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### Abstract

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### Reference

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

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**Running head:** IMA950/Poly-ICLC multipeptide vaccine for glioma

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**Conflict of interest:** The IMA950 peptide vaccine was provided by immatics Biotechnologies GmbH, Germany.

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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 in HLA-A2<sup>+</sup> 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 MHC class I and 2 MHC class II peptides) i.d. and poly-ICLC i.m. After protocol amendment, IMA950 and poly-ICLC were mixed and injected s.c. (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 CD4 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 Th1 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.

**Keywords:** Glioma, IMA950, peptide vaccine, poly-ICLC, immune response

**Key points:**

- The IMA950 glioma vaccine combined with poly-ICLC is safe and immunogenic
- 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.

## INTRODUCTION

Malignant (grade III and IV) gliomas are among the most aggressive solid tumors in adults and the overall survival 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.<sup>1,2</sup> New treatments are therefore urgently needed. Immunotherapeutic strategies are now being tested across many malignancies, with some striking results when using immune checkpoint inhibitors.<sup>3-5</sup> For malignant glioma, however, efficacy of checkpoint antibodies alone appears to be limited,<sup>6</sup> potentially due in part to the low mutation burden found in these tumors,<sup>7</sup> 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.<sup>8-10</sup> With this aim, we recently identified a set of HLA-A2-restricted peptides directly eluted from the surface of tumor samples from GBM patients, which were formulated in the IMA950 vaccine.<sup>11</sup> These antigens are over-expressed in the majority of patients with GBM, with little or no expression in healthy tissues, and are immunogenic *in vitro*.<sup>11</sup> 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.<sup>11</sup> 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 nine HLA-A2-restricted CD8 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 two 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,<sup>12-14</sup> but have been shown to be immunogenic in vaccine trials.<sup>15,16</sup> In addition, IMA950 contains an HLA-A2-restricted peptide derived from the HBV core antigen included as a marker of immunization efficacy.

The IMA950 vaccine was investigated in a clinical trial (IMA950-101 study, CRUK, Great Britain, NCT01222221) using GM-CSF as adjuvant, starting vaccination either before or after concomitant radiochemotherapy, and was found to be safe and immunogenic.<sup>15</sup> 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, i.e. poly-ICLC (Polyinosinic-Polycytidylic Acid Stabilized with Polylysine and Carboxymethylcellulose, Hiltonol®). Poly-ICLC has been shown to enhance the efficacy of vaccination in mouse model of glioma<sup>17,18</sup> and to be well tolerated in patients with grade III or IV glioma,<sup>19,20</sup> and trials of peptide vaccination in combination with poly-ICLC in adult and pediatric glioma patients showed induction of strong CD8 T cell responses.<sup>8-10,21</sup> 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 Th1 CD4 T cell responses.

These results are paving the way for combination with immune checkpoint inhibitors for potential clinical benefit.

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## MATERIALS AND METHODS

Additional information on study design, endpoints, analysis of immune responses in PBMC, tumor-infiltrating and skin biopsy-derived lymphocytes, immunohistochemistry, assessment of clinical response and statistical analysis are given in Supplementary material online.

### 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 that 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.<sup>1</sup> The first 6 patients received intradermal (i.d.) injections of 4.96 mg of IMA950 (413  $\mu\text{g}$ /peptide, Immatics Biotechnologies GmbH) and concomitant intra-muscular (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  $\mu\text{g}$  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 (Figure 1).

### Primary and secondary endpoints

Primary endpoints were safety and immunogenicity. Safety was monitored and dose limiting toxicity (DLT) was defined using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Immunogenicity was defined as  $>60\%$  patients displaying a CD8 T 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, overall survival (OS) as well as additional immunological endpoints.

