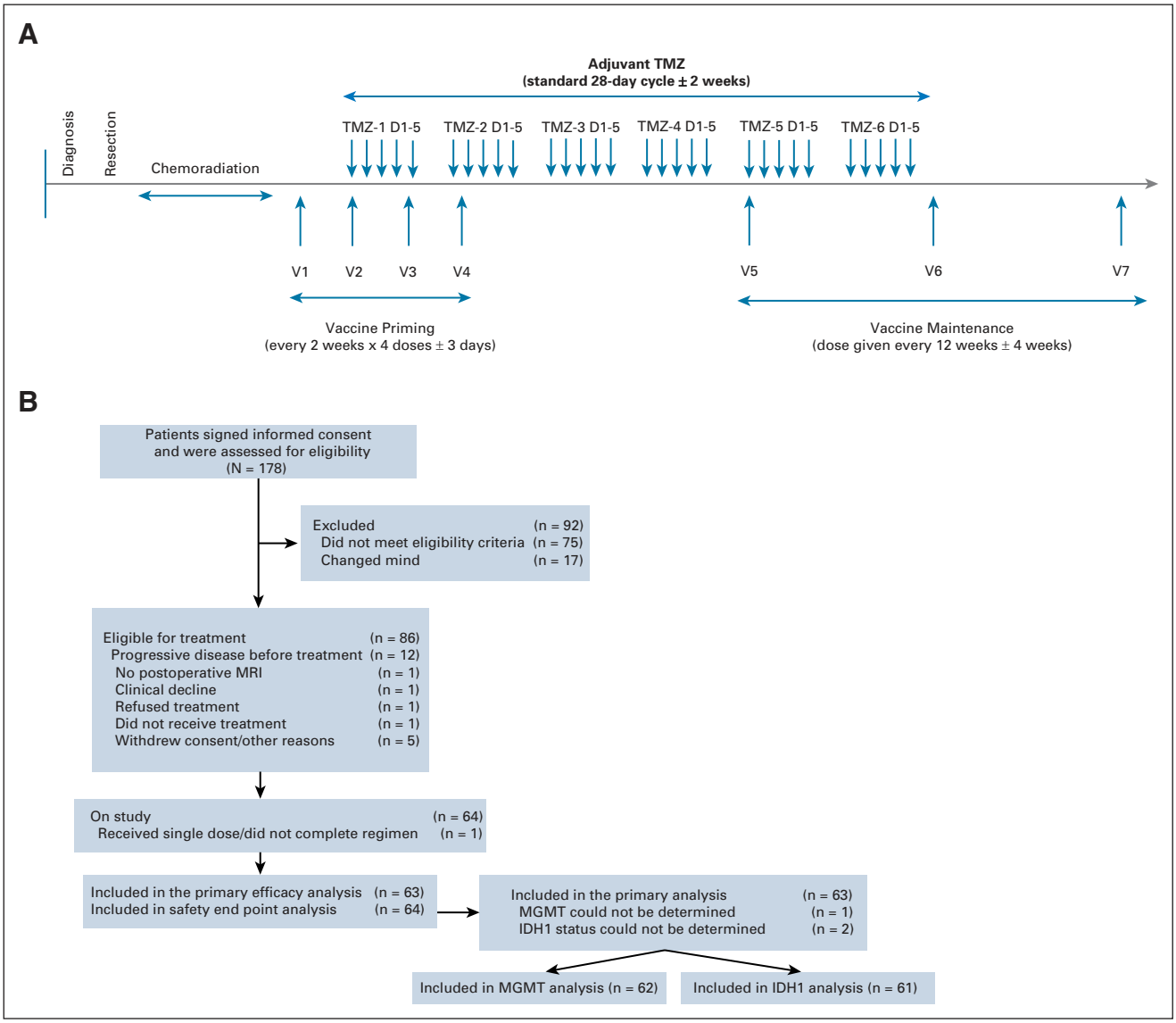

FIG A1. Clinical trial summary. (A) Treatment schedule overview for patients on trial. (B) Recruitment and inclusion of patients on study. Sixty-four patients were recruited and included in the study. One patient who received only a single priming dose of SurVaxM was excluded from efficacy analysis but was included in the final safety analysis. IDH, isocitrate dehydrogenase; MGMT, O-6-methylguanine-DNA methyltransferase; MRI, magnetic resonance imaging; TMZ, temozolomide.

