

Phase I/II trial testing safety and immunogenicity of the multi-peptide IMA950/poly-ICLC vaccine in newly diagnosed adult malignant astrocytoma patients

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Abstract

Background. Peptide vaccines offer the opportunity to elicit glioma-specific T cells with tumor killing ability. Using antigens eluted from the surface of glioblastoma samples, we designed a phase I/II study to test safety and immunogenicity of the IMA950 multi-peptide vaccine adjuvanted with poly-ICLC (polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose) in human leukocyte antigen A2+ glioma patients.

Methods. Adult patients with newly diagnosed glioblastoma ($n = 16$) and grade III astrocytoma ($n = 3$) were treated with radiochemotherapy followed by IMA950/poly-ICLC vaccination. The first 6 patients received IMA950 (9 major histocompatibility complex [MHC] class I and 2 MHC class II peptides) intradermally and poly-ICLC intramuscularly (i.m.). After protocol amendment, IMA950 and poly-ICLC were mixed and injected subcutaneously ($n = 7$) or i.m. ($n = 6$). Primary endpoints were safety and immunogenicity. Secondary endpoints were overall survival, progression-free survival at 6 and 9 months, and vaccine-specific peripheral cluster of differentiation (CD)4 and CD8 T-cell responses.

Results. The IMA950/poly-ICLC vaccine was safe and well tolerated. Four patients presented cerebral edema with rapid recovery. For the first 6 patients, vaccine-induced CD8 T-cell responses were restricted to a single peptide and CD4 responses were absent. After optimization of vaccine formulation, we observed multi-peptide CD8 and sustained T helper 1 CD4 T-cell responses. For the entire cohort, CD8 T-cell responses to a single or multiple peptides were observed in 63.2% and 36.8% of patients, respectively. Median overall survival was 19 months for glioblastoma patients.

Conclusion. We provide, in a clinical trial, using cell surface-presented antigens, insights into optimization of vaccines generating effector T cells for glioma patients.

Trial registration: Clinicaltrials.gov NCT01920191.

Key Points

1. The IMA950 glioma vaccine combined with poly-ICLC is safe and immunogenic.
2. Antitumor T-cell responses are improved by mixing peptides and poly-ICLC.

Importance of the Study

Uniquely, the MHC class I restricted peptides of the IMA950 vaccine were identified as being presented at the glioma cell surface in vivo. This property ensures that the elicited T cells are able to react with tumor cells in vivo. In addition, the IMA950 vaccine uses both MHC class I and II peptides as immunogens.

Generation of an integrated T-cell response should be the aim of future trials, potentially through the identification of multiple tumor-eluted MHC class II peptides. Finally, we highlight, in a clinical study, that modalities of injection of vaccine and adjuvant can profoundly influence immunogenicity.

Malignant (World Health Organization [WHO] grades III and IV) gliomas are among the most aggressive solid tumors in adults and the overall survival (OS) of grade IV glioma (glioblastoma [GBM]) patients treated with surgery, chemotherapy with temozolomide (TMZ), and radiotherapy followed by adjuvant TMZ is 14.6 to 16 months.^{1,2} New treatments are therefore urgently needed. Immunotherapeutic strategies are now being tested across many malignancies, with some striking results when using immune checkpoint inhibitors.³⁻⁵ For malignant glioma, however, efficacy of checkpoint antibodies alone appears to be limited,⁶ potentially due in part to the low mutation burden found in these tumors,⁷ which results in induction of few tumor neoantigen-specific immune responses.

Yet, induction of tumor-specific immune responses can be achieved by therapeutic vaccination with tumor-derived peptides and has been tested with promising results in glioma.⁸⁻¹⁰ With this aim, we recently identified a set of human leukocyte antigen (HLA)-A2 restricted peptides directly eluted from the surface of tumor samples from GBM patients, which were formulated in the IMA950 vaccine.¹¹ These antigens are overexpressed in the majority of patients with GBM, with little or no expression in healthy tissues, and are immunogenic in vitro.¹¹ In addition, and unique to glioma antigens, we showed that they are presented at the peptide level on GBM samples in vivo, ensuring presence of the target for vaccine-elicited T cells.¹¹ Altogether, these antigens provide the opportunity to elicit T-cell responses with broad specificity against GBM cells, limiting the risks of both tumor immune escape and collateral damage to the brain.

The IMA950 vaccine is composed of 9 HLA-A2 restricted cluster of differentiation (CD)8 T-cell epitopes derived from the brevican (BCAN), chondroitin sulfate proteoglycan 4 (CSPG4), fatty acid binding protein 7 (FABP7), insulin like growth factor 2 mRNA binding protein 3 (IGF2BP3), neuronal cell adhesion molecule (NRCAM), neuroligin 4 X-linked (NLGN4X), protein tyrosine phosphatase, receptor type Z1 (PTPRZ1), and tenascin C (TNC) proteins as well as of 2 HLA DR- binding peptides derived from the c-met and survivin proteins, providing CD4 T-cell help. The latter were not eluted from the surface of GBM samples,¹²⁻¹⁴ but

have been shown to be immunogenic in vaccine trials.^{15,16} In addition, IMA950 contains an HLA-A2 restricted peptide derived from the hepatitis B virus (HBV) core antigen included as a marker of immunization efficacy.

The IMA950 vaccine was investigated in a clinical trial (Cancer Research UK IMA950-101, NCT01222221) using granulocyte-macrophage colony-stimulating factor (GM-CSF) as adjuvant, starting vaccination either before or after concomitant radiochemotherapy, and was found to be safe and immunogenic.¹⁵ In the present study, we replicated one of the arms of the IMA950-101 study, starting vaccination after concomitant radiochemotherapy but using a different adjuvant: polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC; Hiltonol). Poly-ICLC has been shown to enhance the efficacy of vaccination in a mouse model of glioma^{17,18} and to be well tolerated in patients with grade III or IV glioma,^{19,20} and trials of peptide vaccination in combination with poly-ICLC in adult and pediatric glioma patients showed induction of strong CD8 T-cell responses.^{8-10,21} Here, we show that combining IMA950 and poly-ICLC is safe. Practically, we also provide evidence that both vaccine compounds should be mixed before administration to get multi-peptide CD8 T-cell and sustained T helper (Th1) CD4 T-cell responses. These results are paving the way for combination with immune checkpoint inhibitors for potential clinical benefit.

Materials and Methods

Additional information is given in the Supplementary material online on study design, endpoints, analysis of immune responses in peripheral blood mononuclear cells (PBMCs), tumor-infiltrating and skin biopsy-derived lymphocytes, immunohistochemistry (IHC), assessment of clinical response, and statistical analysis.

