Current strategies for vaccination in glioblastoma

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Purpose of review
Immunotherapy is viewed as a promising approach for glioblastoma and, in particular, therapeutic vaccines are being intensively studied. Here, we review results provided by recent clinical trials of glioblastoma vaccination and discuss the required strategies to optimize such approaches.

Recent findings
Two studies showed the feasibility of generating mutation-derived personalized vaccines in the short time frame given by the fast course of disease in glioblastoma. However, one of these demonstrated lack of mutation-derived cell surface presented MHC class I or II peptides in tumors with low mutational burden.

Summary
Whereas glioblastoma vaccines are well tolerated, impact on patient survival has yet to be proven. Combinations with immune checkpoint inhibitors are being tested, but strategies aiming at targeting the tumor microenvironment should be implemented as well. Finally, accurate immunomonitoring should be promoted in order to identify the best vaccine strategies, alone or in combination.

Keywords
glioblastoma, immune response, immunomonitoring, immunotherapy, vaccine

INTRODUCTION

Glioma is a primary tumor of the brain, among which grade IV tumors, also called glioblastoma (GBM), are endowed with a dismal prognosis [1]. Novel therapeutic approaches for GBM are based on combinations in order to target GBM heterogeneity, and on immunotherapy based on the promise of vaccines and adoptive cell therapy using chimeric antigen receptors [2,3]. Cancer vaccines aim at eliciting T-cell responses with tumor cell killing properties [4]. However, the efficacy of cancer vaccines is limited by the poor availability of immunogenic shared tumor-specific antigens, the suboptimal homing of elicited T cells to the tumor and the immunosuppressive glioma microenvironment [5].

Vaccine antigens should, ideally, be expressed by tumor cells but not by healthy cells. The reasons for this are two: preventing destruction of normal tissues by vaccine-elicited T cells and, in case the antigen is new to the immune system, inducing T cells that bear a high-affinity TCR. Such tumor antigens arise whenever a tumor-specific mutation gives rise to a T-cell epitope (this is a peptide binding to the patient's HLA allele) and when the generated epitope (called a neoantigen) is distant enough in structure from the corresponding wild type epitope to be seen as foreign by the immune system. The former has been shown to occur quite infrequently, as it is estimated that only a few percentage of mutations are presented as neopeptides at the cell surface [6–8]. Then, if antigen-specific T-cell responses are elicited, T cells have to home to the tumor, a process that is less efficient than for pathogen-induced immune responses [9]. Finally, taken that the elicited T cells have reached the tumor bed, the immunosuppressive milieu they encounter prevents efficient effector functions to take place [10]. In addition to these universal features, GBM is endowed with particular characteristics that further complicate therapeutic vaccination approaches. First, GBM is located in the central nervous system (CNS), which is bathed in immunosuppressive molecules, further limiting the occurrence of efficient immune responses [5]. Second, GBM is not highly mutated, except for rare hypermutated subtypes [11,12], therefore, not providing numerous neoantigens to incorporate in vaccine approaches. If tumor-associated

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KEY POINTS

- Most studies published in the last 18 months have used peptide vaccines or tumor or dendritic cell/tumor vaccines.
- Mutation-derived peptides presented at the GBM cell surface are very infrequent, questioning the use of neoantigen vaccines in GBM.
- Immunomonitoring should be systematically performed in order to learn from vaccine studies.

antigens (TAA), which are usually less tumor-selective, are used, the risk of collateral destruction of brain cells can potentially lead to severe side effects. It has to be noted, however, that up to now, no autoimmune effects of GBM vaccines have been observed [13]. Whether this is because of the limited efficacy of vaccines and whether we should fear these reactions when vaccines become more efficient remains to be elucidated. Finally, T-cell homing to the brain has been shown to be less efficient as for other sites [14,15]. Despite all these potential obstacles, interesting and promising observations have been made during the last 18 months in the field of GBM vaccines.

