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

Hideho Okada1,2,3,4,5, Lisa H. Butterfield4,5,6, Ronald L. Hamilton1,7, Aki Hoji1,3,8, Masashi Sakaki1,4, Brian J. Ahn1,5, Gary Kohanbash1,3, Jan Drappatz1,3,9, Johnathan Engh1,3, Nduka Amankolor1,3, Mark O. Lively10, Michael D. Chan10, Andres M. Salazar11, Edward G. Shaw10, Douglas M. Potter1,2, and Frank S. Lieberman1,3,9

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 or chemotherapy but with prior RT; and (iii) recurrent patients.

Experimental Design: GAA were IL13Rα2, 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-γ 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-γ 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.

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 anti-glioma 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
Translational Relevance

World Health Organization (WHO) grade 2 low-grade gliomas (LGG) are slow-growing primary brain tumors with very high risk of progression following conventional therapies. More than 50% of these patients eventually develop aggressive high-grade gliomas (HGG), and most patients eventually die of the disease. Development of immunotherapeutic approaches, such as vaccines, may be particularly appropriate because patients with LGG are likely not to be as immunocompromised as patients with HGG, and the slower growth rate of LGG (in contrast with HGG) should allow sufficient time to administer multiple immunizations, which may induce high levels of antigenic immunity. We evaluated synthetic peptides for human leukocyte antigen (HLA)-A2–restricted cytotoxic T-cell (CTL) epitopes derived from glioma-associated antigens (GAA) in patients with high-risk low-grade gliomas. Our results with safety and robust inductions of GAA-specific CD8+ T-cell responses support further development of this approach.

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 three 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, antero-posteriorly, 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, Hiltocon; 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 CTCAEv3.0. 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.

Peptides

HLA-A2–restricted peptides used in this study were ALPGFILY (IL13Rα2_A5P3:1A9V; ref. 7), TLADFDPRV (EphA2_A5R8:89Y; ref. 9), LMLGEFLKL (Survivin_b3_A4G5:1A9V; ref. 11); YMFNNLPY (WT1_A12:1A4V; ref. 10) admixed with AQYIKANSKFIGITEL (TetA830) (ref. 18). The peptides were produced using automated solid-phase synthesis by NeoMPS (PolyPeptide Group). Peptides were tested in multiple quality-assurance assays, including purity, sterility, identity, potency, pyrogenicity, and stability.

vaccinations with synthetic peptides for GAA epitopes emulsified in Montanide-ISA-51 every 3 weeks for eight courses as well as intramuscular administration of poly-ICLC (5, 6) in WHO grade 2 gliomas with high risk for recurrence. GAs for these peptides are IL13Rα2 (7, 8), EphA2 (9), Wilms’ tumor gene product 1 (WT1; ref. 10), and Survivin (11), all of which contain HLA-A2–restricted CTL epitopes (7–11). Whereas IL13Rα2 (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 interestingly, high-level expression of Survivin was associated with 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 tetanus toxoid peptide (TetA830) was included in the normal glial cells contained in the tumor specimens (15).
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-isolated PBMC samples from each patient were evaluated simultaneously following 
in vitro stimulation with irradiated autologous dendritic cells loaded with wild-type IL13Rα2, EphA2, WT1, Survivin, EphA2, and TET. A positive ELISPOT response was defined as a ≥2-fold increase in spot-forming T cells (CD8+ cells for GAA, CD4+ cells for TET, and EphA2) 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 post-vaccine 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...
discussed in the result section. Two-sided P values ≤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 (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 vaccine. 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 GAAs

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 GAAs by IFN- \( \gamma \) ELISPOT assays (Table 2). Positive IFN- \( \gamma \) responses against at least three of the four GAA 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 GAAs 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 GAA epitopes combined (\( P = 0.031 \)). The Epha2 epitope also demonstrated the same trend but without statistical significance (\( P = 0.095 \)). Interleukin (IL)-5 ELISPOT assays were performed to assess type II adaptive immune responses against the vaccine-targeted GAAs 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>
<thead>
<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>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytopenia</td>
<td>3 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site reactions</td>
<td>15 (65)</td>
<td>8 (35)</td>
<td></td>
</tr>
<tr>
<td>Constitutional symptoms</td>
<td>8 (35)</td>
<td>12 (52)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Fatigue (lethargy, malaise, asthenia)</td>
<td>11 (44)</td>
<td>5 (22)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>11 (44)</td>
<td>3 (13)</td>
<td></td>
</tr>
<tr>
<td>Body ache</td>
<td>10 (44)</td>
<td>6 (26)</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>8 (35)</td>
<td>9 (39)</td>
<td></td>
</tr>
<tr>
<td>Insomnia</td>
<td>2 (9)</td>
<td>2 (9)</td>
<td></td>
</tr>
<tr>
<td>Light headed/dizziness</td>
<td>4 (17)</td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>1 (4)</td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>2 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaphoresis</td>
<td>1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>3 (13)</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>5 (22)</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>3 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermatological</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>2 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry skin</td>
<td>1 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary/upper respiratory</td>
<td>2 (9)</td>
<td>2 (9)</td>
<td></td>
</tr>
<tr>
<td>Rhinitis/runny nose</td>
<td>2 (9)</td>
<td></td>
<td>1 (4)</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>2 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurological</td>
<td>5 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizure</td>
<td>5 (22)</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, hypercholesterolemia, etc.</td>
<td>6 (26)</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 = 0.095; 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). No statistically significant association was observed between IFNγ ELISPOT and prior use of TMZ (cohort 3), age (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 responses than cohort 3 patients 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 oligoastroctoma 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 must be seen at least two consecutive postvaccine time points against the same antigen[s]. assuring consistency of immune response for determining immunological responders using PBMC-based immune assays.

