

Induction of CD8⁺ T-Cell Responses Against Novel Glioma–Associated Antigen Peptides and Clinical Activity by Vaccinations With α -Type 1 Polarized Dendritic Cells and Polyinosinic-Polycytidylic Acid Stabilized by Lysine and Carboxymethylcellulose in Patients With Recurrent Malignant Glioma

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ABSTRACT

Purpose

A phase I/II trial was performed to evaluate the safety and immunogenicity of a novel vaccination with α -type 1 polarized dendritic cells (α DC1) loaded with synthetic peptides for glioma-associated antigen (GAA) epitopes and administration of polyinosinic-polycytidylic acid [poly(I:C)] stabilized by lysine and carboxymethylcellulose (poly-ICLC) in HLA-A2⁺ patients with recurrent malignant gliomas. GAAs for these peptides are EphA2, interleukin (IL)-13 receptor- α 2, YKL-40, and gp100.

Patients and Methods

Twenty-two patients (13 with glioblastoma multiforme [GBM], five with anaplastic astrocytoma [AA], three with anaplastic oligodendroglioma [AO], and one with anaplastic oligoastrocytoma [AOA]) received at least one vaccination, and 19 patients received at least four vaccinations at two α DC1 dose levels ($1 \times$ or 3×10^7 /dose) at 2-week intervals intranodally. Patients also received twice weekly intramuscular injections of 20 μ g/kg poly-ICLC. Patients who demonstrated positive radiologic response or stable disease without major adverse events were allowed to receive booster vaccines. T-lymphocyte responses against GAA epitopes were assessed by enzyme-linked immunosorbent spot and HLA-tetramer assays.

Results

The regimen was well-tolerated. The first four vaccines induced positive immune responses against at least one of the vaccination-targeted GAAs in peripheral blood mononuclear cells in 58% of patients. Peripheral blood samples demonstrated significant upregulation of type 1 cytokines and chemokines, including interferon- α and CXCL10. Nine (four GBM, two AA, two AO, and one AOA) achieved progression-free status lasting at least 12 months. One patient with recurrent GBM demonstrated sustained complete response. IL-12 production levels by α DC1 positively correlated with time to progression.

Conclusion

These data support safety, immunogenicity, and preliminary clinical activity of poly-ICLC-boosted α DC1-based vaccines.

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INTRODUCTION

Early phase studies of glioma vaccines have shown feasibility and encouraging preliminary clinical activity.¹⁻⁹ The ultimate success of glioma vaccines likely depends critically on the further refinement of strategies to promote type 1 immunity¹⁰⁻¹² and to

target multiple glioma-associated antigen (GAA) epitopes, given the marked antigenic heterogeneity of gliomas.^{13,14}

To this end, this study employed two novel strategies based on our own preclinical studies. First, we implemented culture methods for α -type 1 polarized DCs (α DC1) that are able to produce high

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levels of interleukin (IL) -12 and induce long-lived type 1 T-cell responses against tumor-associated antigens more efficiently than standard mature DCs.^{15,16} Second, we incorporated administration of the immunoadjuvant polyinosinic-polycytidylic acid [poly(I:C)] stabilized by lysine and carboxymethylcellulose (poly-ICLC), which has been shown to be safe through extensive evaluations in patients with malignant glioma,¹⁷⁻¹⁹ to enhance the efficacy of GAA-targeting vaccinations, as we demonstrated in glioma-bearing mice.^{20,21}

The HLA-A2-restricted epitopes included two that we had previously identified, an IL-13R α 2-derived analog peptide (IL-13R α 2_{345-353:1A9V})^{22,23} and EphA2₈₈₃₋₈₉₁,^{24,25} as well as two additional epitopes, YKL-40₂₀₁₋₂₁₀^{26,27} and gp100_{209-217:M2}.²⁸

Thus, this study is the first to evaluate α DC1 loaded with GAA epitopes in combination with poly-ICLC in humans. We hypothesized that this regimen would prove to be safe, and would induce potent antiglioma immune responses.

