HUMAN VACCINES & IMMUNOTHERAPEUTICS
2018, VOL. 14, NO. 2, 255–268
https://doi.org/10.1080/21645515.2017.1388481

Taylor & Francis
Taylor & Francis Group

REVIEW



# Vaccination in the immunotherapy of glioblastoma

Ziren Kong, Yu Wang, and Wenbin Ma

Department of Neurosurgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

**ABSTRACT**

Glioblastoma remains one of the most common central nervous system tumors with an extremely poor prognosis. Recently, rapid progress in immunotherapy has provided new options for the treatment of glioblastoma. Vaccination, the primary method of immunotherapy, stimulates the body's tumor-specific immune response by the injection of foreign antigens. Peptide vaccines involve the injection of tumor-specific antigens, such as EGFRvIII or heat-shock proteins. Cell-based vaccines, which primarily include dendritic cell vaccines and tumor cell vaccines, involve injections of ex vivo-modified cells. Despite the encouraging results of phase I/II clinical trials, no successful phase III clinical trials involving glioblastoma immunotherapy, including glioblastoma vaccinations, have been reported to date. In this review, the authors summarize the published outcomes of glioblastoma vaccine therapy, explore its future prospects based on ongoing clinical trials, and discuss combined therapy as a future direction for glioblastoma treatment.

**ARTICLE HISTORY**
Received 25 May 2017
Revised 10 September 2017
Accepted 2 October 2017

**KEYWORDS**
Glioblastoma; immunotherapy; vaccination; peptide vaccine; cell-based vaccine

Glioblastoma multiforme (GBM) is an extremely malignant grade IV central nervous system (CNS) tumor,[1] accounting for 30% of all gliomas with an incidence of approximately 3.19 patients per 100,000 people annually.[2,3]

Stupp et al. proposed the following standard treatment for GBM: patients underwent tumor resection, followed by radiochemotherapy (during the radiotherapy, the tumors were irradiated at a dose of 2 Gy/day, 5 times per week for 6 weeks, and the total dose was 60 Gy; during the chemotherapy, temozolomide (TMZ) was administered at 75 mg per square meter of body surface area daily, 7 days per week from the first to the final day of radiotherapy) and six cycles of adjuvant TMZ (150 to 200 mg per square meter for 5 days during each 28-day cycle).[4] The prognosis of GBM patients remains poor: The median overall survival (OS) is 14.6∼16.7 months with the current standard-of-care therapy.[5–8] Treatment- and prognosis-related molecular markers, such as the methylation of O6-methylguanine-DNA methyltransferase (*MGMT*), 1p/19q co-deletion, mutation of *IDH*, mutation of the *TERT* promoter, overexpression of epidermal growth factor receptor (EGFR), and new therapies such as immunotherapy, are continuously emerging.[5,9]

Immunotherapy is defined by the National Cancer Institute (NCI) as 'a type of biological therapy that uses substances to stimulate or suppress the immune system to help the body fight cancer or other diseases'.[10] Immunotherapy is mainly divided into active immunotherapy and passive immunotherapy. Active immunotherapy elicits a tumor-specific immune response through an injection of foreign antigens, primarily via a vaccine injection (including peptide and cell-based vaccines), whereas passive immunotherapy, including antibody therapy and adoptive immunotherapy, achieves anti-tumor responses through an injection of novel immunemodulating biologics (e.g., antibodies) instead of directly activating the body's immune system.[11] Recently, immunotherapy has become a new antineoplastic regimen in addition to surgery, radiotherapy, chemotherapy and targeted therapy in cancers such as melanoma and non-small cell lung cancer, yet none immunotherapy approaches have been approved for clinical use to treat GBM. This review aims to summarize the current clinical and experimental outcomes of vaccination-based active immunotherapy for GBM and, thus, provide a reference for the clinical management of GBM.

## Immunology of the central nervous system (CNS)

### *Previously immune-privileged site*

The CNS was traditionally considered an immune-privileged site due to its lack of a lymphatic system and its separation from the blood circulatory system by the blood-brain barrier.[12] However, several studies have revealed the mechanism underlying the circulation of immune cells and systems in the CNS. Microglia constitute one type of CNS immune cell, accounting for 5% to 20% of all glial cells and playing important roles in innate and acquired immunity (e.g., antigen presentation).[13] Interestingly, Fonseca et al. investigated the aggregation of abundant microglia and macrophages in GBM and hypothesized that microglia promoted tumor progression by interacting with the tumor.[14] Activated T-lymphocytes, which are located in cervical lymph nodes, can penetrate the blood-brain barrier, enter the CNS and contribute to the interchange between the

**CONTACT** Wenbin Ma mawb2001@hotmail.com
Authors' postal address: Peking Union Medical College Hospital (East), No.1 Shuaifuyuan Wangfujing Dongcheng District, Beijing 100730, China.

CNS and lymphatic system.[15] The immune system can also communicate with the brain parenchyma via cerebrospinal fluid (CSF) and interstitial fluid (ISF) and can transport antigens and immune cells.[16]

### *Immunosuppression in GBM*

Although the CNS has a unique immune mechanism and sequestration, immunotherapy for GBM continues to face immunosuppression. GBM cells create immunosuppressive microenvironments mainly by secreting certain cytokines and expressing membrane proteins.[17]

GBM can inhibit immune system attacks by secreting cytokines. The tumor cell secretes chemokines (e.g., CCL2), which recruit regulatory T cells (Treg) and inhibit the proliferation and activity of effector T cells and antigen-presenting cells (APCs) through anti-inflammatory cytokines, e.g., interleukin 10 (IL-10) and transforming growth factor beta (TGF-$\beta$).[18,19] In addition, tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) can inhibit the immune response in the GBM microenvironment.[19]

GBM can directly or indirectly attenuate the function of immune cells by expressing membrane proteins. By expressing the FASL, B7 and PDL1 receptors, GBM cells bind to negative regulatory factors of immune functions (e.g., FAS, CTLA-4 and PD-1), thereby inhibiting the activity of T cells or NK cells and promoting the immune escape of GBM.[20–23] The downregulation of classical MHC class I molecules and the overexpression of certain non-classical MHC class I molecules (e.g., HLA-G and HLA-E) can inhibit the cytotoxicity of NK cells and cytotoxic T cells, which contributes to tumor immune escape.[24,25]

Additionally, the irregular vascular structure of GBM and the low perfusion of the tumor could result in a reduction in immune and treatment responses.[26]

## Origins of vaccine immunotherapy and non-specific vaccines

The earliest report of a cancer treatment using a vaccine was reported in 1891 by William B. Coley, who injected streptococcal organisms into a patient with inoperable cancer and observed the disappearance of the tumor.[27] Bacillus Calmette-Guerin, which was developed and licensed for the treatment of superficial urothelial carcinoma of the bladder,[28] was applied to the treatment of GBM in 1980,[29] and soon after, another clinical study reported that this therapy could prolong patient survival.[30] Subsequently, physicians treated glioma patients concurrently with ImuVert (which is derived from the bacterium *Serratia marcescens*) and radiotherapy, and those patients tolerated this therapeutic regimen well with a certain degree of efficacy.[31] At the turn of the century, infecting human tumor cells with Newcastle Disease Virus (NDV) (act as an oncolytic virus in addition to immunomodulation) resulted in the upregulation of HLA, which induced the production of interferon and ultimately resulted in apoptosis; NDV was then applied for treating patients with recurrent GBM.[32,33] These non-specific vaccines achieved an anti-tumor response by stimulating the immune system, which was similar to a postoperative infection as previously reported.[34] The existing vaccine studies exploring GBM are listed in Table 1.

## Peptide vaccines

Tumor-specific antigens are antigens that are present on tumor cells and generally absent from normal tissues. These antigens are often proteins that are encoded by mutant genes in the tumor, which are relatively conserved among different types of cancers and patients and can serve as targets for immunotherapy.[35] Peptide vaccines induce immune responses through an injection of tumor-specific antigens, which leads to the destruction of tumor cells.[36]

### *EGFRvIII-targeted peptide vaccines*

EGFR expression plays a key role in tumorigenesis. EGFR, along with ErbB-2 (HER2), ErbB-3 (HER3) and ErbB-4 (HER4) constitute the ErbB family of receptor tyrosine kinases[37] and form dimers when activated.[38] The ErbB family is closely related to many downstream pathways (e.g., the PI3K/AKT/mTOR and RAS/RAF/MEK pathways), and the mutation or continuous activation of these pathways results in the development of GBM.[39] EGFR is overexpressed in 50% to 60% of patients with GBM,[40] but EGFR-targeted drugs, such as nimotuzumab, do not prolong the progression-free survival (PFS) or OS of patients.[41]

EGFRvIII (type III epidermal growth factor receptor mutation), which is present in 20% to 30% of patients with GBM, is formed due to the deletion of exons 2∼7 of EGFR, which leads to an extracellular truncation of EGFR and allows EGFR to be continuously activated in the absence of ligand.[40]

The earliest study investigating an EGFRvIII-targeted peptide vaccine was reported in 1997 by Moscatello et al., who obtained the peptide vaccine PEP-3-KLH (generic name: Rindopepimut, also known as CDX-110) by conjugating PEP-3, which is a 14-amino-acid peptide that comprises an EGFRvIII-specific mutant part (amino acid sequence: LEEKKGNYVVTDHC), to keyhole limpet hemocyanin (KLH).[42] They induced the corresponding antibody and observed an immune response in cytotoxic T cells in a mouse model.[42] Heimberger et al. observed that the PEP-3-KLH vaccine significantly prolonged the median survival time in an EGFRvIII-expressing murine brain tumor model.[43,44] In 2008, Schmittling et al. first reported that the PEP-3-KLH vaccine could induce newly diagnosed patients with GBM to produce an EGFRvIII-specific antibody.[45] Sampson et al. found a significant inverse correlation between the frequency of Treg and PEP-3-KLH-stimulated humoral immunity during combined treatment with the anti-IL-2R$\alpha$ MAb daclizumab, TMZ and PEP-3-KLH, which explains the role of the PEP-3-KLH vaccine.[46] Saraswathula et al. observed that the administration of the vaccine plus TMZ did not stimulate regulatory B cells and further inhibited the secretion of IL-10 and TGF-$\beta$ to a certain extent, which disinhibited the activation of effector T cells and APCs.[47]

The first clinical trial investigating the PEP-3-KLH vaccine was reported in 2009. Twelve patients with newly diagnosed GBM received three consecutive intradermal vaccinations with PEP-3-KLH and dendritic cell injections. The toxicity was minimal and limited to grade 2 toxicities. Although some patients showed a small increase in the erythrocyte sedimentation rate (33% of patients) and rheumatoid factor level (10% of patients)