PEPTIDE VACCINES

Peptide vaccines have used TAA and tumor-specific antigens (TSA), usually incorporating multiple antigens in order to prevent tumor escape.

VACCINES INCORPORATING TUMOR-ASSOCIATED ANTIGENS

Most trials targeting TAA used MHC class I-restricted epitopes, two trials adding MHC class II-binding peptides [16,17] to allow for an integrated CD4 and CD8 T-cell response [18]. The only trial enrolling patients with newly diagnosed glioma tested the IMA950 peptide cocktail with poly-ICLC (Polyinosinic-Polycytidylic Acid Stabilized with Polylysine and Carboxymethylcellulose). This phase I/II trial for HLA-A2$^+$ grade III and IV glioma patients demonstrated safety and immunogenicity [16]. A significant finding from this study was that the vaccine formulation profoundly influenced elicitation of CD4 and CD8 T-cell responses. Indeed, mixing peptides and adjuvant prior to injection allowed the elicitation of multipeptide CD8 T-cell responses and sustained CD4 T-cell responses, which was not observed when peptides and adjuvant were injected separately [16]. The other strength of the IMA950 vaccine lies in the identification of the antigens by peptide elution from the surface of GBM samples, ensuring peptide presentation at the cell surface [19]. This vaccine is now being tested in combination with the anti-PD1 antibody pembrolizumab in patients with recurrent GBM (NCT03665545, Table 1). Several other peptide cocktails were tested in HLA-A24$^+$ patients with recurrent GBM. A phase III trial of personalized peptide vaccination treated patients with peptides selected based on preexisting peptide-specific IgG levels [20]. Results suggested that vaccination in patients without preexisting immune responses to the vaccine antigens might be detrimental, although the reason for this remains to be determined and is not a common finding [21–23]. A phase I trial tested WT1-derived MHC class I and II peptides and observed CD4 and CD8 T-cell responses lasting up to 1 year after vaccination [17]. Interestingly, authors showed that the WT1 MHC class II epitope was processed and presented in WT1-expressing tumor cells. Finally, two pilot studies tested peptide cocktails including peptides derived from the VEGFR1 and VEGFR2 proteins in order to target tumor angiogenesis and demonstrated CD8 T-cell responses to these peptides [24,25].

VACCINES INCORPORATING TUMOR-SPECIFIC ANTIGENS

Although the phase III clinical trial testing the EGFRvIII mutated peptide in newly diagnosed patients with EGFRvIII$^+$ GBM failed to demonstrate increased survival [26], potentially because of instability of the antigen, many peptide vaccine trials in GBM (or other glioma) are using TSA. Two trials tested a peptide covering the IDH1R132H mutation [27], either in patients with recurrent grade II (RESIST trial, NCT02193347) or newly diagnosed grade III/IV (NOA-016 trial, NCT02454634) IDH1R132H$^+$ gliomas and results are awaited. Preliminary data from the NOA-16 trial showed safety and immunogenicity of the vaccine, with elicitation of IDH1R132H-specific cellular and humoral responses in the majority of patients [28]. This vaccine is now being tested in combination with the anti-PD-L1 antibody avelumab in patients with recurrent grades II, III or IV glioma (NCT03893903, Table 1). Identification of a mutation in the histone 3 gene (*H3.3K27M*), preferentially found in pediatric patients with diffuse intrinsic pontine glioma (DIPG) [29,30] led to initiation of a phase I trial in HLA-A2$^+$ children with newly diagnosed H3.3K27M$^+$ DIPG and non-DIPG glioma testing the H3.3K27M peptide combined with poly-ICLC (NCT02960230, Table 1).