### Radiological and clinical assessment of response

Tumor response was assessed according to the RANO 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 an HLA-A2 negative status, frail postoperative condition or blood/organ function values outside normal ranges. The high percentage of HLA-A2<sup>+</sup> patients (the percentage is 25-35% in the general Swiss population<sup>22</sup>) 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 a 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 (two *IDH1* mutated tumors only), with 4 methylated *MGMT* tumors. According to the updated RTOG RPA classification, the predicted survival for the 16 GBM patients with these characteristics is less than 15 months.<sup>23</sup> Six patients were included in the initial study protocol and 13 in the modified protocol (Figure 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 grade 1 and 2, which were not associated with longer OS as observed previously.<sup>15</sup> 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 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 non-related to the vaccine. One case of grade IV interstitial pneumonia due to pneumocystic infection was diagnosed with eventual favorable outcome. Pseudoprogression was observed in 4 (22%) patients, most frequently after the 4<sup>th</sup> 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, Figure 2A). Vaccinations were neither postponed nor interrupted. The etiology of this reaction remains unclear, TMZ, irradiation or immunotherapy potentially having played a role. Four patients (22%) presented with severe edema, three of them consecutive to disease progression (patients 1, 2 and 11) and one possibly related to the vaccine (patient 4, Table 1). The most intense reaction is illustrated in Figure 2B. This patient (patient 2) presented with somnolence, headache and dizziness arising immediately after the 4<sup>th</sup> vaccination, with a strong T2/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 (Figure 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 six 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 time point in any patient (Figure 3A). In addition, CD8 T cell response to the HBV peptide was induced in one patient only (Figure 3A and S1A), whereas recall responses to CMV and/or EBV were readily detectable in all but one patient (Supplementary Figure S1B), 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 T cell responses were detected in all but one patient (92.3%,  $p=0.002$ , Fisher's exact test as compared to initial vaccination protocol, Figure 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%, Figure 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 BCAN<sub>478-486</sub>, NLGN4X<sub>131-139</sub> and PTPRZ1<sub>1347-1355</sub> peptides (Figure 3C and Supplementary Figures S1C and S2). Finally, tumor peptide-specific CD4 T cell responses were detected in all but two patients (84.6%,  $p=0.001$ , Fisher's exact test as compared to initial vaccination protocol, Figure 3A), with detection of responses to both peptides in 53% of patients (Figures 3D and S3). Even from the first vaccination time point, we were able to detect production of TNF- $\alpha$ , IFN- $\gamma$  and IL-2 and cytokine production was maintained over the vaccination period for both peptides (Figure 3E and S4A-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 two patients, however, IL-17-producing cells were detected, accompanied by IL-5 production in one 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 CD4 T cell responses were observed in the s.c. group (Supplementary Figure S4C). 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 were 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 samples, BIRC5<sub>97-111</sub> or/and MET<sub>651-667</sub>-specific CD4 T cells in 7 out of 9 samples, and NLGN4X<sub>131-139</sub>-specific CD8 T cells in one 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 CXCR3 molecules on vaccine-induced T cells in peripheral blood.  $91.8 \pm 5.9$  % of HBV-specific CD8 T cells detected *ex vivo* co-expressed CD29/CD49d (range 83-98%), whereas CXCR3 was expressed by  $47.4 \pm 19.8$  (range: 21-74%) of cells (Supplementary Figure S5A). 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 S5B). Finally, the majority of BIRC5<sub>97-111</sub>-specific CD4 T cells detected *ex vivo* co-expressed CD29/CD49d, whereas only a minor fraction of these expressed CXCR3 (Supplementary Figure S5C).

### 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 eight proteins from which the IMA950 peptides derive were overexpressed in patients as compared to non-malignant brain samples (Supplementary Figure S6A). All antigens were expressed in >70% of pre-vaccination samples except for FABP7 (55% of samples, Supplementary Figure S6B). Except for patient number 10, all samples expressed a minimum of 4 antigens (mean $\pm$ SD:  $5.9 \pm 1.4$ , Supplementary Figure S6C). 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 as compared to pre-vaccination samples (Supplementary Figure S6B). 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; 95% CI: 19.50-29.23) for the whole cohort and 19 months (range 10-45; 95% CI: 17.25-27.87) for GBM patients (Figure 4A). When calculated from the date of study entry, mOS 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 (Figure 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 (Figure 4C) nor for patients included in the s.c (n=7) or i.m. (n=6) arm of the modified protocol (Figure 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 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.<sup>11</sup> 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 out the 3 anaplastic gliomas were *IDH1* wild type, a feature that is associated with worst patient survival as compared to *IDH1/2* mutated grade III astrocytoma patients.<sup>24</sup> 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.<sup>15,16</sup> 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 DC.<sup>25,26</sup> Reducing the number of vaccination 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<sup>8,9</sup> and to allow injecting at a single site throughout treatment. 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 CD8 T 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,<sup>15</sup> in an effort to assess the impact of the different adjuvants on the generation of immune responses. Nevertheless, comparison of the two 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-peptide 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 mOS of patients in the IMA950/poly-ICLC trial was 19 months for patients with GBM, compared to 15.3 months for patients of the IMA950/GM-CSF trial,<sup>15</sup> despite a lower