**Table 1.** Published results of the vaccination in glioblastoma.

| Year | Pts No. | Disease | WHO grade | Vaccine | Result | Toxicity |
|---|---|---|---|---|---|---|
| 1993[31] | 15 | nAA, nGBM | Grade III, IV | ImuVert | PFS = 7.8 m<br>OS = 18.2 m | 57% grade 3 or 4 flu-like symptoms, 15% grade 3 hypotension |
| 2006[33] | 14 | rGBM | Grade IV | NDV | 1 patient with CR | Grade 1/2 constitutional fever |
| 2009[48] | 12 | nGBM (EGFRvIII) | Grade IV | PEP-3-KLH | PFS = 6.8 m<br>OS = 18.7 m | Limited to grade 2 toxicities; no allergic reactions or serious adverse events |
| 2010[49] | 18 | nGBM (EGFRvIII) | Grade IV | PEP-3-KLH | PFS = 14.2 m<br>OS = 26 m | Limited to grade 2 toxicities, mostly related to injection-site reactions; no autoimmune reactions |
| 2011[50] | 22 | nGBM (EGFRvIII) | Grade IV | PEP-3-KLH | PFS = 15.2 m<br>OS = 23.6 m | Allergic reactions, with grade 3 skin (14%) and gastrointestinal allergies (5%) |
| 2015[51] | 65 | nGBM (EGFRvIII) | Grade IV | PEP-3-KLH | PFS = 12.3 m<br>OS = 24.6 m | Mild-to-moderate injection-site reactions occurred in nearly all patients (≥86%). Fatigue (26%), rash (17%), nausea (12%), pruritus (62%), and headache (8%) of any grade also occurred. Grade 3 or 4 events were relatively rare and limited to single patients. |
| 2015[53](retracted) | 36 | rGBM (EGFRvIII) | Grade IV | PEP-3-KLH | OS = 11.3 m | Frequent grade 1–2 injection-site reactions. 25% ≥grade 3 adverse events, including back pain (6%), convulsion (11%), fall (3%), headache (3%), and hypertension (3%). |
| 2016[56] | 371 | nGBM (EGFRvIII) | Grade IV | PEP-3-KLH | OS = 17.4 m | Mostly limited to grade 2 toxicities. 80% of patients showed injection-site reactions (primarily erythema, pruritus, and rash). Fatigue, nausea, headache, and constipation also occurred in ≥20% of patients. 5% of patients showed brain edema. |
| 2013[72] | 12 | rHGG | Grade III, IV | HSPPC-96 | OS = 11.0 m | Mild injection-site erythema and/or induration (50%) related to vaccine |
| 2014[73] | 41 | rGBM | Grade IV | HSPPC-96 | OS = 10.0 m | The toxicity associated with the vaccine was minimal and mostly related to injection-site reactions (41%). 2% grade 3 constitutional events (fatigue) related to vaccine |
| 2016[84] | 9 | rGBM, recurrent anaplastic glioma (survivin-positive) | Grade III, IV | SurVaxM | PFS = 4.1 m<br>OS = 20.2 m | 67% experienced injection-site reaction (all grade 1). Myalgia (22%), lymphopenia (33%), and leukopenia (33%) of grade 1 or 2 were also observed. |
| 2001[92] | 7 | nGBM | Grade IV | DCs vaccine | OS = 15.2 m | No autoimmune reactions. Fever (14%), nausea (14%) and vomiting (14%), and enlarged lymph nodes (14%) were observed. |
| 2004[93] | 8 | rGBM | Grade IV | DCs vaccine | OS = 31.1 m | No autoimmune reactions. Headache (38%), fatigue (25%), erythema (12%), and seizures (25%) were observed and limited to grade 2 toxicities. |
| 2007[94] | 7 | rAA, rGBM | | DCs vaccine | – | Skin reactions (28%) and headache (14%) were observed and limited to grade 2 toxicities. |
| 2005[95] | 12 | nGBM, rGBM | Grade IV | DCs vaccine | PFS = 19.9 m<br>OS = 35.8 m | Fever and/or flu-like symptoms (33%), nausea and vomiting (25%), and enlarged lymph nodes (17%) were observed and limited to grade 2 toxicities. |
| 2008[96] | 13 | nAA, rAA, nGBM, rGBM | Grade III, IV | DCs vaccine | OS = 11.6 m | No adverse events related to the vaccination process |
| 2008[97] | 34 | nGBM, rGBM | Grade IV | DCs vaccine | Time to death = 21.4 m in responders | Limited to grade 2 toxicities. 3% had enlarged lymph nodes. |
| 2010[98] | 8 | nGBM | Grade IV | DCs vaccine | PFS = 18 m<br>OS = 24 m | 12% grade 4 ischemic stroke. Other adverse events were limited to grade 2. |
| 2011[99] | 16 | nGBM, rGBM | Grade IV | DCs vaccine | OS = 12.7 m (for nGBM)<br>OS = 32.2 m (for rGBM) | No reports of severe constitutional symptoms or injection-site reactions. 52.9% of patients showed lymphopenia, and 47.1% of patients had elevated levels of serum AST/ALT. |
| 2011[100] | 23 | nGBM, rGBM | Grade IV | DCs vaccine | PFS = 15.9 m<br>OS = 31.4 m | No autoimmune reactions. Nausea/vomiting (14%), headache (5%), fatigue (14%), diarrhea (9%), fever (5%), and injection-site reaction (18%) were limited to grade 2 toxicities. |
| 2011[101] | 10 | nGBM | Grade IV | DCs vaccine | PFS = 9.5 m<br>OS = 28 m | 10% of patients had grade 2 unilateral neck pain. |
| 2012[102] | 13 | nGBM | Grade IV | DCs vaccine | PFS = 11.92 m<br>OS = 17 m | 15% of patients developed mild fever, and 8% of patients had red papules. All disappeared without special treatment. |
| 2012[103] | 18 | nGBM | Grade IV | DCs vaccine | PFS = 8.5 m<br>OS = 31.9 m | Transient abnormal liver function (6%), lymphopenia (6%), pancytopenia (6%), and increased intracranial pressure (6%). |
| 2012[104] | 77 | nGBM | Grade IV | DCs vaccine | PFS = 10.4 m<br>OS = 18.3 m | 39% of patients developed a series of adverse events (grade 3/4/5), including 23.3% of patients with hematological adverse events. |
| 2012[105] | 9 | rAA, rAO, rGBM | Grade III, IV | DCs vaccine | – | 11% of patients had mild hepatic dysfunction, which was limited to grade 2 toxicities. |
| 2013[106] | 21 | nGBM, rGBM | Grade IV | DCs vaccine | PFS = 16.9 m<br>OS = 38.4 m | Diarrhea (9%), fatigue (27%), flushing (9%), pruritus (18%), rash (18%), redness on skin (9%), and vomiting (9%) were observed and limited to grade 2 toxicities. |

(*continued on next page*)

**Table 1.** (*Continued*)

| Year | Pts No. | Disease | WHO grade | Vaccine | Result | Toxicity |
|---|---|---|---|---|---|---|
| 2013[107] | 7 | nGBM | Grade IV | DCs vaccine | PFS = 23.1 m<br>OS = 25.3 m | Grade 1 or 2 fatigue (86%), anorexia (71%), and headache (57%) were most often reported, and 14% of patients had grade 3 fatigue. |
| 2013[110] | 34 | nGBM, rGBM, newly diagnosed or recurrent anaplastic glioma | Grade III, IV | DCs vaccine | — | Flu-like symptoms, injection-site reactions, and lymphadenopathy were common and limited to grade 2 toxicities. Grade 3 adverse events (i.e., seizure) were rare and likely related to tumor progression. |
| 2014[108] | 13 | rGBM | Grade IV | DCs vaccine | 22% PR<br>56% SD | — |
| 2015[109] | 10 | rAA, rAO, rGBM | Grade III, IV | DCs vaccine | OS = 26 m | Injection-site reactions (100%), fever (60%), and fatigue (30%) were observed and were all limited to grade 2 toxicities. |
| 2017[111] | 31 | nGBM | Grade IV | DCs vaccine | PFS = 12.7 m<br>OS = 23.4 m | No adverse events or toxicities attributable to the DC vaccine were documented. |
| 2007[113] | 12 | nGBM, rGBM | Grade IV | Formalin -fixed tumor vaccine | OS = 10.7 m | No allergic dermatitis or anaphylaxis was observed. Skin irritation (including erythema, induration, and swelling of the inoculation site) and low-grade fever with mild fatigue (50%) were observed and limited to grade 2 toxicities. |
| 2014[114] | 24 | nGBM | Grade IV | Formalin -fixed tumor vaccine | PFS = 8.2 m<br>OS = 22.2 m | Skin irritation (68%, including local erythema, induration, and swelling at the injection site), fever (17%), appetite loss (4%), seizure (8%), and headache (8%) were observed and were all limited to grade 2 toxicities. |
| 2016[125] | 45 | nGBM | Grade IV | IMA950 | OS = 15.3 m | Injection-site reactions were the most common and limited to grade 2 toxicities. Grade 4 neutrophil count decrease (4%), grade 3 fatigue and anaphylaxis (4%) were observed. |
| 2010[122] | 97 | nGBM | Grade IV | Poly-ICLC | OS = 17.2 m | The most frequent grade 3–4 toxicities were neutropenia (20.6%), leukopenia (16.5%), and thrombocytopenia (9%). |

Notes: Pt. No.: patient number; nGBM: newly diagnosed glioblastoma multiforme; rGBM: recurrent glioblastoma multiforme; nAA: newly diagnosed anaplastic astrocytoma; rAA: recurrent anaplastic astrocytoma; rAO: recurrent anaplastic oligodendroglioma; BCG-CW: Bacillus Calmette-Guerin cell wall; HGG: high-grade glioma; NDV: Newcastle Disease Virus; CR: complete response; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

after vaccination, no associated clinical symptoms were observed.[48] In a subsequent phase II clinical trial (ACTIVATE), 18 patients with newly diagnosed EGFRvIII-expressing GBM received a PEP-3-KLH vaccination, and the median PFS and OS in the treatment group were 14.2 and 26 months, respectively, which were significantly longer than those in the control groups who were on TMZ (PFS: 6.3 months; OS: 15.0 months).[49] In a phase II clinical trial (ACT II) investigating PEP-3-KLH vaccination in 22 patients with newly diagnosed EGFRvIII-expressing GBM, the median PFS and OS were 15.2 and 23.6 months, respectively, which was also significantly longer than historical data (PFS: 6.3 months; OS: 15.0 months).[50] Furthermore, the authors observed that when patients were simultaneously treated with TMZ and vaccines, the dose intensity of TMZ caused lymphopenia, while the vaccine resulted in increased tumor-specific lymphocytosis.[50] No autoimmune reactions were documented in ACTIVATE or ACT II, and adverse events in the two trials were mostly limited to grade 2, most of which were related to injection-site reactions (e.g., erythema and pruritus). Some patients in ACT II had allergic reactions, including grade III skin and gastrointestinal allergic reactions.[49,50]

In 2015, a multicenter, single-arm phase II clinical trial (ACT III) investigated the efficacy of PEP-3-KLH plus standard adjuvant TMZ chemotherapy in 65 patients with newly diagnosed EGFRvIII-expressing GBM.[51] As the treatment progressed, the expression of the serum anti-EGFRvIII antibody increased significantly, but no T cell immune response was observed due to the cytotoxic effect of TMZ on immune cells.[51] The median PFS and OS were 12.3 and 24.6 months, respectively, which was similar to the results observed in ACTIVATE and ACT II.[51] The methylation status of the *MGMT* promoter, which was a predominant predictor of GBM prognosis and the response to TMZ,[52] also serves as a prognoses factor in ACT III.[51] A phase II clinical trial (ReACT) investigating PEP-3-KLH plus bevacizumab in patients with recurrent GBM reported an improved prognoses at the Society of Neuro-oncology Annual Meeting in 2015[53]. However, the article was withdrawn due to subsequent data analysis.[54,55] Adverse events in ACT III and ReACT were similar to those in ACTIVATE and ACT II, with mild-to-moderate injection-site reactions occurring in nearly all patients. Grade 3 or 4 events, including back pain, convulsion, headache, and hypertension, were relatively rare and limited to single patients.[51,53]

