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An Update of Vaccine Therapy and Other Immunotherapeutic Approaches for Glioblastoma

David A. Reardon, MD¹, Kai W. Wucherpennig, MD, PhD, Gordon Freeman, PhD, Catherine Wu, MD, E. Antonio Chiocca, MD, PhD, Patrick Y. Wen, MD, William T. Curry Jr., MD, Duane A. Mitchell, MD, PhD, Peter Fecci, MD, PhD, John H. Sampson, MD, PhD, and Glenn Dranoff, M.D.

Abstract

Outcome for glioblastoma (GBM), the most common primary CNS malignancy, remains poor. Overall survival benefit recently achieved with immunotherapeutics for melanoma and prostate cancer support evaluation of immunotherapies for other challenging cancers including GBM. Much historical dogma depicting the CNS as immunoprivileged has been replaced by data demonstrating CNS immunocompetence and active interaction with the peripheral immune system. Several glioma antigens have been identified for potential immunotherapeutic exploitation. Active immunotherapy studies for GBM, supported by preclinical data, have focused on tumor lysate and synthetic antigen vaccination strategies. Results to date confirm consistent safety, including a lack of autoimmune reactivity; however, modest efficacy and variable immunogenicity have been observed. These findings underscore the need to optimize vaccination variables and to address challenges posed by systemic and local immunosuppression inherent to GBM tumors. Additional immunotherapy strategies are also in development for GBM. Future studies may consider combinatorial immunotherapy strategies with complimentary actions.

Keywords

glioblastoma; immunosuppression; immunotherapy; vaccine; dendritic cells

Introduction

The annual incidence of glioblastoma (GBM), the most common malignant primary tumor of the central nervous system, is approximately 3.15 cases per 100,000 in the United States.¹ Extrapolation to the current global population (6.8 billion) projects more than 210,000 new GBM cases diagnosed each year worldwide. Outcome for GBM patients remains dismal despite aggressive, multimodality therapy. Specifically, current “standard of care” therapy including maximum safe resection followed by radiation and temozolomide chemotherapy (XRT/TMZ), achieves a median overall survival (OS) of 14.6 months with less than 10% of patients alive at 5-years.^{2,3} Recurrence is inevitable and salvage therapies remain ineffective.⁴⁻⁶ Evaluation of multiple cytotoxic agents over the past 40 years has failed to substantially increment survival, likely reflecting hurdles posed by de novo and acquired chemotherapy resistance mechanisms, tumor heterogeneity and limited delivery through the blood brain barrier.^{7,8}

¹David A. Reardon, M.D., Clinical Director, Center for Neuro-Oncology; Dana-Farber Cancer Institute; 450 Brookline Avenue, SW 460F; Boston, MA 02215-5450; telephone: 617-632-2166; fax: 617-632-4773; david_reardon@dfci.harvard.edu.

Recent laboratory advances reveal a multiplicity of genetic mutations and aberrantly activated cell signaling pathways in GBM tumors. In addition, detailed gene expression studies have defined GBMs into distinct subclasses.⁹⁻¹³ Nonetheless, these important biologic insights have yet to impact patient outcome in the clinic. Specifically, evaluation of a wide array of biologically-based, targeted therapeutics have yielded disappointing results.¹⁴⁻²⁰ Most recently, anti-angiogenic therapies including bevacizumab, a humanized monoclonal antibody targeting vascular endothelial growth factor (VEGF), have been shown to durably improve radiologic response but only modestly improve survival.²¹⁻²³

Currently only 4 drugs are approved by the US Food and Drug Administration (US FDA) for GBM and none has improved survival more than a few months. Given the overall poor survival, prioritization of quality of life and preservation of neurologic integrity are critical co-considerations for therapies in development for this disease. Clearly, better therapies that extend survival while preserving neurologic function and quality of life are desperately needed for GBM patients.

Recent exciting clinical data has resurged interest in immunotherapies for cancer patients. Specifically, the US FDA approved two specific immunotherapeutics in 2010. First, sipuleucel-T (Provenge; Dendreon Corporation, Seattle, WA) a dendritic cell-based vaccine was approved for metastatic, hormone-resistant prostate cancer based on improved overall survival (OS).²⁴ Second, ipilimumab (Yervoy; Bristol-Myers Squibb, New York City, NY), a humanized MAb targeting the immunomodulatory molecule cytotoxic T-lymphocyte antigen 4 (CTLA-4), was approved for metastatic melanoma,²⁵ a solid tumor with similarly poor response to conventional cytotoxic therapy as GBM. The increased OS achieved by ipilimumab provides encouraging proof-of-concept that blocking endogenous inhibitors of immune activation can improve survival for cancer patients. Clinical trials targeting CTLA-4 are now underway for a wide array of cancers and a placebo-controlled, randomized, Phase II/III study has been designed for newly diagnosed GBM. Recognition of the importance of the immune system to cancer outcome has been further heightened by growing data demonstrating the prognostic impact of immune cell infiltrate on outcome for several cancers.²⁶ For example, immune cell infiltrate is a more robust multivariable predictor of outcome than TNM stage for non-metastatic colorectal cancer.²⁷ Nonetheless, the overall survival benefit achieved by vaccines such as sipuleucel-T or immunomodulatory agents such as ipilimumab are modest and underscore the complex mechanisms of immunoresistance inherent to many tumors, suggesting that combinatorial immunotherapy approaches will likely prove necessary to heighten and broaden the overall impact of immune-based therapeutic strategies for cancer patients.

Immunotherapies can be classified into four major categories. Active immunotherapy includes strategies that directly sensitize the immune system to tumor-specific antigens as exemplified by cancer vaccines. Passive immunotherapy utilizes immune effector molecules such as antibodies to specifically target tumor antigens without direct activation of the immune system. Adoptive strategies, such as adoptive T cell transfer or administration of T cells with chimeric antigen receptors (CARs) utilize patient immune cells that have been manipulated ex vivo to react against tumor antigen prior to reinfusion to the patient. Finally, immunomodulatory strategies, such as ipilimumab aim to enhance general immunoreactivity by augmenting co-stimulatory molecules or blocking inhibitory molecules. The mechanisms of each of these strategies lend themselves to the possibility of potentially complimentary combinatorial immune-based approaches for cancer patients, and some of these are now beginning to be explored in the clinic. In the current review, we will focus on the status of active immunotherapy approaches for GBM patients, and highlight some potentially limiting hurdles. We will also briefly review the status of other immune-based strategies for GBM patients, particularly given their possible synergy with active immunotherapy approaches.