percentage of patients with *MGMT* methylated tumors (15 vs 29). In addition, PFS at 6 and 9 months were 93% and 56% in the current trial as compared to 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 suggests a possible effect of the IMA950/poly-ICLC vaccine. In addition, impressive pseudoprogression was observed in one patient, associated with a prolonged survival (35 months). 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 PD1 blockade 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 EGFRvIII antigen.<sup>27</sup>

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 Project<sup>28</sup> defined three objectives: identifying targets, rules of immunogenicity, and 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.

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## FIGURE LEGENDS

### Figure 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 one 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 48h after DTH. RT, radiotherapy, TMZ: temozolomide, MRI: magnetic resonance imaging.

### Figure 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 towards the left ventricle trigone, d: Dec 2014: stable disease, e: Feb 2015: partial response, f: Jun 2017: long term follow-up imaging shows relapse towards 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 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.

### Figure 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<sup>+</sup> CD8<sup>+</sup> T cells specific for the BCAN<sub>478-486</sub>, NLGN4X<sub>131-139</sub> and PTPRZ1<sub>1347-1355</sub> antigens in the pre-vaccination (T1+T2), post-vaccination 1 (T3+T4), 2 (T5+T6) and 3 (T7+T8) time points. **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- $\alpha$ -secreting CD4<sup>+</sup> T cells specific for the MET<sub>651-667</sub> and BIRC5<sub>97-111</sub> antigens in the pre-vaccination, post-vaccination 1, 2 and 3 time points. . .

### Figure 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).

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**Table 1: Patients demographics and characteristics**

| Patient | Age | Sex | Histology | Localization                 | RTV (cm <sup>3</sup> ) | MGMT status | IDH1/2 status | Mutation analysis (NGS) | Vaccine schedule       | Best response | OS (months) | Pseudo-progression | Cerebral edema |
|---------|-----|-----|-----------|------------------------------|------------------------|-------------|---------------|-------------------------|------------------------|---------------|-------------|--------------------|----------------|
| 1       | 62  | F   | GBM       | right parietal               | na <sup>a</sup>        | UnMeth      | wt            | nd                      | i.d./i.m. <sup>b</sup> | PD            | 19          | No                 | Yes            |
| 2       | 50  | M   | GBM       | right temporal               | 0                      | UnMeth      | wt            | nd                      | i.d./i.m.              | PD            | 10          | No                 | Yes            |
| 3       | 57  | F   | GBM       | right frontal                | 0 <sup>c</sup>         | UnMeth      | wt            | nd                      | i.d./i.m.              | PD            | 18          | No                 | No             |
| 4       | 55  | M   | GBM       | left fronto-insular          | 15.4                   | UnMeth      | wt            | nd                      | i.d./i.m.              | SD            | 21          | No                 | Yes            |
| 5       | 67  | M   | GBM       | left fronto-insular          | 0.16                   | UnMeth      | wt            | nd                      | i.d./i.m.              | PD            | 24          | No                 | No             |
| 6       | 56  | M   | GBM       | left temporal                | 0                      | <b>Meth</b> | wt            | nd                      | i.d./i.m.              | SD            | 41          | No                 | No             |
| 7       | 23  | F   | AA        | right fronto-temporo-insular | 3.5                    | UnMeth      | <b>mut</b>    | nd                      | s.c.                   | SD            | 37          | No                 | No             |
| 8       | 65  | M   | GBM       | left parieto-occipital       | 0.19                   | <b>Meth</b> | wt            | nd                      | i.m.                   | PR            | 35          | Yes                | No             |
| 9       | 61  | M   | GBM       | left frontal                 | 17.1                   | <b>Meth</b> | <b>mut</b>    | nd                      | s.c.                   | CR            | 35          | No                 | No             |
| 10      | 43  | F   | AA        | right temporal               | 0.26                   | UnMeth      | wt            | nd                      | i.m.                   | PD            | 32          | No                 | No             |
| 11      | 68  | M   | GBM       | left parietal                | 0                      | UnMeth      | wt            | none                    | s.c.                   | PD            | 11          | No                 | Yes            |
| 12      | 73  | M   | AA        | left frontal                 | 0                      | UnMeth      | wt            | none                    | i.m.                   | SD            | 30          | No                 | No             |
| 13      | 44  | M   | GBM       | right parieto-occipital      | 0.86                   | UnMeth      | wt            | none                    | s.c.                   | PD            | 13          | No                 | No             |
| 14      | 68  | F   | GBM       | left temporal                | 0.61                   | <b>Meth</b> | wt            | MET+ SMARCB1 mut        | i.m.                   | SD            | 28          | No                 | No             |
| 15      | 63  | M   | GBM       | left precentral frontal      | 1.85                   | UnMeth      | wt            | NOTCH1 del              | i.m.                   | PR            | 18          | No                 | No             |
| 16      | 41  | M   | GBM       | left temporal                | 11.8                   | UnMeth      | wt            | none                    | s.c.                   | PD            | 27          | Yes                | No             |
| 17      | 60  | M   | GBM       | left fronto-temporal         | 0                      | UnMeth      | wt            | none                    | s.c.                   | PD            | 18          | Yes                | No             |
| 18      | 73  | M   | GBM       | left frontal                 | 3.67                   | UnMeth      | wt            | none                    | i.m.                   | PD            | 18          | Yes                | No             |
| 19      | 41  | M   | GBM       | right parieto-temporal       | 0                      | UnMeth      | wt            | PTEN mut                | s.c.                   | PD            | 15          | No                 | No             |