A multicenter, double-arm phase III clinical trial (ACT IV) investigating PEP-3-KLH was recently completed. This clinical trial enrolled 700 patients with newly diagnosed GBM. Patients in the treatment arm were treated with PEP-3-KLH plus TMZ, and individuals in the control arm were treated with KLH plus TMZ, with the patients' OS as the primary efficacy endpoint. However, at the Society of Neuro-oncology Annual Meeting in November 2016, Weller et al. reported a result of experimental failure. PEP-3-KLH was sufficiently safe in ACT IV, with 80% of patients showing grade 1 or 2 injection-site reactions (primarily erythema, pruritus, or rash). Fatigue, nausea, headache, and constipation also occurred in ≥20% of patients but were mostly limited to grade II toxicities. The patients showed a good humoral immune response, but compared to the control group, patients with minimum residual disease (MRD) and all intention-to-treat (ITT) patients exhibited only a small difference in the median OS (PEP-3-KLH vs. control: MRD: 20.1 months vs. 20 months; ITT: 17.4 months vs. 17.4 months). Only patients with bulky disease exhibited a significant difference in the 2-year OS between individuals who received a therapeutic regimen of PEP-3-KLH plus TMZ and the controls (30% vs. 19%, $P = 0.029$).[56] Weller et al. also found that the prognosis of the control group was significantly beyond expectations, which suggested there was an existing problem with the use of historical data as a control in the previous single-arm phase II clinical trials.[56] Although PEP-3-KLH exhibited sufficient safety in the clinical trial, its efficacy in the phase III clinical trial was unsatisfactory. The extent of tumor resection and the threshold for 'EGFRvIII-positive' criteria may be critical factors that impeded the effectiveness of the PEP-3-KLH vaccine.[57] Nevertheless, the current results of ACT IV failed to demonstrate the efficacy of the PEP-3-KLH vaccine, and subgroup analyses of ACT IV are necessary to determine the clinical role of the peptide vaccine.

The failure of ACT IV terminated the development of EGFRvIII-targeted peptide vaccine. Despite, EGFRvIII still serves as a tumor-specific antigen for GBM in many other immunotherapy studies, such as the antibody-drug conjugates (ADCs) AMG 595 and ABT-414, although does not constitute vaccination therapy. In addition, a phase I clinical trial of ADU-623, a live-attenuated Listeria Monocytogenes Strain expressing the EGFRvIII-NY-ESO-1 vaccine, is underway (NCT01967758).[58] The ongoing clinical trials investigating therapies with GBM vaccines are listed in Table 2.[58]

### Heat shock protein vaccines

Heat shock proteins (HSPs) are a family of proteins that respond to temperature changes and are widely distributed in bacteria, plants and animals. Due to their molecular chaperone activity, HSPs can inhibit the biomacromolecular denaturation induced by temperature, oxygen, and ions.[59] The molecular weights of HSPs range from 10 to over 100 kDa and are closely related to the locations of HSPs in the cell.[60] The expression of HSPs is regulated by many pathways,[61,62] and HSPs are overexpressed in many cancers and are associated with cancer cell proliferation, differentiation, invasion and metastasis.[62,63] HSPs can serve as therapeutic targets and prognostic indicators for several tumors.[63–65] HSP27, HSP72, HSP73/HSP70, and HSP90 have been confirmed to be overexpressed in glioblastoma cell lines and mouse models.[66,67]

HSP vaccines are composed of HSPs and autologous tumor-antigenic peptides.[68] Neither individual HSPs nor autologous tumor-antigenic peptides can elicit an immune response, but HSP-peptide complexes (HSPPCs) can mediate endocytosis by binding to APC membrane receptors and activating $CD4^+$ and $CD8^+$ T cells and certain APC signaling pathways to trigger immune responses to tumor-antigenic peptides by antigen presentation.[68,69] Compared to antigen-specific peptide vaccines (e.g., EGFRvIII-targeted vaccines), HSP vaccines can harbor multiple tumor antigens and respond to the poor outcomes induced by tumor heterogeneity and immunoediting, but their specificity for tumor antigens differs from that of antigen-specific peptide vaccines.[68]

**Table 2.** Ongoing clinical trials of the vaccination in glioblastoma.[58]

| Trail code | Disease | WHO grade | Vaccine | Phase | Start time | Status |
|---|---|---|---|---|---|---|
| NCT01967758 | rAA, rGBM | Grade III, IV | ADU-623 | Phase I | 2013 | Recruiting participants |
| NCT00905060 | nGBM | Grade IV | HSPPC-96 | Phase II | 2009 | Completed |
| NCT02122822 | nGBM | Grade IV | HSPPC-96 | Phase I | 2013 | Ongoing, but not recruiting participants |
| NCT02722512 | Newly diagnosed or recurrent pediatric HGG, ependymoma | — | HSPPC-96 | Phase I | 2016 | Recruiting participants |
| NCT01814813 | rGBM | Grade IV | HSPPC-96 | Phase II | 2013 | Recruiting participants |
| NCT03018288 | nGBM | Grade IV | HSPPC-96 | Phase II | 2017 | Recruiting participants |
| NCT02454634 | nAA, nAO, nGBM | Grade III, IV | Derived from IDH1 R132H | Phase I | 2015 | Recruiting participants |
| NCT02960230 | Newly diagnosed DIPG, newly diagnosed glioma | — | K27M peptide | Phase I | 2016 | Recruiting participants |
| NCT02455557 | nGBM | Grade IV | SurVaxM | Phase II | 2015 | Recruiting participants |
| NCT00045968 | nGBM | Grade IV | DCs vaccine (DCVax) | Phase III | 2006 | Ongoing, but not recruiting participants |
| NCT02146066 | nGBM, rGBM | Grade IV | DCs vaccine (DCVax) | Expanded access | 2014 | Currently available |
| NCT00890032 | rGBM | Grade IV | DCs vaccine (brain tumor stem cells mRNA loaded) | Phase I | 2009 | Completed |
| NCT01171469 | rAA, rGBM, recurrent medulloblastoma, recurrent ependymoma | — | DCs vaccine (brain tumor stem cells as antigen) | Phase I | 2010 | Completed |
| NCT02820584 | rGBM | Grade IV | DCs vaccine (tumor stem cell-loaded) | Phase I | 2016 | Not yet open |
| NCT01522820 | rGBM | Grade IV | DCs vaccine (fusion peptide loaded) | Phase I | 2012 | Completed |
| NCT02709616 | nGBM | Grade IV | DCs vaccine (tumor mRNA loaded) | Phase I/II | 2016 | Recruiting participants |
| NCT00639639 | nGBM | Grade IV | DCs vaccine (pp65 RNA loaded) | Phase I | 2006 | Ongoing, but not recruiting participants |
| NCT02465268 | nGBM | Grade IV | DCs vaccine (pp65 RNA loaded) | Phase II | 2016 | Recruiting participants |
| NCT02366728 | nGBM | Grade IV | DCs vaccine (pp65 RNA loaded) | Phase II | 2015 | Recruiting participants |
| NCT01567202 | nGBM, rGBM | Grade IV | DCs vaccine (autogeneic glioma stem-like cells (A2B5+) loaded) | Phase II | 2012 | Recruiting participants |
| NCT01204684 | nAA, rAA, nAO, rAO, nGBM, rGBM | Grade III, IV | DCs vaccine (tumor lysate loaded) | Phase II | 2010 | Ongoing, but not recruiting participants |
| NCT00626483 | nGBM | Grade IV | DCs vaccine (RNA loaded) | Phase I | 2007 | Ongoing, but not recruiting participants |
| NCT01957956 | nGBM | Grade IV | DCs vaccine (tumor lysate loaded) | Phase I | 2013 | Recruiting participants |
| NCT02049489 | rGBM | Grade IV | DCs vaccine (peptide loaded) | Phase I | 2016 | Ongoing, but not recruiting participants |
| NCT02649582 | nGBM | Grade IV | DCs vaccine (Wilms' tumor 1 mRNA loaded) | Phase I/II | 2015 | Recruiting participants |
| NCT01808820 | rAA, rGBM | Grade III, IV | DCs vaccine + tumor lysate boost | Phase I | 2013 | Recruiting participants |
| NCT02010606 | nGBM, rGBM | Grade IV | DCs vaccine (allogenic GBM stem-like cell lysate loaded) | Phase I | 2013 | Recruiting participants |
| NCT03014804 | rGBM | Grade IV | DCs vaccine (tumor lysate loaded)+ nivolumab | Phase II | 2017 | Not yet open |
| NCT01400672 | Recurrent brain stem GBM | Grade IV | Tumor lysate vaccine | Phase I | 2012 | Completed |
| NCT01759810 | rGBM | Grade IV | HSCs, DCs vaccine, CTLs | Phase II/III | 2012 | Enrolling by invitation |
| NCT02754362 | rGBM | Grade IV | TAA, Poly-ICLC, KLH, bevacizumab | Phase II | 2016 | Not yet open |
| NCT02078648 | rGBM | Grade IV | SL-701, poly-ICLC, bevacizumab | Phase I/II | 2014 | Ongoing, but not recruiting participants |
| NCT02546102 | nGBM | Grade IV | ICT-107 | Phase III | 2015 | Recruiting participants |
| NCT01920191 | nGBM | Grade IV | IMA950, Poly ICLC | Phase I/II | 2013 | Completed |
| NCT01222221 | nGBM | Grade IV | IMA950, GM-CSF | Phase I | 2010 | Completed |
| NCT02510950 | nGBM | Grade IV | Personalized peptide vaccine, Poly ICLC | Phase 0 | 2015 | Recruiting participants |

Notes: nGBM: newly diagnosed glioblastoma multiforme; rGBM: recurrent glioblastoma multiforme; nAA: newly diagnosed anaplastic astrocytoma; rAA: recurrent anaplastic astrocytoma; nAO: newly diagnosed anaplastic oligodendroglioma; rAO: recurrent anaplastic oligodendroglioma; HGG: high-grade glioma; TAA: tumor-associated antigen.

