Induction of Robust Type-I CD8\(^+\) T-cell Responses in WHO Grade 2 Low-Grade Glioma Patients Receiving Peptide-Based Vaccines in Combination with Poly-ICLC

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Abstract

Purpose: WHO grade 2 low-grade gliomas (LGG) with high risk factors for recurrence are mostly lethal despite current treatments. We conducted a phase I study to evaluate the safety and immunogenicity of subcutaneous vaccinations with synthetic peptides for glioma-associated antigen (GAA) epitopes in HLA-A2\(^+\) adults with high-risk LGGs in the following three cohorts: (i) patients without prior progression, chemotherapy, or radiotherapy (RT); (ii) patients without prior progression but with prior RT; and (iii) recurrent patients.

Experimental Design: GAA were IL13R\(_{a2}\), EphA2, WT1, and Survivin. Synthetic peptides were emulsified in Montanide-ISA-51 and given every 3 weeks for eight courses with intramuscular injections of poly-ICLC, followed by q12 week booster vaccines.

Results: Cohorts 1, 2, and 3 enrolled 12, 1, and 10 patients, respectively. No regimen-limiting toxicity was encountered except for one case with grade 3 fever, fatigue, and mood disturbance (cohort 1). ELISPOT assays demonstrated robust IFN\(\gamma\) responses against at least three of the four GAA epitopes in 10 and 4 cases of cohorts 1 and 3, respectively. Cohort 1 patients demonstrated significantly higher IFN\(\gamma\) responses than cohort 3 patients. Median progression-free survival (PFS) periods since the first vaccine are 17 months in cohort 1 (range, 10–47+) and 12 months in cohort 3 (range, 3–41+). The only patient with large astrocytoma in cohort 2 has been progression-free for more than 67 months since diagnosis.

Conclusion: The current regimen is well tolerated and induces robust GAA-specific responses in WHO grade 2 glioma patients. These results warrant further evaluations of this approach. Clin Cancer Res; 21(2); 286–94. ©2014 AACR.

Introduction

World Health Organization (WHO) grade 2 low-grade gliomas (LGG) are slow-growing primary brain tumors with an extremely high risk for undergoing transformation into more aggressive and lethal WHO grade 3 or 4 high-grade gliomas (HGG; ref. 1). Even with the combination of available therapeutic modalities [i.e., surgery, radiotherapy (RT), chemotherapy], the invasive growth and resistance to therapy exhibited by these tumor results in recurrence (a majority of cases as HGGs) and death in most patients (1–3). Immunotherapeutic modalities, such as vaccines, may offer safe and effective treatment options for these patients. The slower growth rate of LGGs (in contrast with HGGs) should allow sufficient time for multiple immunizations and hence high levels of antigenia immunity. Because patients with LGGs are likely not as immunocompromised as patients with HGG, they may exhibit greater immunologic response to and benefit from the vaccines. Furthermore, the generally mild toxicity of vaccines may help maintain a higher quality of life than is experienced with current cancer therapy.

On the basis of encouraging data from a phase I vaccine trial targeting multiple human leukocyte antigen (HLA)-A2\(^+\)-restricted GAA cytotoxic T-cell (CTL) epitopes in patients with recurrent HGGs (4), we conducted a pilot study of subcutaneous...
A pan-HLA-DR tetanus toxoid peptide (TetA830) was included in the normal glial cells contained in the tumor specimens (15). Expression levels in HGGs (15). WT1 protein was not detected in grade 2, 3, and 4 astrocytomas (14). Using immunohistochemistry, Uematsu and colleagues have shown high-level expression of Survivin (14) and WT1 (15) are frequently expressed at high levels in grade 2 gliomas with high risk for recurrence. GAAs for these peptides are IL13Ra2 (7, 8), EphA2 (9), Wilm’s tumor gene product 1 (WT1; ref. 10), and Survivin (11), all of which contain HLA-A2-restricted CTL epitopes (7–11). Whereas IL13Ra2 (12) and EphA2 (13) are typically expressed in HGGs, Survivin (14) and WT1 (15) are frequently expressed at high levels in grade 2, 3, and 4 astrocytomas (14, 15). Using immunohistochemistry, Uematsu and colleagues have shown 100% of glioma specimens (n = 29; grades 2–4), but not normal brain tissues, contain Survivin-positive cells (14). Interestingly, high-level expression of Survivin was associated with poor prognosis in patients with grade 2 or 3 astrocytomas (14). Oji and colleagues have shown expression of WT1 protein in 5 of 6 LGG, and in 18 of 18 HGG cases, with a trend of higher expression levels in HGGs (15). WT1 protein was not detected in the normal glial cells contained in the tumor specimens (15). A pan-HLA-DR tanus toxoid peptide (TetA830) was included to enhance general helper CD4+ T-cell response.