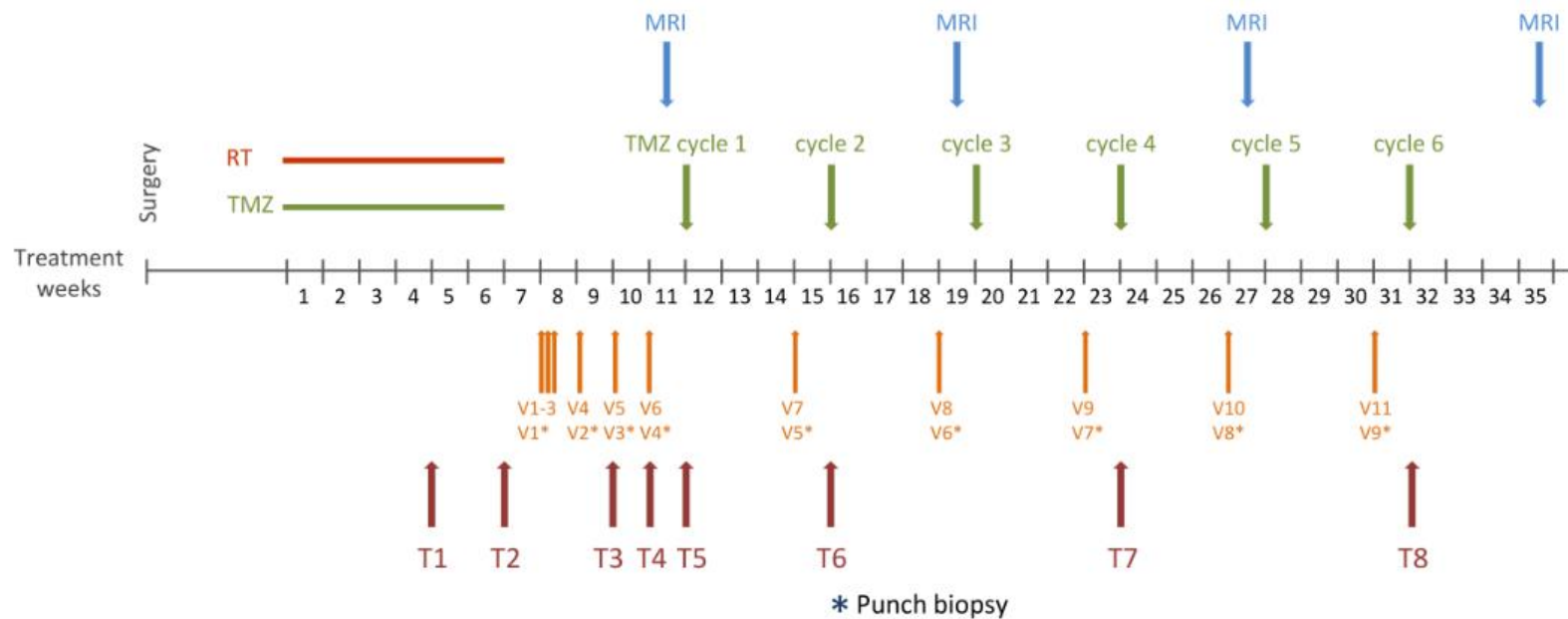
AA: anaplastic astrocytoma; CR: complete response; GBM: glioblastoma multiforme; IDH1/2: isocitrate dehydrogenase 1/2; Meth: methylated; MGMT: O6-methylguanin-DNA-methyltransferase; nd: not done; NGS: next generation sequencing; RTV : residual tumor volume ; SD: stable disease; UnMeth: unmethylated; 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                | Grade |    |   |   | Imputability                | Total number of patients (%) |
|-------------------------|-------|----|---|---|-----------------------------|------------------------------|
|                         | 1     | 2  | 3 | 4 |                             |                              |
| Cerebral edema          | -     | -  | - | 4 | vaccine/adjuvant (1)/PD (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)                      |