HSPPC vaccines mainly use HSPPC-96 (generic name: Vitespen), which is a composite of a tumor antigenic peptide and HSP glycoprotein-96, and clinical trials investigating its efficacy in renal cell carcinoma and melanoma have been performed.[70,71] The first clinical trial investigating HSPPC-96 in autologous tumor-derived peptides was reported in 2013 by Crane et al., who conducted a phase I clinical trial involving 12 patients with high-grade recurrent glioma.[72] The authors obtained non-necrotic tumor tissues from a sample of surgically resected tumors from each patient, and the HSPPC-96 protein was mixed with the tumor antigens, purified and developed into a vaccine.[72] No distinct toxicity was found in the patients after the vaccination, and a significant peripheral immune response specific to the tumor antigens was observed in the peripheral blood and tumor in 11 of 12 patients after the vaccination, which suggests that the HSPPC-96 vaccine was effective.[72]

In 2014, the first phase II clinical trial investigating the HSPPC-96 vaccine in GBM was completed. Investigators performed tumor resections in patients with recurrent GBM and produced the HSPPC-96 vaccine. The vaccine was administered every week for 4 weeks, followed by every 2 weeks thereafter until tumor recurrence; the median PFS and OS reached 19.1 and 42.6 weeks, respectively.[73] Compared to the efficacy of the bevacizumab/CCNU regimen for recurrent GBM (the median PFS and OS were 7.2–24.4 and 24.8–33.1 weeks, respectively),[74–76] HSPPC-96 displayed a certain degree of efficacy.[73] Investigators also studied the relationship between patients' absolute lymphocyte count and their prognosis and found that patients with an above-average lymphocyte count had a significantly better prognosis.[73] The patient's enrollment status (operation tolerance and no relapse during the first follow-up period) may also have affected the improvement in the prognosis.[73] The toxicity associated with the vaccine was minimal, mostly grade 1 or 2 injection-site reactions (erythema or induration). A single-arm, phase II clinical trial investigating HSPPC-96 (NCT00905060) was completed, but the results have not been published.[58] In this trial, 46 patients with newly diagnosed GBM were treated with HSPPC-96 plus TMZ, and the safety profile of this therapy and the patients' OS were considered the primary outcome measures. The results of this trial will provide an important reference for the application of HSPPC-96 and a phase III clinical trial.

To date, several ongoing phase I and II clinical trials have investigated HSPPC-96 (see Table 2).[58] The purpose of the phase I clinical trial (NCT02722512) was to determine the safety profile of the HSPPC-96 vaccine in 20 patients with newly diagnosed high-grade gliomas or recurrent resectable high-grade gliomas and ependymomas. The clinical trial NCT02122822, which was conducted in Beijing Tiantan Hospital in 2013, investigated the safety and effectiveness of an autologous gp96 treatment in 20 patients with GBM. Furthermore, a phase II clinical trial (NCT01814813) investigated the effectiveness of HSPPC-96 when combined with bevacizumab to treat patients with recurrent GBM. In this trial, 165 patients were assigned to the following three groups: HSPPC-96 + concomitant bevacizumab; HSPPC-96 with bevacizumab at progression; and bevacizumab alone, with the patients' OS serving as the primary outcome. The conclusion of this trial may provide insight into resolving the difficulty of treating recurrent GBM. Clinical trial NCT03018288 investigated whether HSPPC-96 improves the efficacy of radiotherapy, TMZ and pembrolizumab during combination therapy. In addition to HSPPC-96, HSP47, which is a glioma-associated antigen, could be a potential target for vaccine therapy.[77] HSP70, however, induced an immune response in a murine model.[78]

### *Peptide vaccines specific to other targets*

*IDH* mutation, the most important molecular marker in gliomas, has important effects on multiple pathways, metabolisms and prognoses of gliomas.[79] *IDH* mutations have been observed in approximately 80% of low-grade gliomas, of which the most common was the *R132H* mutation in *IDH1* (accounting for 70% of all *IDH* mutations).[79] In GBM, mutations in *IDH1* tend to indicate that the tumor has evolved from a low-grade glioma, and a small fraction of primary GBM cases exhibit mutations in *IDH1*.[80] In 2014, Schumacher et al. designed a 15-amino-acid polypeptide targeting *R132H* mutations in IDH1, found that it was present on MHC II and induced mutation-specific $CD4^+$ responses, and detected its antibody in a mouse model.[81] Two phase I clinical trials investigating the IDH1 peptide vaccine (NCT02193347 and NCT02454634) are ongoing, and their aims are to verify the safety of the vaccine and the immune response in patients with gliomas.[58]

Due to the specific location and limited treatment, patients with diffuse intrinsic pontine gliomas (DIPG) exhibit a median OS[82] of less than one year. Since the H3.3 K27M mutation acts as a molecular marker of DIPG, and H3.3-derived peptides that encompass this mutation can induce immune responses in HLA-$A2^+$ mice,[82,83] the phase I clinical trial NCT02960230 investigating H3.3 K27M is currently being conducted.[58] Another phase I clinical trial investigating a long peptide vaccine (SurVaxM) targeting Survivin (BIRC5) was recently completed,[84] and the phase II clinical trial NCT02455557 is underway.[58]

## Cell-based vaccines

Cell-based vaccines have long attracted researchers' attention. Autologous or allogeneic cells are re-injected into patients' bodies after in vitro modifications and trigger specific immune responses for tumor killing. This method mainly comprises dendritic cell (DC) vaccines, tumor cell vaccines and other cell-based vaccines.

### *DC vaccines*

DCs are cells with the strongest antigen presentation in the human body, which stimulate the conversion from innate immunity to acquired immunity. DCs are generated from hematopoietic stem cells, mature during migration to the lymph nodes, and play important roles in the immune response, differentiation, and exogenous/endogenous antigen presentation of lymphocytes.[85]

The production of DC vaccines includes the isolation of DCs from the patient's body, the loading of the tumor antigen, the maturation of DCs via cytokines, and the re-injection of DCs

into the patient's body.[86] Sipuleucel-T is the only approved APC vaccine for the treatment of tumors and has been shown to prolong the median OS in patients with prostate cancer by 4 months.[87] The culture of DCs for a DC vaccine for the treatment of GBM began with CD-14-positive monocytes isolated from the peripheral blood. The monocytes differentiated into immature DCs in the presence of granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4.[88] Tumor antigens, including polypeptides, RNAs, DNAs and tumor lysates, can be loaded onto immature DCs and presented on MHCs,[89] and they mature in the presence of many cytokines (e.g., GM-CSF, IL-4, TNF-$\alpha$ and IL-6).[88] In addition to tumor antigens, DCs loaded with Cytomegalovirus phosphoprotein 65 (pp65) RNA show a significant improvement in lymph node homing after the vaccine site is pre-conditioned with tetanus/diphtheria, which is a potent recall antigen.[90] The migration process and the effects of pp65 DCs are currently being investigated in phase I and II clinical trials (NCT00639639, NCT02465268, and NCT02366728).[58]

The use of DC vaccines in GBM was first reported in 2000 by Liau et al., who enabled a patient with recurrent brainstem glioblastoma to survive for 21 months.[91] In 2001 and 2004, Yu et al. loaded DCs with peptides eluted from the surface of autologous glioma cells and tumor lysates and separately investigated their safety profiles and immune responses,[92,93] and the median OS for the 8 recurrent GBM patients in the 2004 study reached 133 weeks.[93] In 2007, Okada *et al.* loaded tumor lysates into DCs and created a vaccine along with TFG-IL4-Neo-TK-transfected fibroblasts, which produced a better safety profile and a certain immune response.[94] Currently, the results of more than 10 phase I/II trials (see Table 1) have indicated the feasibility of DC vaccines, most of which were tumor lysate-pulsed DCs as well as DCs pulsed with polypeptides and nucleic acids.[95–111] Grade 1 or 2 toxicities, including fever (and/or flu-like symptoms), nausea and vomiting, and enlarged lymph nodes, were the most frequently observed adverse events in these studies. Lymphopenia and hematological adverse events were also reported in some studies and were most likely the result of maintenance TMZ therapy.[95–111] The clinical trial involving the largest number of patients was the HGG-2006 trial, which was published in 2012. Ardon et al. prepared DC vaccines with tumor lysates and treated 77 patients with newly diagnosed GBM.[104] The authors integrated the vaccination into the Stupp regimen as follows: the patients first received 60 Gy of radiotherapy for 6 weeks and daily administration of TMZ, followed by vaccination once per week for 4 weeks; the patients received a 5-day administration of TMZ and were vaccinated once during the subsequent six cycles of a 28-day course.[104] The median PFS and OS were 10.4 and 18.3 months in all patients, respectively, and patients with a lower EORTC RPA classification had significantly better outcomes than patients with a higher EORTC RPA classification; furthermore, patients with a methylated *MGMT* promoter had better outcomes.[104] The adverse events of HGG-2006 were more severe than in other DC vaccines studies, with 38 series adverse events (grade 3/4/5) found in 30 patients (39%), including 19 hematological adverse events in 18 patients.[104] In 2013, by comparing the research results by Prins et al. with the therapeutic effects of autologous tumor lysate (ATL)-pulsed DC vaccination and glioma-associated antigen (GAA) peptide-pulsed DC vaccination, the authors found that both vaccines caused similar adverse events and activated similar numbers of lymphocytes, but significantly higher frequencies of NK cells were activated by the GAA vaccine than those activated by the ATL vaccine.[110] However, patients treated with the ATL-pulsed DC vaccine had significantly better outcomes than those treated with the GAA peptide-pulsed DC vaccine (PFS = 18.1 vs. 9.6 months; OS = 34.4 vs. 14.5 months).[110]

Dozens of clinical trials investigating DC vaccines are ongoing (see Table 2), most of which are phase I or II trials investigating various DC loads.[58] The most noteworthy study is the DCVax clinical trial (a DC vaccine project by Northwest Biotherapeutics), which began in 2006[112] and has entered a phase III clinical trial NCT00045968.[58] In this trial, 348 patients with newly diagnosed GBM underwent surgical resection with concurrent radiotherapy and chemotherapy, and tumor lysate proteins were used to prepare DCVax(R)-L. The treatment cohort was vaccinated on days 0, 10, and 20 and on weeks 8, 16, 32, 48, 72, and 120, and PFS was measured as the primary outcome. Expanded access to DCVax is underway (NCT02146066), which will play a critical role in the application of the DC vaccine for the treatment of GBM.[58]

### *Tumor cell vaccines*

In addition to the re-injection of tumor antigen-loaded immune cells into patients' bodies, investigators have directly injected tumor lysates or fixed tumor cells (as antigens) into patients' bodies.[113,114] Ishikawa et al. reported re-injecting formalin-fixed GBM cells into patients' bodies to treat GBM after the standard Stupp regimen.[113,114] In one study, 24 patients were treated with nGBM and exhibited a median OS of 22.2 months.[114] The authors concluded that the patients with unchanged p53, higher MHC-I expression or a delayed-type hypersensitivity (DTH) response had better outcomes.[113,114] Clinical trial NCT01400672 is currently investigating the treatment effect of imiquimod/tumor lysate vaccine in brain stem glioma.[58]

### *Other cell-based vaccines*

Other immune cells or stem cells are also promising for the treatment of GBM. Bryukhovetskiy et al. reported that cancer stem cells (CSCs) were very difficult to destroy and could recruit other types of stem cells, and they proteomically identified an interaction between CSCs and hematopoietic stem cells (HSCs).[115,116] An animal model has confirmed this hypothesis,[117] and a clinical trial (NCT01759810) investigating concurrent HSCs, DC vaccines and cytotoxic lymphocytes in the treatment of GBM has been conducted.[58] In addition to cell-based vaccines, other cell-based immunotherapies, such as adoptive cellular therapy (ACT), have shown potential feasibility for clinical application.[118]

## Others

In addition to peptide and cell-based vaccines, immune adjuvants are sometimes used in immunotherapy to enhance

efficacy. Problems such as immunoediting must be overcome to allow immunotherapy to have a continuous effect.