CNS Immunocompetence Rather Than Immune Privilege

Enthusiasm for investigation of active immune-based therapies for brain tumor patients was tempered for decades by dogma supporting the CNS as immune privileged.²⁸ This dogma, based primarily on experimental data demonstrating prolonged survival of tissues grafted in the CNS that were otherwise rapidly rejected when grown outside the CNS,²⁹ argued that the CNS lacked immunosurveillance, was relatively immune inert and did not interact effectively with the peripheral immune system. Subsequent data has invalidated much of this dogma by demonstrating that the CNS is immunocompetent and shares dynamic capabilities with the peripheral immune system.³⁰ Specifically, CNS antigens and T cells can access cervical lymphatic tissue,³¹⁻³³ while activated peripheral T cells and antibodies can penetrate or bypass the blood brain barrier to interact with target antigens in the CNS.³⁴⁻⁴⁰ Antigen specific T cells are capable of proliferating and acquiring effector function within the CNS tumor microenvironment,⁴¹ while microglia which serve as the resident macrophage population within the CNS, can function in an immunocompetent manner via expression of class II antigens and T-cell co-stimulatory molecules.⁴²⁻⁵⁰

Active Immunotherapy for GBM: Strategies and Initial Results

Interest in active immunotherapy for GBM has gained momentum as the historical dogma of CNS immune privilege has been replaced by recognition of the reactive capability of the immune system within the CNS. Additionally, a growing number of potentially immunoreactive tumor-associated antigens (TAA) expressed by human gliomas have been identified (Table 1).⁵¹⁻⁶² Two parallel active immunotherapy strategies have emerged for GBM patients including the use of tumor lysates as well as synthetic tumor antigen peptides as vaccination reagents.

Tumor Lysate Vaccines

Early preclinical studies confirmed the anti-tumor benefit of dendritic cells pulsed with GBM cell lysates or eluted peptides.⁶³⁻⁶⁸ The advantages of this approach include the potential availability of a vaccine for every GBM patient and immunogenicity against multiple tumor antigens. Disadvantages include requirement for surgery, a several week delay for vaccine generation, potential overload of the immune system by excessive antigen exposure, and the possibility of autoimmune reactions due to contamination of normal host cells in the vaccine. Clinical studies, evaluating autologous dendritic cells loaded ex vivo with either tumor eluted peptides or tumor cell homogenates, and administered intradermally for both recurrent and newly diagnosed malignant glioma patients, have confirmed the feasibility and safety of this approach.^{60,61,69-80} Specifically, these vaccines have neither elicited autoimmune reactions nor demonstrated evidence of experimental autoimmune encephalitis.⁶⁹ Adverse events have in fact been mild and limited to localized reactions, low-grade fever and headache. In addition, these studies confirmed that tumor lysate vaccines can trigger tumor-specific immune reactions in some patients as measured by increased rates of cytotoxic T cell proliferation, delayed-type hypersensitivity reactivity, positive interferon- γ enzyme-linked immunosorbent spot (ELISPOT) assays, antigen-specific reactivity as assessed by HLA-restricted tetramer staining, and increased intra-tumoral T cell infiltration.

Crane recently reported an innovative adaptation of the tumor lysate vaccine approach in which 12 recurrent GBM patients were vaccinated with tumor peptides bound to the 96 KD heat shock protein complex (HSP96).⁸¹ This approach offers the potential benefit associated with binding the HSP96 complex directly to antigen presenting cells. In this series, no vaccine-related adverse events were reported. Specific peripheral immune responses were noted in 11 patients, and median survival in these patients was 47 weeks. Furthermore, among responding patients who underwent brain biopsy, infiltrative IFN γ -secreting CD4,

CD8 and CD56 cells were detected. Another novel application of the whole tumor approach is the generation of an in situ vaccine generated by application of gene-mediated cytotoxic immunotherapy.⁸² An example of such an approach integrates intratumoral administration of an adenoviral vector expressing the herpes simplex virus thymidine kinase (HSV-tk) gene followed by administration of the anti-herpetic drug, valacyclovir. Valacyclovir is a prodrug that is cleaved by HSV-tk to generate a toxic nucleotide analog, thereby creating a local cytotoxic effect that is restricted to dividing cells. This approach may augment immunogenicity of multiple tumor-associated antigens by directly enhancing tumor cell killing in combination with the immunostimulatory effect of locally expressed HSV-tk. A significant intra-tumoral immune cell infiltrate was recently demonstrated in a phase Ib study among newly diagnosed GBM patients.⁸³

Targeting glioma stem cells is another adaptation of the whole tumor lysate vaccination approach. Glioma stem cells, a subpopulation within GBM tumors that are capable of driving tumor self-renewal, multilineage differentiation and sustained proliferation, can be enriched via neurosphere culture conditions.⁸⁴ In addition, these cells exhibit relative resistance to both chemotherapy and radiation therapy.^{85,86} Furthermore, recent work has demonstrated that glioma stem cells are capable of contributing to local immunosuppression within the tumor microenvironment.⁸⁷ Specifically, glioma stem cells secrete several immunosuppressive cytokines associated with recruitment and polarization of macrophages/microglia including soluble colony-stimulating factor-1 (sCSF-1), transforming growth factor- β 1 (TGF- β 1) and macrophage inhibitory cytokine-1 (MIC-1). Furthermore, conditioned media from glioma stem cells polarize macrophages/microglia to an M2 phenotype, inhibit macrophage/microglia phagocytosis, induce secretion of immunosuppressive cytokines including interleukin-10 and TGF- β 1, and inhibit T cell proliferation.^{87,88} Thus targeting glioma stem cells may represent an attractive therapeutic strategy, however whether such a strategy is more efficacious than glioma lysate approaches remains to be determined. Glioma stem cells have been shown to express glioma-associated antigens, with some antigens demonstrating several fold higher expression levels compared to non-stem cells isolated from the same tumor, as well as class I MHC molecules.^{88,89} Furthermore, vaccination with dendritic cells loaded with glioma stem cell antigens induced significant immunoreactivity as well as survival benefit in rodent orthotopic GBM models.^{89,90} Currently four clinical trials are evaluating strategies to immunize GBM patients with dendritic cells loaded with glioma stem cell antigens (Clinicaltrials.gov NCT00890032, NCT00846456, NCT01171469 and NCT01567202).