PATIENTS AND METHODS

Patients

Patients with recurrent malignant glioma were enrolled with informed consent and approvals by the institutional review board and US Food and Drug Administration (BB-IND#12415). Clinical characteristics of patients are summarized in Table 1 and Appendix Figure A1 (online only). Enrollment criteria included: histologic diagnosis of glioblastoma multiforme (GBM) or anaplastic glioma (AG) including anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), or anaplastic oligoastrocytoma (AOA); up to two previous recurrences; \geq 18 years old; Karnofsky performance status \geq 60; adequate liver and renal function and HLA-A2⁺. Minimum doses of corticosteroid (dexamethasone up to 4 mg/d) were permitted. Twenty-two patients were enrolled and received at least one vaccination. Nineteen of 22 patients completed the scheduled initial four immunizations; three patients (patients 4, 11, and 13) were withdrawn from the protocol due to early tumor progression. Nine patients completed five additional booster vaccinations. Immunologic and safety data are presented on patients who had at least four vaccinations (n = 19), and at least one vaccination (n = 22), respectively.

Characteristic	DC Dose Level (No. of DC/dose)		Total No. of Patients (n = 22)	%
	1 (1 \times 10 ⁷)	2 (3 \times 10 ⁷)		
Received at least one vaccine	11	11	22	
Completed at least four vaccines	10	9	19	86
Female (received at least four vaccines)	5	4	9	47
Median age, years	52	46	48	
Range	37-71	28-63	28-71	
Tumor histology				
AA	3	2	5	23
AO	1	2	3	14
AOA	1	0	1	4
GBM	6	7	13	59
No. of previous recurrences				
0	7	4	11	50
1	2	5	7	32
2	2	2	4	18

Abbreviations: DC, dendritic cells; AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; GBM, glioblastoma multiforme.

Clinical Trial Design

This single institution phase I/II study was designed to assess toxicity and the induction of immune and preliminary clinical responses of vaccinations with GAA-loaded α DC1 and administration of poly-ICLC (Hiltonol; Oncovir, Washington, DC). The first course of vaccines consisted of four ultrasound-guided intranodal (IN) administrations of 1 \times or 3 \times 10⁷ α DC1/injection every 2 weeks rotating between right and left inguinal and axillary lymph node clusters to minimize the potential effects of injection-induced trauma in the microenvironment of the lymph nodes (Data Supplement Fig DS1, online only). The first 10 evaluable patients received 1 \times 10⁷ α DC1/injection (dose level 1); the subsequent nine received 3 \times 10⁷ α DC1/injection (dose level 2). The sample size justification is provided in the Data Supplement (Study Design Parameters; online only). All patients received intramuscular injections with poly-ICLC (20 μ g/kg) twice/wk for 8 weeks starting on day 1. Patients exhibiting stable disease or tumor regression without major adverse events (AEs) after the fourth vaccination were eligible for additional vaccinations. Starting at week 13, these patients were treated with the same dose of additional vaccinations every 4 weeks to a maximum of five vaccine injections and intramuscular poly-ICLC starting on the day of the first additional vaccine and twice/wk (first booster phase). Patients not demonstrating major AEs or tumor progression after the first booster phase were offered the same dose of additional vaccines (every 3 months) and poly-ICLC (every week) for up to 3 years from the first vaccination (second booster phase).

Toxicity Assessment and Stopping Rules

The trial was monitored continuously for treatment-related AEs using the National Cancer Institute Common Toxicity Criteria version 3.0. The following were considered to be a dose-limiting toxicity (DLT) if they were judged possibly, probably, or definitely associated with treatment: \geq grade 2 hypersensitivity; \geq grade 3 nonhematologic/metabolic toxicity; \geq grade 3 hematologic (except for lymphopenia) or metabolic toxicity that did not subside after 4 weeks cessation of poly-ICLC. Stopping rules were implemented such that a dose level was considered excessively toxic, warranting that accrual be halted, if at any time the observed rate of DLT was \geq 33% and at least two DLTs had been observed.

Peptides

HLA-A2-restricted peptides used in these studies were: ALPFGFILV (IL-13R α 2_{345-353:1A9V})²³; TLADFDPV (EphA2₈₈₃₋₈₉₁)²⁴; IMDQVPFVS (GP100_{209-217:M2})²⁵; and SIMTYDFHGA (YKL-40₂₀₁₋₂₁₀). α DC1 were also loaded with aKXVAAWTLKAAZC (a pan-DR epitope [PADRE]), a non-natural epitope optimized for helper T-cell response.³⁰ The peptides were synthesized by automated solid-phase peptide synthesis in the University of Pittsburgh Peptide Synthesis Facility. Peptides were tested in multiple quality-assurance studies including purity, sterility, identity, potency, pyrogenicity, and stability.

