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ORIGINAL REPORT

Antigen-Specific Immune Responses and Clinical Outcome After Vaccination With Glioma-Associated Antigen Peptides and Polyinosinic-Polycytidylic Acid Stabilized by Lysine and Carboxymethylcellulose in Children With Newly Diagnosed Malignant Brainstem and Nonbrainstem Gliomas

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose
Diffuse brainstem gliomas (BSGs) and other high-grade gliomas (HGGs) of childhood carry a dismal prognosis despite current treatments, and new therapies are needed. Having identified a series of glioma-associated antigens (GAAs) commonly overexpressed in pediatric gliomas, we initiated a pilot study of subcutaneous vaccinations with GAA epitope peptides in HLA-A2–positive children with newly diagnosed BSG and HGG.

Patients and Methods
GAAs were EphA2, interleukin-13 receptor alpha 2 (IL-13Rα2), and survivin, and their peptide epitopes were emulsified in Montanide-ISA-51 and given every 3 weeks with intramuscular polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose for eight courses, followed by booster vaccinations every 6 weeks. Primary end points were safety and T-cell responses against vaccine-targeted GAA epitopes. Treatment response was evaluated clinically and by magnetic resonance imaging.

Results
Twenty-six children were enrolled, 14 with newly diagnosed BSG treated with irradiation and 12 with newly diagnosed BSG or HGG treated with irradiation and concurrent chemotherapy. No dose-limiting non-CNS toxicity was encountered. Five children had symptomatic pseudoprogression, which responded to dexamethasone and was associated with prolonged survival. Only two patients had progressive disease during the first two vaccine courses; 19 had stable disease, two had partial responses, one had a minor response, and two had prolonged disease-free status after surgery. Enzyme-linked immunosorbent spot analysis in 21 children showed positive anti-GAA immune responses in 13: to IL-13Rα2 in 10, EphA2 in 11, and survivin in three.

Conclusion
GAA peptide vaccination in children with gliomas is generally well tolerated and has preliminary evidence of immunologic and clinical responses. Careful monitoring and management of pseudoprogression is essential.

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INTRODUCTION

Children with diffuse intrinsic brainstem malignant gliomas have 1-year progression-free survival rates below 25% and median overall survival of 9 to 10 months with current treatment.[1-4] Other than irradiation, no therapy has demonstrated clinical benefit.[3-8] Similarly, children with newly diagnosed nonbrainstem high-grade gliomas have a poor prognosis, with 5-year survival rates around 10% with the use of irradiation and conventional chemotherapy.[9-11]

Accordingly, there is a need for new therapeutic approaches. Cancer vaccines are promising in this regard, because they are designed to induce a systemic immune response against antigens overexpressed by tumor cells. Early-phase clinical trials by us[12-17] and others[18-24] have demonstrated the safety and potential efficacy of subcutaneous or intranodal vaccinations for adults with malignant gliomas. Such approaches may be most effective if applied early in treatment, particularly in patients likely to have robust immunity, such as the pediatric age group.[25-27]

Targeting of multiple glioma-associated antigen (GAA) epitopes helps to address the issue of inter- and intratumoral heterogeneity.

This trial was based on the use of peptide epitopes for three GAAs that we observed to be highly expressed in pediatric glioblastomas and other gliomas: interleukin-13 receptor alpha 2 (IL-13Rα2), EphA2, and survivin.[28] The HLA-A2–restricted cytotoxic T-lymphocyte epitopes included two that we had previously identified, an IL-13Rα2 analog peptide (IL-13Rα2$_{345\text{-}353\text{:}1A9V}$)[29,30] and EphA2$_{883\text{-}891}$,[31] as well as survivin$_{96\text{-}104\text{:}M2}$,[32-34] admixed with a pan–HLA-DR tetanus toxoid peptide (Tet$_{A830\text{-}845}$). The peptides were administered in Montanide, a vaccine delivery vehicle shown in murine models to promote high levels of antigen-specific cytotoxic T-lymphocytes,[35] thus avoiding the need for dendritic cell harvesting. The vaccine was administered concurrently with the immunoadjuvant polyinosinic-polycytidylic acid (poly[I:C]) stabilized by lysine and carboxymethylcellulose (poly-ICLC), which has been shown to enhance efficacy of GAA-targeted vaccinations in glioma-bearing mice[35,36] and to be well tolerated in patients with malignant gliomas.[17,37-39]

To the best of our knowledge, this is the first study to evaluate vaccination with GAA epitopes in conjunction with poly-ICLC for active immunotherapy of children with malignant brain tumors. The primary objectives were to assess tolerability of this regimen and its ability to induce GAA epitope–targeted immune responses.

PATIENTS AND METHODS

Patients

Patients between 1 and 21 years of age with high-risk gliomas were eligible. These included newly diagnosed diffuse intrinsic pontine gliomas defined by magnetic resonance imaging (MRI)[40] or biopsy-confirmed high-grade brainstem gliomas (BSGs) and newly diagnosed biopsy-confirmed nonbrainstem high-grade gliomas (HGGs; ie, glioblastomas and anaplastic astrocytomas). Adequate organ function, absolute lymphocyte count ≥ 500, Karnofsky or Lansky performance score ≥ 60, and HLA-A2–positive status were required. The maximal allowable dexamethasone dose during the week before enrollment was 0.1 mg/kg per day (maximum, 4 mg per day). Patients must have received 50 to 60 Gy involved field fractionated radiotherapy and were stratified on the basis of whether they received chemotherapy during irradiation. Postradiation chemotherapy was not permitted. The trial was conducted under US Food and Drug Administration Investigator New Drug application No. 13624 and Institutional Review Board protocol PRO08030085. Signed informed consent forms approved by the institutional review board were required for HLA screening and for initiation of therapy.