In addition to the IDH1R132H and H3.3K27M antigens, which are shared by a subpopulation of patients with glioma, other studies aimed at

Table 1. Ongoing vaccine studies

Title	Initiated in	Target population	Condition	Nb. patients	Immunogen	Immunomodulator/ other treatments	Phase	Sponsor	NCT number
Peptide vaccines									
TAA									
A Vaccine Trial for Low Grade Gliomas	2015	Pediatric[a]	Recurrent LGG	25	HLA-A2-restricted peptides s.c. + poly-ICLC i.m.		Phase 2	University of Pittsburgh, USA	NCT02358187
SurVaxM Vaccine Therapy and Temozolomide in Treating Patients With Newly Diagnosed Glioblastoma	2015	Adult	Survivin-positive n.d. GBM	64	Survivin 53-67/M57 peptide-KLH/Montanide s.c.+ GM-CSF s.c.		Phase 2	Roswell Park Cancer Institute, USA	NCT02455557
A Study of Varlilumab and IMA950 Vaccine Plus Poly-ICLC in Patients With WHO Grade II Low-Grade Glioma	2016	Adult	Grade II glioma	30	IMA950[b] /poly-ICLC s.c.	± Varlilumab[c]	Phase 1	University of Pittsburgh, USA	NCT02924038
A Toll-like Receptor Agonist as an Adjuvant to Tumor Associated Antigens (TAA) Mixed With Montanide ISA-51 VG With Bevacizumab for Patients With Recurrent Glioblastoma	2016	Adult	Recurrent GBM	30	Peptides (IL-13Rα2, EGFRvIII, EphA2, Her2/ neu, YKL-40)-KLH/ Montanide + poly-ICLC	Bevacizumab	Phase 2	New York University Medical School, USA	NCT02754362
A Study of DSP-7888 Dosing Emulsion in Combination With Bevacizumab in Patients With Recurrent or Progressive Glioblastoma Following Initial Therapy (WIZARD 201G study)	2017	Adult	Recurrent GBM	200	HLA-A2 and A24-restricted + 1 MHC-class II-binding peptides from WT1 i.d.	± Bevacizumab	Phase 2	Boston Biomedical Inc., USA	NCT03149003
Pembrolizumab in Association With IMA950/Poly-ICLC for Relapsing Glioblastoma (IMA950-106 study)	2018	Adult	Recurrent GBM	24	IMA950/poly-ICLC s.c.	± Pembrolizumab[d]	Phase 1/2	Geneva University Hospital, Switzerland	NCT03665545
TSA									
H3.3K27M Peptide Vaccine for Children With Newly Diagnosed DIPG and Other Gliomas	2016	Pediatric	H3.3K27M-positive DIPG and other glioma	29	HLA-A2-restricted H3.3K27M peptide/TT peptide/ Montanide + poly-ICLC		Phase 1	UCSF, USA	NCT02960230
Safety and Immunogenicity of Personalized Genomic Vaccine and Tumor Treating Fields (TTFields) to Treat Glioblastoma	2017	Adult	n.d. GBM	20	Neoantigens (SLP) + poly-ICLC	TTF	Phase 1	Mount Sinai, New York, USA	NCT03223103
Neoantigen-based Personalized Vaccine Combined With Immune Checkpoint Blockade Therapy in Patients With Newly Diagnosed, Unmethylated Glioblastoma	2018	Adult	n.d. MGMT-unmethylated GBM	30	Neoantigens (SLP)/poly-ICLC s.c.	Nivolumab[e] ± Ipilimumab[f]	Phase 1	Washington University School of Medicine, USA	NCT03422094
AMPLIFYing NEOepitope-specific VACcine Responses in Progressive Diffuse Glioma (AMPLIFY-NEOVAC study)	2019	Adult	Recurrent IDH1R132H-positive grade II, III or IV glioma	48	IDH1R132H peptide/ Montanide s.c. + imiquimod	± Avelumab[g]	Phase 1	German Cancer Research Center, Germany	NCT03893903
Heat-shock protein vaccines									
Trial of Heat Shock Protein Peptide Complex-96 (HSPPC-96) Vaccine	2016	Pediatric	n.d. and recurrent HGG and ependymoma	20	HSPPC-96 i.d.		Phase 1	Children's Hospital of Chicago, USA	NCT02722512

Table 1 (*Continued*)

