Published in final edited form as:

Expert Opin Drug Saf. 2017 March ; 16(3): 277–287. doi:10.1080/14740338.2017.1273898.

The Safety of Available Immunotherapy for the Treatment of Glioblastoma

S. Harrison Farber, BS^{1,2,*}, Aladine A. Elsamadicy, BE^{1,2,*}, Fatih Atik, MD^{1,2}, Carter M. Suryadevara, BS^{1,2,3}, Pakawat Chongsathidkiet, MD^{1,2,3}, Peter E. Fecci, MD, PhD^{1,2,3}, and John H. Sampson, MD, PhD^{1,2,3}

¹Duke Brain Tumor Immunotherapy Program, Department of Neurosurgery, Duke University Medical Center, Durham, NC 27710

²The Preston Robert Tisch Brain Tumor Center, Duke University Medical Center, Durham, NC 27710

³Department of Pathology, Duke University Medical Center, Durham, NC 27710

Abstract

Introduction—Glioblastoma (GBM) is the most common malignant primary brain tumor in adults. Current standard of care involves maximal surgical resection combined with adjuvant chemoradiation. Growing support exists for a role of immunotherapy in treating these tumors with the goal of targeted cytotoxicity. Here we review data on the safety for current immunotherapies being tested in GBM.

Areas covered—Safety data from published clinical trials, including ongoing clinical trials were reviewed. Immunotherapeutic classes currently under investigation in GBM include various vaccination strategies, adoptive T cell immunotherapy, immune checkpoint blockade, monoclonal antibodies, and cytokine therapies. Trials include children, adolescents, and adults with either primary or recurrent GBM.

Expert commentary—Based on the reviewed clinical trials, the current immunotherapies targeting GBM are safe and well-tolerated with minimal toxicities which should be noted. However, the gains in patient survival have been modest. A safe and well-tolerated combinatory immunotherapeutic approach may be essential for optimal efficacy towards GBM.

Keywords

Glioblastoma; immunotherapy; clinical trials; safety; adverse events

Correspondence should be addressed to: John H. Sampson, Duke Brain Tumor Immunotherapy Program, Department of Neurosurgery; Duke University Medical Center; Box 3050, Durham, NC 27710. Telephone: (919) 684-3118, Fax: (919)-684-9045, John.Sampson@duke.edu.

*These authors have contributed equally

Work was completed in Durham, NC, USA.

1.0 Introduction

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults, representing almost half of all malignant primary brain tumor diagnoses in the United States (US).¹ GBM, defined as a World Health Organization (WHO) IV astrocytoma, is the deadliest of the histologic subtypes of malignant glial neoplasms. Despite treatment advances over the last few decades, only modest improvements in survival have been realized for these patients. Current standard of care therapies, including maximal gross surgical resection followed by adjuvant radiation and chemotherapy with temozolomide (TMZ), result in a overall survival (OS) of 18 to 21 months, with less than 10% of patients alive at five years.^{2–6} This dismal prognosis for patients with GBM underscores the critical need for novel treatments.

Patient age, performance and mental status, and extent of surgical resection are significant patient- and treatment-related prognostic factors for patients with high grade gliomas; whereas tumor grade and histology are the most significant tumor-related factors for survival.⁷ WHO classification incorporates a multitude of tumor characteristics- cellular morphology, nuclear atypia, mitotic activity, microvascular proliferation, and necrosis- to determine the histological and grading diagnosis of malignant gliomas.⁸ However, recently gliomas are being grouped and categorized based upon distinct classifications of molecular alterations that may more accurately predict survival. These alterations include codeletion of chromosome arms 1p and 19q, somatic mutations of the Krebs cycle enzymes *isocitrate dehydrogenase* (IDH) 1/2, and mutation of the *telomerase reverse transcriptase* (TERT) promotor.⁹

Immunotherapy has garnered increasing support in recent years as a treatment for brain tumors. The immune system has a tremendous capacity for targeting and eliminating tumor cells while sparing normal tissues. Following decades of pre-clinical development and success in other solid and blood-borne cancers, many immunotherapies are now being investigated in patients with GBM. These immunotherapeutic classes include vaccines, adoptive T cell therapy, chimeric antigen receptor (CAR) T cells, immune checkpoint blockade, monoclonal antibodies, and cytokine therapy.¹⁰ As most of these therapies activate the immune system, common side effects related to them are adverse autoreactive immune responses. Minor reactions associated with these treatments reported to date consist of injection-site erythema, pruritus, flushing, headache, and fatigue. Following the increased testing of these therapies in the clinical arena, we continue to gain a better understanding of these and other safety concerns. In this review, we analyze the safety data for currently available immunotherapies for GBM and provide recommendations.

2.0 Vaccine Therapy

A vaccine is a form of active immunotherapy that stimulates an adaptive immune response against target antigens, with the potential for long-term immunologic memory. Ideal target antigens include both tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs). By definition, TAAs are expressed not only by tumor, but also by normal cells. They may, however, be over-expressed by tumor cells creating a targetable, albeit inherently

non-specific, antigen.¹¹ In contrast, TSAs are solely expressed by tumor cells and not expressed on normal tissues. They provide the potential to induce a more potent and specific immune response than TAAs, making them the highest priority targets.^{11,12} TSAs are unfortunately quite rare for solid tumors, particularly ones that are homogeneously expressed. Known TAAs expressed in GBM include IL-13R α 2, HER-2, gp100, survivin, WT1, TRP2, EphA2, SOX2, SOX11, MAGE-A1, MAGE-A3, AIM2, SART1, and tenascin, while examples of TSAs in GBM are EGFRvIII, IDH-1/2 mutations (e.g. R132H), and CMV proteins. Current strategies discussed here include peptide vaccines, dendritic cell vaccines, and heat shock-protein vaccines (See Table 1 for current immunotherapy clinical trials for GBM).

2.1 Peptide Vaccines

Peptide vaccination is a targeted approach in which a chosen protein or peptide antigen is administered directly, often in combination with an adjuvant (e.g. keyhole limpet hemocyanin [KLH]) to increase immunogenicity.^{10,13} Several preclinical CNS tumor models have demonstrated success for this approach.^{14–16} With immune responses directed towards single tumor antigens, the theoretical risk of yielding off-target toxicities against normal tissues is low. Peptide vaccines have undergone the most study in GBM. However, identifying novel GBM-specific antigens to serve as immunotherapeutic targets has been challenging.

One of the most promising and most widely studied TSAs in GBM is the epidermal growth factor receptor variant III (EGFRvIII) mutation. EGFRvIII is a tumor-specific epitope that contains an in-frame deletion of exons 2–7 that is found in approximately 30%–35% of primary GBM patients.^{12,17} Rindopepimut (CDX-110) is a 14mer amino acid peptide that spans the EGFRvIII mutation site conjugated with KLH. In a small single-arm phase II multicenter trial (“ACTIVATE”), 18 patients with newly diagnosed GBM completing standard of care therapy were vaccinated with rindopepimut combined with granulocyte-macrophage colony-stimulating factor (GM-CSF) resulting in a median OS of 26 months.¹⁸ Overall, this vaccine was well-tolerated with minimal toxicity. Adverse events observed in this study were all grade 2 or less occurring in few patients and included numbness, pain, constitutional or GI symptoms, rash, and allergic reactions.¹⁸ Similarly, in another phase II study, “ACT II,” rindopepimut and GM-CSF were given with either standard dose or dose-intensified TMZ to 22 newly diagnosed GBM patients. No difference in survival was seen between the two TMZ cohorts. Overall, the therapy was well-tolerated and adverse events in these patients were again minimal. Grade 3 lymphopenia was noted in the dose-intensified cohort.¹⁹

Following these studies, a randomized phase II trial, “ACT III,” of 65 newly diagnosed EGFRvIII-positive patients with GBM was undertaken. Patients again received rindopepimut combined with GM-CSF following tumor resection and TMZ chemoradiation. The median OS was 24.6 months. Rindopepimut was well tolerated with no additive toxicity over time.²⁰ Observed toxicities were minor and included injection site-erythema, pruritus, swelling, pain, rash, bruising, fatigue, nausea, headache, and hypokalemia.²⁰ Given the wide range of these symptoms, these adverse effects are likely attributed to both a local and

systemic inflammatory response, rather than the antigen-specific effect of the vaccine itself. For example, the most common minor toxicity was injection site-erythema which can be attributed to local reactivity of immune cells harbored within the skeletal muscle.²¹

Based on the positive results of these studies, “ACT IV” (NCT01480479), an international, randomized, double-blind, controlled phase III clinical trial was performed. In ACT IV, all patients with newly diagnosed, surgically resected, EGFRvIII-positive GBM were administered TMZ, with half the patients randomly assigned to receive rindopepimut and half assigned to receive the control KLH. This trial was recently suspended due to an interim analysis by the Data Safety and Monitoring Board which indicated the study would not reach statistical significance for OS. Interestingly, the rindopepimut group performed comparably to prior studies (median OS 20.4 months), but the control arm exceeded expectations (median OS 21.1 months). Further sub-group analyses are ongoing and will be important in assessing the results of this trial.