Synthetic Tumor Antigen Vaccines

An alternative approach has been the administration of synthetic peptides derived from glioma tumor-specific antigens (TSA) and TAAs as vaccination reagents with or without autologous dendritic cells. In preclinical CNS tumor models, tumor antigen peptides have been shown to be immunogenic and associated with anti-tumor activity.^{91,92} In one approach, investigators chose to focus on the mutated epidermal growth factor receptor variant III (EGFRvIII). EGFRvIII is currently the only TSA currently being targeted for GBM vaccination. It is thus a highly attractive immunotherapy target and is present as a tumor-specific cell surface protein in 30-40% of GBM patients⁹³ as well as other cancers.⁹⁴ It is absent on normal tissues, enhances tumorigenicity^{95,96} and appears to convey a negative prognosis for survival.^{97,98} Sampson and colleagues developed an EGFRvIII vaccine for preclinical studies using a synthetic peptide derived from the tumor-specific mutated segment of EGFRvIII (PEP-3) conjugated to keyhole limpet hemocyanin (PEP-3-KLH).

Immunocompetent mice were vaccinated with PEP-3-KLH and then challenged with a syngeneic melanoma line stably transfected with a murine homologue of PEP-3-KLH. Unlike the control mice (treated with KLH alone) where tumors developed in 100%, 70% of

the vaccinated mice never developed palpable tumors. In addition, among mice with established orthotopic intracranial GBM tumors, median overall survival was increased by 173% following vaccination compared to controls and 80% of the vaccinated mice were long-term survivors.⁹⁹ Initial clinical studies evaluating newly diagnosed GBM patients treated with PEP-3-KLH-pulsed dendritic cells or PEP-3-KLH peptide directly, demonstrate that this approach is safe and capable of eliciting both cellular and humoral antigen-specific immune responses.¹⁰⁰⁻¹⁰² In a follow-up phase II study among newly diagnosed GBM patients with EGFRvIII-positive tumors, administration of PEP-3-KLH peptide vaccination after radiotherapy with daily temozolomide was confirmed to be safe with no evidence of autoimmune reactivity.¹⁰³ Of note, patients in this study did not receive adjuvant temozolomide after completion of radiotherapy. Median overall survival was 26.0 months, which was significantly improved to that achieved among matched controls treated with temozolomide chemoradiotherapy alone ($p=0.013$). In this study, the development of either humoral or cellular immune responses appeared to correlate with better outcome. Six of 14 evaluable patients (43%) developed EGFRvIII-specific antibody reactions, and had a median overall survival of 47.7 months compared to 22.8 months for those who did not generate a humoral response. Although only 3 of 17 patients developed EGFRvIII-specific DTH reactivity, the median survival of these three patients had not been reached with a median follow-up of over 50 months, compared to median overall survival of 23.1 months among patients who did not generate a DTH response. In a subsequent phase II study, PEP-3-KLH peptide vaccination was administered monthly during post-radiotherapy adjuvant temozolomide cycles administered using the standard 5-day dosing schedule or a dose-intensified 21-day schedule.¹⁰⁴ Overall tolerance of the vaccine was good, although 3 patients developed, treatable, grade 3 hypersensitivity reactions, and survival significantly surpassed that of appropriately matched historical controls. Importantly, although temozolomide induced significant but expected lymphopenia,¹⁰⁵ all patients demonstrated EGFRvIII-specific immune responses and a subset developed DTH responses. These results suggest that temozolomide-induced lymphopenia does not prevent vaccine-specific immunoreactivity and may not abrogate vaccine-induced anti-tumor benefit. Multicenter trials evaluating PEP-3-KLH (Rindopepimut; Celldex Therapeutics; Needham, MA) are currently ongoing and include a randomized phase II study with bevacizumab among recurrent GBM patients (Clinicaltrials.gov NCT01498328) as well as a placebo-controlled, randomized phase III study for newly diagnosed GBM patients (Clinicaltrials.gov NCT01480479).

A noteworthy observation from the PEP-KLH vaccination experience was the apparent ability of EGFRvIII-positive GBM tumors to recur without EGFRvIII expression,^{103,104} suggesting that PEP-3-KLH vaccination either successfully eradicated EGFRvIII-expressing cells and promoted emergence of EGFRvIII negative clones, or it induced evasion of an initial vaccine antigen-specific immune response by immunoediting.¹⁰⁶ In an effort to avoid such treatment failures, others have turned to vaccines that incorporate multi-valent synthetic peptides generated from TAAs. Okada and colleagues evaluated a novel vaccination strategy consisting of α -type 1 polarized dendritic cells loaded with multiple synthetic glioma-associated antigen peptides (ephrinA2, interleukin-13 receptor- α 2, YKL-40 and gp100) that were administered with the immunoadjuvant poly-ICLC to HLA-A2-positive patients with recurrent malignant glioma.¹⁰⁷ This strategy also proved safe with no grade 3 or 4 toxicities or evidence of autoimmune reactions. Despite the fact that most patients were heavily pre-treated with chemotherapy, 11 of 19 evaluable patients (58%) demonstrated positive ELISPOT or tetramer reactions to vaccine-targeted glioma-associated antigens. Furthermore, evaluation of post-vaccination sera revealed increased immunocytokine levels including interferon- α , CXCL10, interleukin-15, MCP-1 and MIP-1 β . In addition, resected recurrent tumors revealed elevated CXCL10, an important chemokine related to intratumoral CD8+ T-cell trafficking. Two patients (9%) achieved

radiographic responses and 9 patients (41%) remained progression-free for at least 12 months. IL-12 levels produced by α -dendritic cells correlated with time to progression.

Phuphanich and colleagues recently reported results of a similar approach among HLA-A1 or A2-positive GBM patients treated with autologous dendritic cells pulsed with six different glioma associated antigen peptides (HER2, TRP-2, gp100, MAGE-1, IL13R α 2 and AIM-2).¹⁰⁸ Of note, all patient tumors expressed at least three of the target tumor antigens by qRT-PCR, while 75% of patient tumors expressed all six target antigens. Immune responses, as defined by a 1.5 fold increase in post-vaccination CD8⁺ interferon- γ production, were noted in 5 of 15 GBM patients (33%). Among newly diagnosed GBM patients, the median overall survival was 38.4 months. A placebo-controlled, randomized, phase III study evaluating ICT-107 (Immunocellular Therapeutics, Woodland Hills, CA), a vaccine of dendritic cells pulsed with six tumor antigen peptides and integrated with standard temozolomide chemoradiotherapy, has recently completed accrual for newly diagnosed GBM patients (clinicaltrials.gov NCT01280552).