Title	Initiated in	Target population	Condition	Nb. patients	Immunogen	Immunomodulator/ other treatments	Phase	Sponsor	NCT number
Radiation Therapy Plus Temozolomide and Pembrolizumab With and Without HSPPC-96 in Newly Diagnosed Glioblastoma (GBM)	2017	Adult	n.d. GBM	108	HSPPC-96 i.d.	Pembrolizumab	Phase 2	NCI, USA	NCT03018288
A Large-scale Research for Immunotherapy of Glioblastoma With Autologous Heat Shock Protein gp96	2018	Adult	n.d. GBM	150	HSPPC-96 s.c. + cyclophosphamide		Phase 2	Cure&Sure Biotech Co. LTD, China	NCT03650257
CMV-based vaccines									
Vaccine Therapy for the Treatment of Newly Diagnosed Glioblastoma Multiforme (ATTAC-II study)	2015	Adult	n.d. GBM	120	CMV pp65-LAMP[i] mRNA-transfected DCs + GM-CSF + Td boost		Phase 2	University of Florida, USA	NCT02465268
DC Migration Study for Newly-Diagnosed GBM (ELEVATE study)	2015	Adult	n.d. GBM	100	CMV pp65-LAMP mRNA-transfected DCs i.d. ± Td boost	± Basiliximab[h]	Phase 2	Duke University, USA	NCT02366728
Peptide Targets for Glioblastoma Against Novel Cytomegalovirus Antigens (PERFORMANCE study)	2016	Adult	n.d. GBM		PEP-CMV: SLP/Montanide + neutralizing CMV antibody-KLH i.d. + GM-CSF + Td boost		Phase 1	Duke University, USA	NCT02864368
Study to Evaluate Safety, Tolerability, and Optimal Dose of Candidate GBM Vaccine VBI-1901 in Recurrent GBM Subjects	2017	Adult	Recurrent GBM	18	VLP containing pp65 and gB CMV antigens/GM-CSF i.d.		Phase 1	VBI Vaccines Inc., USA	NCT03382977
PEP-CMV in Recurrent MEdulloblastoma/Malignant Glioma (PRiME study)	2017	Pediatric, adult	Recurrent medulloblastoma, recurrent HGG	30	PEP-CMV: SLP/Montanide + neutralizing CMV antibody-KLH i.d. + Td boost		Phase 1	Duke University, USA	NCT03299309
DC Migration Study to Evaluate TReg Depletion In GBM Patients With and Without Varlilumab (DERIVe study)	2018	Adult	n.d. GBM	112	CMV pp65-LAMP mRNA-transfected DCs ± Td	± Varlilumab	Phase 2	Duke University, USA	NCT03688178
Cytomegalovirus (CMV) RNA-Pulsed DCs for Pediatric Patients With Newly Diagnosed WHO Grade IV Glioma, Recurrent Malignant Glioma, or Recurrent Medulloblastoma (ATTAC-P study)	2018	Pediatric, adult	n.d. GBM, recurrent HGG, recurrent medulloblastoma	10	CMV pp65-LAMP mRNA-transfected DCs + GM-CSF + Td		Phase 1	Duke University, USA	NCT03615404
Immunotherapy Targeted Against Cytomegalovirus in Patients With Newly-Diagnosed WHO Grade IV Unmethylated Glioma (I-ATTAC study)	2019	Adult	n.d. MGMT-unmethylated GBM	48	CMV pp65-LAMP mRNA-transfected DCs/GM-CSF i.d. + Td		Phase 2	Duke University, USA	NCT03927222
Tumor vaccines									
Neoadjuvant Evaluation of Glioma Lysate Vaccines in WHO Grade II Glioma	2015	Adult	Grade II glioma	30	GBM6-AD[j]/poly-ICLC s.c.		Phase 1	UCSF, USA	NCT02549833
Adjuvant Dendritic Cell-immunotherapy Plus Temozolomide in Glioblastoma Patients (ADDIT-GLIO study)	2016	Adult	n.d. GBM	20	WT1 mRNA-transfected DCs i.d.		Phase 1/2	University Hospital, Antwerp, Belgium	NCT02649582
Personalized Cellular Vaccine for Recurrent Glioblastoma (PERCELLVAC2 study)	2016	Adult	Recurrent GBM	30	Tumor mRNA-transfected DCs		Phase 1/2	Guangdong Brain Hospital, China	NCT02808364