Vaccine Preparation

For the DC culture, monocytes were obtained from the leukapheresis product and purified by the Elutra System (CardianBCT, Lakewood, CO). The monocytes were cultured in antibiotic-free culture medium (CellGenix Technologie Transfer GmbH, Antioch, IL) supplemented with 1,000 U/mL granulocyte-macrophage colony-stimulating factor and 1,000 U/mL IL-4 in sterile cartridges, using the Replicell System (Astrom Biosciences, Ann Arbor, MI). The immature DC (iDC) were harvested on day 6 and cryopreserved. Before each vaccination, aliquots of frozen iDCs were thawed, further matured, and polarized with clinical grade IL-1 β (10 ng/mL), tumor necrosis factor- α (10 ng/mL), interferon (IFN) - α (3,000 U/mL), IFN- γ (1,000 U/mL), and poly-I:C (20 μ g/mL) at 37°C in 5% CO₂ for 48 hours and loaded with GAA peptides (10 μ g/mL) for 4 to 6 hours. Two hours before harvest, the PADRE peptide was added to the cultures. Criteria for release of α DC1 included: sterility by Gram stain and bacteriologic culture; negative *Mycoplasma*; endotoxin lower than 5.0 EU/kg of body weight; greater than 70% expression of both CD86 and HLA-DR on α DC1.

Enzyme-Linked Immunosorbent Spot Assays

Enzyme-linked immunosorbent spot (ELISPOT) assays were performed as described previously^{5,31} with slight modifications. Briefly, peripheral blood

mononuclear cells (PBMC) samples drawn and cryopreserved at each visit for vaccine (before the vaccine) as well as weeks 0, 9, and 33 were evaluated simultaneously after in vitro stimulation with autologous, irradiated PBMC loaded with wild-type IL-13R α ₃₄₅₋₃₅₃,²² EphA2₈₈₃₋₈₉₁, GP100₂₀₉₋₂₁₇, and YKL-40₂₀₂₋₂₁₁ for a week. A positive ELISPOT response was defined as a two-fold increase in spot-forming T cells over the prevaccine level and at least 10 spots/20,000 cells for at least two consecutive postvaccine time points against any antigen.

Tetramer Assays

Phycoerythrin-conjugated HLA-A*0201/ALPFGFILV (IL-13R α -tetramer), HLA-A*0201/IMDQVPFSV (gp100-tetramer), and HLA-A*0201/TLADFPRV (EphA2-tetramer) were produced by the National Institute of Allergy and Infectious Disease tetramer facility (Emory University Vaccine Center, Atlanta, GA) using the peptide synthesized by the University of Pittsburgh Peptide Production Facility. Fluorescein isothiocyanate-conjugated antihuman CD8 was obtained from BD Biosciences (Sparks, MD). A single timepoint-positive response for a peptide was defined to be (0.1+B)% of all CD8⁺ cells positive by tetramer assay,^{32,33} where B is the percent positive at baseline, which was lower than 0.01% in all cases. A patient was considered to have responded if he/she had two consecutive single timepoint responses for any peptide.

Cytokine and Chemokine Assays

Total RNA samples were obtained from PBMC using the PAXgene Blood RNA System (PreAnalytix, Hombrechtikon, Switzerland). Reverse-transcriptase polymerase chain reaction (RT-PCR) was performed in triplicate, and values were standardized to glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and relative expression of mRNAs was calculated using the $\Delta\Delta C_T$ method.³⁴ The Luminex-based assay was performed in serum samples as previously described.³⁵ Pretested, multiplex plates (Invitrogen, Carlsbad, CA) included standard curves and cytokine standards (R&D Systems, Minneapolis, MN). In situ hybridization with radiolabeled cRNA probe for *CXCL10* was performed as described,³⁶ with autoradiographic exposure times of 14 days.

Radiologic Response Monitoring and Other Clinical End Points

Tumor size was assessed at weeks 9, 17, 25, and 33, and every 3 months thereafter using magnetic resonance imaging (MRI) scans with contrast enhancement. Response was evaluated by McDonald criteria by gadolinium (Gd)-enhanced T1 weighted images, area of signal prolongation on T2 weighted images, or a combination of both, on the basis of the appearance of the pretreatment MRI. Overall survival was defined by the interval from study entry to date of death. MRI scans were used to evaluate time to progression (TTP).