Study Design

Between 2013 and 2017, we conducted a monocentric, single-arm, open-label phase I/II study. This study was

conducted in accordance with the 1975 Declaration of Helsinki. The trial (NCT01920191) was conducted under the control and monitoring of the Swiss regulatory authorities (Swissmedic), as well as of the local institutional review board and ethics committee supervision. Signed informed consent was obtained for HLA screening and inclusion in the study protocol. Patients were eligible if over 18 years old with an HLA-A2 positive status and pathologically confirmed newly diagnosed GBM (a minimum of 16 GBM cases was required and up to 5 additional WHO grade III glioma patients could be enrolled). Patients who underwent biopsy only were allowed. Adequate organ function, minimum absolute lymphocyte count at $1 \times 10^9/L$ prior to radiotherapy, and WHO performance status <2 were required. The maximal allowed dose of dexamethasone was 4 mg per day. Seropositivity for the hepatitis B core antigen was an exclusion criterion. After surgery, all patients received standard therapy.¹ The first 6 patients received intradermal (i.d.) injections of 4.96 mg of IMA950 (413 µg/peptide; Immatic Biotechnologies) and concomitant intramuscular (i.m.) injections of 1.5 mg of poly-ICLC (Hiltonol, Oncovir) at each vaccination. The protocol was amended for the subsequent 13 patients. The latter received either subcutaneous (s.c.) ($n = 7$) or i.m. ($n = 6$) injections of IMA950 (4.96 mg total, 413 µg of each peptide) and poly-ICLC (1.5 mg) mixed together and injected at a single site (thigh). Vaccinations at days 2 and 3 were omitted (Fig. 1).

Primary and Secondary Endpoints

Primary endpoints were safety and immunogenicity. Safety was monitored, and dose limiting toxicity was defined using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Immunogenicity was defined as $>60\%$ of patients

displaying a CD8 T-cell response to a single peptide and $>30\%$ of patients displaying a CD8 T-cell response to more than one peptide. Secondary endpoints were progression-free survival (PFS) at 6 and 9 months, OS, and additional immunological endpoints.

Radiological and Clinical Assessment of Response

Tumor response was assessed according to the Response Assessment in Neuro-Oncology criteria but including the possibility to continue IMA950 vaccination in case of pseudoprogression.

Results

Demographics and Clinical Characteristics

Twenty-seven patients were screened from August 2013 to March 2016 and 19 patients were included, 16 with GBM and 3 with grade III astrocytoma. The main reasons of non-eligibility were HLA-A2 negative status, frail postoperative condition, or blood/organ function values outside normal ranges. The high percentage of HLA-A2+ patients (the percentage is 25–35% in the general Swiss population²²) among the screened patients originates from the fact that glioma patients seen for diagnosis purposes in Geneva are routinely screened for HLA-A2, within the context of a basic research program. A fraction of patients potentially eligible for our study but with known non-HLA-A2 status were not screened. Clinical characteristics are summarized in Table 1. It is important to note that most of the patients suffered from primary GBM (2 isocitrate dehydrogenase 1 [*IDH1*] mutated tumors only), with 4 methylated

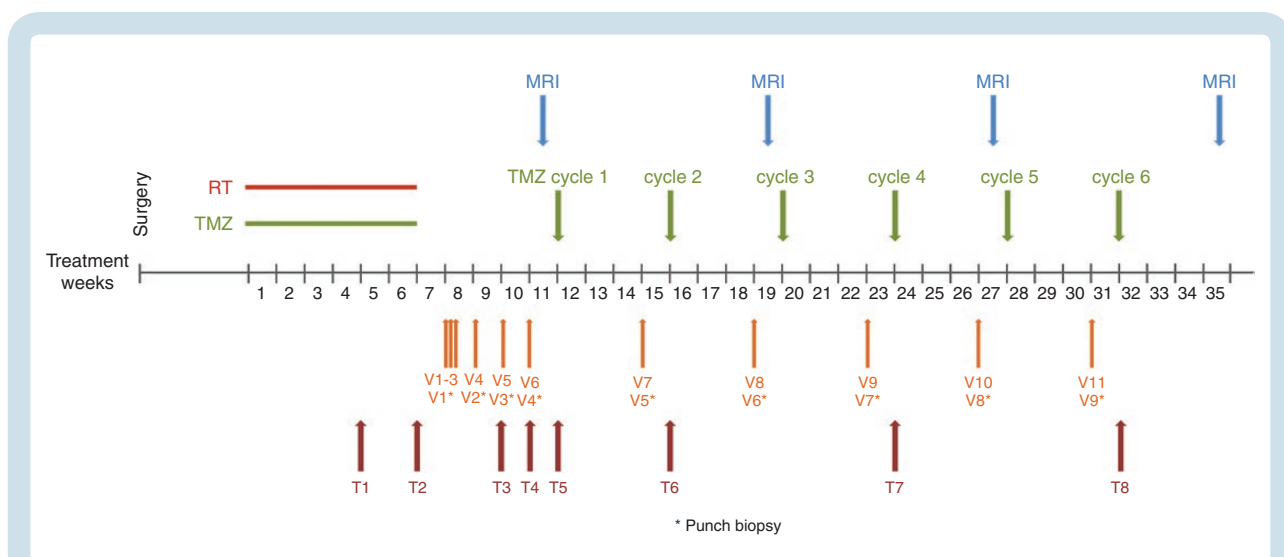


Fig. 1 Study protocol. Patients were screened between surgery and end of radiochemotherapy. Treatment weeks are defined starting from the initiation of radiochemotherapy. Vaccinations were administered over a period of 24 weeks at least 1 week after the end of radiochemotherapy. V: vaccinations in the initial study protocol; V*: vaccinations in the modified study protocol. T1 to T8 represent blood samples collected for immunomonitoring. A punch biopsy was taken 48 h after delayed type hypersensitivity. RT, radiotherapy.