Table 1 (*Continued*)

Title	Initiated in	Target population	Condition	Nb. patients	Immunogen	Immunomodulator/ other treatments	Phase	Sponsor	NCT number
Vaccine Therapy in Treating Patients With Recurrent Glioblastoma	2017	Adult	Recurrent GBM	20	Allogeneic GBM lysate-pulsed DCs		Phase 1	Mayo Clinic, USA	NCT03360708
Adoptive Cellular Therapy in Pediatric Patients With High-grade Gliomas (ACTION study)	2017	Pediatric	HGG	8	Total tumor RNA-transfected DCs/GM-CSF i.d. + Td boost + T cells ± HSC		Phase 1	University of Florida, USA	NCT03334305
Autologous DCs Pulsed With Tumor Lysate Antigen Vaccine and Nivolumab in Treating Patients With Recurrent Glioblastoma	2017	Adult	Recurrent GBM	30	tumor lysate-pulsed DCs (DCVax-L)	± Nivolumab	Phase 2	Jonsson Comprehensive Cancer Center, USA	NCT03014804
Brain Stem Gliomas Treated With Adoptive Cellular Therapy During Focal Radiotherapy Recovery Alone or With Dose-intensified Temozolomide (Phase I) (BRAVO study)	2018	Pediatric, adult	DIPG	21	Total tumor RNA-transfected DCs/GM-CSF i.d. + Td boost + T cells + HSC	± Lymphodepletive Conditioning	Phase 1	University of Florida, USA	NCT03396575
Efficiency of Vaccination With Lysate-loaded DCs in Patients With Newly Diagnosed Glioblastoma (GlioVax study)	2018	Adult	n.d. GBM	136	Tumor lysate-loaded DCs		Phase 2	Heinrich-Heine University, Düsseldorf, Germany	NCT03395587
Autologous DCs Loaded With Autologous Tumor Associated Antigens for Treatment of Newly Diagnosed Glioblastoma	2018	Adult	n.d. GBM	55	Autologous cell line-loaded DCs (AV-GBM-1)/GM-CSF		Phase 2	Aivita Biomedical, Inc., USA	NCT03400917
DC Immunotherapy Against Cancer Stem Cells in Glioblastoma Patients Receiving Standard Therapy (DEN-STEM study)	2018	Adult	n.d. MGMT-unmethylated GBM	60	Autologous tumor Mrna + survivin and hTERTmRNA-transfected DCs i.d.		Phase 2/3	Oslo University Hospital, Norway	NCT03548571
Autologous DCs and Metronomic Cyclophosphamide for Relapsed High-Grade Gliomas in Children and Adolescents	2019	Pediatric	Recurrent HGG	25	Tumor lysate-pulsed DCs + cyclophosphamide		Phase 1/2	Wurzburg University Hospital, Germany	NCT03879512

DCs, dendritic cells; DIPG, diffuse intrinsic pontine glioma; GBM, glioblastoma multiforme; HGG, high-grade glioma; HCS, hematopoietic stem cells; HSPPC-96, heat shock protein peptide complex-96; i.d., intradermal; i.m., intramuscular; LGG, low-grade glioma; MGMT, O6-methylguanine-DNA methyltransferase; n.d., newly-diagnosed; s.c., subcutaneous; Td, tetanus-diphtheria toxin vaccine; TTF, tumor-treating fields.

[a]Pediatric includes patients aged up to 21 years.

[b]IMA950 is composed of nine HLA-A2-restricted peptides and two MHC class II-binding peptides (Dutoit *et al.*, Brain 2012).