RESULTS

Summary of Clinical Toxicities

Treatment-related AEs are listed for all 22 patients in Data Supplement Table DS1 (online only). There were no grade 3 or 4 toxicities, no deaths on study, and no DLT at any dose through the first booster phase. No incidences of autoimmunity were encountered. Toxicity profiles were comparable across dose levels (data not shown). Grade 1 or 2 injection site reactions were the most common (82%). Grade 1 flu-like symptoms, including fatigue (73%), myalgia (32%), fever (23%), chills/rigors (18%), and headache (32%), were common and usually limited to 24 hours after each vaccine. Grade 2 lymphopenia was recorded in one patient (5%).

IL-12 Production by α DC1 and Induction of Epitope-Specific Immune Responses Against GAAs

As presented in Appendix Figure A1, CD40L-induced IL-12 p70 production levels by α DC1 varied substantially between patients, and

positively correlated with TTP ($P = .0255$; Fig 1), but not with IFN- γ ELISPOT response, patients' age, or tumor types (data not shown).

All 19 patients who completed the initial course of four vaccinations had PBMCs available for immunologic monitoring. Insufficient PBMC were obtained from patients 17, 21, and 22 to perform both ELISPOT and tetramer assays, and functional ELISPOT assays were prioritized. The scheduled first four vaccines induced immune reactivity to at least one of the vaccine-targeted GAAs in six of 10 and five of nine patients in dose levels 1 and 2, respectively, by either IFN- γ ELISPOT or tetramer assays (Appendix Fig A1). In patients 6, 7, 8, 16, 19, 20, and 22, some readouts reached the criteria for positive response after booster vaccines (indicated by † in Appendix Fig A1). In summary, 11 (58%) of 19 evaluable patients showed positive responses after the initial four vaccinations, and three additional subjects (patients 8, 19, and 20; 16%) showed positive responses only after booster vaccines.

Positive response rates (either by tetramer or ELISPOT) did not show significant differences across the two α DC1 doses per Fisher's exact test. Furthermore, the magnitudes of ELISPOT response, based on the summation of positive spots from weeks 3 through 9, were comparable across the two α DC1 dose levels (Wilcoxon test). Therefore, we present the time course of IFN- γ ELISPOT responses by combining results from both dose levels (Fig 2A). The gp100 epitope demonstrated the highest magnitude of response among the GAA peptides tested ($P < .001$ against IL-13R α 2-, EphA2-, and YKL-40-derived peptides by Wilcoxon test). For the other epitopes, booster vaccines appeared to improve the induction of specific responses. A temporary decline of responses was typically observed at week 13, which may reflect that some patients who demonstrated positive responses by week 9 did not participate in the booster phase due to tumor progression (patients 2, 9, 18, and 21) or lymphopenia (patient 10), resulting in overall reduction of response when data are pooled for all patients. Patient 10 demonstrated the highest magnitude of IFN- γ ELISPOT responses against IL-13R α 2- and gp100-derived epitopes as well as PADRE (Fig 2B) but tetramer analyses on this patient yielded no responses (Appendix Fig A1). Patient 6, who demonstrated stable disease for longer than 30 months, developed durable and high level responses in tetramer (Fig 2C) and ELISPOT assays.

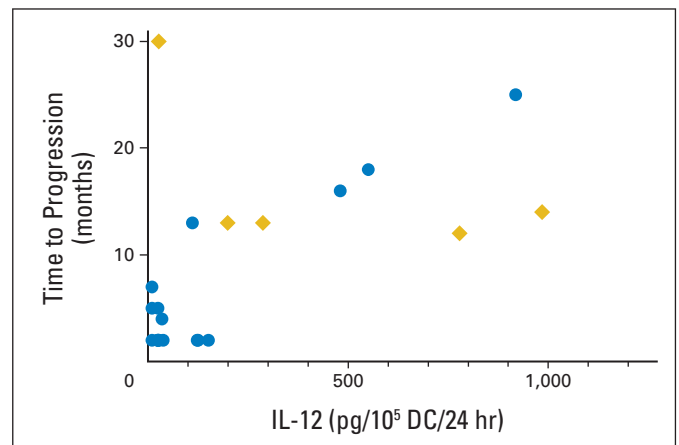


Fig 1. Interleukin (IL)-12 production levels positively correlated with time to progression. $P = .0255$ is based on Cox regression followed by likelihood-ratio test. Circles indicate patients who have already experienced disease progression; diamonds represent patients who have not experienced recurrence to date. DC, dendritic cells.

