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Antigen-specific immunoreactivity and clinical outcome following vaccination with glioma-associated antigen peptides in children with recurrent high-grade gliomas: Results of a pilot study

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Abstract

Purpose—Recurrent high-grade gliomas (HGGs) of childhood have an exceedingly poor prognosis with current therapies. Accordingly, new treatment approaches are needed. We initiated a pilot trial of vaccinations with peptide epitopes derived from glioma-associated antigens (GAAs)

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ETHICAL STATEMENT

Funding sources are as noted above.

Conflicts: Hideho Okada is an inventor in the U.S. Patent Application No. 60,611, 797 (Utility Patent Application) "Identification of An IL-13 Receptor Alpha2 Peptide Analogue Capable of Enhancing Stimulation of Glioma-Specific CTL Response". An exclusive licensing agreement has been completed on this application between University of Pittsburgh and Stemline, Inc. Due to the potential conflicts of interest, Hideho Okada did not solely interpret any data in the current study. Dr. Regina I. Jakacki is currently employed by Astra Zeneca.

Informed consent: Signed IRB-approved informed consent was required both for HLA-A2 screening and initiation of therapy.

overexpressed in these tumors in HLA-A2+ children with recurrent HGG that had progressed after prior treatments.

Methods—Peptide epitopes for three GAAs (EphA2, IL13Rα2, survivin), emulsified in Montanide-ISA-51, were administered subcutaneously adjacent to intramuscular injections of poly-ICLC every 3 weeks for 8 courses, followed by booster vaccines every six weeks. Primary endpoints were safety and T-cell responses against the GAA epitopes, assessed by enzyme-linked immunosorbent spot (ELISPOT) analysis. Treatment response was evaluated clinically and by magnetic resonance imaging.

Results—Twelve children were enrolled, 6 with glioblastoma, 5 with anaplastic astrocytoma, and one with malignant gliomatosis cerebri. No dose-limiting non-CNS toxicity was encountered. ELISPOT analysis, in ten children, showed GAA responses in 9: to IL13Rα2 in 4, EphA2 in 9, and survivin in 3. One child had presumed symptomatic pseudoprogression, discontinued vaccine therapy, and responded to subsequent treatment. One other child had a partial response that persisted throughout two years of vaccine therapy, and continues at > 39 months. Median progression-free survival (PFS) from the start of vaccination was 4.1 months and median overall survival (OS) was 12.9 months. Six-month PFS and OS were 33% and 73%, respectively.

Conclusion—GAA peptide vaccination in children with recurrent malignant gliomas is generally well tolerated, and has preliminary evidence of immunological and modest clinical activity.

Keywords

astrocytoma; glioma; immunotherapy; pediatric brain tumor; vaccine therapy

INTRODUCTION

Children with malignant gliomas have high rates of disease progression after initial therapy with irradiation and adjuvant chemotherapy, with five-year survival rates less than 20%[1–3]. After progression, the likelihood of prolonged survival is low, with poor response rates to numerous conventional[4, 5] and molecularly targeted[6, 7] agents.

Accordingly, there is a need to identify new therapeutic approaches to promote tumor cell killing by targeting distinctive features of tumor cells. Cancer vaccines, designed to induce systemic immunity against antigens overexpressed by tumor cells, are promising in this regard. Pilot clinical trials by us [8–12] and others [13–18] have demonstrated the safety and potential efficacy of peripheral vaccinations for adults with malignant gliomas. Vaccine approaches may be even more effective if applied in clinical scenarios where patients are likely to have intact immunity, such as the pediatric age group,[19–21] as we have recently demonstrated in a pilot trial for children with newly diagnosed brainstem gliomas.[22]