### Immune adjuvants

An immune adjuvant is a substance that does not initially trigger an immune response but enhances the acquired immune response or changes the type of immune response. If injected with a vaccine, the body's immune response can be enhanced, and the seroconversion rate increases in populations with reduced responsiveness, which simultaneously allows for the use of smaller antigen doses and fewer immunizations.[119] Immune adjuvants also alter the type of immune response and increase the generation of memory lymphocytes, the speed of the initial response, and the specificity of the response.[119] Common immune adjuvants include the double-stranded RNA derivative Poly-ICLC, oligonucleotide chain CpG, and imiquimod for Toll-like receptors (TLRs) 3, 9 and 7/8.[120] In the treatment of GBM, oligonucleotide CpG has been confirmed to enhance the immune response in an animal model.[121] Moreover, poly-ICLC plus radiochemotherapy increased the median OS in patients with newly diagnosed GBM to 17.2 months,[122] and this treatment has been used in several clinical trials (NCT02754362, NCT02078648, NCT01920191, and NCT02510950). Imiquimod has been used in several clinical trials investigating DC and tumor cell vaccines (NCT01171469, NCT01204684, NCT01808820, and NCT01400672).[58]

### Immunoediting

Immunoediting refers to the remodeling of tumor cells to promote their motions, invasions and metastases while eliminating by the immune system.[123,124] The relationship between tumors and the immune system is believed to consist of the following three phases: during the 'elimination phase', the tumor cells are killed by the immune system; during the 'equilibrium phase', there is a state of equilibrium between the tumor cells and the immune system; and during the 'escape phase', the tumor cells evade the surveillance of the immune system and eventually form tumors.[123,124] Immunoediting is one of the greatest obstacle in tumor immunotherapy. In phase II clinical trials investigating the EGFRvIII-specific peptide vaccine PEP-3-KLH (ACTIVATE, ACT II and ACT III), 82%, 91.6% and 66.7% of patients lost the EGFRvIII mutation at recurrence, respectively, which suggests that these patients were no longer sensitive to the PEP-3-KLH vaccine.[49–51] Multitarget immunotherapy or multidrug therapy may be a solution to the immunoediting problem: clinical trial NCT02078648 investigated the feasibility of SL-701, poly-ICLC plus bevacizumab for the treatment of GBM; DCs loaded with six tumor-associated antigens (TAAs), including HER2, TRP-2, gp100, MAGE-1, IL13R$\alpha$2 and AIM-2, in the ICT-107 vaccine, which produced good phase I results (median PFS in newly diagnosed GBM was 16.9 months, and median OS in newly diagnosed GBM was 38.4 months) and is currently being investigated in a phase III clinical trial (NCT02546102).[58,106] Although clinical trials investigating the IMA950 multi-peptide vaccine plus poly-ICLC or GM-CSF in GBM are ongoing (NCT01920191 and NCT01222221),[58] a clinical trial in the UK investigating IMA950 did not find a difference in prognosis between multi-tumor-associated peptide responders and single-tumor-associated peptide responders.[125] The clinical trial NCT02510950 involved designing polypeptide vaccines for all individual patients to increase the intensity and specificity of the immune response, but this trial is still in the stage of dose exploration and safety experiments.[58] In addition to mixed polypeptides, researchers have proposed that tumor patients could be treated with vaccine therapy in combination with vaccine adjuvants and immune checkpoint-specific antibodies (e.g. ipilimumab for anti-CTLA-4 antibodies, pembrolizumab and nivolumab for anti-PD-1 antibodies, atezolizumab and avelumab for anti-PD-L1 antibodies),[126,127] and several clinical trials investigating combined therapies are currently ongoing (NCT01176474, NCT02515227, and NCT02897765).[58] However, no such clinical trials for the treatment of GBM have been conducted to date.

## Summary and prospects

GBM, which was once considered an immune-privileged site and harbored an immunosuppressive microenvironment, remains a cancer with an extremely poor prognosis and quality of life in patients. Immunotherapy is increasing in applicability as a therapeutic regimen for the treatment of GBM in addition to surgery, radiotherapy, chemotherapy and targeted therapy. The failure of the phase III clinical trial investigating EGFRvIII (ACT IV) set back the prospects for immunotherapy, although several phase I/II clinical trials investigating active immunotherapy (dominated by vaccinations) have shown some promising results. The study and popularization of immunotherapy, especially cell-based vaccines, requires robust infrastructure, including hospitals with sufficient experience in the management of glioma and research centers with adequate investigations in immunotherapy. In addition, expenses may be a barrier to the study and adoption of immunotherapy (especially cell-based vaccines) due to the vaccines being produced individually.

In addition to the resistance of vaccine therapy in GBM that is caused by the tumor heterogeneity and immunoediting, vaccine therapy continues to face many challenges. Patients require the surgical removal or biopsy of the lesion to identify their molecular pathology, prepare the vaccine, and increase the tumor response to the vaccination. Therefore, it is difficult to administer therapeutic vaccines to patients without indication based on surgery or biopsy. Molecular imaging can help identify molecular markers of the tumor to a certain extent (e.g., PET imaging of D-2HG levels to determine whether *IDH* mutations are present in the patient) and may help patients with surgical limitations in terms of vaccine therapy.

Chemotherapy was once thought to inhibit immune system function. However, researchers studying other tumors recently found that although TMZ chemotherapy significantly reduced the number of T lymphocytes, the ratio of $CD8^+$ T cells to total T cells increased.[128] TMZ chemotherapy also generated chemokines (e.g., CXCL9 and CXCL10), which resulted in significant T-cell accumulation in metastatic melanoma, but not in other cancers, such as skin tumors.[129] The complex relationship between chemotherapy and the immune system may also affect the efficacy of immunotherapy.

Despite the great success achieved in other cancers, immunotherapy for the treatment of glioblastoma continues to face difficulties. The phase III clinical trial CheckMate 143 (NCT02017717) failed to demonstrate improved OS with nivolumab (anti-PD-1 monoclonal antibody) compared to bevacizumab in patients with recurrent GBM, highlighting the obstacle of passive immunotherapy in glioblastoma.[130] Although non-phase III clinical trials investigating immunotherapy for GBM have succeeded, studies investigating immunotherapy will continue to be conducted (ongoing clinical trials investigating vaccination in GBM are summarized in Table 2). Two ongoing phase III clinical trials investigating GBM vaccines, i.e., the DCVax (NCT00045968 and NCT02146066) and ICT-107 vaccines (NCT02546102), as well as the ADC drug ABT-414 (NCT02343406 and NCT02573324) and the phase III clinical trial CheckMate 498 (NCT02617589, investigating nivolumab in combination with radiotherapy ± TMZ in patients with newly diagnosed GBM),[58] represent a silver lining for GBM immunotherapy. Combinations of different therapies, including combinations of different vaccination strategies, combinations of vaccinations with immune checkpoint blockade, and combinations of surgical resection, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, are possible future directions for GBM treatment.

## Disclosure of potential conflicts of interest

The authors report no conflict of interest.

## Funding

This work was supported by the National Health and Family Planning Commission of the People's Republic of China under Grant number 201402008; and Chinese Academy of Medical Sciences under Grant number 2016-I2M-2-001.

## References

1. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 world health organization classification of tumors of the central nervous system: a summary. Acta Neuropathol. 2016;131:803–20. doi:10.1007/s00401-016-1545-1. PMID:27157931.
2. Jiang T, Mao Y, Ma W, Mao Q, You Y, Yang X, Jiang C, Kang C, Li X, Chen L, et al. CGCG clinical practice guidelines for the management of adult diffuse gliomas. Cancer lett. 2016;375:263–73. doi:10.1016/j.canlet.2016.01.024. PMID:26966000.
3. McNeill KA. Epidemiology of Brain Tumors. Neurol Clin. 2016;34:981–98. doi:10.1016/j.ncl.2016.06.014. PMID:27720005.
4. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352:987–96. doi:10.1056/NEJMoa043330. PMID:15758009.
5. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005;352:987–96. doi:10.1056/NEJMoa043330. PMID:15758009.
6. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, Armstrong TS, Wefel JS, Won M, Blumenthal DT, et al. Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. J Clin Oncol. 2013;31:4085–91. doi:10.1200/JCO.2013.49.6968. PMID:24101040.
7. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, Colman H, Chakravarti A, Pugh S, Won M, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med. 2014;370:699–708. doi:10.1056/NEJMoa1308573. PMID:24552317.
8. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, Carpentier AF, Hoang-Xuan K, Kavan P, Cernea D, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med. 2014;370:709–22. doi:10.1056/NEJMoa1308345. PMID:24552318.
9. Delgado-Lopez PD, Corrales-Garcia EM. Survival in glioblastoma: a review on the impact of treatment modalities. Clin Transl Oncol. 2016;18:1062–71. doi:10.1007/s12094-016-1497-x.
10. Dictionary of Cancer Terms. Concept of immunotherapy. National Cancer Institute. [accessed 2017 May 25]. https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=45729.
11. Zhou Q, Wang Y, Ma W. The progress of immunotherapy for glioblastoma. Hum Vaccin Immunother. 2015;11:2654–8. doi:10.1080/21645515.2015.1081727.
12. Sehgal A, Berger MS. Basic concepts of immunology and neuroimmunology. Neurosurg Focus. 2000;9:e1. doi:10.3171/foc.2000.9.6.2. PMID:16817684.
13. Tambuyzer BR, Ponsaerts P, Nouwen EJ. Microglia: gatekeepers of central nervous system immunology. J leukoc Biol. 2009;85:352–70. doi:10.1189/jlb.0608385. PMID:19028958.
14. da Fonseca AC, Amaral R, Garcia C, Geraldo LH, Matias D, Lima FR. Microglia in cancer: For good or for bad? Adv Exp Med Biol. 2016;949:245–61. doi:10.1007/978-3-319-40764-7_12. PMID:27714693.
15. Goldmann J, Kwidzinski E, Brandt C, Mahlo J, Richter D, Bechmann I. T cells traffic from brain to cervical lymph nodes via the cribroid plate and the nasal mucosa. J leukoc Biol. 2006;80:797–801. doi:10.1189/jlb.0306176. PMID:16885505.
16. Carare RO, Hawkes CA, Weller RO. Afferent and efferent immunological pathways of the brain. Brain Behav Immun. 2014;36:9–14. doi:10.1016/j.bbi.2013.10.012.
17. Gieryng A, Pszczolkowska D, Walentynowicz KA, Rajan WD, Kaminska B. Immune microenvironment of gliomas. Lab Invest. 2017;97:498–518. doi:10.1038/labinvest.2017.19. PMID:28287634.
18. Chang AL, Miska J, Wainwright DA, Dey M, Rivetta CV, Yu D, Kanojia D, Pituch KC, Qiao J, Pytel P, et al. CCL2 produced by the glioma microenvironment is essential for the recruitment of regulatory T cells and myeloid-derived suppressor cells. Cancer Res. 2016;76:5671–82. doi:10.1158/0008-5472.CAN-16-0144. PMID:27530322.
19. Razavi SM, Lee KE, Jin BE, Aujla PS, Gholamin S, Li G. Immune evasion strategies of glioblastoma. Front Surg. 2016;3:11. doi:10.3389/fsurg.2016.00011. PMID:26973839.
20. Saggioro FP, Neder L, Stavale JN, Paixao-Becker AN, Malheiros SM, Soares FA, Pittella JE, Matias CC, Colli BO, Carlotti CG Jr, et al. Fas, FasL, and cleaved caspases 8 and 3 in glioblastomas: a tissue microarray-based study. Pathol Res Pract. 2014;210:267–73. doi:10.1016/j.prp.2013.12.012. PMID:24561085.
21. Nduom EK, Wei J, Yaghi NK, Huang N, Kong LY, Gabrusiewicz K, Ling X, Zhou S, Ivan C, Chen JQ, et al. PD-L1 expression and prognostic impact in glioblastoma. Neuro Oncol. 2016;18:195–205. doi:10.1093/neuonc/nov172. PMID:26323609.
22. De Felice F, Musio D, Cassese R, Gravina GL, Tombolini V. New approaches in glioblastoma multiforme: the potential role of immune-check point inhibitors. Curr Cancer Drug Targets. 2016;17:282–89. doi:10.2174/1568009616666160813183738.
23. D'Souza NM, Fang P, Logan J, Yang J, Jiang W, Li J. Combining radiation therapy with immune checkpoint blockade for central nervous system malignancies. Front Oncol. 2016;6:212. PMID:27774435.
24. Kren L, Slaby O, Muckova K, Lzicarova E, Sova M, Vybihal V, Svoboda T, Fadrus P, Lakomy R, Vanhara P, et al. Expression of immune-modulatory molecules HLA-G and HLA-E by tumor cells in glioblastomas: an unexpected prognostic significance? Neuropathology. 2011;31:129–34. doi:10.1111/j.1440-1789.2010.01149.x. PMID:20667016.