Terasaki recently reported results of a phase I study evaluating a novel multi-valent vaccine approach based on pre-existing humoral immunity to HLA-A24-restricted candidate peptides among recurrent GBM patients.¹⁰⁹ Patients were required to demonstrate IgG titers to at least 4 of 14 glioma-associated antigens prior to inclusion. Potential advantages to this approach include that it provides a personalized vaccine specific for each patient with a vaccine is designed to augment pre-existing anti-tumor immunoreactivity. Patients received up to four peptides per vaccination and the recommended phase II dose level was determined to be 3 mg/peptide. The vaccinations were well tolerated, with localized skin reactions up to grade 3 constituted the only significant adverse events. Of note, 2 patients achieved partial responses and the PFS-6 rate was 16.7%.

Antigens associated with cytomegalovirus (CMV) also appear to be attractive vaccination targets for GBM patients. As initially demonstrated by Cobbs¹¹⁰ and subsequently confirmed by others,¹¹¹⁻¹¹³ CMV proteins including IE1, pp65 and late antigens, are detected in nearly all GBM tumors, but are not detectable in surrounding normal brain or brain samples from non-oncologic pathologic conditions. Additional recent work has demonstrated that glioma cancer stem cells also express CMV antigens and secrete CMV IL-10, a viral homolog of IL-10. CMV IL-10 was shown to induce human monocytes, the precursors of CNS macrophages known as microglia, to reflect an M2 immunosuppressive phenotype including down-modulation of the major histocompatibility complex and co-stimulatory molecules as well as increased expression of the B7-H1 immunoinhibitory molecule.¹¹⁴ In addition, US28, a G-protein-coupled receptor-like protein encoded by human CMV, is expressed by a majority of GBM tumors, and can promote GBM angiogenesis, invasion and activation of multiple cellular kinases including STAT3.¹¹⁵ Immunogenicity of CMV antigens was recently confirmed by demonstration of a robust anti-pp65 CD8⁺-T-cell response in a newly diagnosed GBM patient with a CMV pp65 expressing tumor, following administration of autologous dendritic cells pulsed with tumor lysate.¹¹⁶ A phase I study evaluating vaccination of CMV pp65-LAMP loaded dendritic cells with or without autologous T-cell transfer for newly diagnosed GBM patients has recently completed accrual (Clinicaltrials.gov NCT00639639). In addition, the feasibility of expanding CMV-specific T cells from GBM patients for adoptive transfer has recently been reported¹¹⁷ and a clinical trial evaluating the adoptive transfer of CMV-specific cytotoxic T-cells for GBM patients is underway (Clinicaltrials.gov NCT01205334).

Remaining Questions

Although reported active immunotherapy approaches for GBM patients demonstrate evidence of anti-tumor benefit, these outcome data must be interpreted cautiously due to

potential selection bias based on enrollment of small numbers of patients with generally favorable prognostic factors. Of note, correlations between immune response and improved outcome have been observed although with some inconsistency.^{79,80,118,119} Randomized studies have not been reported although a number are now underway and should provide important insights.

Nonetheless, a critical review of reported studies reveals marked variability across multiple aspects of vaccine approaches underscoring that optimization remains to be defined and several important questions have gone unanswered. For example, regarding antigen choice, is there an optimal number or type to incorporate into a vaccine? As described, reported studies have employed a full numeric spectrum from one (EGFRvIII) to innumerable (tumor lysate) as well as a wide array of antigen types. Tumor-associated antigens are often weakly immunoreactive due to co-expression on normal tissues and subsequent host immunotolerance. In contrast, TSA from tumor mutations or viral antigens may generate more robust immunoreactivity. Another important question is whether *ex vivo* sensitization of autologous dendritic cells is necessary, and if so, how are they best loaded with tumor antigens? Additional remaining questions involve vaccine dosing logistics, including whether there are more advantageous choices for frequency, site and route of administration.

Another factor that has not been systematically evaluated is the role of immune adjuvants. Immune adjuvants can enhance vaccine-associated immune responses by a variety of mechanisms including antigen clustering, maintaining an antigen depot, targeting vaccine antigens to specific cell types and stimulating innate immune processes.¹²⁰⁻¹²² For cancer vaccines, many adjuvants are felt to contribute by enhancing dendritic cell maturation.¹²³ This activation signal critically determines the fate of dendritic cells associated with tumor antigens. Without this signal, tolerance due to enhanced Treg activity is promoted, whereas this signal augments anti-tumor immunity by stimulating dendritic cell differentiation as well as processing and presentation of tumor antigens.^{124,125} Several immune adjuvants have been evaluated in malignant glioma vaccine trials to date. Toll-like receptor agonists such as imiquimod and poly-ICLC, as well as synthetic oligodeoxynucleotides containing unmethylated CpG motifs, augment dendritic cell activation and T-cell anti-tumor immunoreactivity.¹²⁶⁻¹²⁹ The use of TLR agonists with tumor lysate loaded dendritic cell vaccination has been shown to be safe with no serious related adverse events.⁷⁷ Keyhole limpet hemocyanin (KLH), a protein derived from the sea mollusk *Megathura crenulata* has been shown to augment both cell-mediated and humoral responses,¹³⁰ and has been incorporated safely into active vaccination studies for GBM patients.^{101,102} GM-CSF, a myeloid cytokine, has been utilized to enhance vaccine immunogenicity based on its ability to promote dendritic cell maturation and function.¹³¹ GM-CSF administration strategies have included co-administration of recombinant protein with vaccines as exemplified by Provenge, the recently FDA-approved vaccine for prostate cancer patients,²⁴ and via gene-transduced tumor cells.¹³²⁻¹³⁴ The role of GM-CSF as an immune adjuvant has been controversial as it has been shown to augment immune responses when administered at low doses, but can also promote immunosuppression at higher doses.^{135,136} Nonetheless, it remains unclear whether immune adjuvants are necessary, and if so, how to best integrate them with vaccine reagents.