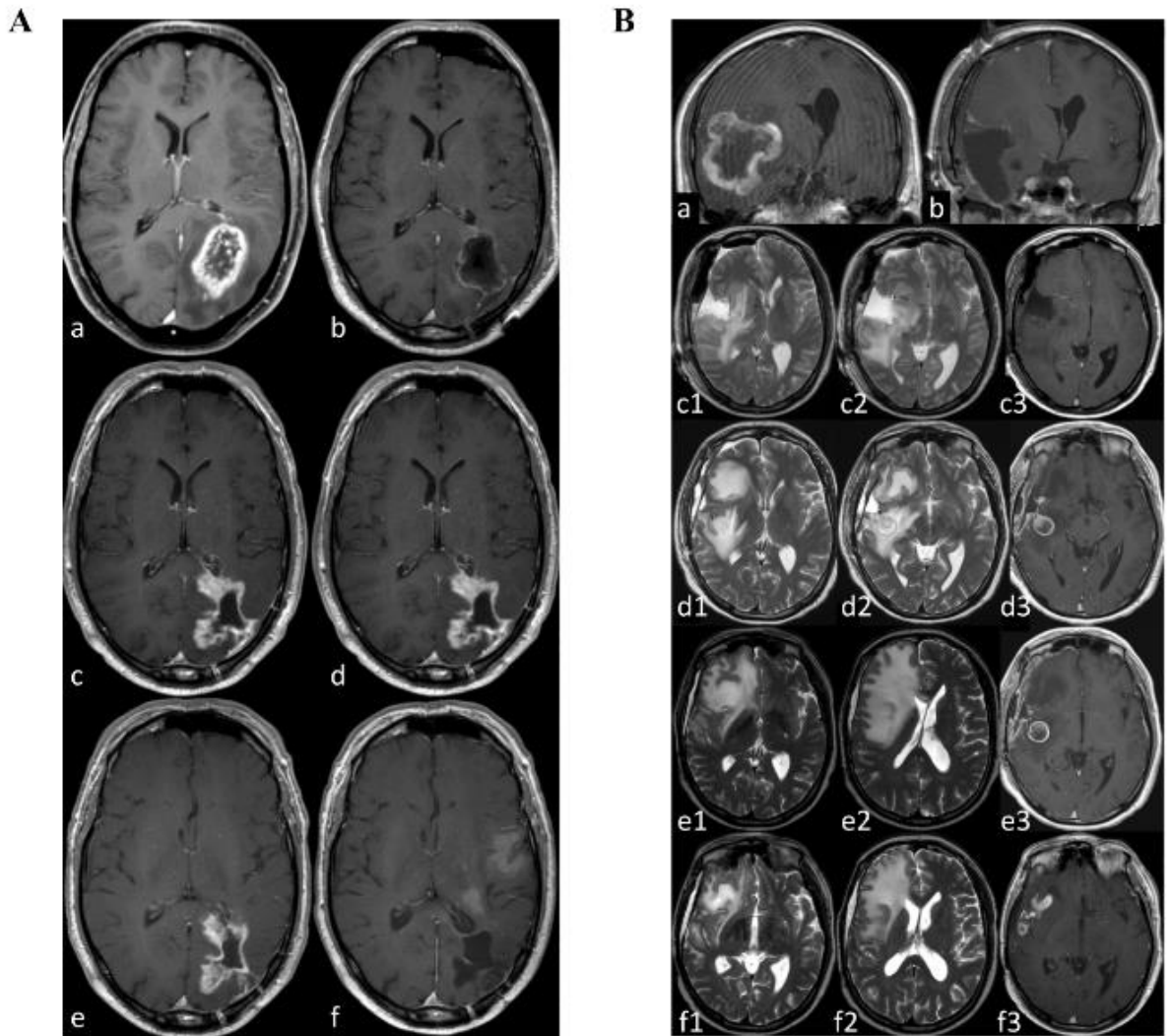
PD: progressive disease, TMZ: temozolomide