Table 1 Patient demographics and characteristics

Patient	Age	Sex	Histology	Localization	RTV (cm ³)	MGMT Status	IDH1/2 Status	Mutation Analysis (NGS)	Vaccine Schedule	Best Response OS, mo	Pseudoprogression	Cerebral Edema
1	62	F	GBM	Right parietal	na ^a	Unmeth	wt	nd	i.d./i.m. ^b	PD	No	Yes
2	50	M	GBM	Right temporal	0	Unmeth	wt	nd	i.d./i.m.	PD	No	Yes
3	57	F	GBM	Right frontal	0 ^c	Unmeth	wt	nd	i.d./i.m.	PD	No	No
4	55	M	GBM	Left fronto-insular	15.4	Unmeth	wt	nd	i.d./i.m.	SD	No	Yes
5	67	M	GBM	Left fronto-insular	0.16	Unmeth	wt	nd	i.d./i.m.	PD	No	No
6	56	M	GBM	Left temporal	0	Meth	wt	nd	i.d./i.m.	SD	No	No
7	23	F	AA	Right fronto-temporo-insular	3.5	Unmeth	mut	nd	s.c.	SD	No	No
8	65	M	GBM	Left parieto-occipital	0.19	Meth	wt	nd	i.m.	PR	Yes	No
9	61	M	GBM	Left frontal	17.1	Meth	mut	nd	s.c.	CR	No	No
10	43	F	AA	Right temporal	0.26	Unmeth	wt	nd	i.m.	PD	No	No
11	68	M	GBM	Left parietal	0	Unmeth	wt	None	s.c.	PD	No	Yes
12	73	M	AA	Left frontal	0	Unmeth	wt	None	i.m.	SD	No	No
13	44	M	GBM	Right parieto-occipital	0.86	Unmeth	wt	None	s.c.	PD	No	No
14	68	F	GBM	Left temporal	0.61	Meth	wt	MET + SMARCB1 mut	i.m.	SD	No	No
15	63	M	GBM	Left precentral frontal	1.85	Unmeth	wt	NOTCH1 del	i.m.	PR	No	No
16	41	M	GBM	Left temporal	11.8	Unmeth	wt	None	s.c.	PD	Yes	No
17	60	M	GBM	Left fronto-temporal	0	Unmeth	wt	None	s.c.	PD	Yes	No
18	73	M	GBM	Left frontal	3.67	Unmeth	wt	none	i.m.	PD	Yes	No
19	41	M	GBM	Right parieto-temporal	0	Unmeth	wt	PTEN mut	s.c.	PD	No	No

AA: anaplastic astrocytoma; CR: complete response; GBM: glioblastoma multiforme; IDH1/2: isocitrate dehydrogenase 1/2; Meth: methylated; MGMT: 06-methylguanine-DNA-methyltransferase; nd: not done; NGS: next generation sequencing; PD: progressive disease; PTEN: phosphatase and tensin homolog; RTV: residual tumor volume; SD: stable disease; Unmeth: unmethylated; wt: wild type. a: this patient underwent biopsy only and no MRI scan was performed post-surgery. b: i.d. refers to peptide injection, i.m. to poly-ICLC injection. In the first 6 patients, peptides and adjuvant were injected separately. c: unquantifiable residue in the millimeter range.

Table 2 Adverse events

Symptoms	CTCAE Grade				Imputability	Total Number of Patients (%)
	1	2	3	4		
Cerebral edema	—	—	—	4	Vaccine/adjuvant (1)/progressive disease (3)	4 (22)
Injection site reaction	6	3	1	0	Vaccine/adjuvant	10 (53)
Flu-like syndrome	4	0	0	0	Vaccine/adjuvant	4 (21)
Lymphopenia	5	9	1	0	TMZ	15 (79)
Thrombopenia	4	0	1	1	TMZ	6 (32)
Nausea/vomiting	2	2	1	0	TMZ	5 (26)
Mucositis	4	0	0	0	TMZ /radiation	4 (21)
Interstitial pneumonia	0	0	0	1	TMZ/radiation/dexamethasone	1 (5)
Alopecia	0	19	0	0	Radiation	19 (100)
Seizure	6	2	1	0	Tumor	9 (47)
Neurological defect	3	0	3	0	Tumor	6 (32)
Headache	6	1	0	0	Vaccine/adjuvant/radiation	7 (37)
Fatigue	8	4	0	0	Vaccine/adjuvant/radiation	12 (63)

O⁶-methylguanine-DNA methyltransferase (*MGMT*) tumors. According to the updated Radiation Therapy Oncology Group recursive partitioning analysis classification, the predicted survival for the 16 GBM patients with these characteristics is less than 15 months.²³ Six patients were included in the initial study protocol and 13 in the modified protocol (Fig. 1). Patients received a median of 9 vaccinations (range, 4 to 11).

Safety Data

Overall the treatment was well tolerated, with most symptoms and side effects imputable to irradiation and TMZ or to the tumor itself (Table 2). We observed frequent (53%) inflammatory reactions at injection sites, mostly CTCAE grades 1 and 2, which were not associated with longer OS as observed previously.¹⁵ Some patients reported headache (37%), fatigue (63%), and flu-like syndrome (21%) that lasted generally 48 hours after each vaccination. These were not different in patients vaccinated with the initial protocol or the s.c. or i.m. arm of the modified protocol. No signs of autoimmunity were observed in any patient. Nine cases (47%) of seizures were observed, among which 6 (31%) were brief partial seizures unrelated to the vaccine. One case of grade 4 interstitial pneumonia due to pneumocystic infection was diagnosed with eventual favorable outcome. Pseudoprogression was observed in 4 (22%) patients, most frequently after the fourth vaccination (Table 1). One patient in particular presented with worsening of the heterogeneous contrast-enhanced lesion without clinical symptoms and eventual regression with complete response (Patient 8, Fig. 2A). Vaccinations were neither postponed nor interrupted. The etiology of this reaction remains unclear; TMZ, irradiation, or immunotherapy potentially played a role. Four patients (22%) presented with severe edema, 3 of them consecutive to disease progression (Patients 1, 2, and 11) and 1 possibly related to the vaccine (Patient 4, Table 1). The most intense reaction

is illustrated in Fig. 2B. This patient (Patient 2) presented with somnolence, headache, and dizziness arising immediately after the fourth vaccination, with a strong T2/fluid attenuated inversion recovery (FLAIR) edema surrounding a small contrast-enhanced lesion. He was treated with high-dose steroids with progressive tapering over 10 days. Symptoms rapidly resolved and control MRI showed decrease of the edema with minimal tumor progression (Fig. 2B). In general, there was no association between residual tumor volume and extent of cerebral edema, nor was occurrence of edema increased upon modification of the vaccine formulation.