Our rationale is to offer both immunotherapeutic and immunoprophylactic potential to reduce the risk of tumor recurrence, which could translate into improved survival. Therapeutically, this approach could suppress the expansion of indolently growing neoplastic LGG cells. Prophylactically, it could prevent the growth of glioma cells that undergo anaplastic transformation. The primary objectives were to assess tolerability of this novel regimen, and its potential for inducing GAA-targeted immune responses.

**Patients and Methods**

**Patients**

HLA-A2+ adults (≥18 years of age) with WHO grade 2 LGG who met the following criteria were enrolled with informed consent and approvals by the institutional review board (IRB) and U.S. Food and Drug Administration (FDA; BB-IND#13624).

Enrollment criteria included: cohort 1 (with no prior RT) and cohort 2 (with prior RT; both cohorts in UPCI 07-057; ClinicalTrials.gov Identifier: NCT00795457); histologically diagnosed WHO grade 2 astrocytoma or oligoastrocytoma that had not progressed since the initial surgery/biopsy, but with at least one of the following high-risk factors: (i) age ≥ 40 years; (ii) incomplete resection [postoperative MRI showing >1 cm residual disease, based on the maximum dimension of residual T2 or fluid-attenuated inversion-recovery (FLAIR) abnormality from the edge of the surgical cavity either laterally, anteroposteriorly, or supero-inferiorly]; or (iii) the preresection tumor size is ≥4 cm (the maximum preoperative tumor diameter, based on the axial and/or coronal T2 or FLAIR MR images) as each of these conditions represents an independent risk factor for WHO grade 2 LGG patients (16, 17). Cohort 3 (UPCI 08-135, NCT00874861): histologically diagnosed WHO grade 2 glioma with recurrence. Patients were required to have a Karnofsky performance status of ≥60; to have adequate liver and renal function; and to be off corticosteroids for at least 4 weeks before study enrollment.

**Study design**

Patients received subcutaneous injections of GAA-derived HLA-A’0201–restricted peptides (300 µg/peptide/dose) and a pan-HLA-DR–binding tetanus toxoid peptide (TetA830; 200 µg/dose) emulsified in Montanide ISA-51 (Seppic) and concurrent intramuscular injections of poly-ICLC (20 µg/kg, Hilto; Oncovir, Inc.), every 3 weeks for eight vaccines. Participants were evaluated for adverse events (AE), regimen-limiting toxicities (RLT), and treatment response by clinic visits, laboratory testing, and MR imaging. At 15, 18, 21, and 24 weeks after starting vaccination, immune response was assessed by ELISPOT assay on peripheral blood mononuclear cells (PBMC). Patients demonstrating no clinical or radiological progression (per RECIST criteria) without RLT had the option of continuing to receive vaccination at 12-week intervals for up to 2 years after initial vaccination. For such patients, additional immunologic and MRI evaluations were obtained at 12-week intervals.