In the current study, we evaluated relative magnitudes of IFNγ and IL5 ELISPOT responses as readouts of type I and type II adaptive immune responses (21). These are appropriate to compare, as the ELISPOT measures the frequency of cytokine-producing cells per number of cells plated, but we also recognize that 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.

We have previously demonstrated that tumor-specific type I T cells, which predominantly secrete IFNγ (22), but not type II T cells can efficiently traffic into brain tumor sites and mediate effective therapeutic efficacy (23) via type I chemokine CXCL10 (23–26) and an integrin receptor VLA-4 (6, 27–30). However, cancers, including gliomas, secrete numerous type II cytokines (31–33) 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 indicated that poly-ICLC promotes type I polarization of T-cell responses against vaccine-targeted GAAa. Although limited numbers of cases were evaluated for IL5 ELISPOT, our data further support the ability of our vaccine regimen for promoting type I (i.e., IFNγ-driven) GAA-specific T-cell responses. While our strategy emphasizes promotion of type I responses, it has also been reported that coordinated T-cell and humoral responses against NY-ESO-1 antigen 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 |
|-------------------------------|----------------|------------|-----------|-----------------|
| **Comparison**                  | **P** | **Groups** | **Median** | **IQR** | **Method** |
| IFNγ ELISPOT in cohorts 1 and 3 | 0.030 | Cohort 1  | 81.5      | 40.2–185 | Wilcoxon test |
| EphA2                           | 0.095 | Cohort 1  | 33.6      | 6.00–37.5 | (median values are spots/10e5 cells) |
| WT1                             | 0.0098 | Cohort 1 | 262.0      | 132–331 | Spearman test |
| Survivin                        | 0.45  | Cohort 1  | 112.0     | 59.2–128 |             |
| All 4 GAAa                      | 0.031 | Cohort 1  | 224.0     | 147–260 |             |
| Tetanus                         | 0.021 | Cohort 1  | 139.0     | 27.0–41.4 |             |
| IFNγ and IL5 ELISPOT (all cohorts combined) | **0.0020** | IFNγ  | 81.5     | 30.8–120 |             |
| EphA2                           | **0.0059** | IFNγ  | 210.0     | 41.5–341 |             |
| WT1                             | **0.014** | IFNγ  | 109.0     | 40.5–266 |             |
| Survivin                        | **0.067** | IFNγ  | 23.0      | 8.67–67.6 |             |
| Tetanus                         | **0.32** | IFNγ  | 36.8      | 11.8–110 |             |
| Age and IFNγ ELISPOT            | **0.46** | IFNγ  | 0.17      |             | Spearman test |
| Baseline tumor size and overall IFNγ ELISPOT response | **0.21** | IFNγ  | 0.20      |             |             |

**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 eliminating missing data; this procedure was carried out for each of the four antigens. To assess the association of tumor size and PFS with ELISPOT response, the means for the four antigens were summed to give a single number for each patient. Bold, P values <0.05.

Abbreviations: CV, coefficient of variation; IQR, interquartile range; Rho is the same as r value.
positive immunoreactivity at least for IL13Rα2 in 3 cases (patients 3, 6, and 8 in cohort 1). All cases showed a peptide/T cell-driven study. Future trials would help identify the role for humoral responses in the tumor environment. Okada et al. mentioned findings contrast from previous observations as more appropriate response criteria for brain tumor immunotherapy need to be developed.

Postvaccine tissues were available for assessing antigen expression in 3 cases (patients 3, 6, and 8 in cohort 1). All cases showed positive immunoreactivity at least for IL13Rα2 and Survivin (Supplementary Fig. S2). Among these cases, both pre-and postvaccine tissues were available from only 1 case (patient 3 in cohort 1). Prevaccine tumor sections showed diffuse immunoreactivity for IL13Rα2 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 (EGFRviii) (38), 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 (IDH1) 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 IDH1(R132H) 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, Roche, and Stemline and is a consultant/advisory board member for Novacure and Roche. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

Conception and design: H. Okada, R.L. Hamilton, A.M. Salazar, E.G. Shaw, D. M. Potter, F.S. Lieberman

Development of methodology: H. Okada, M. Sakaki, E.G. Shaw

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. Amanakul, 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, 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. Amanakul, M.D. Chan

Study supervision: H. Okada, F.S. Lieberman

**Acknowledgments**

The authors thank Shari Reynolds and Rebecca Bishop for regulatory management; physicians who referred their patients; Jennifer Mabold, C.R.N.P. and Clinical Translational Science Institute for patient care; and patients and their families.

**Grant Support**

This research was supported by NIH grants R21CA133859 and P01 CA132714, the Pennsylvania Department of Health, and the Musella Foundation. This project used UPCI-shared resources (Clinical Research Services, Immunological Monitoring and Cellular Products Laboratory, Biostatistics Facility) that are supported in part by NIH P30CA047904.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 15, 2014; revised October 7, 2014; accepted October 31, 2014; published OnlineFirst November 25, 2014.


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


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-14-1790

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2014/11/26/1078-0432.CCR-14-1790.DC1

Cited articles
This article cites 45 articles, 21 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/21/2/286.full.html#ref-list-1

Citing articles
This article has been cited by 11 HighWire-hosted articles. Access the articles at:
/content/21/2/286.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.