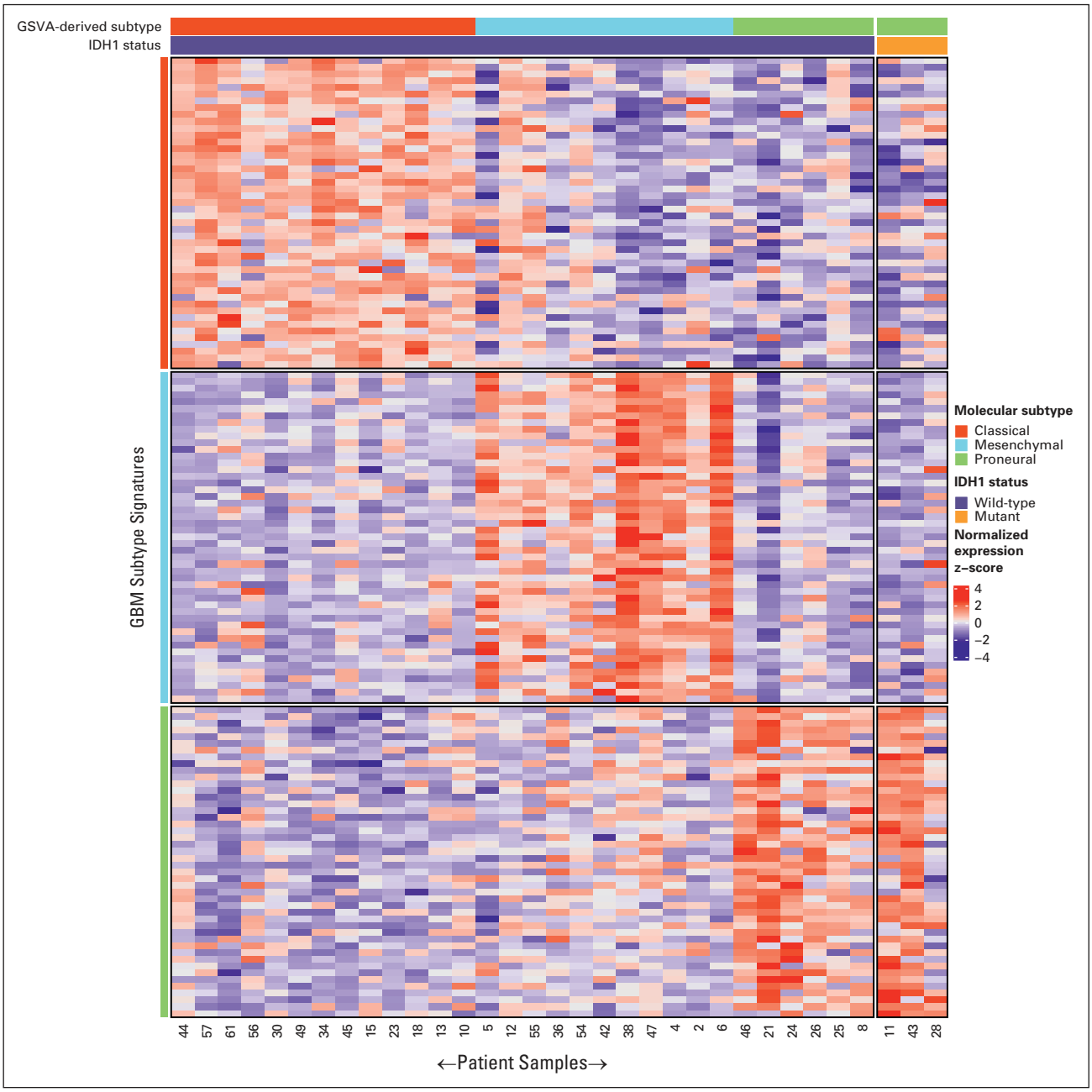

FIG A2. Molecular profiling analysis of patient tumors. Thirty-three patient tumors were assessed by RNAseq and assigned to subtypes on the basis of gene expression as described in the study by Wang et al.[23] GBM, glioblastoma; GSVA, gene set variation analysis; IDH, isocitrate dehydrogenase.