[c]Varlilumab is a fully human IgG1 monoclonal anti-CD27 antibody.

[d]Pembrolizumab is a humanized IgG4 monoclonal anti-PD1 antibody.

[e]Nivolumab is a fully human IgG4 monoclonal anti-PD1 antibody.

[f]Ipilimumab is a fully human IgG1 monoclonal anti-CTLA4 antibody.

[g]Avelumab is a fully human IgG1 monoclonal anti-PD-L1 antibody.

[h]Basiliximab is a chimeric mouse-human monoclonal antibody to the α chain of the IL-2 receptor.

[i]LAMP is a short peptide chimeric antigen from lysosome-associated membrane protein 1.

[j]GBM6-AD is an allogeneic GBM cell line.

targeting patient-specific mutations, with the goal of personalized tumor targeting. In a phase I/Ib trial, eight newly diagnosed GBM patients with unmethylated MGMT promoter were vaccinated with a cocktail of up to 20 peptides predicted to bind their respective HLA alleles, together with poly-ICLC [31▪]. T-cell responses were induced only in the two patients who did not receive dexamethasone in the vaccine priming phase, illustrating the difficulty to induce immune responses in patients who often require steroids to reduce cerebral inflammation. In these two patients, a thorough immunomonitoring revealed that vaccine-induced T cells were able to migrate to the tumor site in the brain but that these tumor-infiltration lymphocytes (TIL) expressed multiple checkpoint molecules, suggesting importance of combining with immune checkpoint inhibitors [31▪]. However, this study did not formally demonstrate presentation of mutation-derived peptides on the tumor cell surface *in vivo*. As suggested above [7], this might strongly limit the potential of such vaccines. This issue was indeed tested in another trial including 15 HLA-A2$^+$-restricted and HLA-A24$^+$-restricted newly diagnosed GBM patients, in which patients were vaccinated first with a set of personalized TAA and then with mutation-containing peptides together with GM-CSF and poly-ICLC [32▪▪]. In that trial, peptide elution was performed from each patient's tumor, allowing, on one hand, to identify patient-tailored tumor-presented TAA and, on the other hand, to validate presentation of mutation-derived peptides at the GBM cell surface. Strikingly, of the 643 mutations identified for the whole cohort, none was identified in the HLA class I and II peptidomes of the respective patients by high-sensitivity mass spectrometry [32▪▪]. This important result confirmed previous reports [6,33] and suggested that, for tumors with low mutation burdens such as GBM, exploitation of TAA might be more suitable. Importantly, these two studies were a proof-of-concept of the feasibility of identifying personalized (neo)antigens in a short time frame that still allowed vaccination to take place in the first 9 months after diagnosis.

TUMOR VACCINES

Several trials of tumor vaccination have been published in the last 18 months, using either tumor lysates [34], tumor-derived heat-shock proteins [35] or tumor-loaded DCs [36–44]. Interim results from the placebo-controlled phase III DCVax-L trial in patients with newly diagnosed GBM unfortunately did not report awaited results, such as PFS, which was the primary endpoint of the study [36]. Furthermore, multiple methodological flaws in the analysis prevent interpretation of the data, as discussed in depth elsewhere [45]. A phase II randomized trial performed a detailed analysis of biomarkers associated with overall survival (OS) in newly diagnosed GBM patients receiving tumor-loaded DCs. This showed that prevaccination blood CD8$^+$ T-cell counts and Granzyme B production in response to the vaccine were significantly correlated with OS [38]. However, these results have to be taken with caution, as no multiple testing corrections were applied. Another study testing a heat shock protein peptide complex-96 vaccine in newly diagnosed GBM patients showed that high levels of postvaccine immune responses were associated with longer survival [35], although no raw data were presented. Finally, a study for newly diagnosed GBM patients showed that vaccination with CMV pp65 RNA-loaded DCs after adoptive transfer of CMV pp65-specific CD8 T cells increased the frequency of polyfunctional CMV-specific CD8 T cells, which was not observed in patients receiving physiological saline [42]. These increases in polyfunctional CMV-specific CD8 T cells correlated with OS.