The current trial incorporated peptide epitopes for three glioma-associated antigens (GAAs) that we demonstrated were highly expressed in pediatric gliomas, IL13Rα2, EphA2, and survivin.[23] The human leukocyte antigen (HLA)-A2-restricted cytotoxic T-lymphocyte (CTL) epitopes included an interleukin-13 receptor (IL-13Rα2) analog peptide ($IL\text{-}13R\alpha2_{345–353:1A9V}$)[24, 25] and $EphA2_{883–891}$[26], both of which were identified by

our group, as well as $Survivin_{96–104:M2}$ [27–29], admixed with a pan-HLA-DR tetanus toxoid (TT) peptide ($Tet_{A830–845}$) as an emulsion in a mineral oil base (Montanide), which has been shown in murine models and clinical trials to induce high levels of antigen-specific CTLs,[22, 30] obviating the requirement for harvested dendritic cells as a delivery vehicle. The vaccine was administered adjacent to the immunoadjuvant polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose (poly-ICLC), which has been shown to promote the activity of tumor antigen vaccination in rodent glioma models[30, 31] and to be well tolerated in patients with malignant glioma[22, 32–35] and as an adjunct to peptide vaccination in our pilot trial for children with newly diagnosed high-grade and brainstem gliomas[22].

The primary objective of the study was to evaluate the safety and immunological efficacy of vaccination with GAA epitopes in combination with poly-ICLC in pediatric patients with recurrent malignant gliomas. We hypothesized that this regimen would prove safe, and induce anti-glioma immune responses and, potentially, clinical responses.

PATIENT AND METHODS

Patients

Patients between 1 to <22 years of age with recurrent biopsy-confirmed high-grade gliomas (e.g., glioblastoma, anaplastic astrocytoma) that had progressed after prior therapy were eligible for screening. Adequate liver, renal and other organ function, Karnofsky or Lansky performance status > 60, and HLA-A2+ status were required, and patients must have recovered from the effects of prior therapies. At least 3 weeks must have elapsed between the last dose of myelosuppressive therapy (at least 1 week from the last dose of non-myelosuppressive therapy) and initiation of vaccine therapy. The maximal allowable dexamethasone dose during the week before beginning vaccination was 0.1 mg/kg/d (maximum 4 mg/d). The trial was conducted under FDA IND# 13624 and IRB protocol PRO08030085, and was registered with ClinicalTrials.gov (No. NCT01130077). The results in the Strata for newly diagnosed brainstem and high-grade gliomas were previously reported.[22] Signed IRB-approved informed consent was required both for HLA-A2 screening and initiation of therapy.

Study Design

Patients received subcutaneous injections of an emulsion consisting of GAA-derived HLA-A*0201-restricted peptides admixed with a pan-HLA-DR tetanus toxoid (TT) peptide ($Tet_{A830–845}$) in Montanide ISA-51 (Seppic) with adjacent intramuscular injections of the immunoadjuvant poly-ICLC (30 μg/kg, Hiltonol, Oncovir, Inc), a toll-like receptor ligand, every three weeks for a total of 8 vaccines. Aqueous solution (400 μl) containing each HLA-A2-restricted GAA peptide (300 μg/peptide) and the TT peptide (200 μg) was mixed 1:1 with Montanide ISA-51 to form an emulsion (total volume/injection 800 μl). Participants were evaluated for adverse events, regimen-limiting toxicity (RLT), and treatment response by clinic visits, laboratory testing, and MR (magnetic resonance) imaging. Immune response was assessed at 6, 15, and 21 weeks after starting vaccination by ELISPOT assays on peripheral blood mononuclear cells (PBMCs), which were sampled to coincide with MRI

scans. Patients demonstrating radiological response (e.g., partial response (PR) or stable disease (SD)) without RLT from the vaccine could continue to receive vaccination at six-week intervals for up to 2 years from the initial vaccine. For such patients, ongoing immunological and MRI evaluation were scheduled to occur at 12-week intervals, in conjunction with every other visit for vaccine administration.

Toxicity Assessment and Stopping Rules

The trial was monitored continuously for treatment-related adverse events using National Cancer Institute Common Toxicity Criteria version 3.0. The following were considered to be RLTs if they were judged to be at least possibly related to treatment: ≥ Grade 2 hypersensitivity or allergic reaction; ≥ Grade 3 non-hematological toxicity; > Grade 3 hematological toxicity that recurred despite 33% poly-ICLC dose reduction or did not resolve to ≤ Grade 1 by the time the next dose was due. The stopping rule for halting accrual for excessive toxicity was an observed rate of RLT of ≥ 33%, provided at least 2 RLTs had been observed.