**Toxicity assessment and stopping rules**

Each trial was monitored for treatment-related AEs using NCI CTCAE 3.0. The following were considered to be RLTs: ≥grade 2 hypersensitivity or allergic reaction; ≥grade 3 nonhematologic toxicity; ≥grade 3 hematologic toxicity that recurred despite 50% 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, warranting accrual be halted, if at any time the observed rate of RLT was ≥33% and at least two RLTs had been observed.
Enzyme-linked immunosorbent spot assays

Enzyme-linked immunosorbent spot (ELISPOT) assays were performed on PBMCs obtained and cryopreserved before vaccination (week 0), at weeks 15, 18, 21, and 24 as described previously (4, 19, 20) with minor modifications. Batched Ficoll-separated PBMC samples from each patient were evaluated simultaneously following in vitro stimulation with irradiated autologous dendritic cells loaded with wild-type IL13Ra1-45, -53, EphA2-885, -891, Survivin-660-104, WT1-26-113, and Tet2-839-845. A positive ELISPOT response was defined as a ≥2-fold increase in spot-forming T cells (CD8+ cells for GAs, CD4+ cells for Tet2) over the pre-vaccine level and ≥50 spots/100,000 cells at any of two consecutive postvaccine time points against the same antigen[s] (weeks 15, 18, 21 and 24). Also, the number of postvaccine spots was required to be at least double that at baseline, and at least three times the standard-deviation of the prevaccine value.

Radiological response monitoring

Tumor size was assessed before vaccination (week 0) and at weeks 12 and 24, and q12 weeks thereafter using MRI scans. Response was evaluated according to RECIST criteria using T2-weighted FLAIR images.

Statistical methods

This pilot study was designed to assess safety and immunologic efficacy in patients who met eligibility criteria described in Patients and Methods. Each cohort was intended to enroll 9 patients, and was to be analyzed separately to provide a point estimate of immune response as assessed by the ELISPOT assay. A cohort was considered worthy of further investigation if there were at least four ELISPOT responses among 9 patients. Patients who received fewer than four vaccines were replaced by other patients for primary endpoint analyses. Each statistical analysis is

Table 2. Demographics and clinical and immunological responses for each patient

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<th>Cohort</th>
<th>ID</th>
<th>Gender</th>
<th>Age, y</th>
<th>Tumor type</th>
<th>Tumor size</th>
<th>Previous Tx</th>
<th>Vac, n</th>
<th>IFNγ ELISPOT</th>
<th>PFS</th>
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<td>&gt;132</td>
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NOTE: PFS (since the first vaccine), periods from diagnosis to the 1st vaccine and OS (overall survival since diagnosis) are described in months. For analyses of IFNγ ELISPOT assays, only the data from Weeks 0, 15, 18, 21 and 24 were used. The Week 0 spot numbers were subtracted from spot numbers for the 4 post-vaccine assays, and if the results were <0, they were set to 0. For each patient, the mean of these 4 corrected spot numbers/1×10^5 cells was computed and shown, after eliminating missing data; this procedure was carried out for each of the 4 antigens. Positive responses are highlighted with yellow per the definition described in the patients and methods section. Age is patient age at the time of study entry. Tumor size (mm²) is based on two dimensional, perpendicular measurement of T2-FLAIR abnormal signal at the baseline. Previous Tx are prior non-surgical treatments. Abbreviations: A, astrocytoma; BCNU, bis-chloroethylnitrosourea; Dx to 1st v, periods from diagnosis to the first vaccine; F, female; M, male; NA, samples not available due to early progression or assay failure; OA, oligoastrocytoma; O, oligodendroglioma; RADO01, everolimus; RT, radiation therapy; Survivin; Tet, Tetanus; TMZ, temozolomide; Tx, therapy; Vac, vaccine.
discussed in the result section. Two-sided P values \( \leq 0.05 \) were considered statistically significant.