Another promising TSA is the IDH-1 R132H mutation. The prevalence of this mutation in primary GBM (~6%) is much lower than that in low grade glioma and secondary GBM (>50%).²² Unlike EGFRvIII, investigation of IDH-1 in humans is relatively new. Currently there are two phase I clinical trials determining the safety and tolerability of an IDH-1 (R132H) peptide vaccine in patients with either IDH1-R132H-mutated Grade III–IV gliomas (“NOA-16,” NCT02454634) or in recurrent grade II gliomas (“RESIST,” NCT02193347). A pre-clinical study demonstrated anti-tumor efficacy in a human MHC class II transgenic orthotopic mouse model of glioma.²³ In this study the vaccine was well tolerated with only a few isolated adverse events, such as extramedullary hematopoiesis.²³

Other work has targeted multiple glioma-associated antigens (GAAs) in GBM and pediatric gliomas. Pollack *et al.* tested GAA peptide vaccination in children with glioma targeting the epitopes EphA2, IL-13Ra2, and survivin given with poly I:C (NCT01130077). This study’s primary objective of safety found that the therapy was well-tolerated. No dose-limiting CNS toxicity or instances of grade 3 or higher systemic toxicity or autoimmunity were encountered in this study.²⁴ Other work by this group loading DCs with synthetic GAA peptide epitopes in GBM is presented below. Finally, ongoing trials are also studying vaccination targeting multiple TAAs in GBM. One multicenter phase I/II clinical trial (NCT02078648) will determine the safety and efficacy of SL-701, a subcutaneously injected multivalent vaccine targeting IL-13Ra2, survivin, and EphA2 in adult patients with recurrent GBM. Similarly, a phase I/II clinical trial (NCT01920191) incorporating an 11-TAA peptide vaccine (IMA950) treatment for newly diagnosed GBM patients was recently completed and results should soon be reported.

These current therapeutic approaches are varied based on their antigens of interest. Specifically, one must weigh targeting single versus numerous antigens. Single antigen modalities may fall short in efficacy as the antigen may not be homogeneously expressed throughout the tumor. Moreover, targeting a single antigen can lead to immunologic escape, as has been previously documented.¹⁸ These risks must, then, be balanced with the risk of autoimmunity when targeting multiple antigens. Understanding this balance will be key as the safety of peptide vaccination is further examined in GBM.

2.2 Dendritic Cell Vaccines

Dendritic cells (DCs) are professional antigen presenting cells (APCs) that link the innate and adaptive immune systems.²⁵ DCs constantly survey the periphery, and upon antigen encounter, engulf and process these proteins into peptides to be presented on the cell surface via binding of their MHC molecules. DCs then traffic to lymph nodes and these peptides are presented to naïve CD8 and CD4 T cells for the induction of an adaptive cellular immune response.²⁶ DC-based vaccination strategies are currently under investigation using DCs generated *ex vivo*, most commonly from monocyte precursors.²⁷ DCs may be loaded, such as by pulsing or electroporation, with a variety of antigenic forms, including peptide antigens, protein antigens, tumor antigen RNA, or whole tumor lysate.²⁸

An early phase I trial by Liau *et al.* established the safety and feasibility of autologous DC vaccines pulsed with tumor peptides in GBM patients.²⁹ Since, several TAA-loaded DC vaccines have been tested for GBM include those targeting: EphA2, HER-2, IL13R α 2, GP100, Mage-1, survivin, hTERT, Trp-1, Aim-2, WT1, SOX2, SOX11, MAGE-A1, MAGE-A3, and SART1.¹⁰ In a completed phase I/II clinical trial of 22 HLA-A2 patients with recurrent malignant gliomas, Okada *et al.* elicited an immune response in 58% of patients after administration of α -type 1 polarized DCs loaded with synthetic GAA epitopes-EphA2, IL13R α 2, YKL-40, and GP100 when combined with poly-ICLC as an immunoadjuvant.³⁰ Moreover, there were no adverse events post-administration of the vaccination, and 41% of patients were progression-free after 12-months.³⁰ Analogously, in another completed phase I clinical trial of 21 HLA-A1/HLA-A2-positive patients (17 patients with newly-diagnosed GBM), Phuphanich *et al.* demonstrated that 33% of patients responded to the vaccine in the newly-diagnosed GBM cohort after the administration of ICT-107, a vaccine consisting of autologous DCs pulsed with six synthetic peptide epitopes: HER2, TRP-2, GP100, MAGE-1, IL13R α 2, and AIM-2.³¹ These patients demonstrated a median OS of 38.4 months. All adverse events post-vaccination were minor and included fatigue (33%), flushing (8%), pruritus (17%), rash (17%), skin redness (8%), vomiting (8%), and diarrhea (8%).³¹ In a completed placebo-controlled phase II study (NCT01280552), ICT-107 was found to increase PFS by 2 months. This therapy was deemed safe, and no difference in adverse events between the treatment (n = 80) and control (n = 43) groups was seen, with the exception of intracranial hemorrhage (5.0% vs. 0.0%) and increased ICPs (2.5% vs. 0.0%).³² The safety and efficacy of this therapy will be further tested in a subsequent phase III randomized, double-blind, controlled study of ICT-107 with maintenance TMZ in newly-diagnosed GBM following resection and concomitant TMZ chemotherapy (NCT02546102).

The presence of human cytomegalovirus (HCMV) antigens including phosphoprotein 65 (pp65) and immediate early 1 (IE1) have been detected within GBM, thus creating targetable TSAs for DC vaccination.^{12,33,34} This approach is very exciting in the context of safe and efficacious CMV-targeting in immunocompromised patients undergoing bone marrow transplantation.^{12,35} Our own group recently published a randomized phase I/II clinical trial in which newly diagnosed GBM patients were vaccinated with CMV pp65 mRNA-pulsed DCs with vaccine site preconditioning using tetanus toxoid (Td) or unpulsed DCs ("ATTAC," NCT00639639).³⁶ In this trial of 12 patients with GBM, patients that were randomized to the Td cohort had an increased PFS (range 15.4 – 47.3 months) and OS

(range 20.6 – 47.3 months) compared to the unpulsed DC cohort. Moreover, no patients experienced any vaccine or Td-related adverse events.³⁶ This therapeutic modality will continue to be tested in a higher-powered phase II trial (“ELEVATE,” NCT02366728). It should be noted that this modality is directed towards non-self antigens, and thus, should have a theoretical advantage with regard to safety and autoimmunity.