Induction of Vaccine Peptide-Specific CD4 and CD8 T-Cell Responses

All patients were available for immunologic analysis. In the first 6 patients, in which the peptides were given i.d. and poly-ICLC i.m. in close vicinity, no peptide-specific CD4 T-cell responses were detected at any timepoint in any patient (Fig. 3A). In addition, CD8 T-cell response to the HBV peptide was induced in 1 patient only (Fig. 3A and Supplementary Figure 1A), whereas recall responses to cytomegalovirus and/or Epstein-Barr virus were readily detectable in all but 1 patient (Supplementary Figure 1B), demonstrating the overall response capacity of the patients' PBMC and suggesting that the vaccination formulation was suboptimal. At that point, we decided to modify vaccine formulation and amended the protocol to include the following changes that were accepted by the local and national authorities: (i) mixing of peptides and adjuvant before injection, (ii) omission of vaccinations V2 and V3, and (iii) injection at a single site. Since the optimal route of injection could not be judged from the existing literature, we took the opportunity to compare s.c. (7 patients) and i.m. (6 patients) injections.

Protocol modification resulted in striking improvement in vaccination efficiency. Firstly, HBV-specific CD8

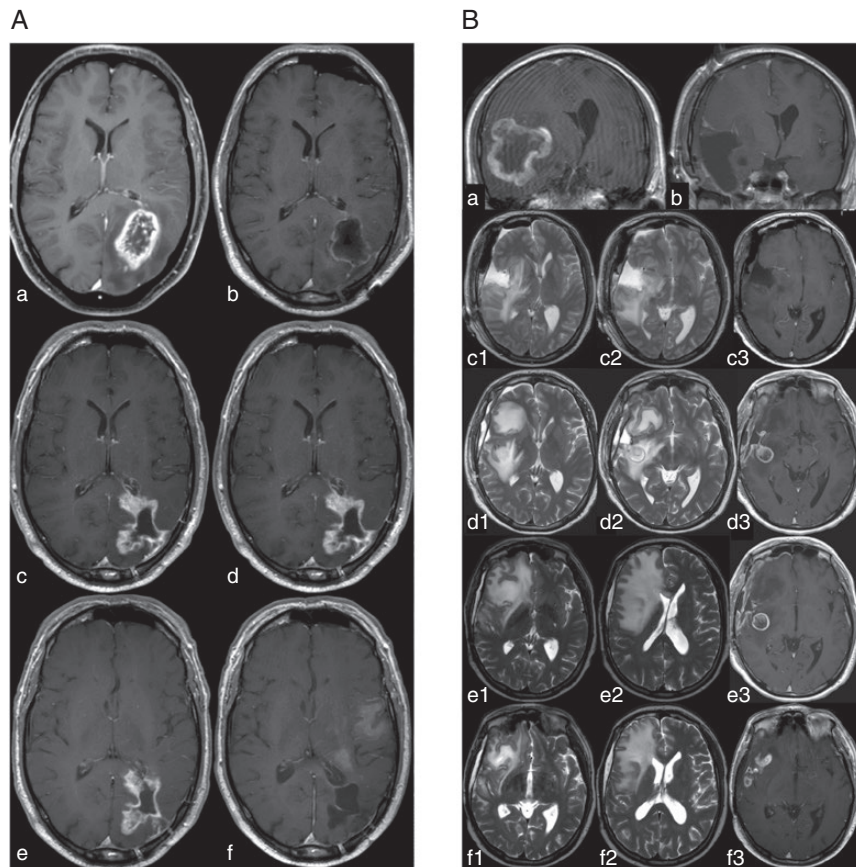


Fig. 2 Illustrative local injection site reactions and MR response patterns. (A) Axial plane post gadolinium T1-weighted imaging MRI shows impressive radiological pseudoprogression after vaccination number 4 in Patient 8, with eventual complete local response. a: Jun 2014: left parieto-occipital lesion before initial gross total resection, b: immediate post-surgical MRI shows hemorrhagic changes with methemoglobin rim surrounding the resection cavity, c: Oct 2014: local relapse after V4 toward the left ventricle trigone, d: Dec 2014: stable disease, e: Feb 2015: partial response, f: Jun 2017: long term follow-up imaging shows relapse toward left insula and internal capsule but local complete response at the original surgical bed. (B) Illustrative example of peritumoral edema after 4 immunizations in Patient 2. Jun 2013: coronal T1 post-contrast images show right temporal lesion before (a) and after (b) surgical removal. Axial fast spin echo (FSE) T2 (c1–c2, 2 different anatomical levels) and T1 (c3) images post-surgery. Oct. 2013: d1–d2 FSE T2 images denote prominent edema after vaccination number 4, with minimal tumor progression on T1 post-contrast image (d3). Nov 2013: e1–e3 show the same pattern of vaccine-induced edema after vaccination number 6. Jan 2014: f1–f3: right temporal heterogeneously enhancing multifocal tumor relapse.

T-cell responses were detected in all but 1 patient (92.3%, $P = 0.002$, Fisher's exact test compared with initial vaccination protocol; Fig. 3A). Secondly, whereas the percentage of patients displaying tumor peptide-specific CD8 T-cell response was moderately improved (69.2% vs 50.0% in the initial protocol), the percentage of patients with CD8 T-cell response to multiple tumor peptide almost tripled (46.2% vs 16.7%; Fig. 3B). Overall, the primary endpoint of vaccine immunogenicity was reached, as the percentage of patients in the whole cohort displaying CD8 T-cell responses to one or multiple tumor peptides was 63.2% and 36.8%, respectively. In the GBM only cohort ($n = 16$), 62.5% and 31.3% of patients displayed CD8 T-cell responses to one or multiple tumor peptides, respectively. Patients mounted CD8 T-cell responses to a maximum of 3 tumor peptides, the majority of responses being directed against the BCAN₄₇₈₋₄₈₆, NLGN4X₁₃₁₋₁₃₉, and PTPRZ1₁₃₄₇₋₁₃₅₅ peptides (Fig. 3C and Supplementary Figures 1C and 2). Finally,

tumor peptide-specific CD4 T-cell responses were detected in all but 2 patients (84.6%, $P = 0.001$, Fisher's exact test compared with initial vaccination protocol; Fig. 3A), with detection of responses to both peptides in 53% of patients (Fig. 3D and Supplementary Figure 3). Even from the first vaccination timepoint, we were able to detect production of tumor necrosis factor alpha (TNF- α), interferon gamma (IFN- γ), and interleukin (IL)-2, and cytokine production was maintained over the vaccination period for both peptides (Fig. 3E and Supplementary Figure 4A–B). In the majority of patients, we observed production of type 1 cytokines only, without production of IL-4, IL-5, or IL-17. In 2 patients, however, IL-17-producing cells were detected, accompanied by IL-5 production in 1 patient (data not shown). Finally, we were not able to determine an optimal route of vaccination with regard to elicitation of both CD4 and CD8 T-cell responses, since higher multi-peptide CD8 T-cell responses were observed in the i.m. group, and higher multi-peptide