**Results**

Demographics and clinical characteristics

Between February 2009 and December 2011, 12, 1, and 10 eligible patients were enrolled in cohorts 1, 2, and 3, respectively (Table 1). Twenty-one of 23 patients completed the scheduled initial eight immunizations; 2 patients (1 in cohort 1 and 2 in cohort 3) were withdrawn from the protocol due to early tumor progression (Table 2). Four patients completed six additional booster vaccinations. Immunologic and safety data are presented on patients who had at least four vaccinations (21 patients; Table 2), and at least one vaccination (23 patients; Table 3), respectively.

**Summary of systemic toxicities**

The primary objective of this study was to assess safety, given that this was the first such trial in patients with WHO grade 2 glioma (Table 3). Principal toxicities included grade 1 and 2 injection site reactions (100%) and flu-like symptoms (fatigue, myalgias, fever, headache), which were usually limited to 48 hours after each vaccine and were controlled with acetaminophen or ibuprofen. Grade 1 leukopenia developed in 3 patients in cohort 3. No instances of autoimmunity were encountered. No RLT has been encountered except for 1 case in cohort 1 who presented with grade 3 fever and fatigue following the seventh vaccination. The symptoms subsided by the use of over-the-counter nonsteroidal anti-inflammatory drug by the next day.

**Induction of epitope-specific immune responses against GAs**

All but 2 patients (one in cohort 1 and one in cohort 3), who had disease progression before the first postvaccine PBMC sampling on week 15, had PBMCs available for immunologic analysis. In 10 of 11, 1 of 1, and 5 of 9 evaluable patients in cohorts 1, 2, and 3, respectively, vaccination induced immune reactivity to at least one of the vaccine-targeted GAs by IFN-\( \gamma \) ELISPOT assays (Table 2). Positive IFN-\( \gamma \) responses against at least three of the four GAs epitopes were observed in 9 of 11, and 3 of 9 cases in cohorts 1 and 3, respectively. Nine of 10 in cohort 1 but only 1 of 9 in cohort 3 responded to the Tet peptide. The time course and magnitude of the IFN-\( \gamma \) ELISPOT responses in these immunologically evaluable patients in cohorts 1 and 3 are summarized in Fig. 1.

When magnitude of IFN-\( \gamma \) ELISPOT responses was compared against each of the four GAs between cohorts 1 and 3 (Table 4), cohort 1 patients demonstrated a significantly higher magnitude of IFN-\( \gamma \) response than cohort 3 patients for IL13R\( \alpha \)2 (\( P = 0.030 \)), WT1 (\( P = 0.0098 \)), and Tetanus (\( P = 0.0211 \)) epitopes as well all four GAs epitopes combined (\( P = 0.0311 \)). The Epha2 epitope also demonstrated the same trend but without statistical significance (\( P = 0.0959 \)). Interleukin (IL)-5 ELISPOT assays were performed to assess type II adaptive immune responses against the vaccine-targeted GAs in 6 (patients 2–7), 1, and 6 (patients 1, 3, 4, 6–8) in cohort 1, 2, and 3, respectively (Table 4). In corresponding cases, IFN-\( \gamma \) responses were significantly higher than IL5 responses in each of IL13R\( \alpha \)2, Epha2, and WT1 epitopes (\( P = 0.0020, 0.0059, 0.014 \)). The Survivin (\( P = 0.067 \)) but not the Tetanus (\( P = 0.32 \)) epitope showed a similar trend.

We also evaluated possible associations between baseline IFN-\( \gamma \) ELISPOT values and PFS or subsequent vaccine responses (i.e., whether preexisting baseline responses contribute to vaccine effects) as shown in Supplementary Table S1. There was a positive association between the baseline response against EphA2 and PFS (\( P = 0.046 \)), but not with any other vaccine-targeted antigen. There were no associations between baseline responses and subsequent responses in any of the vaccine-targeted antigens.