Autologous whole tumor lysate-loaded DC vaccines incorporate unfractionated tumor antigens to create a therapy that is customized to each patient’s tumor, regardless of the capacity to identify the individual antigens.^{11,12} The seminal preclinical work by Liao *et. al* demonstrated that autologous tumor lysate-loaded DC vaccines can induce specific cytotoxic T lymphocytes against intracranial tumors.³⁷ In principle, this method permits targeting of many TSAs and TAAs, with the aim of stimulating a broader immune response. The concomitant fear, however, would be for autoimmune or inflammatory toxicity given the accompanying normal self-antigens that are included in the tumor lysate.¹² Nevertheless, work from Yu *et. al* showed that autologous DCs loaded with autologous tumor-lysate are safe in patients with GBM.³⁸ Moreover, the safety profile for tumor-lysate vaccines has since been confirmed in various clinical studies.^{39–42} The most common side effects have included headache, mild vaccine site reactions, and low-grade fever.¹¹

An ongoing phase II trial is comparing the most effective adjuvants to be co-administered with whole tumor-lysate vaccines (NCT01204684). Moreover, multiple trials are currently examining DCVax-L (Northwest Biotherapeutics)—autologous whole tumor lysate pulsed DCs—including a double blind, randomized, placebo controlled phase III trial (NCT00045968; NCT02146066). The phase I study of 22 patients with GBM and other high-grade gliomas resulted in a mean OS between 16 and 38.4 months for newly diagnosed GBM and between 9.6 and 35.9 months for recurrent GBM.⁴³ The vaccine was considered well tolerated, with vaccine-related adverse effects commonly being mild (grade 2).⁴³ Results from the phase III trial will be of much interest.

2.3 Heat Shock Protein Vaccines

Heat-Shock Proteins (HSPs) are intracellular, highly conserved chaperone proteins that function under adverse stress-inducing events. They have a role in protein folding, trafficking, and homeostasis.⁴⁴ HSPs also function in regulating immune responses and are overexpressed, through transcriptional upregulation, under stress-inducing environments, such as in GBM.^{44,45} Of all known HSPs, heat shock protein-peptide complex-96 (HSPPC-96), derived from resected tumor specimens and composed of autologous antigenic peptides chaperoned by HSP glycoprotein-96 (HSP gp-96), has demonstrated to be the least toxic and most easily purified for optimal use in vaccines.⁴⁴ In a phase I trial in which 12 patients with recurrent GBM were administered HSPPC-96 derived from the autologous resected tumor, 11 of 12 patients demonstrated a significant peripheral immune response to the vaccine, as well as tumor site-specific responses in these responders. The vaccine was well tolerated with only six patients experiencing injection site reactions.⁴⁶ In an open-label, single-arm, phase II clinical trial, 41 patients received 6 doses of the HSPPC-96 vaccine after gross total resection of recurrent GBM, resulting in a median OS of 42.6 weeks.⁴⁷ Overall, the vaccine was well tolerated with only mild adverse events attributed to the

vaccine – fatigue (29%) and injection site reaction (41%).⁴⁷ Following these promising results, both with safety and efficacy, there is an ongoing randomized phase II clinical trial that is evaluating the OS difference between HSPPC-96 and bevacizumab versus bevacizumab alone in patients with resected recurrent GBM (NCT01814813).

3.0 Adoptive T cell immunotherapy

Adoptive lymphocyte transfer (ALT) is an antigen-specific therapeutic approach that involves *ex vivo* antigen-driven expansion of autologous lymphocytes followed by transfer into patients.^{48,49} One version of this therapy depends on the harvest and use of tumor-infiltrating lymphocytes (TILs) derived from the tumor specimen, which can be technically challenging. More commonly, then, T cells are isolated from peripheral blood mononuclear cells (PBMCs), expanded *ex vivo* against tumor antigens, and administered either systemically or directly into tumor cavities. For example, one phase I study assessed the safety and tolerability of autologous CMV-specific T cell therapy for 19 patients with recurrent GBM.⁵⁰ CMV-specific T cells were expanded from 13 patients, and this therapy was well tolerated in combination with chemotherapy. Adverse events were most commonly mild (grade 1), while one patient developed a grade 3 seizure. A few grade 2 symptoms including anxiety, seizure, lymphopenia, and abnormal liver function tests were also noted.⁵⁰ The study required three intravenous infusions consisting of 25 to 40×10^6 autologous CMV-specific T cells in sterile saline that were coordinated with the patients' chemotherapy regimen.⁵⁰ The dosing regimen of 25 to 40×10^6 cells was well tolerated by the patients.⁵⁰ However, increased dosing administration to 10^{10} cells has been reported to cause serious adverse reactions, such as respiratory distress, after intravenous injection.⁵¹ Overall, the safety and feasibility of ALT has been shown in various other clinical trials that have incorporated ALT for patients with GBM (NCT01082926; NCT00331526; NCT01588769; NCT00003185; NCT00730613).

3.1 CAR T cells

ALT therapy has now evolved to leverage advances in gene engineering and retroviral delivery. Patient-derived T cells can be engineered with antigen-specific T cell receptors (TCRs) or tumor-specific chimeric antigen receptors (CARs) to confer target recognition independent of and in addition to naturally occurring TCRs.⁴⁹ The best studied of these T cell modifications are CARs. CARs are synthetic receptors that couple the single chain Fv fragment of a monoclonal antibody with various T cell signaling molecules, thus endowing T cells with the antigen-specific recognition of the humoral compartment, the intracellular signaling required for cytotoxicity, and the co-stimulation necessary for sustained activity. As such, CAR T cells recognize target antigens without a need for MHC peptide presentation, circumventing one major mechanism of tumor immune escape—MHC down-regulation.⁵² CAR T cell therapy has demonstrated promising results and FDA approval for hematological cancers is expected shortly.^{53,54}

Although clinical evaluation of CARs targeting GBM remains in its infancy, several phase I clinical trials are currently underway. Two phase I studies will assess the safety of HER2-targeting CARs in patients with GBM (NCT02442297; NCT01109095). In a separate study

evaluating the direct intracranial delivery of IL13R α 2-CARs in a first-in-human pilot trial in 3 patients with recurrent or refractory GBM (NCT02208362), Brown *et. al* reported promising results of a transient anti-glioma response observed in two patients following the infusion of IL13R α 2-CARs.⁵⁵ All patients received intracavitary infusions which were well tolerated with a cumulative T-cell doses ranging from 9.6 –10.6 $\times 10^8$ cells in 11–12 doses.⁵⁵ The grade 3 adverse events reported after intracavitary delivery were headaches and neurological symptoms (shuffling gait and tongue deviation).⁵⁵ Two phase I/II trials are currently underway utilizing EGFRvIII-CARs in patients with EGFRvIII-positive tumors (NCT01454596; NCT02209376). These studies are critically important to characterizing the safety profile and feasibility of CAR therapy for GBM. An upcoming study will soon begin enrolling patients for a phase I trial using EGFRvIII-CARs in patients with newly diagnosed GBM (NCT02664363). Outcomes of this study will further define the safety of this modality including determination of maximum tolerated dose and dose limiting toxicities.

Importantly, cytokine release syndrome (CRS) and tumor lysis syndrome (TLS) are serious adverse events that have been observed with different immunotherapies, but more recently with the use of ALT and CAR-T cell therapy.^{53,56} CRS is a non-antigen-specific toxicity associated with an increased release of cytokines into the circulation from activated lymphocytes and myeloid cells. TLS is associated with tumor cells releasing their contents into the bloodstream ensuing severe electrolyte abnormalities, such as hyperkalemia, hyperphosphatemia, and hypocalcemia.⁵⁷ Both are severe and can result in life-threatening sequelae. Cases of CRS and TLS have been described after ALT and CAR-T cell therapy mostly in leukemia patients.^{53,56} As the use of these immunotherapies continues in GBM patients, it will be vitally important to monitor and identify these potentially life-threatening syndromes.