25. Wastowski IJ, Simoes RT, Yaghi L, Donadi EA, Pancoto JT, Poras I, Lechapt-Zalcman E, Bernaudin M, Valable S, Carlotti CG Jr, et al. Human leukocyte antigen-G is frequently expressed in glioblastoma and may be induced in vitro by combined 5-aza-2′-deoxycytidine and interferon-gamma treatments: results from a multicentric study. Am J Pathol. 2013;182:540–52. doi:10.1016/j.ajpath.2012.10.021. PMID:23219427.
26. Sorensen AG, Emblem KE, Polaskova P, Jennings D, Kim H, Ancukiewicz M, Wang M, Wen PY, Ivy P, Batchelor TT, et al. Increased survival of glioblastoma patients who respond to antiangiogenic therapy with elevated blood perfusion. Cancer Res. 2012;72:402–7. doi:10.1158/0008-5472.CAN-11-2464. PMID:22127927.
27. McCarthy EF. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. Iowa Orthop J. 2006;26:154–8. PMID:16789469.
28. Kresowik TP, Griffith TS. Bacillus Calmette-Guerin immunotherapy for urothelial carcinoma of the bladder. Immunotherapy. 2009;1:281–8. doi:10.2217/1750743X.1.2.281. PMID:20046960.
29. Bergquist BJ, Mahaley MS Jr, Steinbok P, Dudka L. Treatment of a brain tumor with BCG cell wall preparation. Surg Neurol. 1980;13:197–201. PMID:6245476.
30. Mahaley MS Jr, Bigner DD, Dudka LF, Wilds PR, Williams DH, Bouldin TW, Whitaker JN, Bynum JM. Immunobiology of primary intracranial tumors. Part 7: Active immunization of patients with anaplastic human glioma cells: a pilot study. J Neurosurg. 1983;59:201–7. doi:10.3171/jns.1983.59.2.0201. PMID:6864286.
31. Black KL, Ciacci JD, Ammirati M, Selch MT, Becker DP. Clinical trial of Serratia marcescens extract and radiation therapy in patients with malignant astrocytoma. J Clin Oncol. 1993;11:1746–50. doi:10.1200/JCO.1993.11.9.1746. PMID:8394880.
32. Washburn B, Schirrmacher V. Human tumor cell infection by Newcastle Disease Virus leads to upregulation of HLA and cell adhesion molecules and to induction of interferons, chemokines and finally apoptosis. Int J Oncol. 2002;21:85–93. PMID:12063554.
33. Freeman AI, Zakay-Rones Z, Gomori JM, Linetsky E, Rasooly L, Greenbaum E, Rozenman-Yair S, Panet A, Libson E, Irving CS, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol Ther. 2006;13:221–8. doi:10.1016/j.ymthe.2005.08.016. PMID:16257582.
34. De Bonis P, Albanese A, Lofrese G, de Waure C, Mangiola A, Pettorini BL, Pompucci A, Balducci M, Fiorentino A, Lauriola L, et al. Postoperative infection may influence survival in patients with glioblastoma: simply a myth? Neurosurgery. 2011;69:864–8;discussion 8-9. doi:10.1227/NEU.0b013e318222adfa. PMID:21900810.
35. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348:69–74. doi:10.1126/science.aaa4971. PMID:25838375.
36. Tsukahara T, Hirohashi Y, Kanaseki T, Nakatsugawa M, Kubo T, Sato N, Torigoe T. Peptide vaccination therapy: Towards the next generation. Pathol Int. 2016;66:547–53. doi:10.1111/pin.12438. PMID:27435148.
37. Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, Carotenuto A, De Feo G, Caponigro F, Salomon DS. Epidermal growth factor receptor (EGFR) signaling in cancer. Gene. 2006;366:2–16. doi:10.1016/j.gene.2005.10.018. PMID:16377102.
38. Nevoltris D, Lombard B, Dupuis E, Mathis G, Chames P, Baty D. Conformational nanobodies reveal tethered epidermal growth factor receptor involved in EGFR/ErbB2 predimers. ACS Nano. 2015;9:1388–99. doi:10.1021/nn505752u. PMID:25603171.
39. Bai H, Harmanci AS, Erson-Omay EZ, Li J, Coskun S, Simon M, Krischek B, Özduman K, Omay SB, Sorensen EA, et al. Integrated genomic characterization of IDH1-mutant glioma malignant progression. Nat Genet. 2016;48:59–66. doi:10.1038/ng.3457. PMID:26618343.
40. Heimberger AB, Suki D, Yang D, Shi W, Aldape K. The natural history of EGFR and EGFRvIII in glioblastoma patients. J Transl Med. 2005;3:38. doi:10.1186/1479-5876-3-38. PMID:16236164.
41. Westphal M, Heese O, Steinbach JP, Schnell O, Schackert G, Mehdorn M, Schulz D, Simon M, Schlegel U, Senft C, et al. A randomised, open label phase III trial with nimotuzumab, an anti-epidermal growth factor receptor monoclonal antibody in the treatment of newly diagnosed adult glioblastoma. Eur J Cancer. 2015;51:522–32. doi:10.1016/j.ejca.2014.12.019. PMID:25616647.
42. Moscatello DK, Ramirez G, Wong AJ. A naturally occurring mutant human epidermal growth factor receptor as a target for peptide vaccine immunotherapy of tumors. Cancer Res. 1997;57:1419–24. PMID:9108438.
43. Heimberger AB, Archer GE, Crotty LE, McLendon RE, Friedman AH, Friedman HS, Bigner DD, Sampson JH. Dendritic cells pulsed with a tumor-specific peptide induce long-lasting immunity and are effective against murine intracerebral melanoma. Neurosurgery. 2002;50:158–64;discussion 64-6. PMID:11844246.
44. Heimberger AB, Crotty LE, Archer GE, Hess KR, Wikstrand CJ, Friedman AH, Friedman HS, Bigner DD, Sampson JH. Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors. Clin Cancer Res. 2003;9:4247–54. PMID:14519652.
45. Schmittling RJ, Archer GE, Mitchell DA, Heimberger A, Pegram C, Herndon JE 2nd, Friedman HS, Bigner DD, Sampson JH. Detection of humoral response in patients with glioblastoma receiving EGFRvIII-KLH vaccines. J Immunol Methods. 2008;339:74–81. doi:10.1016/j.jim.2008.08.004. PMID:18775433.
46. Sampson JH, Schmittling RJ, Archer GE, Congdon KL, Nair SK, Reap EA, Desjardins A, Friedman AH, Friedman HS, Herndon JE 2nd, et al. A pilot study of IL-2Ralpha blockade during lymphopenia depletes regulatory T-cells and correlates with enhanced immunity in patients with glioblastoma. PloS One. 2012;7:e31046. doi:10.1371/journal.pone.0031046. PMID:22383993.
47. Saraswathula A, Reap EA, Choi BD, Schmittling RJ, Norberg PK, Sayour EJ, Herndon JE 2nd, Healy P, Congdon KL, Archer GE, et al. Serum elevation of B lymphocyte stimulator does not increase regulatory B cells in glioblastoma patients undergoing immunotherapy. Cancer Immunol Immunother. 2016;65:205–11. doi:10.1007/s00262-015-1784-3. PMID:26759007.
48. Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Herndon JE 2nd, Lally-Goss D, Paolino A, Reardon DA, Friedman AH, Friedman HS, et al. An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. Mol Cancer Ther. 2009;8:2773–9. doi:10.1158/1535-7163.MCT-09-0124. PMID:19825799.
49. Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, Gilbert MR, Herndon JE 2nd, McLendon RE, Mitchell DA, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. J Clin Oncol. 2010;28:4722–9. doi:10.1200/JCO.2010.28.6963. PMID:20921459.
50. Sampson JH, Aldape KD, Archer GE, Coan A, Desjardins A, Friedman AH, Friedman HS, Gilbert MR, Herndon JE, McLendon RE, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. Neuro Oncol. 2011;13:324–33. doi:10.1093/neuonc/noq157. PMID:21149254.
51. Schuster J, Lai RK, Recht LD, Reardon DA, Paleologos NA, Groves MD, Mrugala MM, Jensen R, Baehring JM, Sloan A, et al. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. Neuro Oncol. 2015;17:854–61. doi:10.1093/neuonc/nou348. PMID:25586468.
52. Thon N, Thorsteinsdottir J, Eigenbrod S, Schuller U, Lutz J, Kreth S, Belka C, Tonn JC, Niyazi M, Kreth FW. Outcome in unresectable glioblastoma: MGMT promoter methylation makes the difference. J Neurol. 2016;264:350–8. doi:10.1007/s00415-016-8355-1. PMID:27921166.
53. Reardon DA, Desjardins A, Schuster J, Tran DD, Fink KL, Nabors LB, Bota DA, Lukas RV, Desjardins A, Ashby LS, et al. ReACT: Long-Term survival from a randomized Phase II study of Rindopepimut (CDX-110) plus Bevacizumab in relapsed Glioblastoma. Neuro Oncol. 2015;17:v109–v. doi:10.1093/neuonc/nov218.08.
54. Gatson NT, Weathers SP, de Groot JF. ReACT Phase II trial: a critical evaluation of the use of rindopepimut plus bevacizumab to treat EGFRvIII-positive recurrent glioblastoma. CNS Oncol. 2016;5:11–26. doi:10.2217/cns.15.38. PMID:26670466.