Peptides

The HLA-A2–restricted peptides that were administered in this study consisted of ALPFGFILV (IL-13Rα2$_{345–353:1A9V}$)[25]; TLADFDPRV (EphA2$_{883–891}$)[26]; and LMLGEFLKL (Survivin$_{96–104:M2}$)[27–29]; admixed with a pan-DR helper epitope AQYIKANSKFIGITEL (Tet$_{A830–845}$)[36, 37]. The peptides were synthesized by automated solid-phase peptide synthesis by NeoMPS (PolyPeptide Group, San Diego, CA). Peptides were tested in multiple quality-assurance studies including purity, sterility, identity, potency, pyrogenicity and stability.

ELISPOT assays

Enzyme-linked immunosorbent spot (ELISPOT) assays were performed as described previously[22, 35] on PBMCs obtained and cryopreserved before vaccination (Week 0), and at Weeks 6, 15, 21, and q12 weeks. Briefly, batched cryopreserved Ficoll-isolated PBMC samples were evaluated following *in vitro* stimulation with IL-13Rα$_{345–353}$, EphA2$_{883–891}$, and Survivin$_{96–104}$ peptides, 10 ng/ml IL-7, and 20 IU/ml IL-2. IFN-γ responses by purified CD8+ and CD4+ T cells were tested against T2 cells pulsed with GAA peptides or PBMCs pulsed with Tet$_{A830–845}$, respectively. A positive ELISPOT response was defined as >2-fold increase in net spot-forming T-cells (after background subtraction) (CD8+ cells for GAAs, CD4+ cells for Tet$_{A830–845}$) over the pre-vaccine level and at least 50 spots/100,000 cells.

Immunohistochemical analysis

In cases with available tissue, expression of the target proteins was determined as previously described [22, 23]. Paraffin-embedded tissue sections were deparaffinized in xylene, and rehydrated in graded concentrations of ethanol. Endogenous peroxidase activity was quenched and antigen retrieval was performed. Non-specific antibody binding was blocked and sections were incubated with monoclonal antibodies against human EphA2 (1:50; Santa Cruz, sc-10746), IL-13Rα2 (1:500; Abcam, ab55275), and Survivin (1:100, sc-10811), followed by EnVision+ System-HRP polymer (Dako). Specimens were graded as positive if

they had antigen expression in 20–60% of tumor cells (2) or > 60% of tumor cells (3), versus negative if they had no staining (0) or staining in less than 20% of tumor cells (1), as previously described[22, 23].

Radiological response monitoring: Tumor size was assessed at Weeks 6, 15, 21, and every 12 weeks subsequently by MRI. More frequent scans were obtained if clinically warranted as described below. Response was evaluated by gadolinium-enhanced T1-weighted images, T2-weighted images, or both, based upon the appearance of the tumor on pretreatment MRI. Complete response (CR) was defined as complete disappearance of all enhancing tumor sustained for at least 6 weeks, with no new lesions; stable or improved non-enhancing (T2/FLAIR) lesions; off corticosteroids (or on physiologic replacement doses only); and stable or improved clinically. For non-enhancing tumors, complete disappearance of all T2/FLAIR signal was required. Partial response (PR) was defined as a >50% decrease compared with baseline in the products of the maximal perpendicular diameters of all measurable enhancing lesions (or T2/FLAIR areas) sustained for at least 6 weeks; no new lesions, on stable or declining corticosteroid doses. Minor response was defined as a 25 to < 50% decrease in tumor dimensions compared to baseline, with the above caveats. Progression was defined as >25% increase in sum of the products of perpendicular diameters of the enhancing tumor or T2/FLAIR signal compared with the smallest tumor measurement obtained either at baseline or best response, not explained by pseudoprogression; on stable or increasing doses of corticosteroids; any new lesion. Stable disease (SD) did not qualify for any other above categories.

Management of Immunological Pseudoprogression

In our previous vaccine trials in adults with gliomas[35, 38] and children with newly diagnosed brainstem and high-grade gliomas[22], a subset of participants had pseudoprogression, characterized by transient increase in the size or contrast enhancement of the tumor secondary to intratumoral immune response, followed by tumor stabilization or regression. We therefore incorporated detailed guidelines for pseudoprogression management in this cohort.