4.0 Immune Checkpoint Blockade

Antigen-specific T cells facilitate cellular immunity as the effectors of ordered immune responses. The sequential series of this active response begins with clonal selection and secondary lymphoid tissue activation and proliferation, followed by localization of these T cells to target sites, initiation of their effector functions, and recruitment of additional immune cells and signaling molecules.¹¹ Immune checkpoints are intrinsic regulators of these immune responses that ensure appropriate activation and attenuation of T cell signaling to preserve responses at an appropriate level, thus maintaining tolerance and preventing autoimmunity.¹³ These mechanisms can negatively impact anti-tumor immune responses allowing cancers to avoid immunosurveillance; therefore, their blockade may be exploited to boost anti-tumor immunity.⁵⁸ There are two popular and widely studied immune checkpoint molecules currently being tested in GBM: cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death 1 (PD-1). Use of these immune checkpoint inhibitors in GBM is a promising approach, due to the efficacy seen with their use for other malignancies resulting in FDA approval.^{59,60}

4.1 CTLA-4

CTLA-4 is a molecule expressed on T cells during early stages of T cell activation, and primarily located within secondary lymphoid tissue.^{10,11} It is also constitutively expressed on CD4+ CD25+ FoxP3+ regulatory T cells (Treg).⁵⁸ Normally, its expression is upregulated on the surface of T cells following antigen binding. CTLA-4 competes with CD28 for binding to the ligands CD80 and CD86 which are expressed on APCs and necessary for T cell costimulation.⁶¹ However, CTLA-4 has a much higher affinity for these ligands than CD28. Therefore, by decreasing the availability of CD80/86, T cells are unable to receive the necessary costimulatory signaling following antigen binding. As such, blockade of CTLA-4 with the monoclonal antibody, ipilimumab, removes this checkpoint leading to enhanced T cell activation.^{10,62,63}

Although knowledge of the role and safety of ipilimumab in GBM remains at an early stage, it has already received FDA approval for metastatic melanoma.⁵⁹ In a completed phase II clinical trial assessing tumor response to ipilimumab in melanoma patients with brain metastases who were neurologically asymptomatic (n=51) and symptomatic (n=21) (NCT00623766), 18% of patients in the asymptomatic and 5% in the symptomatic cohort achieved disease control after 12 weeks.⁶⁴ The most common grade 3 adverse events in the asymptomatic cohort were diarrhea (12%) and fatigue (12%), and in the symptomatic cohort were dehydration (10%), hyperglycemia (10%), and elevated serum aspartate aminotransferase (10%).⁶⁴ Only one patient in each cohort experienced a grade 4 adverse event (confusion).⁶⁴ The immune-related adverse events were considered to be diarrhea, rash, pruritus, and elevated liver enzymes.⁶⁴ Currently, ipilimumab is being tested in patients with GBM in a phase III clinical trial (NCT02017717), *CheckMate-143*, discussed below.

4.2 PD-1

Contrary to CTLA-4, PD-1 functions to inhibit the activity of T cells in peripheral tissues to limit autoimmunity. Its expression is induced on T cells upon activation, and it is also highly expressed on Tregs. In the presence of the tumor microenvironment, PD-1 inhibits the host immune system from properly responding to the tumor insult.⁶³ PD-1 blockade reverses this inhibitory strain on the T cell. Of note, PD-1 is also present on various other cells including B cells, macrophages, and dendritic cells. Moreover, PD-1's ligands, PD-L1 and PD-L2, are expressed in a variety of immune cells and myeloid cells, respectively.¹⁰ Of interest, the expression of PD-L1 has been found to be expressed in pathological conditions and more importantly increased in GBM after the loss of tumor suppressor phosphatase and tensin homolog (PTEN).⁶⁵⁻⁶⁷ Nivolumab (anti-PD-1 antibody) is now approved by the FDA in melanoma, non-small cell lung carcinoma, and renal cell carcinoma. Common adverse events observed with nivolumab are fatigue, pruritus, nausea, diarrhea, rash, vitiligo, constipation, and asthenia.⁶⁰ *CheckMate-143* is an ongoing-multicenter phase III clinical trial determining the safety and efficacy of nivolumab (anti-PD-1) versus bevacizumab and of nivolumab with or without ipilimumab in GBM patients (NCT02017717). We eagerly await the results of *CheckMate-143* for this promising combinatorial approach against GBM.

5.0 Monoclonal Antibodies

Monoclonal antibodies have been used in various malignancies for some time, with many now approved by the FDA. Following such success, their use is now being examined in GBM, as well. They work by targeting specific antigens, similar to previously described modalities. Monoclonal antibodies can target specific-mutated ligands and receptors that enhance proliferation in GBM.⁶⁸ One well-studied target is the vascular endothelial growth factor (VEGF) family (i.e., VEGF-A, -B, -C, -D) and their tyrosine kinase receptors (VEGFR-1, -2, -3) that activate intracellular signaling cascades thus promoting growth and angiogenesis in GBM.^{68,69} Bevacizumab (anti-VEGF-A) is a humanized immunoglobulin G1 (IgG1) antibody that was approved by the FDA in May 2009 as a first-line treatment of recurrent GBM patients.⁷⁰ However, results from phase III clinical trials have shown increased PFS, but not OS, with bevacizumab plus radiotherapy/TMZ in newly diagnosed GBM patients.^{71–73} Compared to patients receiving placebo plus radiotherapy/TMZ (n=461), those receiving bevacizumab plus radiotherapy/TMZ (n=461) experienced significantly higher rates of serious adverse events grade 3 or higher (66.8% vs. 51.3%).⁶³ Even so, there is promise for bevacizumab to be incorporated as part of a combination treatment regimen for GBM.^{72,73}

Likewise, monoclonal anti-EGFR antibodies, such as mAb-806, cetuximab, and Y10, have shown significant promise in pre-clinical models, reducing tumor volumes and cell proliferation, while increasing OS in tumor-bearing mice.^{68,74–76} However, when translating these pre-clinical results into clinical trials, the findings were difficult to replicate. In a phase II trial of cetuximab in patients with recurrent high-grade glioma, 55 patients (28 with and 27 without EGFR amplification) were administered cetuximab, resulting in unfavorable PFS of <6 months in 50/55 patients, with only 5 patients having a PFS >9 months.⁷⁷ The most common reported cetuximab-related adverse events included (grade 2 and grade 3): skin toxicity (18%), thrombocytopenia (10%), fatigue (5%), and confusion/diminished consciousness (5%).⁷⁷ There is a great deal of interest in the use of monoclonal antibodies in treating GBM as there are currently over 40 clinical trials testing bevacizumab in combination with different mAbs (i.e., onartuzumab – NCT01632228), drugs (i.e., irinotecan – NCT00921167), and administration devices (i.e., NovoTTF – NCT02343549).

Important side effects that have been noted with the use of monoclonal antibodies and tyrosine kinase inhibitors (TKIs) are labeled on/off-target toxicities. These toxicities occur when antigen-specific targets are expressed on non-tumor tissue, thus ensuing an autoimmune attack on both normal and tumor tissue. Toxicities such as dermatological rash, diarrhea, and vascular hemorrhage have all been observed with the use of these agents.⁷⁸ Clinicians should assess the risk-benefit ratio between the related side effects and anti-tumor efficacy when determining optimal therapies.

6.0 Cytokine Therapy

Cytokines are small proteins that play an important role in coordination of the immune system. They are produced in innate and adaptive immune responses upon encounter of both foreign microbes and tumor antigens. Release of individual cytokines often results in varied

effects on assorted cell types, a phenomenon known as pleiotropism.^{79,80} Their resulting effects can be either suppressive or stimulatory on the immune response, and therefore their modulation has the potential to be used therapeutically. There are several cytokine subtypes such as Interferons (IFNs), Interleukins (ILs), and hematopoietic growth factors. To date the two cytokine-based therapies approved by the FDA in cancer are high dose IL-2 for the treatment of metastatic melanoma and renal cell carcinoma, and IFN- α for stage III melanoma as an adjuvant treatment.⁷⁹ Given its role in enhancing T cell proliferation, high dose IL-2 has led to significant autoimmune reactions including thyroiditis and vitiligo.⁸¹ Constitutional symptoms are commonly encountered with IFN- α treatment in melanoma. Other toxicities include neuropsychiatric issues, hepatotoxicity, neutropenia, thrombocytopenia, lymphopenia, and other autoimmune reactions.^{79,82}