Another critical question is how to optimally integrate vaccination strategies with standard therapies for either newly diagnosed or recurrent GBM patients. Temozolomide combined with radiotherapy followed by adjuvant temozolomide is the current standard of care for newly diagnosed GBM patients based on a survival advantage compared to radiation therapy alone.³ Although temozolomide chemoradiotherapy is associated with significant lymphopenia in some GBM patients,¹⁰⁵ growing evidence supports combining active immunotherapeutics with temozolomide based on the ability of temozolomide to enhance

tumor immunogenicity via several mechanisms. First, lymphodepletion can enhance immune reactivity in some cancer settings,^{137,138} possibly due to homeostatic expansion of critical T and B lymphocyte cell populations that regulate immune tumor control.^{139,140} Temozolomide can also preferentially increase CD8+ T cells and deplete regulatory T cells,^{104,141-143} in a manner similar to cyclophosphamide and other chemotherapeutics.^{140,144,145} Second, temozolomide can enhance antigen-specific T cell responses following dendritic cell vaccination suggesting that temozolomide-induced tumor cell apoptosis may act to increase T-cell cross-priming against tumor associated antigens.^{141,146} In addition, lymphodepletion, as commonly achieved by temozolomide can diminish homeostatic cytokine sinks, leading to enhanced immunogenic responses.¹⁴⁷ Finally, temozolomide can modulate glioma chemokine production leading to altered tumor CD4+ infiltration and improved survival.¹⁴⁸⁻¹⁵⁰

Bevacizumab, a humanized monoclonal antibody that blocks tumor angiogenesis by inhibiting vascular endothelial growth factor (VEGF), is an approved therapeutic for recurrent GBM patients in many countries based on durable radiographic response rates.¹⁵¹ In addition, phase III registration trials evaluating bevacizumab among newly diagnosed GBM patients have recently completed accrual. Preliminary results of the AVAglio study were recently reported.¹⁵² In this study, bevacizumab significantly improved progression-free survival but did not prolong overall survival, although patient follow-up is not mature and the impact of cross-over to bevacizumab among control patients at recurrence is unclear. Growing data demonstrates that VEGF contributes to the immunosuppressive ability of tumors.¹⁵³⁻¹⁵⁶ Specifically, VEGF can inhibit dendritic cell maturation and antigen presentation, induce apoptosis of CD8+ T cells, enhance Treg activity and diminish infiltration of T cells across tumor endothelium.¹⁵⁷⁻¹⁶⁰ The rationale underlying the combination of immunotherapy approaches with anti-angiogenic agents is based on the ability of VEGF inhibition to diminish immunosuppressive features of tumors¹⁶¹⁻¹⁶³ and enhance the anti-tumor activity of immunotherapies.¹⁶¹⁻¹⁶⁵ These factors are the basis of an ongoing trial combining the EGFRvIII peptide vaccine Rindopepomit (Celldex Therapeutics, Needham, MA) plus bevacizumab among recurrent GBM patients (Clinicaltrials.gov: NCT01498328).

Another area of great interest but marked uncertainty is the identification of biomarkers including clinical, immunologic or molecular factors that may predict patients who are most likely to benefit from active immunotherapy strategies. Among clinical factors, a fundamental issue that remains unclear is whether there is a specific time during the course of therapy when immunotherapy approaches are most likely to be of benefit. Specifically, are treated patients with recurrent disease capable of achieving an adequate immune response, or should immunotherapies be prioritized for those with newly diagnosed and untreated disease where the immune system is more intact. Several additional clinical factors remain controversial. For example, younger patients have been shown in some studies to achieve better anti-tumor immunity as well as overall outcome following vaccination therapies.^{74,75,166,167} In addition, patients with lower tumor burden, such as those who have undergone a gross total or near total resection may be more likely to respond to vaccine immunotherapy approaches than patients who undergo less substantial resections.^{74,75,81} Along these lines, a recently reported recursive partitioning analysis model identified age, histologic grade, degree of resection and performance status as relevant predictors of outcome among recurrent malignant glioma patients treated with dendritic cell-based vaccination therapy.¹⁶⁸

Regarding potentially relevant molecular genetic biomarkers, detailed gene expression profiling studies demonstrate that GBM tumors can be subclassified into 3-4 subtypes.^{12,13} A recent analysis suggests that patients with the mesenchymal gene expression profile, a

signature characterized by overexpression of many pro-inflammatory genes, have increased CD3+ and CD8+ tumor-infiltrating lymphocytes as well as increased survival following tumor lysate pulsed dendritic cell vaccination compared to other GBM subtypes; however these findings have not been validated.⁷⁷

The role of immune response biomarkers remains another area of uncertainty. A wide array of assays to assess various types of immune responses has been evaluated in immunotherapy clinical trials for GBM patients.^{60,73-75,78-80,134,169} Although some results have been encouraging, definitive evaluation of these response parameters has been hindered by significant variability in methodology and characterization of response. Furthermore, it is unlikely that a single immune system response parameter will reliably correlate with outcome across all active immunotherapy studies given the complexity of localized and systemic factors impacting the immune response following active vaccination. Hierarchical clustering of immune parameters represents an alternative strategy to evaluate subsets of patients more likely to benefit from immunotherapy approaches, but experiences to date are mixed most likely due to small numbers of assessed patients.^{78,79} An intriguing biomarker that warrants further investigation is the detection of pre-existing tumor antigen-specific immune responses as this has been shown to correlate with response in some studies.^{75,76,109,170}

Overcoming Hurdles

Profound immunosuppression is a hallmark of many complex cancers including GBM.¹⁷¹⁻¹⁷³ Immunosuppression represents a critical tumor adaptation that contributes to poor outcome by fostering tumor immunotolerance rather than rejection. Immunosuppressive adaptations in GBM can be classified into systemic and local mechanisms. Systemically, GBM patients exhibit decreased T-cell responsiveness, increased circulating CD4+/CD25+/FoxP3+ regulatory T cells (Tregs) and diminished immunoglobulin levels.¹⁷³⁻¹⁷⁷ Local immune inhibitory factors within the GBM microenvironment include: downregulation of MHC molecules;^{178,179} impaired T cell function due to physical factors such as hypoxia;¹⁸⁰ secretion of immunoinhibitory cytokines such as transforming growth factor- β ,^{181,182} vascular endothelial growth factor,^{159,160,183} prostaglandin E2,¹⁸⁴ interleukin-10,^{185,186} and lectin-like transcript-1;^{178,187-191} immunosuppressive activity of microglia/tumor associated macrophages which can account for up to 40% of glioma mass;^{192,193} and increased levels of infiltrating Tregs.^{100,178,188,194-196} In addition, malignant gliomas express Fas ligand which can induce apoptosis of activated T cells.^{197,198}

Strategies to counteract some of the adaptive immunosuppressive mechanisms exhibited by GBM tumors include immunomodulatory therapeutics and adoptive T cell approaches. A variety of immunomodulatory monoclonal antibodies are in development for cancer therapy including those that either block inhibitory receptors or stimulate activating receptors of cytotoxic T cells (Figure 2). FDA approval of ipilimumab, a fully human IgG1 monoclonal antibody targeting CTLA-4, for advanced melanoma,²⁵ including those with CNS metastases,^{199,200} has stimulated interest in exploring various immunomodulatory strategies for cancer patients including those with GBM. Preclinical studies confirm that CTLA-4 blockade enhances survival among immunocompetent VM/Dk mice bearing orthotopic SMA-560 glioma tumors.²⁰¹ A phase II/III randomized, placebo-controlled study of ipilimumab for newly diagnosed GBM has been developed and is planned to initiate accrual soon.