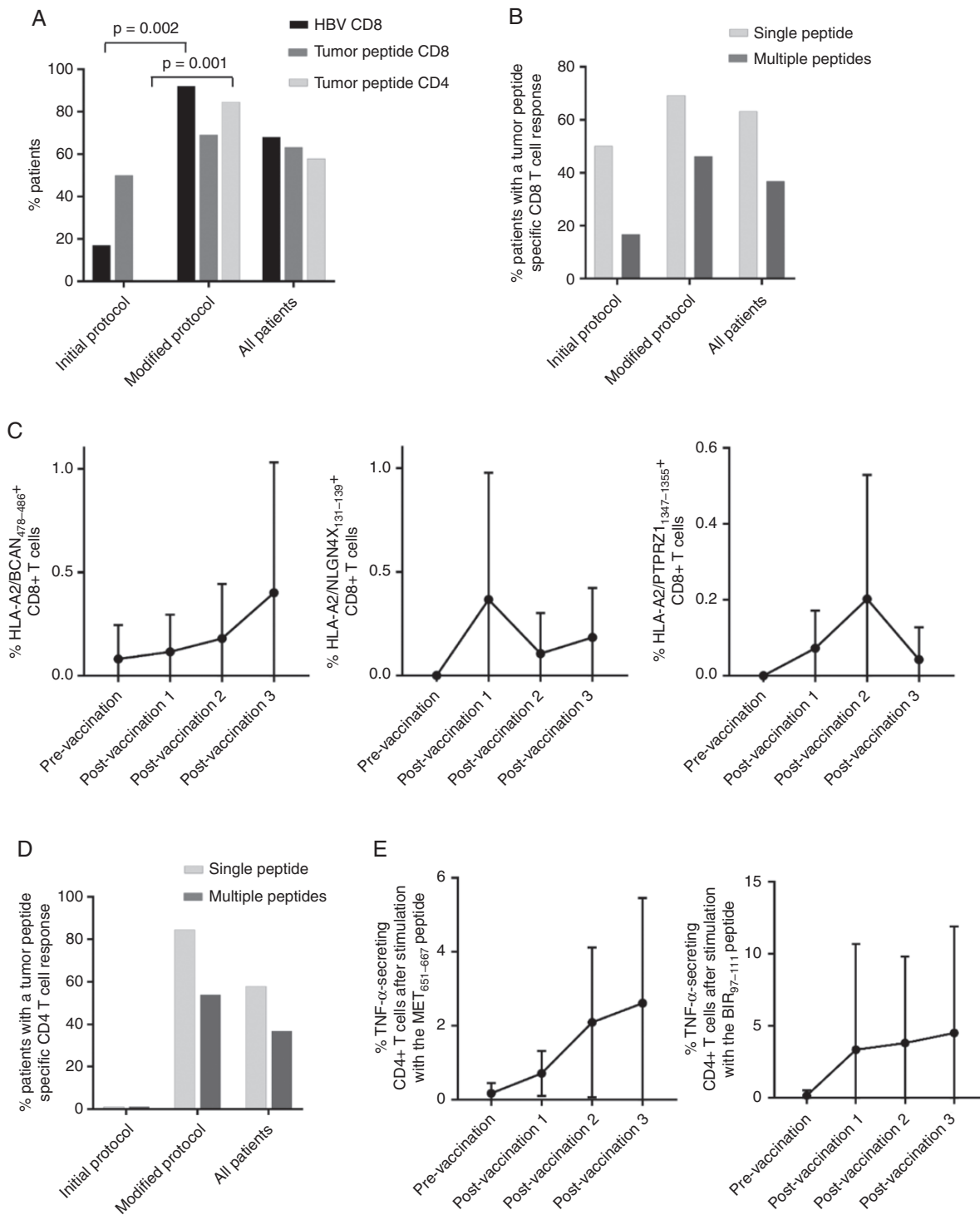


Fig. 3 Vaccine-induced CD8 and CD4 T-cell responses before and after protocol amendment. (A) Percentage of patients with an HBV-, tumor antigen-specific CD8 or CD4 T-cell response in the initial ($n = 6$)/modified protocol ($n = 13$) and for all patients. (B) Percentage of patients with a tumor antigen-specific CD8 T-cell response to one or multiple peptides in the initial ($n = 6$)/modified protocol ($n = 13$) and for all patients. (C) Mean \pm SD of the percentage of HLA-A2/peptide multimer+ CD8+ T cells specific for the BCAN₄₇₈₋₄₈₆, NLGN4X₁₃₁₋₁₃₉, and PTPRZ1₁₃₄₇₋₁₃₅₅ antigens in the pre-vaccination (T1 + T2), post-vaccination 1 (T3 + T4), 2 (T5 + T6), and 3 (T7 + T8) timepoints. (D) Percentage of patients with a tumor antigen-specific CD4 T-cell response to one or multiple peptides in the initial ($n = 6$)/modified protocol ($n = 13$) and for all patients. (E) Mean \pm SD of the percentage of TNF- α -secreting CD4+ T cells specific for the MET₆₅₁₋₆₆₇ and BIRC5₉₇₋₁₁₁ antigens in the pre-vaccination, post-vaccination 1, 2, and 3 timepoints.

CD4 T-cell responses were observed in the s.c. group (Supplementary Figure 4C). Steroids were used after the start of vaccination in 7 patients. No correlation between steroid intake and elicitation of vaccine-specific CD4 and/or CD8 T-cell responses was found. There was additionally no correlation between the presence, breadth, or magnitude of CD4 or CD8 T-cell responses and presence of cerebral edema. For Patient 4, who had cerebral edema in absence of progressive disease, CD8 T-cell responses were detected at the time of edema manifestation. However, whether a causality link can be established would have required analysis of T cells at the tumor site, which was not available at the time of edema manifestation. Skin biopsy-derived T cells were obtained in 11 of the 19 patients and contained variable proportions of CD4 (range, 0.5–88%) and CD8 (range, 4–98%) T cells. We detected HBV-specific CD8 T cells in all but one sample, BIRC5₉₇₋₁₁₁ or/and MET₆₅₁₋₆₆₇ specific CD4 T cells in 7 of 9 samples, and NLGN4X₁₃₁₋₁₃₉ specific CD8 T cells in 1 sample (data not shown). Finally, we did not detect vaccine-specific T cells in 5 tumor samples analyzed after vaccine administration. In order to understand whether the absence of antigen-specific T cells in the tumor bed was due to inadequate homing, we tested expression of $\alpha 4\beta 1$ integrin (CD29/CD49d) and C-X-C chemokine receptor type 3 (CXCR3) molecules on vaccine-induced T cells in peripheral blood. The majority (91.8 ± 5.9%) of HBV-specific CD8 T cells detected ex vivo coexpressed CD29/CD49d (range, 83–98%), whereas CXCR3 was expressed by 47.4 ± 19.8 (range, 21–74%) of cells (Supplementary Figure 5A). In addition, the majority of tumor antigen-specific T-cell responses tested after in vitro amplification expressed the CD29/CD49d and CXCR3 molecules (Supplementary Figure 5B). Finally, the majority of BIRC5₉₇₋₁₁₁ specific CD4 T cells detected ex vivo coexpressed CD29/CD49d, whereas only a minor fraction of these expressed CXCR3 (Supplementary Figure 5C).