If pseudoprogression was suspected following the initiation of vaccination, and the patient was neurologically worse, sufficient to warrant initiation of dexamethasone, subsequent doses of vaccine and poly-ICLC were held. Re-imaging was performed at 4-week intervals until it was determined whether the clinical and imaging changes reflected pseudoprogression or true progression. Once the subject was clinically stable and on <0.1 mg/kg/day decadron for > 1 week, a repeat MRI was performed and the patient could restart treatment with 67% of the poly-ICLC dose (i.e., 20 μg/kg), as long as the MRI changes had improved or resolved.

If the repeat MRI and/or clinical status had not improved, a biopsy or resection would be considered to definitively differentiate between pseudoprogression (i.e., inflammatory/lymphocytic infiltration or necrosis comprising the majority of the specimen) and true tumor progression (where the majority of the resected specimen consisted of tumor). If a biopsy was not deemed to be clinically indicated or safe, the patient was taken off vaccine therapy.

Statistical Methods

This pilot study was designed to enroll children with recurrent high-grade gliomas to assess safety and immunological efficacy. The treatment approach was considered worthy of further investigation if there were at least 5 ELISPOT responses observed among evaluable subjects. In addition, we planned to stop accrual if the rate of RLT was > 33%, and at least 2 RLTs were observed. Patients with disease progression during the first two courses of therapy were replaced for RLT analysis. Survival functions were estimated using the product-limit (Kaplan-Meier) method, and compared by log-rank tests. Greenwood's method was used to calculate confidence regions for the survival function estimates.

RESULTS

Demographics and Clinical Characteristics

Between November 2009 and September 2015, 69 children with recurrent malignant gliomas were screened for HLA-A2 status, of which 26 (38%) were HLA-A2+. Fourteen of these patients did not enroll, because of progression prior to enrollment (3), ineligible histology (1), metastatic disease (2), poor performance status (1), intracranial hemorrhage (1), travel difficulties (2) or choosing other therapies (4). Twelve children were enrolled (Table 1), 6 with glioblastoma, 5 with anaplastic astrocytoma, and 1 with malignant gliomatosis cerebri. Patients received 2–20 courses of therapy (median 5).

Summary of Systemic Toxicities

The primary objective of this study was to assess safety, given that this was a component of the first such trial in children. Principal toxicities included Grade 1 and 2 injection site reactions in all 12 patients and flu-like symptoms (such as fatigue, myalgias, fever, chills, headache), which were present in various combinations in virtually all patients, but generally Grade 1 in severity, lasting less than 48 hours after each vaccine and well controlled with acetaminophen or ibuprofen. Grade 1gastrointestinal toxicity was observed in 5 children and Grade 1 anemia and lymphopenia in one each. None of the patients exhibited evidence of autoimmunity.

Pseudoprogression

One child (Patient 4) with a progressive HGG had presumed symptomatic pseudoprogression after the fourth vaccine, with enlargement in tumor size and enhancement that stabilized but did not regress after interruption of protocol therapy. Because repeat resection was not felt to be clinically warranted, vaccine therapy was halted, and the child was withdrawn from the study. The child then received bevacizumab and had a partial response to this agent, ultimately surviving for a year after initial institution of vaccine therapy. This overall course was presumed to be secondary to pseudoprogression.

Induction of Epitope-Specific Immune Responses against GAAs

All but one patient (Patient 1), who had disease progression before completion of the second vaccine cycle, had PBMCs available for immunological analysis. Patient 3's samples failed analysis. In 9 of 10 evaluable patients (90%), vaccination induced immune reactivity to at

least one of the vaccine-targeted GAAs by IFN-γ ELISPOT assays: to IL13Rα2 in 4, EphA2 in 9 and survivin in 3 (Table 1). Three children also had positive responses to the Tet epitope. The time course and magnitude of the ELISPOT responses are summarized in Figure 1A. In some patients, immune responses peaked and then spontaneously dropped prior to or in association with disease progression, possibly reflecting loss of immune response to the vaccine. However, in others responses were maintained over a long interval as illustrated in Figure 1B, in which Patient 6 demonstrated a very prominent response to EphA2 that persisted during the entire 2 year course of vaccination, and also responded to the Tet. Patient 4, who had presumed pseudoprogression, had a particularly strong response to all three vaccine antigens, as well as to the Tet epitope. Because of the small number of patients, it was not possible to demonstrate an association between ELISPOT reactivity and outcome, or between Tet and GAA reactivity.