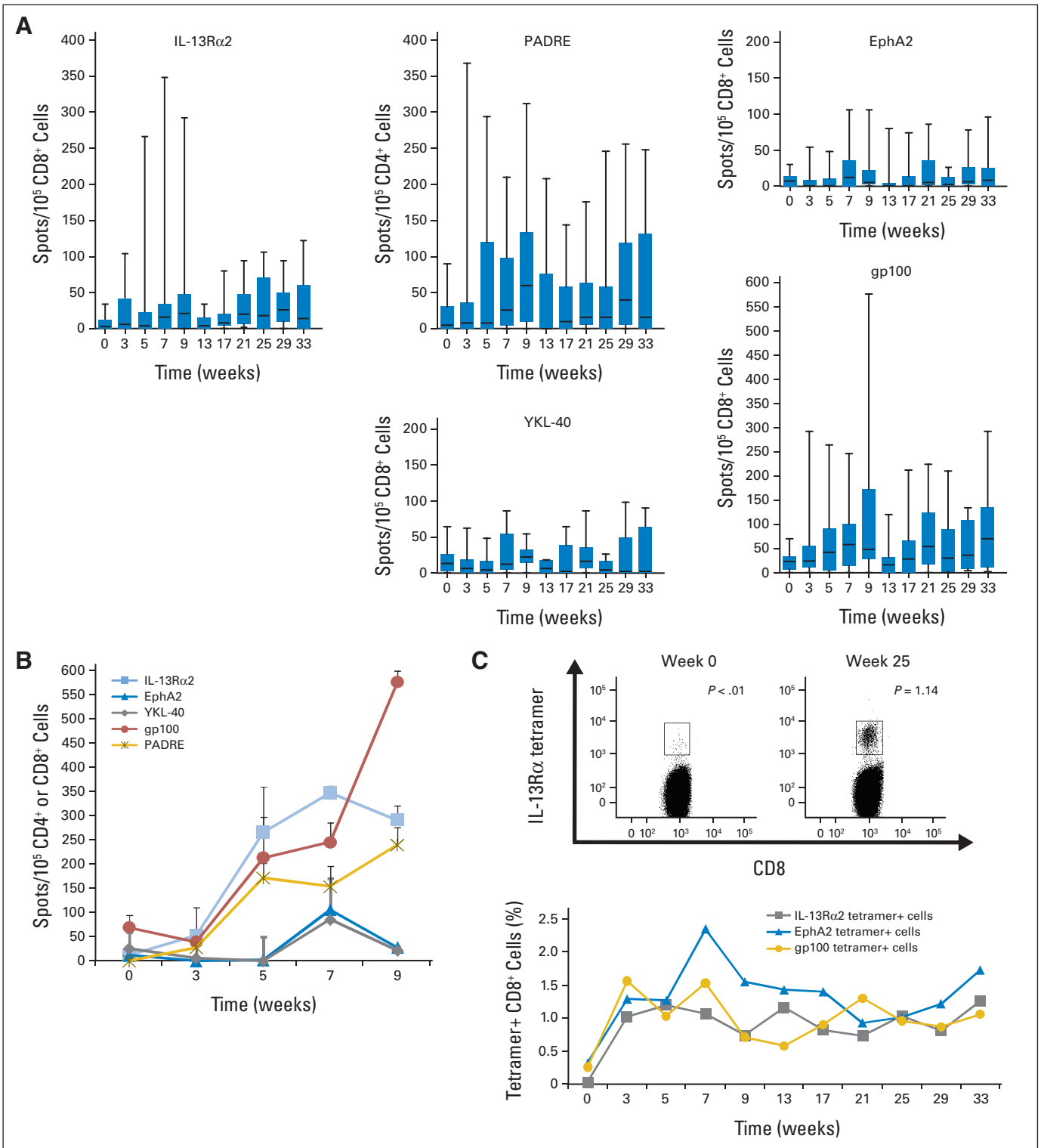


Fig 2. Glioma-associated antigen (GAA)-specific T-cell responses evaluated by interferon (IFN)- γ enzyme-linked immunosorbent spot (ELISPOT) and tetramer analyses. (A) Time course for IFN- γ ELISPOT assays for all evaluated patients with box plots (boxes = 25th to 75th percentiles; vertical lines = minimum to maximum). Numbers at the bottom of each time point in the panel for YKL-40 are the number of assessable patients at the time shown. These numbers also pertain to the other GAAs and a pan-DR epitope (PADRE). (B) IFN- γ ELISPOT analysis for patient 10. (C) Patient 6 showed durable tetramer responses, which were analyzed for up to 33 weeks. Examples of histograms for positive tetramer responses against the interleukin (IL)-13R α 2-epitope are shown.

Induction of Type-1 Cytokine and Chemokine Responses

RT-PCR analyses of PBMC (Appendix Fig A2A, A2B, online only) revealed upregulation of mRNA expression for several type 1 cytokines and chemokines, specifically *IFN- α 1*, *CXCL10*, and *TLR3*, at both postfirst vaccine and postfourth vaccine. *IFN- γ* was found to be upregulated after the fourth vaccine, but not after the first vaccine, suggesting that the *IFN- γ* upregulation may be associated with the induction of adaptive, rather than innate, immune response. *CCL22*, which is known to attract regulatory T cells,³⁷ and *CCL5* levels decreased in paired analyses of postfirst vaccine samples. Perforin, Granzyme B, cyclo-oxygenase2, and forkhead box protein p3 levels did not change significantly (data not shown).

A panel of cytokines and chemokines was evaluated at protein levels in available prevaccine and postvaccine serum samples from five patients (Appendix Fig A2C). Among them, *IFN- α* , *CXCL10*, *IL-15*, *MCP-1*, and *MIP-1 β* were significantly upregulated in postvaccine sera. *IL-17* was under detectable ranges in both RT-PCR and serum analyses (data not shown).