**Clinical outcomes**

Although the primary goal of this study was to provide an analysis of safety and immunoreactivity, preliminary outcome data were obtained (Table 2 and Fig. 2). Median PFS periods since the first vaccine are 17 months (cohort 1; range, 3–42+) and 12 months (cohort 3; range, 3–37+; Fig. 2). Because patients in cohorts 1 and 2 were allowed to enter the study at any time following diagnosis as long as they did not have recurrence, there was a considerable variability in the time period between diagnosis and the first vaccine (Table 2). The median PFS since diagnosis is 21 months for cohort 1. In cohort 1, 3 patients still remain progression free (37, 42, and 47 months, respectively, to date; Table 2). The only patient with large astrocytoma in cohort 2

Table 3. Adverse events (\( N = 23 \))

<table>
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<tr>
<th>Adverse event</th>
<th>Grade 1, ( n(%) )</th>
<th>Grade 2, ( n(%) )</th>
<th>Grade 3, ( n(%) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood/bone marrow</td>
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<td>1 (4)</td>
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<td>Leukocytopenia</td>
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</tr>
<tr>
<td>Injection site reactions</td>
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<td>8 (33)</td>
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<tr>
<td>Redness, induration, pruritis, pain</td>
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<tr>
<td>Constitutional symptoms</td>
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<tr>
<td>Fatigue (lethargy, malaise, asthenia)</td>
<td>8 (35)</td>
<td>12 (52)</td>
<td>1 (4)</td>
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<tr>
<td>Fever</td>
<td>11 (48)</td>
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<tr>
<td>Body ache</td>
<td>10 (44)</td>
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<td>Light headed/dizziness</td>
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<td>2 (9)</td>
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</tr>
<tr>
<td>Rhinitis/runny nose</td>
<td>2 (9)</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>2 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>3 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizure</td>
<td>5 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>3 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (neuropathy, ataxia)</td>
<td>3 (13)</td>
<td>4 (17)</td>
<td></td>
</tr>
<tr>
<td>Metabolic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia, hyponatremia,</td>
<td>6 (26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypercholesterolemia, etc.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>3 (13)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NOTE: All AEs were possibly, probably, or definitely related to the vaccine and/or poly-ICLC administration. The numbers represent the number of patients (of 23) experiencing a particular event at any point during the treatment period, with the highest grade reported for any single individual. No grade 4 events observed related to treatment.*
has been progression free for more than 45 months since the first vaccine (Supplementary Fig. S1). In cohort 3, there is 1 patient who is progression free to date at 41 months since the first vaccine. Among patients who completed at least eight vaccines, 7 of 10, 1 of 1, and 7 of 9 in cohorts 1 to 3, respectively, are alive to date (29–58, 67, and 52–164 months, respectively, since diagnosis for cohorts 1–3, respectively; Table 2 and Fig. 2). There was 1 patient in each of cohorts 1 and 3 who had to be taken off the study after four vaccines due to rapid tumor progression. Both cases were found to have recurred with glioblastoma upon resection of the recurrent tumor. No patients had a partial or complete response. We also evaluated median PFS and OS in each of the pathologic types with small sample numbers. In regard to biologic and clinical correlates, although no statistically significant association between IFNγ ELISPOT response and PFS was observed, given the modest numbers of patients on this trial, a trend was observed in cohort 3 (P = 0.00095; Table 4). Although we had hypothesized that baseline tumor size might be negatively associated with IFNγ ELISPOT response and PFS, no trends were observed to support this (Table 4), or lymphopenia (not shown). In regard to associations between immune responses and genetic markers, such as chromosome 1p/19q deletion, p53, and isocitrate dehydrogenase (IDH) mutations, our attempts were challenged because 13 of the total 23 cases were biopsied or resected for pathologic diagnosis before 2010, before implementation of IDH mutation analyses even in major medical centers, and 16 of the total 23 cases were referred from institutions from distant areas (Table 1).

**Discussion**

This is, to our knowledge, the first clinical study of peptide-based vaccination using novel GAA-derived epitopes and adjuvant poly-ICLC in “high-risk” WHO grade 2 LGGs. Our findings demonstrate tolerability and immunologic activity of this approach.