Immunohistochemical Analysis

Tissue from prior resections was available for assessing antigen expression in eight children, six of whom were evaluable for all three GAAs. All eight showed immunoreactivity for at least one vaccine antigen: 6 of 8 evaluable had positive staining for IL13Rα2, 4 of 7 for EphA2, and 3 of 6 for survivin. Illustrative results are shown in Figure 1C. Because of the small number of patients, it was not possible to evaluate the association between antigen expression as assessed by immunohistochemistry and ELISPOT reactivity.

Clinical Outcomes

Although the goal of this study was to provide an analysis of safety and tolerability, preliminary efficacy data were obtained. Median progression-free survival (PFS) from the start of vaccination was 4.11 months (95% CI: (2.2,NA)) and median overall survival (OS) was 12.9 months (95% CI: (10.8,NA), Figure 2). Six-month PFS and OS were 33% (95% CI: (0.10,0.65)) and 73% (95% CI: (39%,94%)), respectively. Patient 6 had a partial response that persisted throughout two years of vaccine therapy, and continues at > 39 months (Figure 3). Because of the small numbers of patients in this cohort, an association between ELISPOT response and clinical response was not apparent.

DISCUSSION

This is, to the best of our knowledge, the first clinical report of peptide-based vaccination using a cocktail of GAA-derived epitopes in Montanide, administered in parallel with the immunoadjuvant poly-ICLC in a cohort of children with recurrent childhood high-grade gliomas. Our findings demonstrate reasonable safety of this approach, as well as evidence of immunological responses, and modest clinical activity.

For children with recurrent high-grade gliomas, conventional[4, 5] and molecularly targeted[6, 7] approaches have generally failed to achieve rates of response or 6-month disease stabilization much above 10%. Although recent molecular studies have identified new pharmacological targets for childhood gliomas[39–41], these insights have yet to translate into improvements in outcome. Accordingly, novel treatment strategies, such as immunotherapy, warrant consideration.

The GAA epitopes employed in this vaccine were derived from three proteins known to be highly expressed in pediatric gliomas.[23] The immunohistochemistry data obtained in eight patients in the current trial confirmed that these proteins were commonly overexpressed in the treated tumors, consistent with our preliminary data in archival HGG specimens[23]. Likewise, ELISPOT data demonstrated that the majority of vaccinated patients mounted an immune response against at least one of the target antigens, supporting the rationale for incorporating such epitopes in pediatric glioma vaccine trials.

Although our outcome results for patients with recurrent high-grade gliomas suggest that this vaccination approach has at least a modest degree of activity, it is important to emphasize that this was a pilot study focusing on safety, and the requirement for HLA-A2+ status and other restrictive entry criteria may have influenced outcome, considering that only 12 of 69 patients referred for screening received protocol therapy. However, that all patients had progressive disease that had failed to respond to initial and, in some instances, salvage therapy, the 6- month progression-free survival rate of 33% and overall survival of 73% compare favorably to other studies in this population,[5, 42] and the long-term survival in one child that persists beyond 39 months is intriguing. Nonetheless, the results were less encouraging than those observed in our prior studies with recurrent low-grade gliomas[43], which may reflect differences in the immune milieu in these patients.

In that context, the rate of clinical stability or response was notably lower than the frequency of target overexpression and ELISPOT response, and in some patients immunoreactivity waned over time. This phenomenon may reflect a combination of factors, including the presence of immunosuppressive molecules in the tumor itself, the outgrowth of tumor subclones not expressing targeted antigens[44] or antigen processing components, such as MHC molecules[45, 46], or the development of a hostile systemic immune milieu mediated by regulatory T-cell populations[47] or upregulation of immune checkpoint molecules.[48] Elucidating the factors involved will be critical to optimizing the implementation of immunotherapy strategies for these tumors.