In addition, three of five available tumors resected due to postvaccine radiographic progression expressed mRNA for *CXCL10*, which is a critical chemokine for effective trafficking of $CD8^+$ T-cells to brain tumor sites (Appendix Fig A2D for a representative case).^{10-11,38} These data suggest that this regimen induces systemic, polyfunctional immune responses in generally immunosuppressed patients with malignant glioma.

Clinical Outcomes

Two patients (patients 1 and 20) experienced objective clinical tumor regressions (response rate, 9%). Both patients were nonresponders by ELISPOT, but tetramer responders. Patient 20 with recurrent GBM demonstrated complete response based on disappearance of the Gd-enhanced mass at week 17 postvaccine compared with the baseline MRI, which has been durable and ongoing to date for at least 13 months since initiation of treatment (Appendix Fig A3A, online only). Patient 1 with recurrent GBM exhibited a partial response at week 9. After two booster vaccines, the Gd-enhanced lesion enlarged. Biopsy of the lesion, however, revealed intensive infiltration of $CD8^+$ T cells and $CD68^+$ macrophages and no evidence of mitotically active tumor (Appendix Figs A3B to A3D). Then, this patient received one additional vaccine before exhibiting recurrence at 7 months after the initial vaccine. Nine patients (41%; four and five with GBM and AG, respectively) are progression free for at least 12 months. Five patients are currently progression-free (Appendix Fig A1) and still receiving booster vaccines. Median TTP were 4 and 13 months for GBM and AG, respectively (Data Supplement Fig DS2, online only).

DISCUSSION

To our knowledge, this is the first clinical evaluation of α DC1-based vaccines loaded with novel GAA-derived peptides, in combination with poly-ICLC. Our findings demonstrate safety and immunogenicity as well as preliminary efficacy of the approach.