Study Design

Patients received subcutaneous injections of GAA-derived HLA-A*0201–restricted peptides and Tet$_{A830\text{-}845}$ emulsified in Montanide ISA-51 (Seppic, Fairfield, NJ) and concurrent intramuscular injections of the toll-like receptor ligand poly-ICLC (30 μg/kg; Hiltonol, Oncovir, Washington, DC), every 3 weeks (defined as a course) for eight vaccines. Participants were evaluated for adverse events, regimen-limiting toxicities (RLTs), and treatment response by clinic visits, laboratory testing, and MRI. Immune response was assessed 6, 15, and 21 weeks after starting vaccination by enzyme-linked immunosorbent spot (ELISPOT) assay on peripheral blood mononuclear cells (PBMCs); MRI scans were done at the same time points. Patients demonstrating complete response, partial response (PR; > 50% decrease in maximum cross-sectional tumor area), minor response (25% to 50% decrease in maximum tumor area), or stable disease without RLTs could continue vaccination courses every 6 weeks for up to 2 years after initial vaccination, with additional immunologic and MRI evaluations obtained at 12-week intervals.

Toxicity Assessment and Stopping Rules

The trial was monitored for treatment-related adverse events by using National Cancer Institute Common Toxicity Criteria 3.0. The following were considered to be RLTs: grade ≥ 2 hypersensitivity or allergic reaction, grade ≥ 3 nonhematologic toxicity, or grade ≥ 3 hematologic toxicity that recurred despite a 33% poly-ICLC dose reduction or did not resolve to grade ≤ 1 by the time the next dose was due. Stopping rules were implemented such that the treatment was considered excessively toxic, if at any time the observed rate of RLTs was ≥ 33% and at least two RLTs had been observed in any stratum.

Peptides

HLA-A2–restricted peptides used in this study were ALPFGFILV (IL-13Rα2$_{345\text{-}353\text{:}1A9V}$),[30] TLADFDPRV (EphA2$_{883\text{-}891}$),[31] and LMLGEFLKL (survivin$_{96\text{-}104\text{:}M2}$),[32-34] admixed with AQYIKANSKFIGITEL (Tet$_{A830\text{-}845}$).[41,42] The peptides were produced by using automated solid-phase synthesis by NeoMPS (PolyPeptide Group, San Diego, CA). Peptides were tested in multiple quality-assurance studies that included assessment of purity, sterility, identity, potency, pyrogenicity, and stability.

ELISPOT Assays

ELISPOT assays were performed on PBMCs obtained and cryopreserved before vaccination (week 0), at weeks 6, 15, 21, and every 12 weeks thereafter. Batched PBMC samples from each patient were evaluated simultaneously following a 7-day in vitro stimulation with IL-13Rα$_{345\text{-}353}$, EphA2$_{883\text{-}891}$, and survivin$_{96\text{-}104}$ peptides, 10 ng/mL IL-7, and 20 IU/mL IL-2. Interferon gamma (IFN-γ) responses by purified CD8$^+$ and CD4$^+$ T cells were tested against T2 cells pulsed with GAA peptides or PBMCs pulsed with Tet$_{A830\text{-}845}$, respectively. A positive ELISPOT response was defined as more than two-fold increase in net spot-forming T cells (after background subtraction; CD8$^+$ cells for GAAs and CD4$^+$ cells for Tet$_{A830\text{-}845}$) over the prevaccine level and at least 50 spots per 100,000 cells. Serum cytokine and chemokine responses were assessed in parallel (see "Methods" in the Appendix [online only]).

Immunohistochemical Analysis

In patients with available tissue, expression of the target proteins was determined as described,[28] with minor modifications, specifically using monoclonal antibodies against human EphA2 (1:50; Santa Cruz Biotechnology, Dallas, TX, sc-10746), IL-13Rα2 (1:500; Abcam, Cambridge, MA, ab55275), survivin (1:100; sc-10811), and EnVision+ System HRP polymer (Dako, Carpinteria, CA). Specimens were graded as positive if they had antigen expression in 20% to 60% of tumor cells (2) or more than 60% of tumor cells (3) versus negative if they had no staining (0) or staining in less than 20% of tumor cells (1).

Radiologic Response Monitoring

Tumor size was assessed before vaccination (week 0) and at weeks 6, 15, 21, and every 12 weeks thereafter using MRI scans with and without contrast enhancement. More frequent scans were obtained if clinically warranted. Response was evaluated by gadolinium-enhanced T1-weighted images, T2-weighted images, or both, based on the appearance of the pretreatment MRI.

Management of Immunologic Pseudoprogression

A concern regarding therapeutic vaccines in patients with brain tumors is the development of pseudoprogression,[17,43] characterized by transient increased edema and/or contrast enhancement of the tumor, secondary to intratumoral immune response, followed by stabilization or regression and symptomatic improvement. Given that pseudoprogression could lead to significant clinical deterioration, particularly in children with brainstem gliomas, we incorporated detailed management guidelines for possible pseudoprogression in the protocol, taking into account that the distinction from true progression was generally made in retrospect.