Emerging data also implicate enhanced activity of the inhibitory immunoregulatory molecules programmed death-1 (PD-1) and its associated binding partners PD-L1 (Figure 3) and PD-L2 as pivotal mediators of tumor immune evasion for many cancers including

GBM.²⁰² Indeed, recent analyses have confirmed that PD-L1 is expressed by a high percentage of GBM cell lines²⁰³ and primary tumors.²⁰³⁻²⁰⁷ In addition, PD-L1 expression correlates with glioma grade and is detected on CD133+ GBM stem cells.²⁰⁷ Furthermore, inactivating mutation of the phosphatase and tensin homolog (PTEN) tumor suppressor, which occurs in 36% of GBM tumors,⁹ has been associated with upregulation of PD-L1 expression and immunoresistance.²⁰⁸ Targeting PD-1 and PD-L1 has recently generated encouraging evidence of anti-tumor activity among advanced solid tumor cancer patients.^{209,210}

A third immunomodulatory approach for GBM patients includes strategies to decrease the number or activity immunosuppressive Treg cells. Preclinical studies using an immunocompetent, orthotopic GBM model demonstrated anti-tumor activity associated with Treg depletion.²¹¹⁻²¹³ Alternatively, inhibition of IL-2 activation of Tregs has shown promise.^{211,214} A clinical evaluation of this approach is underway utilizing daclizumab (Zenapax; Hoffman-La Roche Incl, Nutley, NJ), a monoclonal antibody against the IL-2 receptor, in combination with active vaccination against EGFRvIII in a phase I/II clinical trial (Clinicaltrials.gov NCT00626015). Adoptive cellular immunotherapy, involving administration of tumor-specific T cells has shown promise in several cancer indications including melanoma, GBM and CNS metastases.²¹⁵⁻²²² Adoptively transferred, tumor-specific T cells have been shown to accumulate within intracranial tumors in preclinical models.³⁸ An attractive variation of this approach involves the administration of T cells engineered to express chimeric antigen receptors (CARs).²²³⁻²²⁵ T cells expressing CARs have been developed to target several GBM antigens including EGFRvIII,^{226,227} IL13RαR,²²⁸ human epidermal growth factor receptor 2 (HER2),²²⁹ and CMV antigens.^{117,230} Clinical trials evaluating adoptive transfer of CAR-expressing T cells targeting either HER2 (Clinicaltrials.gov NCT01109095) or EGFRvIII (Clinicaltrials.gov NCT01454596) are underway for GBM patients.

Finally, an attractive therapeutic approach involves combinations of immune-based therapies. In particular, adding active antigen-specific vaccination that sensitizes the immune system against tumor specific antigens to approaches that enhance overall immunoreactivity is appealing as long as non-specific or autoimmune activity is not significantly enhanced. One approach in evaluation as described above involves blocking the activation of Tregs with daclizumab, a monoclonal antibody against the IL-2 receptor, combined with active vaccination (Clinicaltrials.gov NCT 00626015). Another approach under study involves adoptive transfer of expanded CMV-specific T cells in combination with active vaccination against CMV antigens (Clinicaltrials.gov NCT 00693095). Finally, preclinical evaluation of CTLA-4 blockade in combination with active vaccination has generated encouraging anti-tumor data supporting the clinical development of such approaches for GBM patients.²³¹ Recent phase I studies in prostate cancer, have confirmed the safety of combining CTLA-4 blockade with active immunotherapy vaccination.^{232,233} Nonetheless, response assessment following any type of immunotherapy approach will likely warrant careful consideration based on the possibility that cancer specific immune responses may generate radiographic changes mimicking tumor progression or that a subset of patients with true early progressive disease, manifest by either enlargement of existing lesions or the appearance of new lesions, may subsequently achieve anti-tumor benefit upon generation of an adequate immune response.²³⁴ These considerations form the basis of the recommendation to integrate specific immune-related response criteria into immunotherapy clinical trials rather than relying on traditional Response Evaluation Criteria in Solid Tumors (RECIST) or World Health Organization (WHO) response criteria.^{235,236} The observation of an analogous pattern of radiographic pseudoprogression among some patients following combined radiotherapy plus temozolomide chemotherapy,²³⁷ was part of the basis for the

development of the Radiologic Assessment in Neuro-Oncology (RANO) criteria among GBM patients.²³⁸

Conclusion

Novel therapeutic approaches are critically needed to improve outcome for patients with GBM, the most common malignant tumor of the central nervous system. The generation of a specific immune response offers a potential dual phase anti-tumor benefit. First, attack by immune targeting cells can initially contribute to tumor cell elimination. Second, the memory capability of an immune response may prevent future recurrence from establishing. Immunotherapy approaches have recently achieved proof-of-concept in other challenging cancers. Historically, enthusiasm for the pursuit of these approaches for patients with primary brain tumors has been dampened by dogma of the immune privileged status of the CNS. More recently, much of that dogma has been discarded as growing data supports immunocompetence within the CNS. A variety of active vaccination strategies based primarily on either whole tumor lysate or specific tumor antigen sensitization approaches have generated consistent safety data as well as preliminary yet encouraging immunogenicity and efficacy data. Adoptive T cell and immunomodulatory therapies are also in development. Nonetheless, many variables need to be systematically assessed and the elucidation of biomarkers to predict outcome will be critical if these approaches are to be optimized for GBM patients. Finally, mechanisms of immunosuppression associated with GBM will require better understanding and possibly therapeutic intervention to maximize vaccination efficacy. Such efforts offer substantial promise, including the possibility of combining complimentary immunotherapy approaches to achieve potentially synergistic anti-tumor benefit.