Expression of the IMA950 Antigens in Pre- and Post-Vaccination Tumor Samples

Pre-vaccination tumor samples from 18 patients were available for analysis of IMA950 antigen expression by IHC. The 8 proteins from which the IMA950 peptides derive were overexpressed in patients compared with nonmalignant brain samples (Supplementary Figure 6A). All antigens were expressed in >70% of pre-vaccination samples except for FABP7 (55% of samples; Supplementary Figure 6B). Except for Patient 10, all samples expressed a minimum of 4 antigens (mean ± SD: 5.9 ± 1.4; Supplementary Figure 6C). Seven patients underwent a second surgery upon progression and were available for IHC analysis. There were no major changes in antigen expression in recurrent samples compared with pre-vaccination samples (Supplementary Figure 6B). We did not observe correlation between tumor antigen expression and antigen-specific CD8 T-cell responses.

Clinical Outcome

The disease control rate was 42% in the whole cohort ($n = 19$), and 31.2% in the GBM cohort ($n = 16$). Median OS from surgery was 21 months (range, 10–45 mo; 95% CI:

19.50–29.23) for the whole cohort and 19 months (range, 10–45 mo; 95% CI: 17.25–27.87) for GBM patients (Fig. 4A). When calculated from the date of study entry, median OS was 19 and 17 months for the whole cohort and for GBM patients only, respectively. PFS was 84% and 63% at 6 and 9 months, respectively, for the whole cohort and 81% and 63% for GBM patients only. When calculated from study entry, PFS was 68% and 58% at 6 and 9 months, respectively, for the whole cohort and 69% and 56% for GBM patients only. The median PFS from surgery was 10 and 9.5 months for the overall cohort and GBM patients, respectively (Fig. 4B), and 9 and 9 months for the whole cohort and for GBM patients only, respectively, if calculated from the date of study entry. There was no difference between the OS of patients vaccinated with the initial ($n = 6$) or modified ($n = 13$) formulation (Fig. 4C) or for patients included in the s.c ($n = 7$) or i.m. ($n = 6$) arm of the modified protocol (Fig. 4D). Similarly, no differences in PFS were observed between patients vaccinated with the different vaccine injection modalities (not shown).

Discussion

This is the first study evaluating the combination of the IMA950 vaccine and poly-ICLC in patients with malignant astrocytoma. The originality of this peptide set is the validated peptide presentation on GBM samples, ensuring presence of the target for potential T-cell recognition and killing.¹¹ Our findings demonstrate feasibility, tolerability, and immunogenicity of this combination in this bad prognosis patient population (elderly patients, predominant wild-type *IDH1/2*, and unmethylated *MGMT*). It is worth noting that 2 of the 3 anaplastic gliomas were *IDH1* wild-type, a feature that is associated with worst patient survival compared with *IDH1/2* mutated grade III astrocytoma patients.²⁴ Both endpoints of safety and immunogenicity were reached.

Within the total cohort, the IMA950/poly-ICLC vaccine was immunogenic, with 63.2% of patients displaying a tumor peptide-specific CD8 T-cell response and 36.8% of patients displaying multi-peptide CD8 T-cell responses, whereas 57.9% and 36.8% of patients displayed CD4 T-cell responses specific for one or two tumor peptides, respectively. Strikingly, we observed a remarkable difference in immunogenicity between the first and second vaccine formulations. With the first vaccine formulation ($n = 6$), the absence of tumor peptide-specific CD4 T-cell responses and the detection of HBV-specific T cells in one patient only spoke for vaccine inefficacy, as similar studies using this marker peptide revealed positive responses in 50–60% of patients.^{15,16} The rationale for mixing peptides and adjuvant in the modified protocol was to favor presentation of the peptides and uptake of adjuvant by the same dendritic cell.^{25,26} Reducing the number of vaccinations in the induction phase was decided in order to prevent potential activation-induced cell death associated with repetitive stimulation of the same T cells. The resulting weekly vaccination schedule was thought to limit the risk of severe side effects at the site of injection^{8,9} and to allow injecting at a single site throughout treatment.