If tumor enlargement and/or increased enhancement was noted on an MRI scan, and the patient was neurologically worse, sufficient to warrant initiation of or increase in steroid dose, subsequent doses of vaccine and poly-ICLC were held. Imaging and clinical assessments were performed at 4-week intervals thereafter, until it was determined whether the changes reflected pseudoprogression or true progression. If the patient improved clinically with dexamethasone doses that could be weaned to ≤ 0.1 mg/kg per day for ≥ 1 week and the MRI changes improved or resolved, the patient was determined to have pseudoprogression and

Table 1. Demographics and Clinical and Immunologic Responses for Each Patient

Patient ID	Age (years)	Sex	Tumor Type	Histology, Resection Extent	Chemotherapy*	ELISPOT Responses				Best Response	Pseudoprogression	OS
						IL-13Rα2	EphA2	Survivin	Tet			
1	9.0	F	BSG		None	NA	NA	NA	NA	PD		8.3
2	13.6	F	BSG		None	NA	NA	NA	NA	PD		8.6
3	6.1	F	BSG		None	**68**	14	2	**1,480**	SD		9.6
4	7.1	F	BSG		None	**846**	**140**	**322**	**560**	SD	Yes	18.4
5	5.6	F	BSG		None	22	12	14	**534**	SD		13.0
6	7.3	F	HGG	GBM, STR	TMZ	**742**	**256**	42	**456**	SD		10.8
7	7.2	M	BSG		SAHA	**474**	**340**	6	**396**	SD		15.7
8	9.6	M	HGG	GBM, GTR	TMZ	**78**	**102**	26	**182**	CCR		25.1
9	5.2	M	BSG		None	14	**180**	4	**162**	SD		10.9
10	7.8	F	BSG		None	24	26	38	208	SD	Yes	19.5
11	10.2	F	BSG		None	**248**	28	6	**84**	PR	Yes	19.5
12	17.9	M	BSG		None	**344**	**188**	**314**	0	SD	Yes	11.3
13	16.6	M	HGG	GBM, STR	TMZ	**240**	**138**	42	2	PR		20.3
14	4.7	M	BSG	AA, biopsy	TMZ	18	**72**	30	**348**	SD	Yes	> 38.0
15	3.3	M	BSG		TMZ	0	4	0	32	SD		23.0
16	3.1	M	BSG		0	AF	AF	AF	AF	SD		23.6
17	2.2	M	BSG		Bev	16	8	12	10	SD		13.3
18	9.3	M	BSG		TMZ	10	**64**	34	18	SD		8.5
19	11.7	M	HGG	AA, biopsy	TMZ	**54**	**84**	**74**	28	MR		26.0
20	7.8	M	BSG		TMZ	24	44	40	34	SD		6.3
21	4.4	F	BSG		None	18	12	14	0	SD		17.5
22	9.2	F	BSG		None	26	26	42	8	SD		12.4
23	9.7	F	BSG		None	**608**	**60**	30	**222**	SD		13.6
24	16.1	F	BSG		None	2	10	0	**66**	SD		10.0
25	13.6	M	HGG	GBM, GTR	TMZ	PA	PA	PA	PA	CCR		> 14.7
26	11.5	F	HGG	GBM, biopsy	TMZ	PA	PA	PA	PA	SD		> 13.1

NOTE. Results of maximal interferon gamma enzyme-linked immunosorbent spot (ELISPOT) reactivity (net spots per 10^5 cells after background subtraction) are shown for each peptide epitope. Values ≥ 50 were considered positive and, in all but one instance, they reflected a two-fold increase compared with prevaccine. The sole exception, patient 10, responded strongly to tetanus toxoid at baseline so did not fulfill the criterion for a two-fold increase. Positive responses are shown in **bold**. NA indicates that samples were not available because of early progression before week 6.

Abbreviations: AA, anaplastic astrocytoma; AF, assay failure; Bev, bevacizumab; BSG, brainstem glioma; CCR, continued complete response (ie, prolonged disease-free status after surgical resection); GBM, glioblastoma; GTR, gross total resection; HGG, high-grade glioma; ID, identification number; IL-13Rα2, interleukin-13 receptor α2; MR, minor response; OS, overall survival; PA, pending analysis; PD, progressive disease; PR, partial response; SAHA, suberoylanilide hydroxamic acid; SD, stable disease; STR, subtotal resection; Tet, tetanus toxoid; TMZ, temozolomide.

*Chemotherapy was administered concurrently with irradiation.

could restart vaccine treatment with 67% of the poly-ICLC dose (ie, 20 μg/kg). Conversely, if the repeat MRI scan was unchanged or worse, and the patient's clinical status had not improved despite increased dexamethasone doses, the patient was taken off study for tumor progression.

Statistical Methods

This pilot study was designed to have 12 patients with newly diagnosed BSG or HGG receiving either radiation alone or radiation with concurrent chemotherapy to assess safety and immunologic efficacy. Each stratum was to be analyzed separately to provide a point estimate of immune response as assessed by the ELISPOT assay. A stratum was considered worthy of further investigation if there were at least five ELISPOT responses among 12 patients. In addition, we planned to stop accrual to a stratum if the rate of RLTs was greater than 33%, and at least two RLTs were observed. Patients with disease progression during the first two courses of therapy were replaced by other patients for RLT analysis. Survival was assessed by using the Kaplan-Meier method, and survival curves were compared using the Cox proportional hazards method. Two-sided P values ≤ .05 were considered statistically significant.

RESULTS

Demographics and Clinical Characteristics

Between May 2009 and December 2012, 84 newly diagnosed patients were screened for HLA status, among which 42 were HLA-A2–positive; 16 did not enroll because of inability to wean from dexamethasone (four), poor performance status (two), metastatic disease (two), ineligible diagnoses or irradiation doses (five), or opting for other treatments (three). Twenty-six patients were enrolled (Table 1), including 14 (all BSGs) treated with irradiation alone, and 12 treated with irradiation and concurrent chemotherapy, of whom six had BSG and six had nonbrainstem HGG; the latter groups were pooled for toxicity and immunologic analyses. Survival outcome in the BSG and HGG groups were also assessed independently. Two patients in the radiotherapy only group with early disease progression who were thus not evaluable for RLT were replaced.