Supplementary Material

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Key Issues

- Outcome for glioblastoma patients remains poor and innovative treatment approaches are desperately needed;
- Immunotherapy approaches are in development for malignant glioma patients as growing data supports immunocompetence of the CNS and capability to interact effectively with the peripheral immune system;
- Malignant gliomas express a multitude of antigens that are potentially targetable for immune therapies;
- Glioblastoma is associated with systemic and local microenvironment immunosuppression;
- Vaccines for glioblastoma, utilizing whole cell lysates or synthetic antigens, have been very well tolerated to date with no evidence of CNS autoimmunity, and have generated evidence of immunogenicity and anti-tumor efficacy;
- Several variables require systematic investigation in order to optimize vaccine immunotherapy for glioblastoma patients;
- Additional immune based strategies including immunomodulatory reagents and adoptive cell transfer and under investigation for patients with glioblastoma;
- Combinatorial immune-based treatment approaches offer exciting therapeutic potential for malignant glioma patients

Expert Commentary

Outcome for patients with glioblastoma, the most common malignancy of the central nervous system (CNS), remains dismal despite aggressive surgery and cytotoxic therapies. Immunotherapy approaches have recently demonstrated exciting anti-tumor activity in a number of challenging oncology indications. Enthusiasm for immune-based therapies for primary brain tumor patients has historically been tempered by dogma arguing that the CNS is immune privileged. More recent data has invalidated much of this dogma and demonstrates that the CNS is capable of effectively interacting with the peripheral immune system. Taking advantage of the expression of a multitude of glioma-associated antigens, several vaccine strategies have been initiated, utilizing both whole cell lysate and synthetic antigen approaches, for malignant glioma patients. All of the vaccines evaluated to date have demonstrated favorable toxicity profiles and a lack of CNS-directed autoimmune reactions. Encouraging, although somewhat variable evidence of anti-tumor immunogenicity as well as efficacy, have been observed in these trials. At least two major factors likely contribute to the overall modest and inconsistent immune responses and anti-tumor benefit observed to date. First, completed studies have been small and incorporate a wide array of variable treatment components including type/number of tumor antigens, use of immune adjuvants, timing and dosing of vaccinations, and the use of autologous dendritic cells. Patient selection has been quite variable as well including several important considerations such as status of underlying tumor (newly diagnosed versus recurrent), the extent of underlying tumor (gross total resection versus residual bulky tumor burden) and concurrent usage of corticosteroids. Careful and systematic evaluation of these pertinent variables will likely be required in order to optimize anti-tumor benefit associated with vaccine therapies. Second, glioblastoma is associated with significant immunosuppression, both systemically as well as within the tumor microenvironment, as an adaptive mechanism to induce immune tolerance and evade immune rejection. Ongoing efforts seek to better delineate contributors underlying mechanisms of immunosuppression that may subsequently be therapeutically exploited. Key considerations in this regard include regulators of immune checkpoint responses such as cytotoxic T lymphocyte antigen (CTLA-4) and programmed death -1 (PD-1) and its associated ligand (PD-L1), which is known to be highly upregulated and expressed on glioblastoma tumor cells. An additional critical area of investigation also includes the identification of correlative biomarkers that may predict patient subsets more likely to respond to immune-based strategies as well as provide an early yet reliable read-out of response or treatment failure. A further challenge rests in the assessment of response to guide treatment continuation or termination as immunotherapies are increasingly recognized to be associated with delayed responses or robust inflammatory reactions that can mimic tumor progression. Beyond vaccine approaches, several additional immune-based treatment strategies are in early development for malignant glioma patients and warrant further investigation including blockade of immune checkpoint regulators, inhibiting the impact of immunoinhibitory cytokines such as TGF- β , depletion of inhibitory Tregs, and the adoptive transfer of tumor-specific T cells including those engineered to express chimeric antigen receptors (CARs). Given the spectrum of immune-based treatment reagents in development, the evaluation of approaches that combine potentially complimentary therapeutics, such as vaccination to elicit a tumor-specific immune response with an inhibitor of immune checkpoint response, offer great promise for meaningful anti-tumor benefit for patients with malignant glioma.

5-Year View

Harnessing the immune system to provide effective and specific anti-tumor responses offers the exciting and unique capability of providing both treatment of currently active

tumors as well as the prevention of future tumor recurrence. A variety of vaccination approaches are in various stages of clinical development for malignant glioma patients based on encouraging, albeit preliminary, evidence of therapeutic benefit. In the next 1-2 years, data from ongoing randomized trials will better clarify the potential for these reagents in this disease indication. Nonetheless much work remains to optimize vaccination approaches including the systematic evaluation of treatment and patient characterization variables that may impact overall response and anti-tumor benefit. A major area of focus will continue to be the identification of biomarkers, including imaging parameters, circulating factors, immune response correlatives, and tumor markers, that will permit optimal stratification of patients and early determination of patients likely to achieve long-term benefit. Parallel clinical development of other immune-based treatment strategies beyond vaccines that are designed to overcome mediators of tumor-induced immunosuppression or induce more potent anti-tumor immune responses are expected to gain momentum over the next few years. As these therapeutics become better defined, their combination with effective anti-tumor vaccination strategies, will likely become the focus of future treatment approaches.

Figure 1.
Immunosuppression among glioblastoma patients is mediated by systemic and local (microenvironment) factors.

Figure 2.
Immunomodulatory receptors on T cells that may be targeted to enhance T cell activation.

Figure 3. PD-L1 is upregulated by GBM cells to diminish cytotoxic T cell activity (A). Blockade of either PD-1 or PD-L1 by monoclonal antibodies may enhance cytotoxic T cell activity (B).

Table 1

Potential glioma tumor-associated antigens for immunotherapy

Antigen	Frequency	HLA-Locus Restriction	Citation
Aim-2		A1	1,2
Art1 (antigen recognized by T cells 1)			3
Art4 (antigen recognized by T cells 4)			3
B-catenin			4
BMI1			5
B-cyclin		A2	6
Cathepsin B			7
Cav-1			8
CD74			9
COX-2			10
EGFRvIII			11,12
Ephrin A2/Eck		A2	13,14
EZH2 (enhancer of zeste homolog 2)			15
Fra-1/Fos1 1			3
Gage		A2	16
Ganglioside/GD2			17
Gli-1			4
GnT-V			18
GP100		A2	19,20
Her-2/Neu		A2	19,20
Interleukin 13 receptor 2 α		A2	21,22
Ki-67			23
Ku70/Ku80			24
LICAM (human L1 cell adhesion molecule)			25
Livin/Livin β			26
Mage-1		A2	16,19,20,27
Mage-3			28
MART-1 (melanoma antigen recognized by T cells)			29