In our IFNγ ELISPOT analyses, cohort 1 patients demonstrated significantly higher magnitudes of response against IL13Rα2, WT1, Tetanus-epitopes, and overall four GAA epitopes combined (Fig. 1 and Table 4). These data strongly suggest that WHO grade 2 astrocytoma or oligoastrocytoma patients without prior treatment other than surgery may be a particularly suitable group of patients for vaccine treatments. Furthermore, because our recent pilot study of GAA-peptide vaccines in combination with poly-ICLC in newly diagnosed pediatric glioma patients (20) used the same vaccine schedule (i.e., every 3 weeks for 8 vaccines) and methods for IFNγ ELISPOT assays, we made a preliminary comparison of IFNγ ELISPOT data between cohort 1 patients in the current study and those in the pediatric study (Supplementary Table S2). Cohort 1 patients demonstrated significantly higher magnitudes of response against EphA2 (P = 0.00095) and Survivin (P = 0.0031), although data could be confounded by other factors, such as the use of RT in the pediatric patients. The current study enrolled only 1 patient in cohort 2 due to paucity of patients who were interested in the
study. This was somewhat surprising but may suggest that those patients who receive upfront RT may tend to receive chemotherapy as well. Nonetheless, the current study supports further development of vaccine approaches in WHO grade 2 adult LGG patients.

We observed considerable levels of interpatient variability in IFNγ ELISPOT data (Fig. 1). This is likely, at least partially, due to different frequencies of antigen-reactive precursor CD8+ T cells among patients. We also noted some fluctuations of responses along the time course in individual patients as also seen in our previously published studies (4, 20). This could be possibly due to one or both of the following events: (i) migration of GAA-reactive T cells in systemic circulation (i.e., detectable in PBMC) to the tumor tissue and/or lymph nodes, as possible memory T-cell development; and (ii) an induction of tolerance and/or exhaustion of GAA-reactive T cells. Nonetheless, our positive response criteria (Patients and Methods) require elevated spot numbers indicated that poly-ICLC promotes type I polarization of T-cell immunity (23) via type I chemokine CXCL10 (24) and an integrin receptor VLA-4 (6, 27–29) that promote tumor proliferation (34, 35) and immune escape (36). Our preclinical studies (5, 6) and prior phase I/II clinical study in recurrent WHO grade 3/4 HGG patients (4) have reported that coordinated T-cell and humoral responses against vaccine-targeted GAA antigens contribute to superior outcome patients (37), but the observation could also relate to the high immunogenicity of NY-ESO-1. Expanding assessments of serological responses in