Summary of Systemic Toxicities

The primary objective of this study was to assess safety, given that this was the first such trial in children. Principal toxicities included grade 1 and 2 injection-site reactions (100%) and flu-like symptoms (fatigue, myalgias, fever, chills, and headache; 92%), usually limited to 24 to 48 hours after each vaccine and controlled with acetaminophen and/or ibuprofen, and grade 1 GI symptoms (31%). Grade 1 leukopenia developed in four patients (15.4%). No

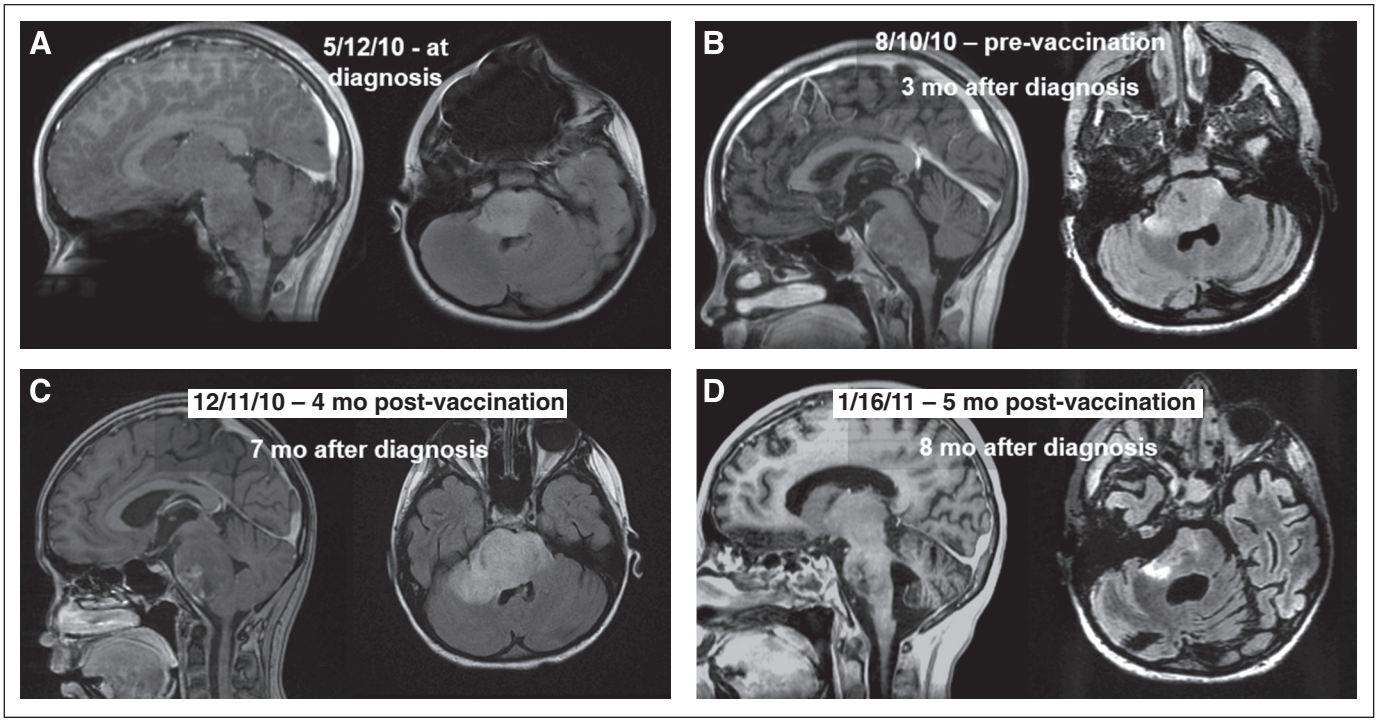

Fig 1. Magnetic resonance imaging (MRI) results in a responding patient (patient 11), with transient pseudoprogression. In each panel, sagittal T1-weighted gadolinium-enhanced images are on the left, and axial fluid-attenuated inversion recovery images are on the right. (A) MRI scan at diagnosis. (B) MRI scan before vaccine therapy (3 months after diagnosis and start of irradiation). Tumor is unchanged from baseline. (C) Four months after the start of vaccination, the patient had tumor enlargement, increased enhancement, and symptomatic worsening. High-dose dexamethasone was started. (D) The MRI scan shows dramatic reduction of enhancement and mass effect 1 month later (1/16/11). Dexamethasone was weaned, and vaccination was subsequently resumed. The partial response status was then maintained until 18 months after diagnosis.

grade 3 or higher systemic toxicities or instances of autoimmunity were encountered.

Pseudoprogression

Two children with BSGs developed acute neurologic worsening several months after beginning vaccination: one with central hyperventilation and fatigue, and another with lower cranial neuropathies resulting in aspiration pneumonia, which necessitated intubation. MRI scans showed increased tumor size and enhancement, with subsequent clinical and radiographic improvement on high-dose dexamethasone, consistent with pseudoprogression; in one child, the improvement culminated in a prolonged objective response that was maintained until 18 months after diagnosis (Fig 1). Three other children with BSG had probable pseudoprogression with less abrupt onset of neurologic changes (eg, seventh nerve palsy and ataxia, worsened sixth nerve palsy, and torticollis with neck pain, respectively) and imaging that worsened during vaccination followed by radiographic stabilization and/or prolonged survival, including one child (patient 14) who subsequently had dramatic disease regression after coming off study and receiving palliative chemotherapy. Four of five children with BSG and pseudoprogression survived at least 18 months after diagnosis versus two of 15 without pseudoprogression, suggesting that this may represent an efficacy signal.