Figure 1



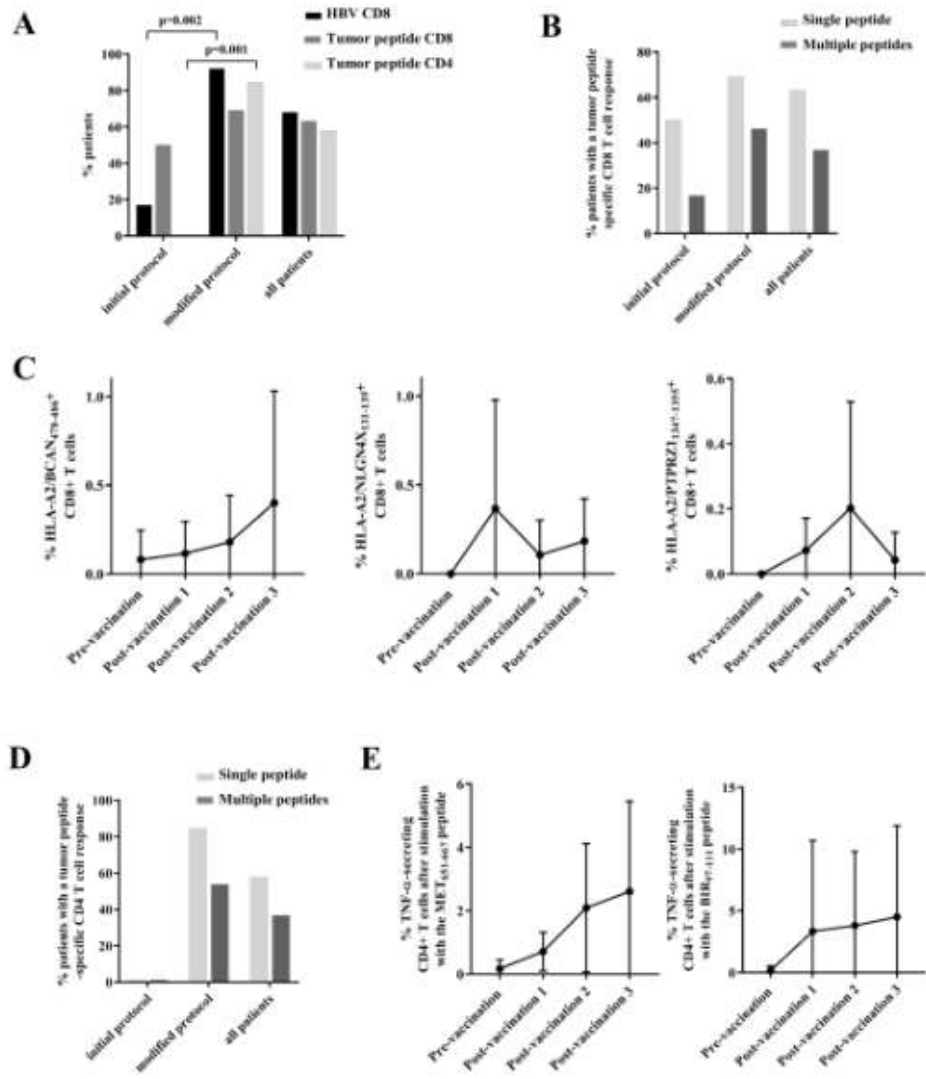
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**Figure 2**