### Table 4. Summary of statistical analyses

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P</th>
<th>Groups</th>
<th>Median</th>
<th>IQR</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ ELISPOT in cohorts 1 and 3</td>
<td>0.030</td>
<td>Cohort 1</td>
<td>81.5</td>
<td>40.2-185</td>
<td>Wilcoxon test (median values are spots/10E5 cells)</td>
</tr>
<tr>
<td>IL13Rx2</td>
<td>0.095</td>
<td>Cohort 3</td>
<td>13.3</td>
<td>6.00-37.5</td>
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</tr>
<tr>
<td>EphA2</td>
<td>0.0098</td>
<td>Cohort 2</td>
<td>32.0</td>
<td>27.3-210</td>
<td></td>
</tr>
<tr>
<td>WT1</td>
<td>0.031</td>
<td>Cohort 1</td>
<td>224</td>
<td>147-260</td>
<td></td>
</tr>
<tr>
<td>Survivin</td>
<td>0.021</td>
<td>Cohort 3</td>
<td>20.5</td>
<td>3.00-154</td>
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</tr>
<tr>
<td>All 4 GAs</td>
<td>0.021</td>
<td>Cohort 1</td>
<td>139</td>
<td>21.0-318</td>
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<tr>
<td>Tetanus</td>
<td>0.0020</td>
<td>IFNγ</td>
<td>81.5</td>
<td>30.8-120</td>
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<tr>
<td>IL5 ELISPOT (all cohorts combined)</td>
<td>0.0059</td>
<td>IFNγ</td>
<td>210</td>
<td>41.5-341</td>
<td></td>
</tr>
<tr>
<td>IL13Rx2</td>
<td>0.014</td>
<td>IFNγ</td>
<td>109</td>
<td>40.9-266</td>
<td></td>
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<tr>
<td>EphA2</td>
<td>0.067</td>
<td>IFNγ</td>
<td>69.0</td>
<td>34.9-273</td>
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<tr>
<td>WT1</td>
<td>0.32</td>
<td>IFNγ</td>
<td>36.8</td>
<td>11.8-110</td>
<td></td>
</tr>
<tr>
<td>Survivin</td>
<td>0.24</td>
<td>IFNγ</td>
<td>23.0</td>
<td>2.75-43.5</td>
<td></td>
</tr>
<tr>
<td>Tetanus</td>
<td>0.46</td>
<td>IL5</td>
<td>36.8</td>
<td>23.2-276</td>
<td></td>
</tr>
</tbody>
</table>

### Comparison

<table>
<thead>
<tr>
<th>P</th>
<th>CV for Cox or rho for Spearman</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNγ and IL5 ELISPOT</td>
<td>0.95</td>
<td>0.0000662</td>
</tr>
<tr>
<td>Cohort 3</td>
<td>0.95</td>
<td>0.00261</td>
</tr>
<tr>
<td>PFS for cohorts 1 and 3</td>
<td>0.26</td>
<td>0.612</td>
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<tr>
<td>Baseline tumor size and PFS</td>
<td>0.24</td>
<td>0.00118</td>
</tr>
<tr>
<td>Age and IFNγ ELISPOT</td>
<td>0.46</td>
<td>0.17</td>
</tr>
<tr>
<td>Baseline tumor size and overall IFNγ ELISPOT response</td>
<td>0.21</td>
<td>0.20</td>
</tr>
</tbody>
</table>

NOTE: For analyses of IFNγ ELISPOT assays, only the data from weeks 0, 15, 18, 21, and 24 were used. The week 0 spot numbers were subtracted from spot numbers for the four postvaccine assays, and if the results were <0, they were set to 0. For each patient, the mean of these four corrected spot numbers was computed, after applying coefficients of variation; IQR, interquartile range; Rho is the same as r value.

Abbreviations: CV, coefficient of variation; IQR, interquartile range; Rho is the same as r value.

These measures are only examples of one type I cytokine and one type II cytokine. To capture a more comprehensive picture of type I versus type II immunoskewing, in our future analyses, a broader cytokine analysis would need to be performed to measure the total type I and type II cytokines (and other types), perhaps by a multiplexed Luminex assay for antigen-stimulated lymphocyte supernatants.
positive immunoreactivity at least for IL13R in 3 cases (patients 3, 6, and 8 in cohort 1). All cases showed absence of WT1. In contrast, postvaccine tumor sections showed diffuse immunoreactivity for IL13R and EphA2, heterogeneous expression of Survivin, but absent expression of WT1. In contrast, postvaccine tumor sections obtained at the time of recurrence as WHO grade 3 anaplastic astrocytoma demonstrated diffuse and high-level expression of all four antigens. These findings contrast from previous observations in patients receiving peptide-vaccines targeting epidermal growth factor receptor (EGFR) expression, in which recurrent tumors showed absence of EGFRviii expression. Infiltration of CD8+ T cells was evaluated in patients 6 and 8 and found to be sparse (Supplementary Fig. S2). Two of the 3 patients (Patients #3 and #6) showed positive IFNγ ELISPOT responses against all four GAAs, suggesting that the systemically induced GAA-specific CD8+ effector T cells in these 2 patients may have failed to (i) sufficiently traffic to the tumor site (39) and/or (ii) mediate cytotoxic effects against GAA-expressing glioma cells for a variety of reasons, including the lack of antigen-processing components (40) and local immunosuppression in the tumor environment (41). Although we recognize the importance of overcoming these issues, we also think that these observations from 2 cases do not necessarily provide us with any conclusion about the vaccine efficacy. This is because, in these cases, the recurrent tumor has already acquired resistance and/or escaped from the vaccine response. On the other hand, the tumor from patients who display sustained positive clinical response or stable disease will never be evaluated unless we implement prospective studies to evaluate the tumors following the study interventions.