Induction of Epitope-Specific Immune Responses Against GAAs

All but two patients who had disease progression before completion of the second vaccine course had PBMCs available for immunologic analysis. One child had samples that were not evaluable, and two had samples that are pending analysis. In 13 of 21 evaluable patients, vaccination induced immune responses to at least one vaccine-targeted GAA epitope by IFN-γ ELISPOT assays (Table 1): to IL-13Rα2 in 10, to EphA2 in 11, and to survivin in three. In three of three patients with adequate PBMC samples, we also detected $CD4^+$ and $CD8^+$ T-cell IFN-γ responses to full-length GAA proteins presented by autologous dendritic cells (data not shown). Twelve patients responded to the Tet peptide, including one with a strong baseline response who did not meet criteria for a two-fold increase. Six of 11 evaluable patients who received radiotherapy alone exhibited positive immune responses (response rate, 0.55; 95% CI, 0.23 to 0.83) as did seven of 10 who received radiation and chemotherapy (response rate, 0.70; 95% CI 0.35 to 0.93).

The time course and magnitude of the ELISPOT responses in 15 patients who had samples at baseline and at the 6-, 15-, and 21-week time points are summarized in Figure 2A. The IL-13Rα2 epitope demonstrated the highest magnitude of responses. Although some patients demonstrated sustained positive responses to one or more antigens that increased over multiple courses of vaccination, in others, immune responses peaked at 6 to 15 weeks, reflecting that a subset of patients at longer times may have developed immune tolerance against vaccine-targeted GAAs or, conversely, had begun high-dose steroid therapy because of pseudoprogression, as illustrated by the course in patient 11. This patient (whose MRI scans are shown in Fig 1) demonstrated a prominent response to IL-13Rα2, which peaked around the onset of pseudoprogression (time point A in Fig 2B), diminished after administration of high-dose dexamethasone, and