The safety and efficacy of cytokine therapy in GBM is still being evaluated. One small phase II clinical trial assessing peginterferon α -2b (PEG-Intron) alone and together with thalidomide in patients with gliomas—4 with GBM and 3 with anaplastic glioma—has completed (NCT00047879). The adverse events noted in the GBM group included elevated AST, hyperglycemia, and seizures; while patients in the anaplastic glioma cohort experienced nausea, vomiting, thrombocytopenia, muscle weakness, and headache. In two completed single-arm phase II trials in 63 adults with GBM, patients were treated with standard of care TMZ combined with either short-acting (IFN) or long-acting (pegylated) interferon α -2b (PEG). PFS at six months was 31% for 29 evaluable patients in the IFN study and 38% for 26 evaluable patients in the PEG study.⁸³ The Grade 3/4 adverse events observed in the IFN (n=33) and PEG (n=29) groups, respectively, were leukopenia (35%, 38%), thrombocytopenia (18%, 21%), fatigue (18%, 18%), pulmonary embolism/thrombosis (0%, 17%), nausea/vomiting (6%, 0%), headache (0%, 7%), and incontinence (0%, 7%).⁸³ There is only one phase III trial (NCT01765088) utilizing cytokine therapy that is currently recruiting patients with newly diagnosed high-grade gliomas. This study will determine the efficacy of adjuvant TMZ with or without the addition of IFN- α , and is expected to reach completion in 2017.

7.0 Conclusion

GBM is the most common malignant primary brain tumor in adults. Despite advances in standard of care therapy, where side effects are common, gains in patient survival have been modest. Immunotherapy has emerged in recent years as a novel modality with the potential to provide new-found hope in this disease. Immunotherapeutic classes currently under investigation in GBM include various vaccination strategies, adoptive T-cell immunotherapy, immune checkpoint blockade, monoclonal antibodies, and cytokine therapies. As our knowledge of these platforms is improved and as research findings are translated to the clinic, we continue to gain a better understanding of the safety and efficacy of these treatments. To date, many of these therapies have been well-tolerated in early clinical trials with minimal adverse events. We look forward to their continued evaluation in larger studies.

8.0 Expert Opinion

While there are many more treatment options available for patients with GBM now than ever before, improvements in survival have been modest at best. Efficacy associated with current therapies has been impermanent. The observed therapeutic failure is multi-factorial, resulting from various elements such as the invasive nature of GBM within the brain and the resistance of GBM to radiation therapy. Moreover, current therapies are also limited by their safety. Complete surgical resection of a primary brain lesion is complex and very difficult when located in eloquent brain areas. Additionally, the normal CNS is less able to tolerate cytotoxic therapies compared to other tissues. Lastly, corticosteroids are frequently used to offset neurologic symptoms brought on by peri-tumoral edema, yet long-term use of corticosteroids results in substantial side effects.

Many barriers remain which prevent advances for the treatment of GBM. This disease imposes a substantial economic encumbrance on the healthcare system as current therapies for brain tumors rank among the most expensive treatments; one year of treatment has been shown to cost over \$180,000 dollars in the United States.^{84,85} Another barrier remains in our inadequate understanding of basic immunobiology and the factors leading to immune dysfunction such as tolerance and exhaustion. Moreover, while immunotherapy offers great potential in GBM, more targeted therapies are still needed. As previously mentioned, TSAs are ideal candidates for ensuring safety for patients given their highly specific expression on tumor tissue alone. Responses against these antigens should be greater than those seen against over-expressed self-antigens (TAAs), while the risk of autoimmunity should be greatly reduced with them. Relatively few TSAs have currently been identified in GBM. In addition to the R132H mutation in IDH1 and CMV antigens discussed above, two mutations (C250T and C228T) have been identified in the TERT protein.⁸⁶ Therapies specifically targeting these antigens will be vital to developing safe and efficacious treatments. EGFRvIII remains the best-studied TSA to date in GBM. Based on the cumulative results from pre-clinical and clinical studies, targeting of this antigen has been shown to improve survival with minimal toxicity. We were disappointed with the negative results of the ACT IV trial; however, we are interested to see subgroup analyses to further understand trends in these data. Moreover, future EGFRvIII-targeted approaches using BiTEs and CARs represent novel classes of therapy with much potential.

Another exciting area of treatment in GBM is that of immune checkpoint blockade. Great promise has been seen with these drugs in treating both solid and blood-borne cancers. Given the extremely immunosuppressive nature of GBM, checkpoint blockade is less likely to prove effective as monotherapy, yet clinical success may be seen when these are combined with other active immunotherapies. Interestingly, as many immunotherapies depend upon activation of the immune system, they may show delayed antitumor responses. As such, traditional endpoints used in clinical trials may not adequately assess the efficacy of some of these modalities. Checkpoint blockade provides a prime example of this, as a phase III trial of tremelimumab (targeting CTLA-4) was initially halted during an interim analysis as no improvement in survival was seen. When reviewed at two years, however, a survival difference was observed.⁸⁷ Appropriate criteria and endpoints must be incorporated

into future trials to appropriately assess immunotherapies. Safety of these treatments and their propensity to induce auto-immunity will remain of chief importance.

To reach the goal of finding safe and efficacious therapies for GBM, investigational and novel strategies will continually be needed and encouraged. One such example is that of tumor-treating fields (TTFs). This approach entails the use of transcutaneous delivery of low-intensity intermediate frequency alternating electric fields (AEFs). This therapy is hypothesized to work via disruption of spindle formation during cellular division leading to mitotic arrest, and has been shown to work *in vitro* and *in vivo*.⁸⁸ An interim analysis from a multi-center randomized trial using a combinatorial approach with maintenance TMZ chemotherapy has recently been reported (NCT00916409).⁸⁹ The trial was terminated based on the positive results in the interim analysis, which showed an increased median PFS in the intent-to-treat population of 7.1 months in the TMZ and TTFs group compared to four months in the TMZ group. The median OS was also increased to 20.5 months compared to 15.6 months. The delivery of this local therapy was not associated with increased toxicity systemically or an increased seizure risk compared to patients who received TMZ alone. However, these patients did have a higher incidence of scalp irritation, anxiety, confusion, insomnia, and headaches.⁸⁹

Ultimately, moving toward more targeted treatments of GBM will be key. Combining active immunotherapeutic modalities with checkpoint inhibitors will likely be necessary to maintain robust clinical responses in these patients. We expect over the next five years to see increasing use of these combinations of therapies. Moreover, advances in other investigational treatment modalities, such as TTFs, provide new avenues for rendering safe and efficacious treatments. Finally, an increasing understanding of the basic biology of these tumors and the differences between the various molecular subtypes of GBM should augment our success in developing more efficacious and safe therapies for this disease.

References

1. Ostrom QT, Gittleman H, Liao P, et al. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2007–2011. *Neuro-oncology*. Oct; 2014 16(Suppl 4):iv1–63. [PubMed: 25304271]
2. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *The New England journal of medicine*. Mar 10; 2005 352(10):987–996. [PubMed: 15758009]
- 3**. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *The Lancet. Oncology*. May; 2009 10(5):459–466. Comment: Current standard of care treatment protocol for patients with glioblastoma. [PubMed: 19269895]
4. Finlay JL, Boyett JM, Yates AJ, et al. Randomized phase III trial in childhood high-grade astrocytoma comparing vincristine, lomustine, and prednisone with the eight-drugs-in-1-day regimen. *Childrens Cancer Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Jan; 1995 13(1):112–123. [PubMed: 7799011]
5. Grossman SA, Ye X, Piantadosi S, et al. Survival of patients with newly diagnosed glioblastoma treated with radiation and temozolomide in research studies in the United States. *Clinical cancer research : an official journal of the American Association for Cancer Research*. Apr 15; 2010 16(8):2443–2449. [PubMed: 20371685]