55. Retraction. CNS Oncol. 2016;5:110. doi:10.2217/cns.16.1. PMID:26732311.
56. Weller M, Butowski N, Tran D, Recht L, Lim M, Hirte H, Ashby L, Mechtler L, Goldlust S, Drappatz FIJ, et al. ACT IV: An international, double-blind, Phase 3 trial of Rindopepimut in newly diagnosed, EGFRvIII-expressing Glioblastoma. Neuro Oncol. 2016;18: vi17–vi8. doi:10.1093/neuonc/now212.068.
57. Elsamadicy AA, Chongsathidkiet P, Desai R, Woroniecka K, Farber SH, Fecci PE, Sampson JH. Prospect of rindopepimut in the treatment of glioblastoma. Expert Opin Biol Ther. 2017;17:507–13. doi:10.1080/14712598.2017.1299705. PMID:28274144.
58. ClinicalTrials.gov. National Institutes of Health (NIH). [accessed 2017 May 25]. https://www.clinicaltrials.gov/.
59. Lindquist S, Craig EA. The heat-shock proteins. Annu Rev Genet. 1988;22:631–77. doi:10.1146/annurev.ge.22.120188.003215. PMID:2853609.
60. Jee H. Size dependent classification of heat shock proteins: a mini-review. J Exerc Rehabil. 2016;12:255–9. doi:10.12965/jer.1632642.321. PMID:27656620.
61. Jacob P, Hirt H, Bendahmane A. The heat shock protein/chaperone network and multiple stress resistance. Plant Biotechnol J. 2016;15:405–14. doi:10.1111/pbi.12659.
62. Calderwood SK, Gong J. Heat shock proteins promote cancer: It's a protection racket. Trends Biochem Sci. 2016;41:311–23. doi:10.1016/j.tibs.2016.01.003. PMID:26874923.
63. Ciocca DR, Calderwood SK. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones. 2005;10:86–103. doi:10.1379/CSC-99r.1.
64. Kaigorodova EV, Bogatyuk MV. Heat shock proteins as prognostic markers of cancer. Curr Cancer Drug Targets. 2014;14:713–26. doi:10.2174/1568009614666140926122846. PMID:25258164.
65. Shevtsov M, Multhoff G. Heat shock protein-peptide and HSP-based immunotherapies for the treatment of cancer. Front Immunol. 2016;7:171. doi:10.3389/fimmu.2016.00171. PMID:27199993.
66. Hermisson M, Strik H, Rieger J, Dichgans J, Meyermann R, Weller M. Expression and functional activity of heat shock proteins in human glioblastoma multiforme. Neurology. 2000;54:1357–65. doi:10.1212/WNL.54.6.1357. PMID:10746610.
67. Graner MW, Cumming RI, Bigner DD. The heat shock response and chaperones/heat shock proteins in brain tumors: surface expression, release, and possible immune consequences. J Neurosci. 2007;27:11214–27. doi:10.1523/JNEUROSCI.3588-07.2007. PMID:17942716.
68. Ampie L, Choy W, Lamano JB, Fakurnejad S, Bloch O, Parsa AT. Heat shock protein vaccines against glioblastoma: from bench to bedside. J Neuro Oncol. 2015;123:441–8. doi:10.1007/s11060-015-1837-7. PMID:26093618.
69. See AP, Pradilla G, Yang I, Han S, Parsa AT, Lim M. Heat shock protein-peptide complex in the treatment of glioblastoma. Expert Rev Vaccines. 2011;10:721–31. doi:10.1586/erv.11.49. PMID:21692695.
70. Wood CG, Mulders P. Vitespen: a preclinical and clinical review. Future Oncol. 2009;5:763–74. doi:10.2217/fon.09.46. PMID:19663726.
71. Tosti G, Cocorocchio E, Pennacchioli E, Ferrucci PF, Testori A, Martinoli C. Heat-shock proteins-based immunotherapy for advanced melanoma in the era of target therapies and immunomodulating agents. Expert Opin Biol Ther. 2014;14:955–67. doi:10.1517/14712598.2014.902928. PMID:24670226.
72. Crane CA, Han SJ, Ahn B, Oehlke J, Kivett V, Fedoroff A, Butowski N, Chang SM, Clarke J, Berger MS, et al. Individual patient-specific immunity against high-grade glioma after vaccination with autologous tumor derived peptides bound to the 96 KD chaperone protein. Clin Cancer Res. 2013;19:205–14. doi:10.1158/1078-0432.CCR-11-3358. PMID:22872572.
73. Bloch O, Crane CA, Fuks Y, Kaur R, Aghi MK, Berger MS, Butowski NA, Chang SM, Clarke JL, McDermott MW, et al. Heat-shock protein peptide complex-96 vaccination for recurrent glioblastoma: a phase II, single-arm trial. Neuro Oncol. 2014;16:274–9. doi:10.1093/neuonc/not203. PMID:24335700.
74. Clarke JL, Ennis MM, Yung WK, Chang SM, Wen PY, Cloughesy TF, Deangelis LM, Robins HI, Lieberman FS, Fine HA, et al. Is surgery at progression a prognostic marker for improved 6-month progression-free survival or overall survival for patients with recurrent glioblastoma? Neuro Oncol. 2011;13:1118–24. doi:10.1093/neuonc/nor110. PMID:21813511.
75. Gorlia T, Stupp R, Brandes AA, Rampling RR, Fumoleau P, Dittrich C, Campone MM, Twelves CC, Raymond E, Hegi ME, et al. New prognostic factors and calculators for outcome prediction in patients with recurrent glioblastoma: a pooled analysis of EORTC Brain Tumour Group phase I and II clinical trials. Eur J Cancer. 2012;48:1176–84. doi:10.1016/j.ejca.2012.02.004. PMID:22464345.
76. Heiland DH, Masalha W, Franco P, Machein MR, Weyerbrock A. Progression-free and overall survival in patients with recurrent Glioblastoma multiforme treated with last-line bevacizumab versus bevacizumab/lomustine. J Neuro Oncol. 2016;126:567–75. doi:10.1007/s11060-015-2002-z. PMID:26614518.
77. Wu ZB, Cai L, Qiu C, Zhang AL, Lin SJ, Yao Y, Xu J, Zhou LF. CTL responses to HSP47 associated with the prolonged survival of patients with glioblastomas. Neurology. 2014;82:1261–5. doi:10.1212/WNL.0000000000000290. PMID:24623841.
78. Shevtsov MA, Pozdnyakov AV, Mikhrina AL, Yakovleva LY, Nikolaev BP, Dobrodumov AV, Komarova EY, Meshalkina DA, Ischenko AM, Pitkin E, et al. Effective immunotherapy of rat glioblastoma with prolonged intratumoral delivery of exogenous heat shock protein Hsp70. Int J Cancer. 2014;135:2118–28. doi:10.1002/ijc.28858. PMID:24691976.
79. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, Kos I, Batinic-Haberle I, Jones S, Riggins GJ, et al. IDH1 and IDH2 mutations in gliomas. N Engl J Med. 2009;360:765–73. doi:10.1056/NEJMoa0808710. PMID:19228619.
80. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, et al. An integrated genomic analysis of human glioblastoma multiforme. Science. 2008;321:1807–12. doi:10.1126/science.1164382. PMID:18772396.
81. Schumacher T, Bunse L, Pusch S, Sahm F, Wiestler B, Quandt J, Menn O, Osswald M, Oezen I, Ott M, et al. A vaccine targeting mutant IDH1 induces antitumour immunity. Nature. 2014;512:324–7. doi:10.1038/nature13387. PMID:25043048.
82. Hennika T, Becher OJ. Diffuse intrinsic pontine glioma: Time for cautious optimism. J Child Neurol. 2016;31:1377–85. doi:10.1177/0883073815601495. PMID:26374787.
83. Hou Y, Kohanbash G, Okada K, Shrivastav S, Smith-Cohn M, Nicolaides T, Mueller S, Carcaboso A, Pollack I, Okada H. Novel and shared neoantigen for glioma T cell therapy derived from histone 3 variant H3.3 K27M mutation. J ImmunoTher Cancer. 2015;3:P445. doi:10.1186/2051-1426-3-S2-P445.
84. Fenstermaker RA, Ciesielski MJ, Qiu J, Yang N, Frank CL, Lee KP, Mechtler LR, Belal A, Ahluwalia MS, Hutson AD. Clinical study of a survivin long peptide vaccine (SurVaxM) in patients with recurrent malignant glioma. Cancer Immunol Immunother. 2016;65:1339–52. doi:10.1007/s00262-016-1890-x.
85. Mildner A, Jung S. Development and function of dendritic cell subsets. Immunity. 2014;40:642–56. doi:10.1016/j.immuni.2014.04.016. PMID:24837101.
86. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer. 2012;12:265–77. doi:10.1038/nrc3258. PMID:22437871.
87. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363:411–22. doi:10.1056/NEJMoa1001294. PMID:20818862.
88. Nair S, Archer GE, Tedder TF. Isolation and generation of human dendritic cells. In: Coligan JE, Bierer B, Margulies DH, Shevach EM, Strober W, eds. Curr Protoc in Immunol. Milton (Australia): Wiley, 2012;Chapter 7:Unit7.32. doi:10.1002/0471142735.im0732s99. PMID:23129155.
89. Strome SE, Voss S, Wilcox R, Wakefield TL, Tamada K, Flies D, Chapoval A, Lu J, Kasperbauer JL, Padley D, et al. Strategies for