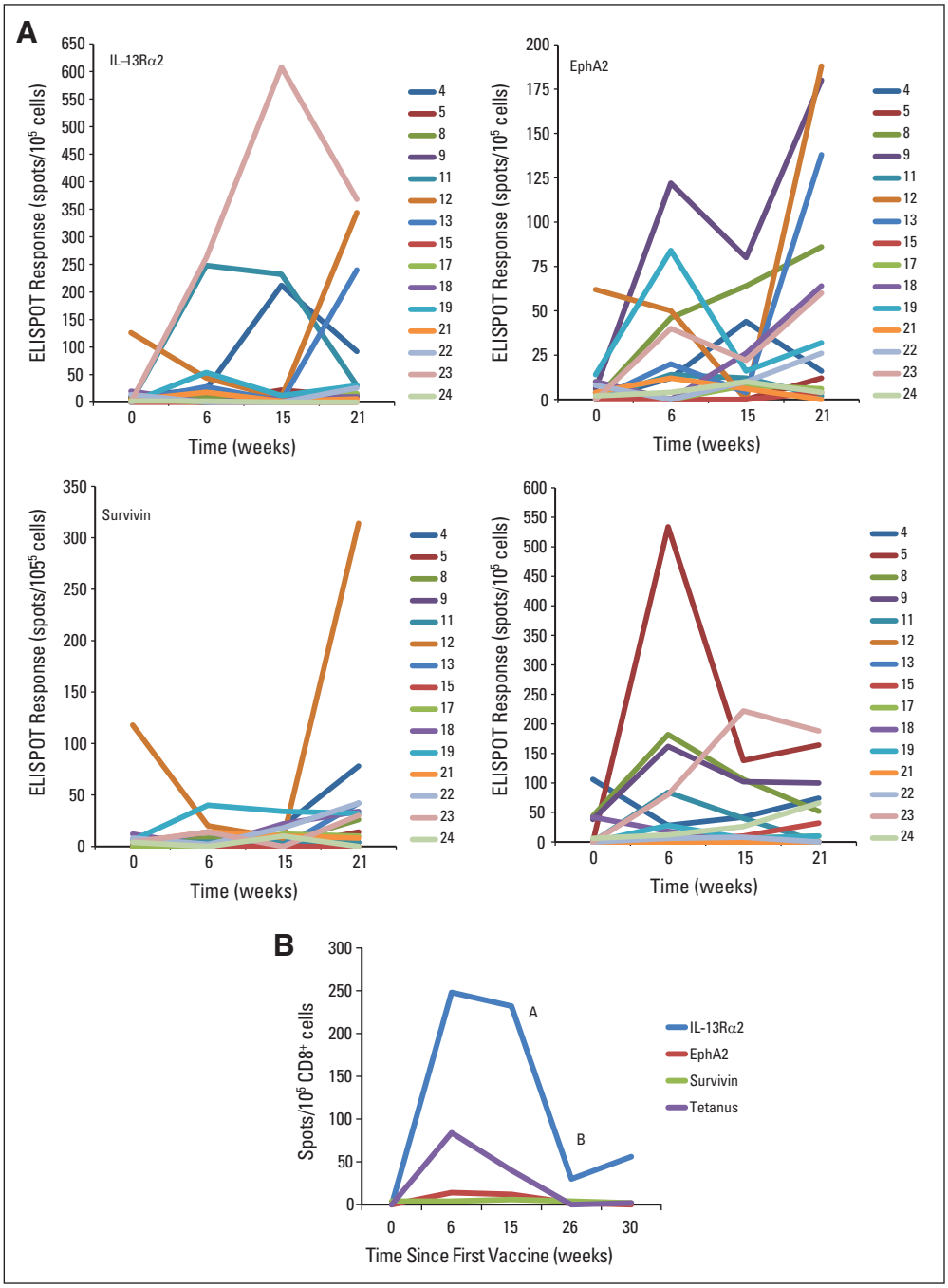

Fig 2. (A) Time course of glioma-associated antigen epitope-specific T-cell responses evaluated by interferon-γ enzyme-linked immunosorbent spot (ELISPOT) analyses in 15 patients who had samples available at week 0 (prevaccine) and at the week 6, 15, and 21 time points. Points represent net values after background subtraction. Patients who exhibited progression and did not have data at the week 15 or 21 time points are not shown. (B) ELISPOT results for patient 11, showing a dramatic response to interleukin 13 receptor alpha 2 (IL-13Rα2). The 12/11/10 scan from Figure 1 corresponds to time point A. High-dose steroids were started and the scan and symptoms rapidly and dramatically improved. ELISPOT positivity was diminished after initiation of high-dose dexamethasone (time point B), with subsequent recovery on weaning of dexamethasone.

began to recover when the dexamethasone dose was tapered (time point B in Fig 2B). Four other patients lost immunoreactivity before exhibiting disease progression. Serial analysis of cytokine and chemokine responses in 22 children noted frequent elevations in IL-15 in post- versus prevaccination samples but no statistically significant associations with ELISPOT response (see "Results" in the Appendix and Appendix Fig A1 [online only]).

Immunohistochemical Analysis

Tumor tissue was available for assessing antigen expression in five children, two of whom (patients 11 and 20) had postmortem tissue that was obtained several months after they had gone off study. All five showed strong immunoreactivity (grade 2 or 3) for at least one vaccine antigen: to IL-13Rα2 in five, EphA2 in four, and survivin in four. Illustrative results for patients 11 and 13 are shown in Figure 3.

Clinical Outcomes

Although a primary study goal was to assess safety and tolerability, preliminary outcome data were obtained (Fig 4). Median survival was 13.3 months from diagnosis (95% CI, 10.9 to 20.3 months) in the overall cohort: 12.7 months among the 20 children with BSG, with no

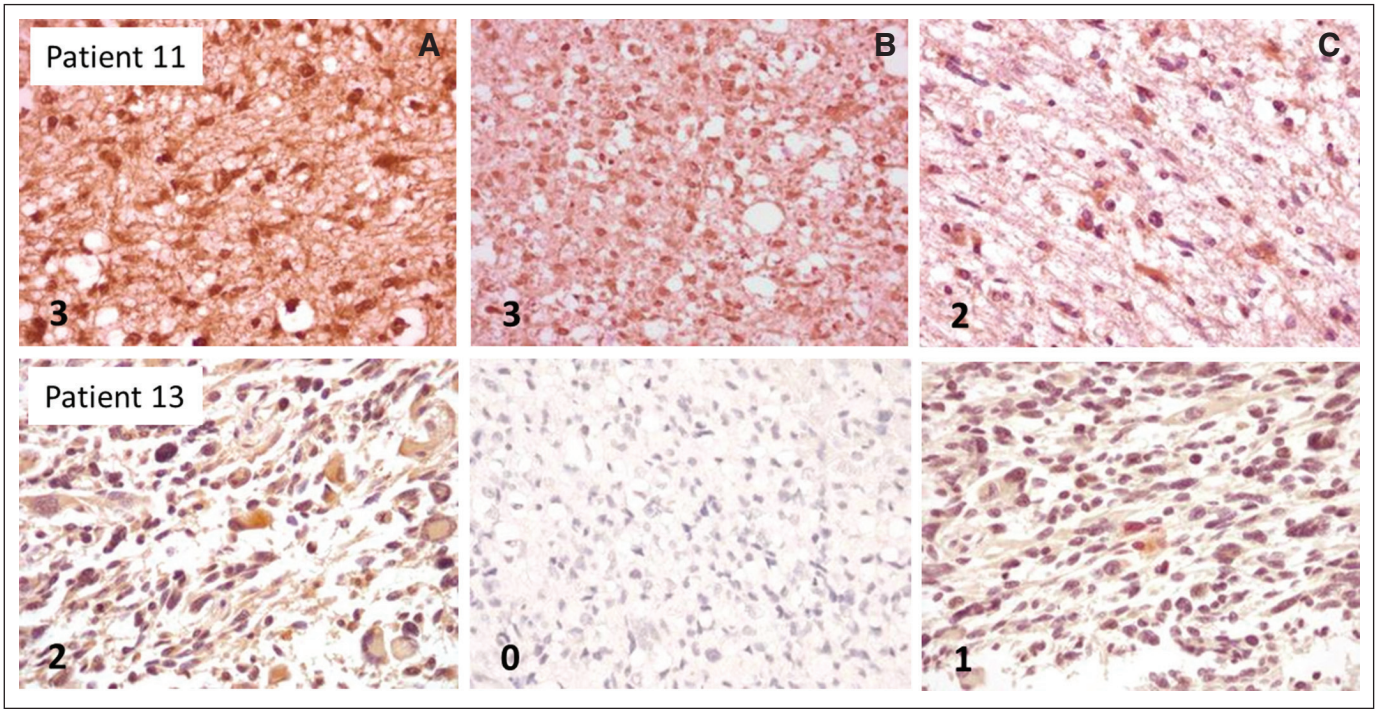

Fig 3. Immunohistochemistry results for antigen expression (column A, IL13Rα2; column B, EphA2; column C, survivin) in patients 11 (postmortem specimen) and 13 (pretreatment specimen), illustrating the range of antigen expression observed. Patient 11 had diffuse immunoreactivity for all three antigens, whereas patient 13 had absent expression of EphA2 and low-level expression of survivin in a minority of cells. High levels of expression of interleukin 13 receptor alpha 2 were observed, which was one antigen to which the patient mounted a strong enzyme-linked immunosorbent spot assay response, highlighting the importance of including multiple antigen epitopes in the vaccine cocktail.