The incidence of possible immunological pseudoprogression in this cohort (1 of 12 patients) is lower than we previously observed in a cohort of children with newly diagnosed brainstem gliomas[22], and may reflect distinctive features of tumor response in these two locations. The difficulty we experienced in confirming this diagnosis in the one potentially affected patient highlights the challenges in diagnosing and managing this phenomenon and calls attention to the need for systematically evaluating the utility of advanced imaging techniques, such as a MR spectroscopy and diffusion/perfusion imaging[49, 50], as a way to facilitate this determination.

In summary, this trial demonstrated acceptable safety and promising immunoreactivity in response to peptide vaccination. Although clinical activity was observed, there is clearly room for improvement in our immunotherapy approach in these challenging tumors, perhaps by broadening the panel of antigen epitopes included in the vaccine or by incorporating strategies to prevent immune resistance or escape, such as the use of immune checkpoint inhibitors[48] that can potentiate and maintain immune response to the vaccine.

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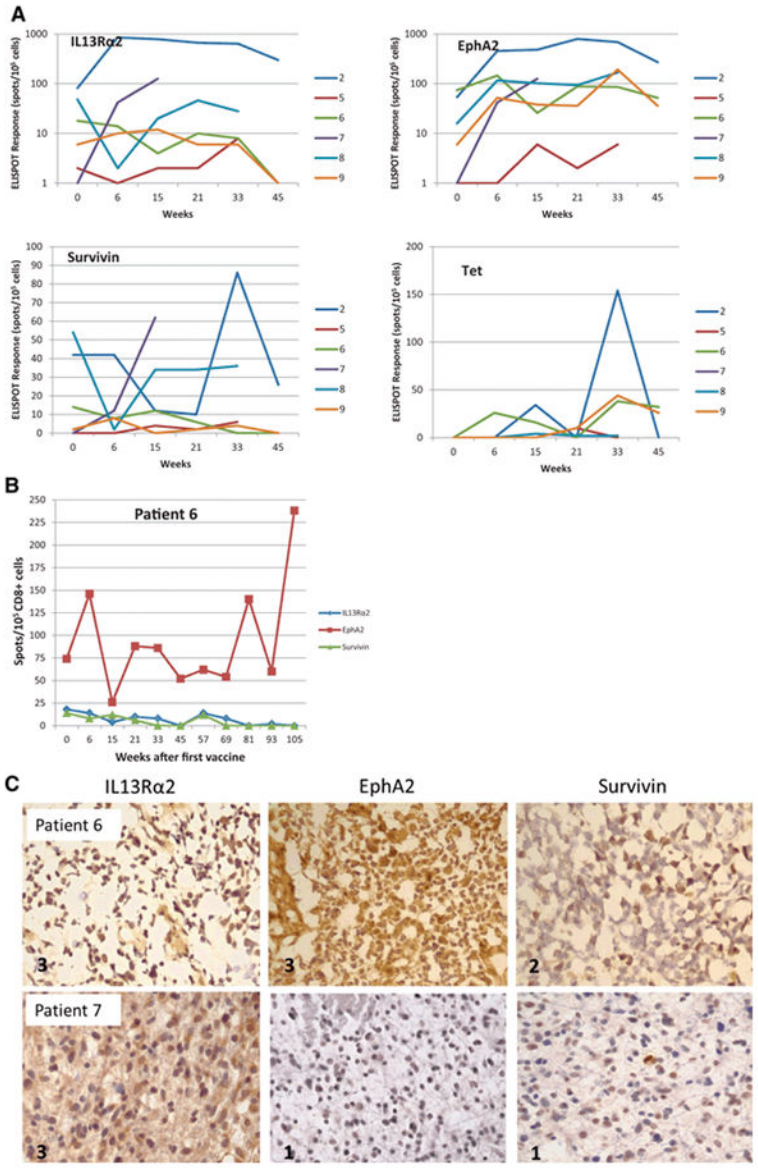

Figure 1.

A. Time course of glioma-associated antigen (GAA) epitope-specific T-cell responses evaluated by interferon-γ enzyme-linked immunosorbent spot (ELISPOT) analyses in patients that had samples available at week 0 (pre-vaccine) and at the week 6 and 15 time points. Patients lacking pre-vaccine or post-vaccine samples were excluded. Points represent net values after background subtraction. A positive ELISPOT response was defined as >2-fold increase in net spot-forming T-cells (after background subtraction) (CD8+ cells for GAAs, CD4+ cells for $Tet_{A830–845}$) over the pre-vaccine level and at least 50 spots/100,000 cells. IL13Rα and EphA2 are plotted using a logarithmic scale for ELISPOT response to better illustrate the range of values obtained, with a value of 1 used to represent a lack of ELISPOT reactivity (e.g., raw values of 0 or 1).