6. Johnson DR, Ma DJ, Buckner JC, Hammack JE. Conditional probability of long-term survival in glioblastoma: a population-based analysis. *Cancer*. Nov 15; 2012 118(22):5608–5613. [PubMed: 22569786]
7. Buckner JC. Factors influencing survival in high-grade gliomas. *Seminars in oncology*. Dec; 2003 30(6 Suppl 19):10–14.
8. Louis DN, Ohgaki H, Wiestler OD, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta neuropathologica*. Aug; 2007 114(2):97–109. [PubMed: 17618441]
9. Eckel-Passow JE, Lachance DH, Molinaro AM, et al. Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. *The New England journal of medicine*. Jun 25; 2015 372(26):2499–2508. [PubMed: 26061753]
10. Fecci PE, Heimberger AB, Sampson JH. Immunotherapy for primary brain tumors: no longer a matter of privilege. *Clinical cancer research : an official journal of the American Association for Cancer Research*. Nov 15; 2014 20(22):5620–5629. [PubMed: 25398845]
11. Reardon DA, Freeman G, Wu C, et al. Immunotherapy advances for glioblastoma. *Neuro-oncology*. Nov; 2014 16(11):1441–1458. [PubMed: 25190673]
12. Sampson JH, Mitchell DA. Vaccination strategies for neuro-oncology. *Neuro-oncology*. Nov; 2015 17(Suppl 7):vii15–vii25. [PubMed: 26516221]
13. Neagu MR, Reardon DA. An Update on the Role of Immunotherapy and Vaccine Strategies for Primary Brain Tumors. *Current treatment options in oncology*. Nov.2015 16(11):54. [PubMed: 26454859]
14. Reardon DA, Wucherpennig KW, Freeman G, et al. An update on vaccine therapy and other immunotherapeutic approaches for glioblastoma. *Expert review of vaccines*. Jun; 2013 12(6):597–615. [PubMed: 23750791]
15. Liu G, Akasaki Y, Khong HT, et al. Cytotoxic T cell targeting of TRP-2 sensitizes human malignant glioma to chemotherapy. *Oncogene*. Aug 4; 2005 24(33):5226–5234. [PubMed: 15897911]
16. Prins RM, Odesa SK, Liao LM. Immunotherapeutic targeting of shared melanoma-associated antigens in a murine glioma model. *Cancer research*. Dec 1; 2003 63(23):8487–8491. [PubMed: 14679014]
17. Xu LW, Chow KK, Lim M, Li G. Current vaccine trials in glioblastoma: a review. *Journal of immunology research*. 2014; 2014:796856. [PubMed: 24804271]
18. Sampson JH, Heimberger AB, Archer GE, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Nov 1; 2010 28(31):4722–4729. [PubMed: 20921459]
19. Sampson JH, Aldape KD, Archer GE, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. *Neuro-oncology*. Mar; 2011 13(3):324–333. [PubMed: 21149254]
- 20*. Schuster J, Lai RK, Recht LD, et al. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. *Neuro-oncology*. Jun; 2015 17(6):854–861. Comment: Demonstration of efficacy and safety with the use rinodpepimut targeting EGFRvIII in a phase II clinical trial. [PubMed: 25586468]
21. Liang F, Ploquin A, Hernandez JD, et al. Dissociation of skeletal muscle for flow cytometric characterization of immune cells in macaques. *Journal of immunological methods*. Oct.2015 425:69–78. [PubMed: 26099800]
22. Ichimura K, Pearson DM, Kocalkowski S, et al. IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. *Neuro-oncology*. Aug; 2009 11(4):341–347. [PubMed: 19435942]
23. Schumacher T, Bunse L, Pusch S, et al. A vaccine targeting mutant IDH1 induces antitumour immunity. *Nature*. Aug 21; 2014 512(7514):324–327. [PubMed: 25043048]
24. Pollack IF, Jakacki RI, Butterfield LH, et al. Antigen-specific immune responses and clinical outcome after vaccination with glioma-associated antigen peptides and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in children with newly diagnosed malignant

- brainstem and nonbrainstem gliomas. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Jul 1; 2014 32(19):2050–2058. [PubMed: 24888813]
25. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *The Journal of experimental medicine*. May 1; 1973 137(5):1142–1162. [PubMed: 4573839]
 26. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. *Annual review of immunology*. 2000; 18:767–811.
 27. Nair S, Archer GE, Tedder TF. Isolation and generation of human dendritic cells. *Current protocols in immunology / edited by John E. Coligan ... [et al.]*. Nov.2012 Chapter 7(Unit7):32.
 28. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. *Nature reviews. Cancer*. Apr; 2012 12(4):265–277. [PubMed: 22437871]
 29. Liao LM, Prins RM, Kiertscher SM, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. *Clinical cancer research : an official journal of the American Association for Cancer Research*. Aug 1; 2005 11(15):5515–5525. [PubMed: 16061868]
 30. Okada H, Kalinski P, Ueda R, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Jan 20; 2011 29(3):330–336. [PubMed: 21149657]
 31. Phuphanich S, Wheeler CJ, Rudnick JD, et al. Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. *Cancer immunology, immunotherapy : CII*. Jan; 2013 62(1):125–135. [PubMed: 22847020]
 32. Wen PY, Reardon DA, Phuphanich S, et al. A randomized, double-blind, placebo-controlled phase 2 trial of dendritic cell (DC) vaccination with ICT-107 in newly diagnosed glioblastoma (GBM) patients. *Journal of Clinical Oncology*. May 20.2014 32(15)
 33. Lucas KG, Bao L, Bruggeman R, Dunham K, Specht C. The detection of CMV pp65 and IE1 in glioblastoma multiforme. *Journal of neuro-oncology*. Jun; 2011 103(2):231–238. [PubMed: 20820869]
 34. Soderberg-Naucler C, Johnsen JI. Cytomegalovirus in human brain tumors: Role in pathogenesis and potential treatment options. *World journal of experimental medicine*. Feb 20; 2015 5(1):1–10. [PubMed: 25699229]
 35. Fuji S, Kapp M, Grigoleit GU, Einsele H. Adoptive immunotherapy with virus-specific T cells. Best practice & research. *Clinical haematology*. Sep; 2011 24(3):413–419. [PubMed: 21925094]
 36. Mitchell DA, Batich KA, Gunn MD, et al. Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. *Nature*. Mar 19; 2015 519(7543):366–369. [PubMed: 25762141]
 37. Liao LM, Black KL, Prins RM, et al. Treatment of intracranial gliomas with bone marrow-derived dendritic cells pulsed with tumor antigens. *Journal of neurosurgery*. Jun; 1999 90(6):1115–1124. [PubMed: 10350260]
 38. 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 research*. Jul 15; 2004 64(14):4973–4979. [PubMed: 15256471]
 39. De Vleeschouwer S, Fieuws S, Rutkowski S, et al. Postoperative adjuvant dendritic cell-based immunotherapy in patients with relapsed glioblastoma multiforme. *Clinical cancer research : an official journal of the American Association for Cancer Research*. May 15; 2008 14(10):3098–3104. [PubMed: 18483377]
 40. Yamanaka R, Homma J, Yajima N, et al. Clinical evaluation of dendritic cell vaccination for patients with recurrent glioma: results of a clinical phase I/II trial. *Clinical cancer research : an official journal of the American Association for Cancer Research*. Jun 1; 2005 11(11):4160–4167. [PubMed: 15930352]
 41. Fadul CE, Fisher JL, Hampton TH, et al. Immune response in patients with newly diagnosed glioblastoma multiforme treated with intranodal autologous tumor lysate-dendritic cell vaccination