With regard to the manufacturing of α DC1, since the publication of the original α DC1 preparation method¹⁶ and during optimization studies for α DC1 production from patients with glioma, lipopolysaccharide was eliminated to achieve the US Food and Drug Administration–mandated endotoxin level on vaccine release. Our preclinical

studies also indicated that prostaglandin E_2 should not be used for optimal type-1 maturation of DC.^{39,40} Although suppressed immune status,⁴¹ as well as decreased numbers and functions of monocytes,^{42,43} have been reported in patients with glioma, we had no major difficulties generating adequate numbers of α DC1. Although the ability to produce *IL-12* varied substantially among the patients, surface maturation markers, such as *CD83*, were all strongly positive in all DC preparations (data not shown), supporting that these cells were properly matured. Based on our data showing a positive correlation between α DC1 production of *IL-12* and TTP, further studies are warranted to determine which factors in patients with cancer influence the *IL-12* production of α DC1, and whether *IL-12* production is a surrogate measure of DC potency and vaccine efficacy in future trials.

This study is the first to document in vivo induction of specific $CD8^+$ T-cell responses against three of four GAA epitopes, *IL-13R α 2*_{345-353:1A9V},^{23,44,45} *EphA2*₈₈₃₋₈₉₁,^{24,46} and *YKL-40*₂₀₁₋₂₁₀.^{26,27} With regard to the expression of these GAAs in our series, although resection of the recurrent tumor is not often clinically indicated in these patients, we summarized available immunohistochemistry data for seven patients in Data Supplement Table DS2. These preliminary data suggest that expression of gp100 may be very low in primary high-grade gliomas, consistent with published results.⁴⁷ We included the gp100 peptide to assess the potency of the vaccine regimen because this was the only epitope that was previously demonstrated for its potent immunogenicity in humans.^{29,48} Now that the immunogenicity of the other GAAs have been shown, we may not include the gp100 epitope in future designs due to its low expression levels in primary gliomas.

We still have to elucidate the mechanisms underlying the observed divergence in tetramer versus ELISPOT responses. This may be because ELISPOT assays were performed following 1 week restimulation of PBMC whereas tetramer assays were performed without restimulation. Two clinical responders (patients 1 and 20) demonstrated tetramer but not ELISPOT responses. Although we cannot make conclusive comments with only two patients as to whether tetramer responses would better correlate with clinical success than ELISPOT, we are currently developing multicolor flow-cytometric analyses for evaluation of polyfunctional tetramer-reactive T cells (eg, tetramer-positive cells that also express multiple effector molecules, such as *IFN- γ* , *TNF- α* , and *IL-2*).⁴⁹ These new technologies may lead us to better understand critical immune response indicators that may help us to predict clinical responses.

Although analyses of sentinel lymph nodes have reported higher positive rates and magnitudes of vaccine-induced responses than PBMCs,^{50,51} we focused our analyses on PBMCs to avoid invasive lymph node biopsy and to save the central site of vaccine-induced responses. Although our data did not clearly demonstrate correlations between GAA-specific responses and clinical outcomes, this may be because α DC1 vaccines promote the T-cell expression of receptors against tumor-associated chemokines,^{11,15,52} thereby facilitating their translocation from the circulation to tissues.

Our RT-PCR and serum analyses indicate upregulation of some type-1 cytokines and chemokines by the current regimen. This observation is intriguing, given that our preclinical studies have demonstrated critical contributions of these molecules to the efficacy of glioma vaccines.^{10-12,20,38} Further studies are warranted to determine if one or more of these molecules can serve as biomarkers of response in patients receiving glioma vaccines.

The choice of DC administration route may critically impact the efficacy of vaccines. We employed ultrasound-guided IN administration based on previous studies showing feasibility⁵³ and superior T-cell response induction⁵⁴ of the IN route of DC administration compared with intravenous and intradermal routes. In our study, IN administration was feasible and well-tolerated, warranting further development with this approach.

The postvaccine biopsy on patient 1 after the increase of Gd-enhancing signals revealed remarkable immune cell infiltration, but not mitotically active tumor cells, suggesting that the condition was pseudotumor progression. This implies that MRI-based evaluation of TTP requires particularly careful interpretation of imaging data and, potentially, pathologic confirmation of true versus pseudotumor progression.

Although the relatively high number of patients with AG probably contributed to the high rate of 12-month progression-free survival, our preliminary data on TTP in patients with GBM are not inferior to the results of studies that provided the basis for recent US Food and Drug Administration approval of the use of bevacizumab in these patients.⁵⁵⁻⁵⁷ These data, particularly the complete response in a patient with GBM, support larger studies of α DC1-based vaccines loaded with novel GAA epitopes.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about

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