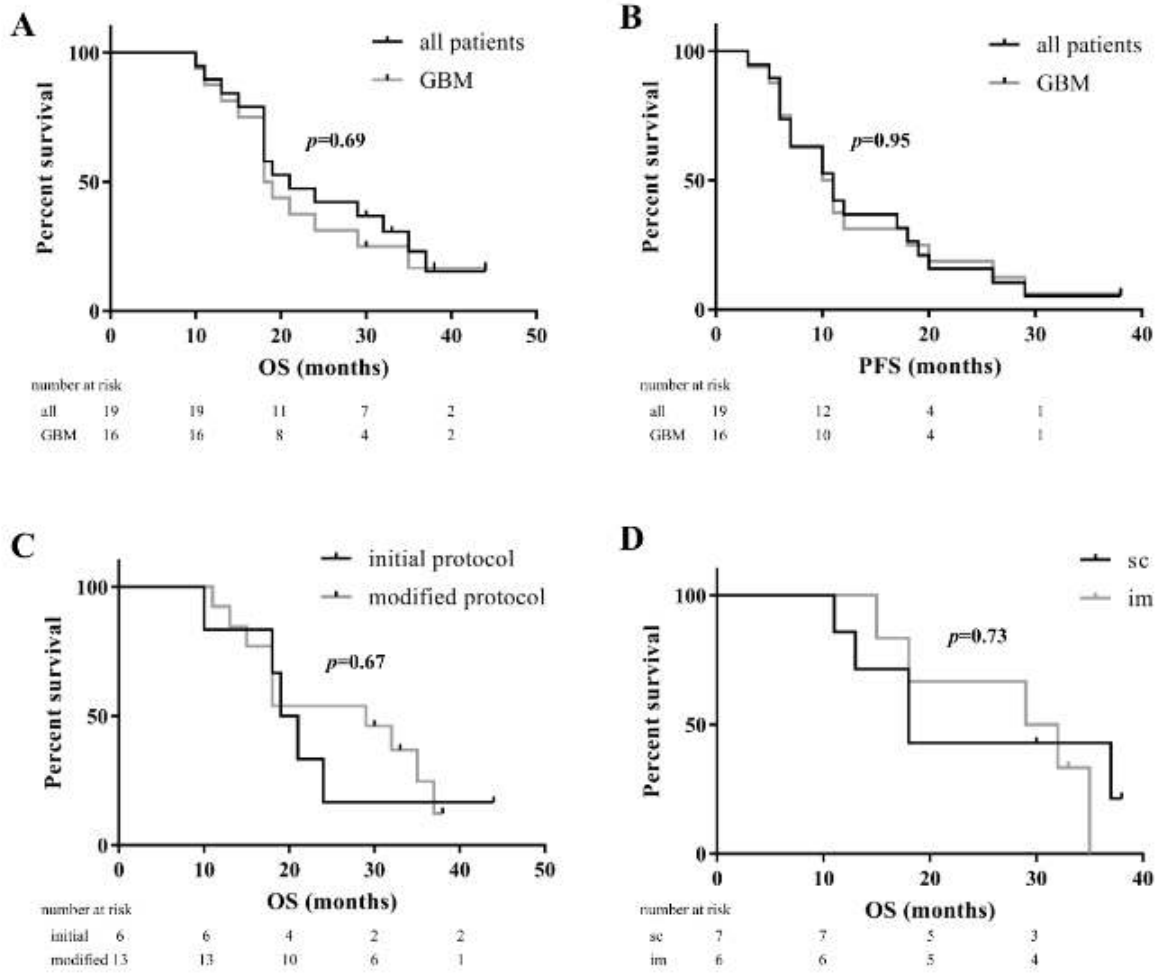


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Figure 3



**Figure 4**



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