- after radiation chemotherapy. *Journal of immunotherapy*. May; 2011 34(4):382–389. [PubMed: 21499132]
- 42*. Ardon H, Van Gool SW, Verschuere T, 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 immunology, immunotherapy : CII*. Nov; 2012 61(11):2033–2044. Comment: Early safety profile results of autologous dendritic cell-based immunotherapy in patients with glioblastoma. [PubMed: 22527250]
43. Polyzoidis S, Ashkan K. DCVax(R)-L--developed by Northwest Biotherapeutics. *Human vaccines & immunotherapeutics*. 2014; 10(11):3139–3145. [PubMed: 25483653]
44. Ampie L, Choy W, Lamano JB, Fakurnejad S, Bloch O, Parsa AT. Heat shock protein vaccines against glioblastoma: from bench to bedside. *Journal of neuro-oncology*. Jul; 2015 123(3):441–448. [PubMed: 26093618]
45. Christians ES, Zhou Q, Renard J, Benjamin IJ. Heat shock proteins in mammalian development. *Seminars in cell & developmental biology*. Oct; 2003 14(5):283–290. [PubMed: 14986858]
46. Crane CA, Han SJ, Ahn B, et al. Individual patient-specific immunity against high-grade glioma after vaccination with autologous tumor derived peptides bound to the 96 KD chaperone protein. *Clinical cancer research : an official journal of the American Association for Cancer Research*. Jan 1; 2013 19(1):205–214. [PubMed: 22872572]
47. Bloch O, Crane CA, Fuks Y, et al. Heat-shock protein peptide complex-96 vaccination for recurrent glioblastoma: a phase II, single-arm trial. *Neuro-oncology*. Jan; 2014 16(2):274–279. [PubMed: 24335700]
48. Yee C, Thompson JA, Byrd D, et al. Adoptive T cell therapy using antigen-specific CD8+ T cell clones for the treatment of patients with metastatic melanoma: in vivo persistence, migration, and antitumor effect of transferred T cells. *Proceedings of the National Academy of Sciences of the United States of America*. Dec 10; 2002 99(25):16168–16173. [PubMed: 12427970]
49. Suryadevara CM, Verla T, Sanchez-Perez L, et al. Immunotherapy for malignant glioma. *Surgical neurology international*. 2015; 6(Suppl 1):S68–77. [PubMed: 25722935]
- 50*. Schuessler A, Smith C, Beagley L, et al. Autologous T-cell therapy for cytomegalovirus as a consolidative treatment for recurrent glioblastoma. *Cancer research*. Jul 1; 2014 74(13):3466–3476. Comment: Demonstration of efficacy and safety with the use of autologous T-cell therapy approach in patients with recurrent glioblastoma. [PubMed: 24795429]
51. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Molecular therapy : the journal of the American Society of Gene Therapy*. Apr; 2010 18(4):843–851. [PubMed: 20179677]
52. Bonini C, Mondino A. Adoptive T-cell therapy for cancer: The era of engineered T cells. *European journal of immunology*. Sep; 2015 45(9):2457–2469. [PubMed: 26202766]
53. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *The New England journal of medicine*. Aug 25; 2011 365(8):725–733. [PubMed: 21830940]
54. Maude SL, Frey N, Shaw PA, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *The New England journal of medicine*. Oct 16; 2014 371(16):1507–1517. [PubMed: 25317870]
55. Brown CE, Badie B, Barish ME, et al. Bioactivity and Safety of IL13Ralpha2-Redirected Chimeric Antigen Receptor CD8+ T Cells in Patients with Recurrent Glioblastoma. *Clinical cancer research : an official journal of the American Association for Cancer Research*. Sep 15; 2015 21(18):4062–4072. [PubMed: 26059190]
56. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood*. Jul 10; 2014 124(2):188–195. [PubMed: 24876563]
57. Howard SC, Jones DP, Pui CH. The Tumor Lysis Syndrome. *New Engl J Med*. May 12; 2011 364(19):1844–1854. [PubMed: 21561350]
58. Korman AJ, Peggs KS, Allison JP. Checkpoint blockade in cancer immunotherapy. *Advances in immunology*. 2006; 90:297–339. [PubMed: 16730267]

59. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *The New England journal of medicine*. Aug 19; 2010 363(8):711–723. [PubMed: 20525992]
- 60*. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *The New England journal of medicine*. Jan 22; 2015 372(4):320–330. Comment: Demonstration of efficacy and safety with the use of an anti-PD-1 antibody for melanoma that have led way for clinical trials targeting glioblastoma. [PubMed: 25399552]
61. McCoy KD, Le Gros G. The role of CTLA-4 in the regulation of T cell immune responses. *Immunology and cell biology*. Feb; 1999 77(1):1–10. [PubMed: 10101680]
62. Peggs KS, Quezada SA, Chambers CA, Korman AJ, Allison JP. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. *The Journal of experimental medicine*. Aug 3; 2009 206(8):1717–1725. [PubMed: 19581407]
63. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nature reviews. Cancer*. Apr; 2012 12(4):252–264. [PubMed: 22437870]
64. Margolin K, Ernstoff MS, Hamid O, et al. Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. *The Lancet. Oncology*. May; 2012 13(5):459–465. [PubMed: 22456429]
65. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nature medicine*. Jan; 2007 13(1):84–88.
66. Han SJ, Ahn BJ, Waldron JS, et al. Gamma interferon-mediated superinduction of B7-H1 in PTEN-deficient glioblastoma: a paradoxical mechanism of immune evasion. *Neuroreport*. Dec 9; 2009 20(18):1597–1602. [PubMed: 19875977]
67. Berghoff AS, Kiesel B, Widhalm G, et al. Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. *Neuro-oncology*. Aug; 2015 17(8):1064–1075. [PubMed: 25355681]
68. Hernandez-Pedro NY, Rangel-Lopez E, Vargas Felix G, Pineda B, Sotelo J. An update in the use of antibodies to treat glioblastoma multiforme. *Autoimmune diseases*. 2013; 2013:716813. [PubMed: 24294521]
69. Alitalo K, Carmeliet P. Molecular mechanisms of lymphangiogenesis in health and disease. *Cancer cell*. Apr; 2002 1(3):219–227. [PubMed: 12086857]
70. Johnson DR, Leeper HE, Uhm JH. Glioblastoma survival in the United States improved after Food and Drug Administration approval of bevacizumab: a population-based analysis. *Cancer*. Oct 1; 2013 119(19):3489–3495. [PubMed: 23868553]
71. Fu P, He YS, Huang Q, et al. Bevacizumab treatment for newly diagnosed glioblastoma: Systematic review and meta-analysis of clinical trials. *Molecular and clinical oncology*. May; 2016 4(5):833–838. [PubMed: 27123291]
72. Gilbert MR, Dignam JJ, Armstrong TS, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *The New England journal of medicine*. Feb 20; 2014 370(8):699–708. [PubMed: 24552317]
73. Chinot OL, Wick W, Mason W, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. *The New England journal of medicine*. Feb 20; 2014 370(8):709–722. [PubMed: 24552318]
74. Mishima K, Johns TG, Luwor RB, et al. Growth suppression of intracranial xenografted glioblastomas overexpressing mutant epidermal growth factor receptors by systemic administration of monoclonal antibody (mAb) 806, a novel monoclonal antibody directed to the receptor. *Cancer research*. Jul 15; 2001 61(14):5349–5354. [PubMed: 11454673]
75. Patel D, Lahiji A, Patel S, et al. Monoclonal antibody cetuximab binds to and down-regulates constitutively activated epidermal growth factor receptor vIII on the cell surface. *Anticancer research*. Sep-Oct; 2007 27(5A):3355–3366. [PubMed: 17970081]
76. Sampson JH, Crotty LE, Lee S, et al. Unarmed, tumor-specific monoclonal antibody effectively treats brain tumors. *P Natl Acad Sci USA*. Jun 20; 2000 97(13):7503–7508.