significant difference based on use of concurrent chemotherapy during irradiation (data not shown), and 25.1 months among those with HGG. Only two of 26 patients had disease progression during the first two courses of therapy. Among the remaining patients, the best radiographic response was stable disease in 19, PR in two (patients 11 and 13), minor response in one (patient 19), and sustained disease-free status in two patients with HGGs (patients 8 and 25) who had undergone prior gross total resection. Among the five patients with pseudoprogression, one exhibited a subsequent PR and survived for 19.5 months, and the remainder survived for intervals of 18.4, 19.5, 11.3, and more than 38.0 months, respectively. Patient 14, the one BSG patient with an atypical lesion involving not only the pons but also the medulla and cervicomedullary junction, subsequently stopped vaccine therapy and experienced a dramatic PR to subsequent chemotherapy (persisting more than 38 months after diagnosis). Median survival among patients with BSGs with pseudoprogression was 19.5 months versus 10.9 months in those without pseudoprogression. No association was observed between age and sex and survival.

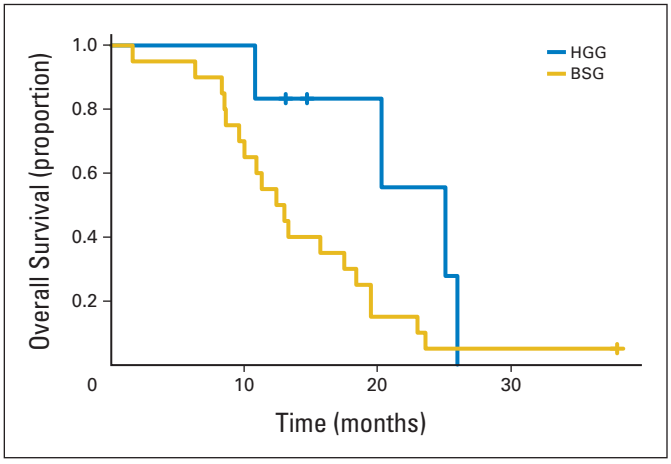

Fig 4. Kaplan-Meier plots of overall survival in 20 patients with brainstem gliomas (BSGs) treated either with (n = 6) or without (n = 14) chemotherapy during irradiation compared with six children with high-grade gliomas (HGGs), all of whom received chemotherapy during irradiation.

DISCUSSION

This is, to the best of our knowledge, the first clinical evaluation of peptide-based vaccination using novel GAA-derived epitopes in an emulsion-based vehicle, administered in conjunction with poly-ICLC for childhood brain tumors. Our findings demonstrate tolerability and immunologic activity of this approach, as well as preliminary evidence of clinical efficacy in a subset of patients.

For children with brainstem gliomas, conventional[2-4,7,8,40,44,45] and molecularly targeted[5,6,46] approaches have failed to increase median overall survival beyond the range of 9 to 12 months. Likewise survival of pediatric nonbrainstem malignant gliomas has been poor with current therapies.[10,11] Although recent molecular studies have identified new pharmacologic targets,[47-50] these insights have yet to translate into improved outcomes. Accordingly, novel treatments, such as immunotherapy, warrant consideration.

The peptide epitopes included in this vaccine were derived from three proteins previously reported to be highly expressed in pediatric gliomas.[28] The immunohistochemistry data obtained in five patients in this trial are consistent with the observation that these proteins are commonly overexpressed in these tumors. Likewise, ELISPOT data demonstrated that more than 50% of vaccinated patients mounted an immune response against at least one target antigen, supporting the use of such epitopes in future pediatric glioma vaccine regimens.

Given the high incidence of target expression and induction of immunoreactivity, the cause of eventual tumor progression remains conjectural but may reflect immune tolerance or escape involving outgrowth of tumor subclones not expressing targeted antigens[24,51] or lacking antigen processing components, such as major histocompatibility complex molecules,[52-54] or a hostile immune milieu mediated by regulatory T-cell populations or tumor-secreted immunosuppressive factors.[55,56] This fits with data in two cases that underwent postmortem evaluations in which high levels of GAA expression within the tumor were noted.

Although our median survival results are nominally superior to those previously reported for these tumors, it should be emphasized that this was a pilot study, and the requirement for HLA-A2-positive status and low or no dexamethasone usage at study entry may have influenced outcome results. Moreover, stringency of eligibility criteria in terms of duration and type of symptoms and imaging features can influence outcome.[57] Thus, inferences cannot be made regarding efficacy of this regimen versus others. Although secondary therapy among patients who discontinued vaccine treatment may have augmented the effects of immunotherapy, the impact of such interventions on survival cannot be assessed.