B. ELISPOT responses in Patient 6, who had a partial response throughout the two-year course of vaccination that has been maintained for more than 39 months. Persistent positive ELISPOT responses to EphA2 were observed throughout the 2-year course of vaccination.
C. Immunohistochemistry results for antigen expression in patients 6 and 7.

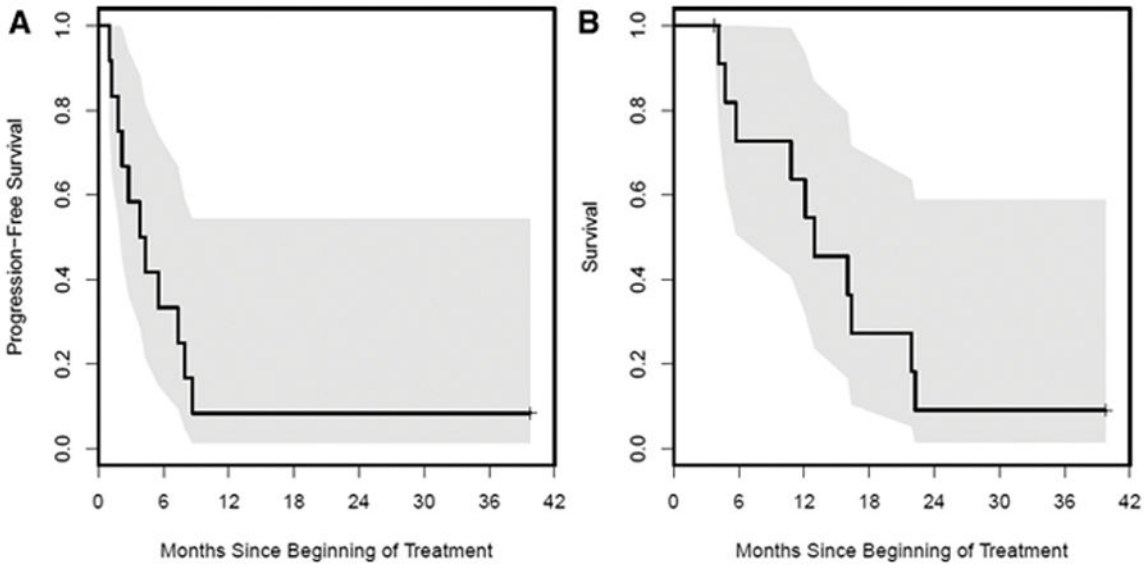

Figure 2.

Kaplan-Meier plots of progression-free (A) and overall (B) survival in 12 recurrent high-grade glioma patients treated on this study.

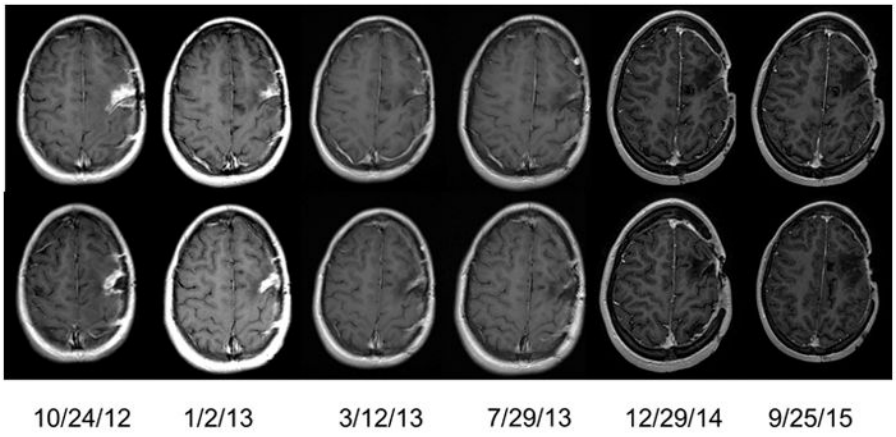

Figure 3.
MRI results (T1-weighted gadolinium enhanced images) over the course of time in patient 6.