In our previous vaccine clinical studies using poly-ICLC (4, 20, 42), a number of cases demonstrated initial imaging changes that can suggest immunotherapy failure, which was followed by improvement by observation alone or dexamethasone treatment. In the current study, however, despite the robust induction of IFNγ response in PBMC, we did not observe any apparent case of such “tumor pseudoprogression.” Nevertheless, this does not preclude a possibility that some patients may have been prematurely withdrawn from the study based on MRI findings suggesting progressive disease. Indeed, patient 6 in cohort 3 has been radiologically and clinically stable without any active antitumor therapy for longer than 33 months as this patient was withdrawn from our study due to radiological progression after the initial eight vaccinations. Novel imaging technologies as well as more appropriate response criteria for brain tumor immunotherapy need to be developed (42).

In regard to common genetic mutations and novel immunotherapy targets in LGG, mutations of the isocitrate dehydrogenase (IDH) metabolic enzymes IDH1 and IDH2 have been found to be frequent and early genetic alterations in astrocytomas and oligodendrogliomas (43). Mutation of IDH1 occurs early in glioma progression, with somatic mutations of the R132 residue of IDH1 identified in the majority (>70%) of grades 2 and 3 astrocytomas and oligodendrogliomas, as well as in secondary GBMs that develop from these LGG (44, 45). It has been recently reported that the IDH1R132H mutation contains an immunogenic epitope suitable for mutation-specific vaccination in the context of major histocompatibility complexes (MHC) class II (46). Further refinement of vaccine-targeted antigens and identification of novel antigens for LGGs are warranted for development of more effective vaccine strategies for LGGs, such as personalized vaccines based on biopsy-based antigen-characterization in each patient.

In summary, the current study demonstrated promising immunoreactivity in high-risk groups of WHO grade 2 LGG patients. These data support larger studies of GAA peptide-based vaccination in patients with LGG, in which clinical efficacy will be assessed as the primary endpoint. However, preliminary data with recurrent tumors suggest that the vaccine regimen may not eliminate tumor cells expressing vaccine-targeted antigens.
Further studies are warranted to understand mechanisms limiting the efficacy of the current approach.

Disclosure of Potential Conflicts of Interest

H. Okada has ownership interest (including patents) in and is a consultant/advisory board member for Stemline Therapeutics, Inc. A.M. Salazar is an employee of and has ownership interest (including patents) in Oncovir, Inc. F.S. Lieberman reports receiving commercial research grants from Novacure and Roche. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Okada, I.H. Butterfield, R.L. Hamilton, A. Hoji, M. Sakaki, G. Kohanbash, J. Drappatz, J. Engh, N. Amankulor, M.O. Lively, M.D. Chan, E.G. Shaw, F.S. Lieberman
Writing, review, and/or revision of the manuscript: H. Okada, I.H. Butterfield, R.L. Hamilton, A. Hoji, B.J. Ahn, G. Kohanbash, J. Drappatz, F.S. Lieberman
M.O. Lively, M.D. Chan, A.M. Salazar, E.G. Shaw, D.M. Potter, F.S. Lieberman

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Okada, G. Kohanbash, N. Amankulor, M.D. Chan
Study supervision: H. Okada, F.S. Lieberman

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References


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