Overall, all published tumor vaccine trials were well tolerated. Only a minority of studies, however, reported monitoring of vaccine-induced immune responses.

ONGOING CLINICAL TRIALS

Ongoing glioma vaccine trials are testing TAA and TSA peptide ($n = 10$), heat-shock protein ($n = 3$), CMV-based ($n = 8$) and tumor ($n = 11$) vaccines in the adult and pediatric population (Table 1, including trials initiated in 2015 and after). Many trials of peptide vaccines are using either long peptides or a combination of MHC class I and II peptides, and the majority are using poly-ICLC as adjuvant. Four trials are combining peptide vaccines with immune checkpoint molecules, either PD1, CTLA4 or CD27. A significant number of trials are testing the potential of CMV as target in GBM, the majority originating from the same center and using CMV pp65-LAMP-mRNA-transfected dendritic cell (DC) or the PEP-CMV compound (Table 1). Interestingly, some of these studies are performing a tetanus/diphtheria toxin boost to induce inflammation and augment DC draining to lymph nodes, and are assessing migration of DC *in vivo* (NCT02366728, NCT03688178 and NCT03927222). The majority of tumor vaccine trials are testing DCs transfected with tumor or antigen-coding RNA, one study combining with nivolumab.

IMMUNOMONITORING

Most studies testing peptide vaccines have performed monitoring of vaccine-induced responses.

Technological advances now allow sensitive detection of immune responses *ex vivo*, without the need for prior in-vitro amplification [32▪▪]. This is clearly an asset, as ex-vivo analysis may not only assess the presence of specific T cells, but also their function, which is less relevant after *in vitro* culture. Importantly, one trial performed extensive immunomonitoring in a limited number of patients, and showed migration of induced T cells to the brain [31▪]. Unfortunately, a limited number of trials testing tumor vaccines are performing accurate vaccine-specific immunomonitoring, partly because of the lack of identified antigens. This is, however, a very important effort to make, as relying on influence of the vaccine on survival alone may prevent learning from these trials. Indeed, immunomonitoring data could provide us with clues of why vaccines are able or not to influence outcome, and how to move forward to optimize vaccines and design rationale combinations with other immunotherapies or treatments. Another limitation of vaccine immunomonitoring is the usually limited access to postvaccine tumor samples. Consequently, immunomonitoring is performed in the peripheral blood, which might not be representative of what is happening *in situ*. Assessment of vaccine-induced T cells in the brain, as performed in a limited number of studies [16,31▪,32▪▪] will potentially enable us to design modalities to improve T-cell trafficking to the brain. In line with this, two studies (NCT03665545 and NCT03893903) testing peptide vaccines in patients with recurrent GBM have a planned surgery after vaccine initiation.

CONCLUSION

Intense research is ongoing in the field of GBM vaccines, but influence on patient survival has yet to be proven. Potential limitations to the efficacy of vaccines, in GBM but also in other indications, are the nature of the antigen, the lack of knowledge of the best way to vaccinate (where, in what format, at what frequency, how long, using what adjuvant), the lack of efficient homing of T cells to the tumor site and the immunosuppressive microenvironment. It is probable that vaccination alone will not have a major impact on survival, and we should, in addition to finding ways to improve vaccine formulation and T-cell homing to the brain, concentrate on combining with immune checkpoint inhibitors and molecules targeting the glioma microenvironment. In addition, the best way to combine vaccine and other immunotherapies with standard of care (radiotherapy, temozolomide, or bevacizumab) has also to be explored. Altogether, vaccines, which are well tolerated and relatively easy to produce, should be tested in rational designs, accompanied by a thorough monitoring of immune responses in order to bring benefit to patients in the future.

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Conflicts of interest

There are no conflicts of interest..

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- ▪ of special interest
- ▪▪ of outstanding interest

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