77. Neyns B, Sadones J, Joosens E, et al. Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*. Sep; 2009 20(9):1596–1603.
78. Liu S, Kurzrock R. Understanding Toxicities of Targeted Agents: Implications for Anti-tumor Activity and Management. *Seminars in oncology*. Dec; 2015 42(6):863–875. [PubMed: 26615131]
79. Lee S, Margolin K. Cytokines in cancer immunotherapy. *Cancers*. 2011; 3(4):3856–3893. [PubMed: 24213115]
80. Belardelli F. Role of interferons and other cytokines in the regulation of the immune response. *APMIS : acta pathologica, microbiologica, et immunologica Scandinavica*. Mar; 1995 103(3):161–179.
81. Ridolfi L, de Rosa F, Ridolfi R, et al. Radiotherapy as an immunological booster in patients with metastatic melanoma or renal cell carcinoma treated with high-dose Interleukin-2: evaluation of biomarkers of immunologic and therapeutic response. *Journal of translational medicine*. 2014; 12:262. [PubMed: 25245327]
82. Di Trollo R, Simeone E, Di Lorenzo G, Buonerba C, Ascierto PA. The use of interferon in melanoma patients: a systematic review. *Cytokine & growth factor reviews*. Apr; 2015 26(2):203–212. [PubMed: 25511547]
83. Groves MD, Puduvalli VK, Gilbert MR, et al. Two phase II trials of temozolomide with interferon-alpha2b (pegylated and non-pegylated) in patients with recurrent glioblastoma multiforme. *British journal of cancer*. Aug 18; 2009 101(4):615–620. [PubMed: 19672263]
84. Ray S, Bonafede MM, Mohile NA. Treatment Patterns, Survival, and Healthcare Costs of Patients with Malignant Gliomas in a Large US Commercially Insured Population. *American health & drug benefits*. May; 2014 7(3):140–149. [PubMed: 24991398]
85. Kutikova L, Bowman L, Chang S, Long SR, Thornton DE, Crown WH. Utilization and cost of health care services associated with primary malignant brain tumors in the United States. *Journal of neuro-oncology*. Jan; 2007 81(1):61–65. [PubMed: 16773215]
86. Killela PJ, Reitman ZJ, Jiao Y, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proceedings of the National Academy of Sciences of the United States of America*. Apr 9; 2013 110(15):6021–6026. [PubMed: 23530248]
87. Hoos A, Britten C. The immuno-oncology framework: Enabling a new era of cancer therapy. *Oncoimmunology*. May 1; 2012 1(3):334–339. [PubMed: 22737609]
- 88*. Sampson JH. Alternating Electric Fields for the Treatment of Glioblastoma. *Jama*. Dec 15; 2015 314(23):2511–2513. Comment: Novel treatment approach that is rising as alternative approaches to targeting glioblastoma that are efficacious and safe. [PubMed: 26670969]
89. Stupp R, Taillibert S, Kanner AA, et al. Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. *Jama*. Dec 15; 2015 314(23):2535–2543. [PubMed: 26670971]

Article Highlights

1. Various immunotherapeutic platforms are currently in clinical testing including various peptide, dendritic cell, and heat shock protein vaccination strategies, adoptive T-cell transfer, checkpoint blockade, monoclonal antibodies, and cytokine therapies.
2. As these platforms begin to show promise with regard to efficacy, safety is a chief concern as well.
3. Safety concerns include autoimmune reactions, on-target vs. off-target toxicity, tumor lysis syndrome, cytokine storming, dosing thresholds, and route of administration.
4. Peptide vaccine therapy is currently the furthest along in clinical testing with minor systemic adverse reactions such as injection-site erythema, muscle pain, headache and fatigue.
5. Overall, based on current clinical trial data, immunotherapies targeting GBM are safe and well-tolerated with minimal toxicities and should continue to be studied.
6. Combining active immunotherapeutic modalities will be key for future therapies to maintain robust clinical responses while minimizing safety concerns.

Table 1
Summary of Safety Profiles for Current Immunotherapy Clinical Trials for GBM Patients

Class	Studied Therapies	Targets	Phase	Identifier	Acronym	Reported Adverse Events
Peptide Vaccines	Rindopepimut (CDX-110)	EGFRvIII	II	NCT00643097	ACTIVATE	Numbness, constitutional, GI, pain, dermatologic/skin, allergic
	Rindopepimut (CDX-110)	EGFRvIII	II		ACT II	Constitutional, dermatologic (rash, flushing), cardiovascular, leukopenia, GI
	Rindopepimut (CDX-110)	EGFRvIII	II	NCT00458601	ACT III	Injection site-erythema, pruritus, swelling, pain, rash, bruising, fatigue, systemic rash, nausea, headache, hypokalemia
	Rindopepimut (CDX-110)	EGFRvIII	III	NCT01480479	ACT IV	
	IDH1 peptide vaccine	IDH1R132H	I	NCT02454634	NOA-16	
	PEPIDHIM vaccine	IDH1R132H	I	NCT02193347	RESIST	
	GAA Epitope Peptide Vaccine	IL-13Ra, EphA2, survivin		NCT01130077		Grade 1 and 2 injection-site reactions and flu-like symptoms
	SL-701	IL-13Ra, EphA2, survivin	I/II	NCT02078648		
	IMA950	11 tumor-associated antigens	I/II	NCT01920191		
	α -type 1 polarized DCs	EphA2, IL-13Ra, YKL-40, gp100	I/II	NCT00766753		Grade I injection site reaction, grade I flu like symptoms, fatigue, myalgia, fever, chills, headache, grade II lymphopenia
Dendritic Cell Vaccines	ICT-107 (autologous DCS)	HER2, TRP-2, gp100, MAGE-1, IL-13Ra, AIM-2	I	NCT00576641		Fatigue, flushing, pruritus, skin redness, vomiting, diarrhea
	ICT-107 (autologous DCS)	HER2, TRP-2, gp100, MAGE-1, IL-13Ra, AIM-2	II	NCT01280552		
	mRNA-transfected DCs	Autologous cancer stem cells	I/II	NCT00846456		Fatigue, anorexia, pain, nausea, constipation
	mRNA-pulsed DCs	CMV antigen pp65	I	NCT00693095	ERaDICAtc	
	mRNA-pulsed DCs (with Td)	CMV antigen pp65	I	NCT00639639	ATTAC	
	mRNA-pulsed DCs (with Td)	CMV antigen pp65	II	NCT02366728	ELEVATE	
	DCs pulsed with tumor lysate	Antigens derived from patient tumor	II	NCT01204684		
	DC-VaxL	Antigens derived from patient tumor	III	NCT00045968		

Class	Studied Therapies	Targets	Phase	Identifier	Acronym	Reported Adverse Events
Heat Shock Protein Vaccines	HSPPC-96	Heat shock protein	I/II	NCT00293423		Fatigue, injection site reaction
	HSPPC-96	Heat shock protein	II	NCT01814813		
Adoptive T cell Immunotherapy	HCMV-specific CTL	HCMV antigens	I	ACTRN12609000338268		Grade III seizure, grade II anxiety, seizure, lymphopenia, abnormal liver function tests
	CAR T cells	Her2	I	NCT01109095	HERT-GBM	
	CAR T cells	Her2	I	NCT02442297	iCAR	
	CAR T cells	IL-13R α	I	NCT02208362		
	CAR T cells	EGFRvIII	I/II	NCT01454596		
	CAR T cells	EGFRvIII	N/A	NCT02209376		
	CAR T cells	EGFRvIII	I	NCT02664363	ExCel	
Immune Checkpoint Blockade	Ipilimumab	CTLA-4	II	NCT00623766		fatigue, diarrhea, headache, nausea, rash, pruritus, elevated liver enzymes
	Nivolumab, ipilimumab, bevacizumab	PD-1, CTLA-4, VEGF	III	NCT02017717	CheckMate 143	
	Bevacizumab	VEGF	III	NCT00884741		
Monoclonal Antibodies	Bevacizumab	VEGF	III	NCT00943826		
	Cetuximab	EGFR	II			Skin Toxicity, thrombocytopenia, fatigue, confusion/diminished consciousness
	Onartuzumab	MET	II	NCT01632228		
Cytokine Therapy	Pegylated IFN- α 2B (with thalidomide)		II	NCT00047879		Elevated AST, hyperglycemia, seizures, nausea, vomiting, thrombocytopenia, muscle weakness, headache
	Pegylated and non-pegylated IFN- α 2B		II			Leukopenia, thrombocytopenia, fatigue, pulmonary embolism/thrombosis, nausea, vomiting, headache, incontinence
	IFN- γ (with DNX2401)	Tumor selective adenovirus	I	NCT02197169	TARGET-I	