The frequency of pseudoprogression in this cohort (five of 26 patients), which is higher than in our adult study using a dendritic cell-based vaccine,[17] is of interest. All five patients had transient increases in tumor size or enhancement with new or worsening neurologic deficits, and subsequent, often prolonged clinical improvement and/or MRI stabilization or improvement after administration of high-dose dexamethasone or withholding vaccine therapy. The high incidence in this study compared with other recent vaccine trials may in part reflect the sensitivity of the brainstem to manifest neurologic deficits with even slight increases in tumor size. Accurately identifying and managing such patients is essential to avoiding both premature termination of therapy and unacceptable neurologic decline. Three of these children subsequently resumed vaccination and four survived beyond 18 months, including one with a PR who survived 19.5 months, and another with a PR after discontinuing vaccine therapy, persisting more than 3 years after diagnosis. All five children had positive ELISPOT responses. Advanced imaging techniques, such as magnetic resonance (MR) spectroscopy and diffusion imaging,[58] may help to prospectively distinguish patients with pseudoprogression from those with true progression in future trials.

In summary, this study demonstrated promising immunoreactivity and clinical responses in a group of patients with high-risk brain tumors and highlighted challenges in diagnosing and managing immunologic pseudoprogression. These data support larger studies of GAA peptide-based vaccination in children with malignant gliomas, in which feasibility and efficacy will be assessed in the multi-institutional setting.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
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GLOSSARY TERMS

active immunotherapy: induction of an immune response in the host, typically to a particular antigen or set of antigens. This is commonly by means of a vaccine and is in contrast to a passive immunotherapy in which cells, antibodies, or cytokines of the immune systems are passively infused into the host.

epitope: region within an antigen that has the potential to give rise to an antibody response. With respect to protein antigens, epitopes may be defined on the basis of primary, secondary, or tertiary structure of the molecule and, consequently, maybe exposed or hidden within the molecule.

immunotherapy: a therapeutic approach that uses cellular and/or humoral elements of the immune system to fight a disease.

pediatric glioblastoma (pGBM): a highly malignant astrocytoma that occurs in children and young adolescents. In contrast to adult GBM, which is a common type of brain tumor in adulthood, pGBM is a rare type of brain tumor in children.

therapeutic vaccine: a vaccine used for induction of humoral and/or cellular immune responses against an antigen or set of antigens to treat existing disease. In contrast, prophylactic vaccines are used to induce humoral and/or cellular immune responses against an antigen or set of antigens to prevent future disease.

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Appendix

Methods

Serum cytokine and chemokine assays. A Luminex-based assay was performed as previously described[17] in serum samples obtained before vaccination, 6 weeks after vaccination, and at 15 weeks, or the time of maximal enzyme-linked immunosorbent spot (ELISPOT) immunoreactivity. Pretested, multiplex plates (Invitrogen, Grand Island, NY) included standard curves and multiplexed cytokine standards (R&D Systems, Minneapolis, MN). The following cytokines and chemokines were examined: interleukin-1beta (IL-1β), IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/p70, IL-13, IL-15, IL-17, epidermal growth factor, fibroblast growth factor-basic, hepatocyte growth factor, vascular endothelial growth factor, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interferon alfa, interferon gamma, macrophage inflammatory protein-1α (MIP-1α), MIP-1β, inducible protein-10, monokine induced by interferon gamma (MIG), eotaxin, regulated on activation normal T-cell expressed and secreted (RANTES), monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor α (TNF-α), TNF receptor I, TNF receptor II, TNFR superfamily member 10b (also known as DR5), and chemokine (C-X-C motif) ligand 1 (also known as CXCL1 and formerly called GRO-α). Positive responses were defined as $>$ 50% increase in protein expression in post- compared with prevaccine specimens.

Results

Serum cytokine and chemokine responses. Samples were available for serial analysis of cytokine and chemokine responses in 22 children. Several cytokines showed substantial increases between the pre- and postvaccine samples in several patients, specifically IL-15, MIP-1β, and monocyte chemotactic protein-1. IL-15 was increased most consistently, showing positive responses in nine children versus positive responses in six children for MIP-1β and and six for MCP-1. Illustrative results from patient 11 are shown in Appendix Fig A1 (online only). Given the large number of parameters analyzed relative to the numbers of patients, no statistically meaningful correlations between cytokine and/or chemokine and ELISPOT response profiles could be performed. Interestingly, IL-8 was substantially decreased in nine patients and increased in eight others, although an association with ELISPOT response was not apparent.

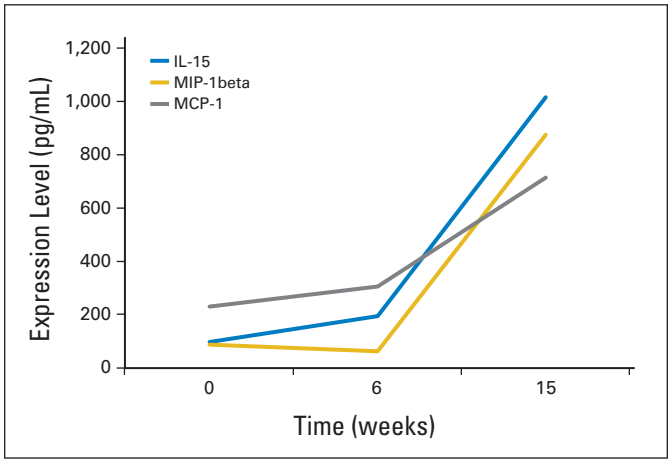

Fig A1. The time course of interleukin-15 (IL-15), macrophage inflammatory protein-1beta (MIP-1β), and monocyte chemotactic protein-1 (MCP-1) expression in serum from patient 22.