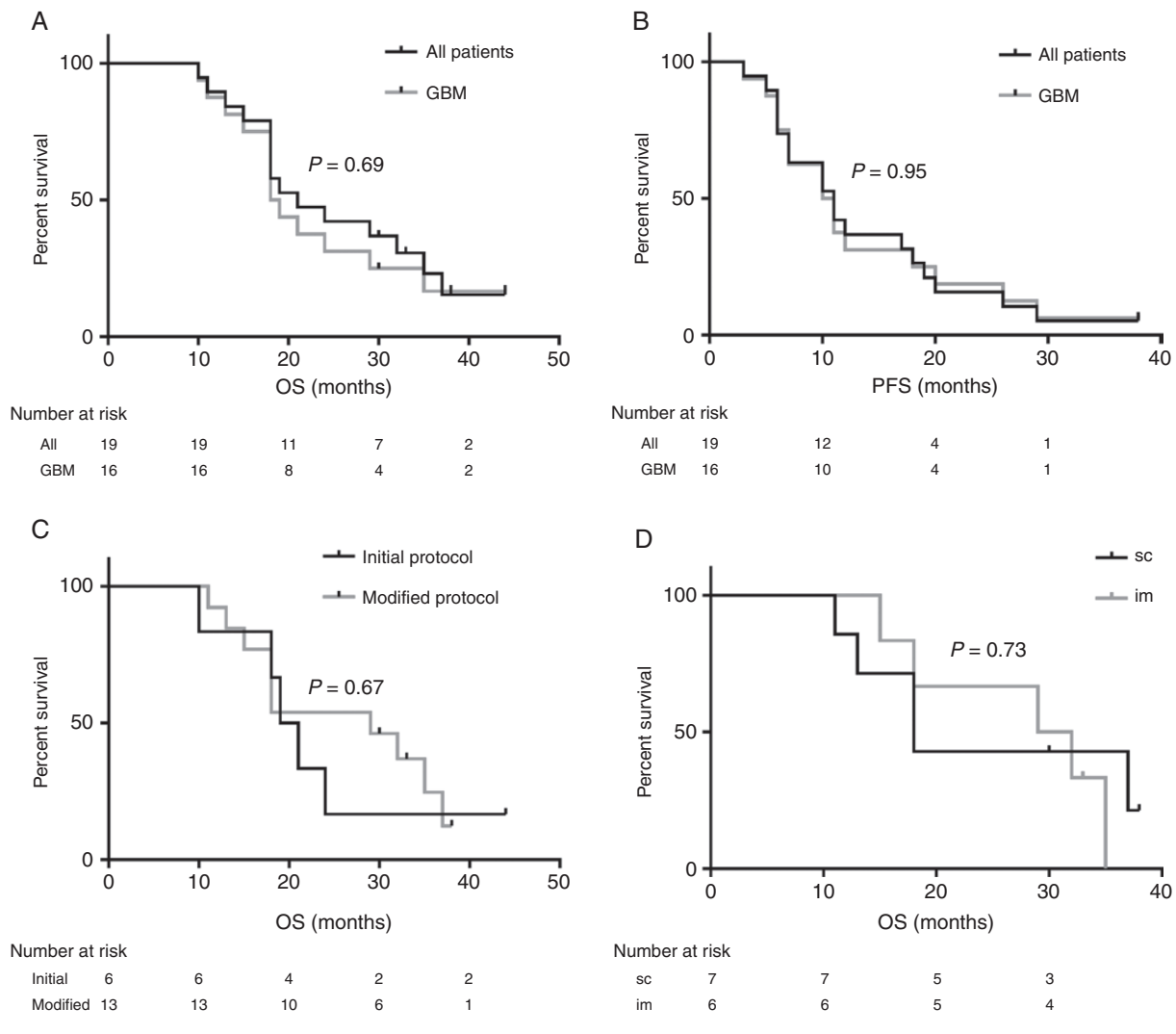


Fig. 4 Patient survival. (A) Percentage surviving patients in the whole ($n = 19$) and GBM-only cohort ($n = 16$). (B) Percentage of patients with PFS in the whole ($n = 19$) and GBM-only cohort ($n = 16$). (C) Percentage surviving patients vaccinated with the initial ($n = 6$) or modified ($n = 13$) protocol. (D) Percentage surviving patients vaccinated in the s.c. ($n = 7$) or i.m. ($n = 6$) arm of the modified protocol. Ticks denote censored patients (5 living patients).

Protocol modification resulted in remarkable increase in immunogenicity, with appearance of HBV-specific CD8 T cells and tumor peptide-specific CD4 T-cell responses in most patients (92.3% and 84.6%, respectively) as well as tripling of CD8T-cell responses to multiple tumor peptides (46.2% patients). Whereas we cannot definitively determine which change in the modified vaccine was the most important, mixing peptide and adjuvant likely played a significant role. By modifying the vaccine formulation in the course of a single clinical trial, immunization efficiency was dramatically improved, paving the way for the design of future clinical trials.

Immunomonitoring of tumor peptide-specific CD8 T-cell responses was performed using protocols similar to the ones used in the IMA950/GM-CSF trial,¹⁵ in an effort to assess the impact of the different adjuvants on the generation of immune responses. Nevertheless, comparison

of the 2 trials has to be made with caution, considering the limited number of GBM patients included in the current trial and the various vaccine injection modalities used. Although the rate of CD8 T-cell responses to single peptides observed in the current trial did not reach that of the IMA950/GM-CSF trial, the rate of multi-epitopic CD8 responses was similar. More importantly, poly-ICLC was able to induce Th1 CD4 T-cell responses mostly sustained during the vaccination period (CD4 T-cell responses were not reported in the IMA950/GM-CSF study). The median OS of patients in the IMA950/poly-ICLC trial was 19 months for patients with GBM, compared with 15.3 months for patients of the IMA950/GM-CSF trial,¹⁵ despite a lower percentage of patients with *MGMT* methylated tumors (15 vs 29). In addition, PFS rates at 6 and 9 months were 93% and 56% in the current trial compared with 74% and 31% in the IMA950/GM-CSF trial. Generation of sustained Th1

CD4 T-cell responses together with multi-peptide CD8 T-cell responses using poly-ICLC as adjuvant might therefore be important for patient outcome.

Patient 4 presented an important and rapidly occurring edema after the first set of vaccinations. This peculiar edema pattern and the time course observed after the start of vaccinations suggest a possible effect of the IMA950/poly-ICLC vaccine. In addition, impressive pseudo-progression was observed in 1 patient, associated with a prolonged survival (35 mo). However interpreting edema, T2 FLAIR, or contrast worsening after immunotherapy remains challenging. As a result, correlating survival to the occurrence of any radiological change is not yet relevant especially in this small cohort.

The observation that the IMA950 antigens are not lost upon disease progression suggests absence of immunoeediting. In line with this, we did not detect tumor-infiltrating vaccine-specific T cells, suggesting inefficient brain homing or retention at the tumor site despite expression of $\alpha 4\beta 1$. Nevertheless, CXCR3 expression was low, which may be a limiting factor. In this regard, whether combining IMA950 with blockade of programmed cell death 1 could promote brain homing will be investigated in an upcoming trial (NCT03665545). Even if the possible absence of immunoeediting is a concern, our results demonstrate that the IMA950 antigens are stable targets that do not fluctuate over time, an issue that was recently brought to light for the epidermal growth factor receptor variant III antigen.²⁷

Altogether, our results advocate for follow-up studies using this vaccine. For instance, IMA950/poly-ICLC is now being tested in patients with grade II glioma in combination with the activating anti-CD27 antibody (NCT02924038). In addition, we are currently preparing a clinical trial in patients with recurrent GBM testing IMA950/poly-ICLC alone or combined with pembrolizumab (NCT03665545). The design of the latter trial will allow functional and molecular analysis of T cells in pre- and post-vaccine tumor samples as well as characterization of tumor microenvironment modulation. In future trials, targeting concomitantly the tumor microenvironment will undoubtedly be required to allow efficient tumor-specific T-cell responses to occur.

Conclusions

The Human Vaccine Project²⁸ defined 3 objectives: identifying (i) targets, (ii) rules of immunogenicity, and (iii) vaccination strategies that generate sustained effector T-cell responses. Our study addressed these objectives and is able to provide the medical community with insights into vaccine formulation, administration, and adjuvant selection that might eventually enable antitumor vaccines to have effective impact on survival of patients suffering from GBM.