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Table 1

Demographics and clinical characteristics of participating patients

Results of maximal interferon gamma enzyme-linked immunosorbent spot (ELISPOT) reactivity (net spots/10^5 cells after background subtraction) are shown for each epitope.

	Clinical Factors					Maximal ELISPOT Responses				Immuno-histochemistry			Response and Outcome			
Patient ID	**Age at start (years)**	**Gender**	**Tumor Histology**	**Tumor Location**	**Prior Therapy**	**I**	**E**	**S**	**T**	**I**	**E**	**S**	**Best Response**	**Vaccines received**	**PFS**	**OS**
1	4.9	F	GBM	Left Frontal	RT w/TMZ	NA	NA	NA	NA	NA	NA	NA	PD	2	1.0	4.2
2	23.3[1]	M	GBM	Right Fronto-Parietal	RT w/TMZ Bev w/irinotecan	**848**	**796**	**86**	**154**	NA	NA	NA	SD	9	7.4	21.9
3	11.5	M	AA	Bithalamic	RT w/Bev Bev w/irinotecan	F	F	F	F	1	**2**	1	SD	5	4.3	5.8
4	14.7	M	GBM	Right Occipital Parietal	RT CPM, CDDP, VCR and SCR	**602**	**618**	**60**	**1346**	1	1	**3**	PR p study	4	2^{PTP}	12.1
5	19.4	F	AA	Right Midbrain Thalamus	RT TMZ	8	6	6	10	**3**	**3**	NA	SD	6	5.5	16.0
6	15.3	F	AA	Left Frontal	RT with SAHA TMZ and Bev Topo/ Celebrex/CPM	14	**238**	12	**58**	**3**	**3**	**2**	**PR**	20	**39.8+**	**39.8+**
7	19.1	M	AA	Left Parieto-Occipital	RT w/TMZ and Bev	**126**	**126**	**62**	0	**3**	1	1	SD	5	3.9	10.8
8	19.9	F	GC	Basal Ganglia/Chiasm	RT w/TMZ and CCNU	46	**168**	36	6	**2**	NA	NA	SD	9	8.0	13.0
9	12.0	F	GBM	Left Thalamus	RT w/cetux and irinotecan TMZ and Bev	12	**232**	8	44	NA	NA	NA	SD	9	8.6	22.3
10	11.8	F	GBM	Right Occipital	RT TMZ	2	**152**	2	4	**3**	0	0	PD	2	1.8	16.4
11	2.3	M	GBM	Holocord	TMZ	12	**290**	6	8	**3**	**3**	**2**	PD	2	1.2	4.8
12	10.9	M	AA	Right Frontal	RT TMZ	**50**	**162**	30	2	**NA**	**NA**	**NA**	SD	4	2.8	3.7+

Values ≥50 were considered positive, and reflected at least a two-fold increase compared to prevaccine. Positive responses are shown in bold. NA indicates specimens that were not available because of progression before completion of the second vaccine course. F indicates samples that failed ELISPOT analysis. Immunohistochemistry: pre-vaccine samples with positive expression (e.g., 2 or 3) of GAA targets are indicated in bold; NA indicates patients for whom evaluable tissue was not available. Abbreviations: ID, Identification number; M, male; F, female; GBM – glioblastoma; AA, anaplastic astrocytoma; GC, grade III glioma with gliomatosis cerebri; RT, radiotherapy; TMZ, temozolomide; Bev, Bevacizumab; CPM, cyclophosphamide; CDDP; Cisplatin; VCR, vincristine; SCR, stem cell reconstitution; SAHA, suberoylanilide hydroxamic acid; topo, topotecan; CCNU, lomustine; Cetux, cetuximab; I, IL13Rα2; E, EphA2; S, survivin; Best Response: PR, partial response; SD, stable disease; PD, progressive disease; OS and PFS are expressed in months; PTP, pseudo-tumor progression, also referred to as pseudoprogression.

[1]Patient 2 was 20 years of age at the time of screening, but received multiple treatment regimens before beginning vaccine therapy, and was thus >22 at that time.