antigen loading of dendritic cells to enhance the antitumor immune response. Cancer Res. 2002;62:1884–9. PMID:11912169.
90. Mitchell DA, Batich KA, Gunn MD, Huang MN, Sanchez-Perez L, Nair SK, Congdon KL, Reap EA, Archer GE, Desjardins A, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. Nature. 2015;519:366–9. doi:10.1038/nature14320. PMID:25762141.
91. Liau LM, Black KL, Martin NA, Sykes SN, Bronstein JM, Jouben-Steele L, Mischel PS, Belldegrun A, Cloughesy TF. Treatment of a patient by vaccination with autologous dendritic cells pulsed with allogeneic major histocompatibility complex class I-matched tumor peptides. Case Report. Neurosurg Focus. 2000;9:e8.
92. Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, Yong WH, Incardona F, Thompson RC, Riedinger MS, et al. Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. Cancer Res. 2001;61:842–7. PMID:11221866.
93. Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ. Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. Cancer Res. 2004;64:4973–9. doi:10.1158/0008-5472.CAN-03-3505. PMID:15256471.
94. Okada H, Lieberman FS, Walter KA, Lunsford LD, Kondziolka DS, Bejjani GK, Hamilton RL, Torres-Trejo A, Kalinski P, Cai Q, et al. Autologous glioma cell vaccine admixed with interleukin-4 gene transfected fibroblasts in the treatment of patients with malignant gliomas. J Transl Med. 2007;5:67. doi:10.1186/1479-5876-5-67. PMID:18093335.
95. Liau LM, Prins RM, Kiertscher SM, Odesa SK, Kremen TJ, Giovannone AJ, Lin JW, Chute DJ, Mischel PS, Cloughesy TF, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clin Cancer Res. 2005;11:5515–25. doi:10.1158/1078-0432.CCR-05-0464. PMID:16061868.
96. Walker DG, Laherty R, Tomlinson FH, Chuah T, Schmidt C. Results of a phase I dendritic cell vaccine trial for malignant astrocytoma: potential interaction with adjuvant chemotherapy. J Clin Neurosci. 2008;15:114–21. doi:10.1016/j.jocn.2007.08.007. PMID:18083572.
97. Wheeler CJ, Black KL, Liu G, Mazer M, Zhang XX, Pepkowitz S, Goldfinger D, Ng H, Irvin D, Yu JS. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. Cancer Res. 2008;68:5955–64. doi:10.1158/0008-5472.CAN-07-5973. PMID:18632651.
98. Ardon H, Van Gool S, Lopes IS, Maes W, Sciot R, Wilms G, Demaerel P, Bijttebier P, Claes L, Goffin J, et al. Integration of autologous dendritic cell-based immunotherapy in the primary treatment for patients with newly diagnosed glioblastoma multiforme: a pilot study. J Neuro Oncol. 2010;99:261–72. doi:10.1007/s11060-010-0131-y. PMID:20146084.
99. Chang CN, Huang YC, Yang DM, Kikuta K, Wei KJ, Kubota T, Yang WK. A phase I/II clinical trial investigating the adverse and therapeutic effects of a postoperative autologous dendritic cell tumor vaccine in patients with malignant glioma. J Clin Neurosci. 2011;18:1048–54. doi:10.1016/j.jocn.2010.11.034. PMID:21715171.
100. Prins RM, Soto H, Konkankit V, Odesa SK, Eskin A, Yong WH, Nelson SF, Liau LM. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. Clin Cancer Res. 2011;17:1603–15. doi:10.1158/1078-0432.CCR-10-2563. PMID:21135147.
101. Fadul CE, Fisher JL, Hampton TH, Lallana EC, Li Z, Gui J, Szczepiorkowski ZM, Tosteson TD, Rhodes CH, Wishart HA, et al. Immune response in patients with newly diagnosed glioblastoma multiforme treated with intranodal autologous tumor lysate-dendritic cell vaccination after radiation chemotherapy. J Immunother. 2011;34:382–9. doi:10.1097/CJI.0b013e318215e300. PMID:21499132.
102. Jie X, Hua L, Jiang W, Feng F, Feng G, Hua Z. Clinical application of a dendritic cell vaccine raised against heat-shocked glioblastoma. Cell Biochem Biophys. 2012;62:91–9. doi:10.1007/s12013-011-9265-6. PMID:21909820.
103. Cho DY, Yang WK, Lee HC, Hsu DM, Lin HL, Lin SZ, Chen CC, Harn HJ, Liu CL, Lee WY, et al. Adjuvant immunotherapy with whole-cell lysate dendritic cells vaccine for glioblastoma multiforme: a phase II clinical trial. World Neurosurg. 2012;77:736–44. doi:10.1016/j.wneu.2011.08.020. PMID:22120301.
104. Ardon H, Van Gool SW, Verschuere T, Maes W, Fieuws S, Sciot R, Wilms G, Demaerel P, Goffin J, Van Calenbergh F, et al. Integration of autologous dendritic cell-based immunotherapy in the standard of care treatment for patients with newly diagnosed glioblastoma: results of the HGG-2006 phase I/II trial. Cancer Immunol Immunother. 2012;61:2033–44. doi:10.1007/s00262-012-1261-1. PMID:22527250.
105. Akiyama Y, Oshita C, Kume A, Iizuka A, Miyata H, Komiyama M, Ashizawa T, Yagoto M, Abe Y, Mitsuya K, et al. alpha-type-1 polarized dendritic cell-based vaccination in recurrent high-grade glioma: a phase I clinical trial. BMC Cancer. 2012;12:623. doi:10.1186/1471-2407-12-623. PMID:23270484.
106. Phuphanich S, Wheeler CJ, Rudnick JD, Mazer M, Wang H, Nuno MA, Richardson JE, Fan X, Ji J, Chu RM, et al. Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. Cancer Immunol Immunother. 2013;62:125–35. doi:10.1007/s00262-012-1319-0. PMID:22847020.
107. Vik-Mo EO, Nyakas M, Mikkelsen BV, Moe MC, Due-Tonnesen P, Suso EM, Sæbøe-Larssen S, Sandberg C, Brinchmann JE, Helseth E, et al. Therapeutic vaccination against autologous cancer stem cells with mRNA-transfected dendritic cells in patients with glioblastoma. Cancer Immunol Immunother. 2013;62:1499–509. doi:10.1007/s00262-013-1453-3. PMID:23817721.
108. Hunn MK, Bauer E, Wood CE, Gasser O, Dzhelali M, Ancelet LR, Mester B, Sharples KJ, Findlay MP, Hamilton DA, et al. Dendritic cell vaccination combined with temozolomide retreatment: results of a phase I trial in patients with recurrent glioblastoma multiforme. J Neuro Oncol. 2015;121:319–29. doi:10.1007/s11060-014-1635-7. PMID:25366363.
109. Sakai K, Shimodaira S, Maejima S, Udagawa N, Sano K, Higuchi Y, Koya T, Ochiai T, Koide M, Uehara S, et al. Dendritic cell-based immunotherapy targeting Wilms' tumor 1 in patients with recurrent malignant glioma. J Neurosurg. 2015;123:989–97. doi:10.3171/2015.1.JNS141554. PMID:26252465.
110. Prins RM, Wang X, Soto H, Young E, Lisiero DN, Fong B, Everson R, Yong WH, Lai A, Li G, et al. Comparison of glioma-associated antigen peptide-loaded versus autologous tumor lysate-loaded dendritic cell vaccination in malignant glioma patients. J Immunother. 2013;36:152–7. PMID:23377664.
111. Inogés S, Tejada S, de Cerio AL-D, Pérez-Larraya JG, Espinós J, Idoate MA, Domínguez PD, de Eulate RG, Aristu J, Bendandi M, et al. A phase II trial of autologous dendritic cell vaccination and radiochemotherapy following fluorescence-guided surgery in newly diagnosed glioblastoma patients. J Transl Med. 2017;15:104. doi:10.1186/s12967-017-1202-z. PMID:28499389.
112. Hdeib A, Sloan AE. Dendritic cell immunotherapy for solid tumors: evaluation of the DCVax(R) platform in the treatment of glioblastoma multiforme. CNS Oncol. 2015;4:63–9. doi:10.2217/cns.14.54. PMID:25768330.
113. Ishikawa E, Tsuboi K, Yamamoto T, Muroi A, Takano S, Enomoto T, Matsumura A, Ohno T. Clinical trial of autologous formalin-fixed tumor vaccine for glioblastoma multiforme patients. Cancer Sci. 2007;98:1226–33. doi:10.1111/j.1349-7006.2007.00518.x. PMID:17517052.
114. Ishikawa E, Muragaki Y, Yamamoto T, Maruyama T, Tsuboi K, Ikuta S, Hashimoto K, Uemae Y, Ishihara T, Matsuda M, et al. Phase I/IIa trial of fractionated radiotherapy, temozolomide, and autologous formalin-fixed tumor vaccine for newly diagnosed glioblastoma. J Neurosurg. 2014;121:543–53. doi:10.3171/2014.5.JNS132392. PMID:24995786.
115. Bryukhovetskiy A, Shevchenko V, Kovalev S, Chekhonin V, Baklaushev V, Bryukhovetskiy I, Zhukova M. To the novel paradigm of proteome-based cell therapy of tumors: through comparative proteome mapping of tumor stem cells and tissue-specific stem cells of

humans. Cell Transplant. 2014;23(Suppl 1):S151–70. doi:10.3727/096368914X684907. PMID:25303679.
116. Bryukhovetskiy IS, Mischenko PV, Tolok EV, Zaitcev SV, Khotimchenko YS, Bryukhovetskiy AS. Directional migration of adult hematopoeitic progenitors to C6 glioma in vitro. Oncol Lett. 2015;9:1839–44. PMID:25789053.
117. Bryukhovetskiy IS, Dyuizen IV, Shevchenko VE, Bryukhovetskiy AS, Mischenko PV, Milkina EV, Khotimchenko YS. Hematopoietic stem cells as a tool for the treatment of glioblastoma multiforme. Mol Med Rep. 2016;14:4511–20. doi:10.3892/mmr.2016.5852. PMID:27748891.
118. Kuramitsu S, Yamamichi A, Ohka F, Motomura K, Hara M, Natsume A. Adoptive immunotherapy for the treatment of glioblastoma: progress and possibilities. Immunotherapy. 2016;8:1393–404. doi:10.2217/imt-2016-0076. PMID:28000534.
119. Coffman RL, Sher A, Seder RA. Vaccine adjuvants: putting innate immunity to work. Immunity. 2010;33:492–503. doi:10.1016/j.immuni.2010.10.002. PMID:21029960.
120. Khong H, Overwijk WW. Adjuvants for peptide-based cancer vaccines. J Immunother Cancer. 2016;4:56. doi:10.1186/s40425-016-0160-y. PMID:27660710.
121. Ouyang M, White EE, Ren H, Guo Q, Zhang I, Gao H, Yanyan S, Chen X, Weng Y, Da Fonseca A, et al. Metronomic doses of temozolomide enhance the efficacy of carbon nanotube CpG immunotherapy in an invasive glioma model. PloS One. 2016;11:e0148139. doi:10.1371/journal.pone.0148139. PMID:26829221.
122. Rosenfeld MR, Chamberlain MC, Grossman SA, Peereboom DM, Lesser GJ, Batchelor TT, Desideri S, Salazar AM, Ye X. A multi-institution phase II study of poly-ICLC and radiotherapy with concurrent and adjuvant temozolomide in adults with newly diagnosed glioblastoma. Neuro Oncol. 2010;12:1071–7. doi:10.1093/neuonc/noq071. PMID:20615924.
123. Ribatti D. The concept of immune surveillance against tumors. The first theories. Oncotarget. 2016;8:7175–80.
124. O' Reilly E, Tirincsi A, Logue SE, Szegezdi E. The janus face of death receptor signaling during tumor immunoediting. Front Immunol. 2016;7:446. doi:10.3389/fimmu.2016.00446. PMID:27843441.
125. Rampling R, Peoples S, Mulholland PJ, James A, Al-Salihi O, Twelves CJ, McBain C, Jefferies S, Jackson A, Stewart W, et al. A cancer research uk first time in human phase I trial of IMA950 (Novel multipeptide therapeutic vaccine) in patients with newly diagnosed glioblastoma. Clin Cancer Res. 2016;22:4776–85. doi:10.1158/1078-0432.CCR-16-0506. PMID:27225692.
126. Sharma P, Allison JP. The future of immune checkpoint therapy. Science. 2015;348:56–61. doi:10.1126/science.aaa8172. PMID:25838373.
127. Hirayama M, Nishimura Y. The present status and future prospects of peptide-based cancer vaccines. Int Immunol. 2016;28:319–28. doi:10.1093/intimm/dxw027. PMID:27235694.
128. Iversen TZ. Immune modulations during chemoimmunotherapy & novel vaccine strategies–in metastatic melanoma and non small-cell lung cancer. Dan Med J. 2013;60:B4774. PMID:24355457.
129. Tan KW, Evrard M, Tham M, Hong M, Huang C, Kato M, Prevost-Blondel A, Donnadieu E, Ng LG, Abastado JP. Tumor stroma and chemokines control T-cell migration into melanoma following Temozolomide treatment. Oncoimmunology. 2015;4:e978709. doi:10.4161/2162402X.2014.978709. PMID:25949877.
130. Reardon DA, Omuro A, Brandes AA, Rieger J, Wick A, Sepulveda J, Phuphanich S, de Souza P, Ahluwalia MS, Vlahovic MLG, et al. Randomized Phase 3 Study Evaluating the Efficacy and Safety of Nivolumab vs Bevacizumab in Patients With Recurrent Glioblastoma: CheckMate 143. 5th Quadrennial Meeting of the World Federation of Neuro-Oncology Societies; 2017 May 7 [accessed 2017 May 25]. http://www.abstractsonline.com/pp8/#!/4277/presentation/151.