Supplementary Material

Supplementary data are available at *Neuro-Oncology* online.

Keywords

glioma | IMA950 | immune response | peptide vaccine | poly-ICLC

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References

1. Stupp R, Hegi ME, Mason WP, et al; European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups; National Cancer Institute of Canada Clinical Trials Group. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009;10(5):459–466.

2. Stupp R, Taillibert S, Kanner A, et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. *JAMA*. 2017;318(23):2306–2316.
3. Motzer RJ, Escudier B, McDermott DF, et al; CheckMate 025 Investigators. Nivolumab versus everolimus in advanced renal-cell carcinoma. *N Engl J Med*. 2015;373(19):1803–1813.
4. Garon EB, Rizvi NA, Hui R, et al; KEYNOTE-001 Investigators. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med*. 2015;372(21):2018–2028.
5. Robert C, Schachter J, Long GV, et al; KEYNOTE-006 investigators. Pembrolizumab versus ipilimumab in advanced melanoma. *N Engl J Med*. 2015;372(26):2521–2532.
6. Reardon DA, Omuro A, Brandes AA, et al. OS10.3 Randomized phase 3 study evaluating the efficacy and safety of nivolumab vs bevacizumab in patients with recurrent glioblastoma: checkmate 143. *Neuro Oncol*. 2017;19(suppl_3):iii21–iii21.
7. Alexandrov LB, Nik-Zainal S, Wedge DC, et al; Australian Pancreatic Cancer Genome Initiative; ICGC Breast Cancer Consortium; ICGC MML-Seq Consortium; ICGC PedBrain. Signatures of mutational processes in human cancer. *Nature*. 2013;500(7463):415–421.
8. Okada H, Butterfield LH, Hamilton RL, et al. Induction of robust type-1 CD8+ T-cell responses in WHO grade 2 low-grade glioma patients receiving peptide-based vaccines in combination with poly-ICLC. *Clin Cancer Res*. 2015;21(2):286–294.
9. Okada H, Kalinski P, Ueda R, et al. 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. *J Clin Oncol*. 2011;29(3):330–336.
10. Pollack IF, Jakacki RI, Butterfield LH, et al. Immune responses and outcome after vaccination with glioma-associated antigen peptides and poly-ICLC in a pilot study for pediatric recurrent low-grade gliomas. *Neuro Oncol*. 2016;18(8):1157–1168.
11. Dutoit V, Herold-Mende C, Hilf N, et al. Exploiting the glioblastoma peptidome to discover novel tumour-associated antigens for immunotherapy. *Brain*. 2012;135(Pt 4):1042–1054.
12. Widenmeyer M, Griesemann H, Stevanović S, et al. Promiscuous survivin peptide induces robust CD4+ T-cell responses in the majority of vaccinated cancer patients. *Int J Cancer*. 2012;131(1):140–149.
13. Schag K, Schmidt SM, Müller MR, et al. Identification of C-met oncogene as a broadly expressed tumor-associated antigen recognized by cytotoxic T-lymphocytes. *Clin Cancer Res*. 2004;10(11):3658–3666.
14. Li YQ, Li A, Glas M, et al. c-Met signaling induces a reprogramming network and supports the glioblastoma stem-like phenotype. *Proc Natl Acad Sci U S A*. 2011;108(24):9951–9956.
15. Rampling R, Peoples S, Mulholland PJ, et al. A cancer research UK first time in human phase I trial of IMA950 (Novel Multi-peptide Therapeutic Vaccine) in patients with newly diagnosed glioblastoma. *Clin Cancer Res*. 2016;22(19):4776–4785.
16. Walter S, Weinschenk T, Stenzl A, et al. Multi-peptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. *Nat Med*. 2012;18(8):1254–1261.
17. Zhu X, Fallert-Junecko BA, Fujita M, et al. Poly-ICLC promotes the infiltration of effector T cells into intracranial gliomas via induction of CXCL10 in IFN- α and IFN- γ dependent manners. *Cancer Immunol Immunother*. 2010;59(9):1401–1409.
18. Zhu X, Nishimura F, Sasaki K, et al. Toll like receptor-3 ligand poly-ICLC promotes the efficacy of peripheral vaccinations with tumor antigen-derived peptide epitopes in murine CNS tumor models. *J Transl Med*. 2007;5:10.
19. Butowski N, Chang SM, Junck L, et al. A phase II clinical trial of poly-ICLC with radiation for adult patients with newly diagnosed supratentorial glioblastoma: a North American Brain Tumor Consortium (NABTC01-05). *J Neurooncol*. 2009;91(2):175–182.
20. Butowski N, Lamborn KR, Lee BL, et al. A North American brain tumor consortium phase II study of poly-ICLC for adult patients with recurrent anaplastic gliomas. *J Neurooncol*. 2009;91(2):183–189.
21. Pollack IF, Jakacki RI, Butterfield LH, et al. Antigen-specific immune responses and clinical outcome after vaccination with glioma-associated antigen peptides and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in children with newly diagnosed malignant brainstem and nonbrainstem gliomas. *J Clin Oncol*. 2014;32(19):2050–2058.
22. Buhler S, Nunes JM, Nicoloso G, Tiercy JM, Sanchez-Mazas A. The heterogeneous HLA genetic makeup of the Swiss population. *PLoS One*. 2012;7(7):e41400.
23. Wee CW, Kim E, Kim N, et al. Novel recursive partitioning analysis classification for newly diagnosed glioblastoma: a multi-institutional study highlighting the MGMT promoter methylation and IDH1 gene mutation status. *Radiother Oncol*. 2017;123(1):106–111.
24. Louis DN, Perry A, Reifenberger G, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol*. 2016;131(6):803–820.
25. Cho HI, Barrios K, Lee YR, Linowski AK, Celis E. BiVax: a peptide/poly-IC subunit vaccine that mimics an acute infection elicits vast and effective anti-tumor CD8 T-cell responses. *Cancer Immunol Immunother*. 2013;62(4):787–799.
26. Sabbatini P, Tsuji T, Ferran L, et al. Phase I trial of overlapping long peptides from a tumor self-antigen and poly-ICLC shows rapid induction of integrated immune response in ovarian cancer patients. *Clin Cancer Res*. 2012;18(23):6497–6508.
27. Weller M, Butowski N, Tran DD, et al; ACT IV trial investigators. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol*. 2017;18(10):1373–1385.
28. Romero P, Banchemareau J, Bhardwaj N, et al. The human vaccines project: a roadmap for cancer vaccine development. *Sci Transl Med*. 2016;8(334):334ps339.