Antigen	Frequency	HLA-Locus Restriction	Citation
MRP3			30
Nestin			31
NY-ESO-1			28
Olig2			32
Prox1 (prospero homeobox protein 1)			33
PSCA (prostate stem cell antigen)			34
Sart-1		A24 and A26	35
Sart-2			3
Sart-3			3
Sox2			36
Sox10 (SRY-related HMG-box 10)			37
Sox11 (SRY-related HMG-box 11)			3
Survivin		A24	38,39
Telomerase reverse transcriptase (hTert)		A24	40
Tyrosinase		A2	27
Tyrosinase-related protein 1		A2	27
Tyrosinase- related protein 2			27,41
UPAR (urokinase-type plasminogen activator receptor)			42
WT-1 (Wilm's tumor protein 1)			43

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Table 2

Glioma lysate pulsed dendritic cell vaccine studies

Citation	Phase	#/Type patients	Tumor status	Dose/Vaccine schedule	Bulky tumor at vaccination	Immune adjuvant	Immunoreactivity	Outcome
Yu, 2001	I	GBM, 7; AA, 2	Newly diagnosed	10^6 DC every 2 weeks $\times$ 3	Not specified	None	CTL activity: 4/7 (57%)	OS: 16.25 months (GBM)
Yamanaka, 2003	I/II	GBM, 7; AG, 3	Recurrent	$1-32 \times 10^6$ DC every 3 weeks $\times$ 4	Yes	None	DTH: 3/6 (50%) ELISPOT: 5/5 (100%)	Minor MRI response: 2/6 (33%)
Yu, 2004	I	GBM, 10; AA, 4	Newly diagnosed, 4; recurrent, 10	10^7-10^8 DC every 2 weeks $\times$ 3	Yes	None	CTL γ IFN: 6/10 (60%)	OS: 133 weeks (recurrent GBM)
Rutkowski, 2004	II	GBM, 7; AG, 3	Recurrent	$2-4 \times 10^6$ DC every 3 weeks (2-7 doses administered)	Yes	None	DTH: 6/8 (75%)	PFS: 3 months; OS: 10.5 months
Liau, 2005	I	GBM, 12	Newly diagnosed, 7; recurrent, 5	$1-10 \times 10^6$ DC every 2 weeks $\times$ 3	Yes	None	DTH: 6/12 (50%)	TTP: 15.5 months; OS: 23.4 months
Yamanaka, 2005	I/II	GBM, 18; AG, 6	Recurrent	$1-32 \times 10^6$ DC every 3 weeks $\times$ 10	Yes	None	DTH: 8/17 (47%) ELISPOT: 7/16 (44%)	PR: 1 (4%) MR: 3 (13%) SD: 10 (42%)
Wheeler, 2008	II	GBM, 34	Newly diagnosed, 11; Recurrent, 23	$1-4 \times 10^7$ DC every 2 weeks $\times$ 3; then 1 dose 4 weeks later	Yes	None	CTL γ IFN: 17/34 (50%)	TTP: 261 days (immune responders)
De Vleeschouwer, 2008	I	GBM, 56	Recurrent	18×10^6 DC every 1-2 weeks $\times$ 3-7 doses	Yes	None	DTH: 2/12 (17%)	PFS: 3 months OS: 9.6 months
Ardon, 2010	I/II	GBM, 8	Newly diagnosed	$1-12 \times 10^6$ DC weekly $\times$ 4; then at 1 st , 2 nd , 3 rd and 6 th cycles	Yes	None	DTH: 3/6 (50%) ELISPOT: 5/8 (63%)	PFS: 18 months OS: 24 months
Prins, 2011	I	GBM, 23	Newly diagnosed, 15; recurrent, 8	$1-10 \times 10^6$ DC every 2 weeks $\times$ 3; booster every 3 months	Unclear	Imiquimod or Poly-ICLC (booster vaccinations only)	None	OS (newly diagnosed): 35.9 months OS (recurrent): 17.9 months
Fadul, 2011	II	GBM, 10	Newly diagnosed	1×10^7 DC every 2 weeks $\times$ 3	Yes	None	ELISPOT: 4/10 (40%)	PFS: 9.5 months OS: 28 months
Ardon, 2012	I/II	GBM, 77	Newly diagnosed	$1-12 \times 10^6$ DC weekly $\times$ 4; then at 1 st , 2 nd , 3 rd and 6 th cycles	Yes	None	None	OS: 18.3 months
De Vleeschouwer, 2012	II	GBM, 95; AG, 22	Recurrent	18×10^6 DC every 1-2 weeks $\times$ 3-7 doses	Yes	None	None	OS: 6-48.4 months (varied by RPA class)

Abbreviations: AA, anaplastic astrocytoma; AG, anaplastic glioma; CTL, cytotoxic T cell; DC, dendritic cells; DTH, delayed type hypersensitivity; ELISPOT, enzyme-linked immunosorbent spot assay; GBM, glioblastoma; MRI, magnetic resonance imaging; OS, overall survival; RPA, recursive partitioning analysis; TTP, time-to progression

Table 3

Glioma antigen peptide vaccine studies

Citation	Phase	#/Type patients	Glioma antigen (s)	Dendritic cells	Vaccine dose schedule	Bulky tumor allowed?	Immune adjuvant	Immunoreactivity	Outcome
Sampson, 2009	I	GBM, newly diagnosed, 12	EGFRvIII	Yes	3-10 × 10 ⁷ DC every 2 weeks × 3	No	KLH/GM-CSF	DTH: 5/9 (56%) T cell proliferation: 10/12 (83%)	TTP: 6.8 months OS: 18.7 months
Sampson, 2010	II	GBM, newly diagnosed, 18	EGFRvIII	No	Every 2 weeks × 3, then monthly	No	KLH/GM-CSF	Antibody response: 6/14 (43%) DTH: 3/17 (18%)	PFS: 14.2 months OS: 26.0 months
Okada, 2011	I/II	13 GBM, recurrent, 13; AG, recurrent, 9	IL-13Rα2; EphA2; GP100; YKL-40	Yes	1-3 × 10 ⁷ DC every 2 weeks × 4, then every 4 weeks × 5	Yes	Poly-I/LC	ELISPOT: 6/10 (60%) Tetramer: 5/9 (56%)	ORR: 2/22 (9%) TTP: 4 months (GBM) and 13 months (AG)
Sampson, 2011	II	GBM, newly diagnosed, 22	EGFRvIII	No	Every 2 weeks × 3, then monthly	No	GM-CSF	Antibody response: 22/22 (100%) DTH: 7/8 (88%)	PFS: 11.8 months; OS: 23.6 months
Terasaki, 2011	I	GBM, recurrent, 12	EGFR; EZH2; Lck; MRP3; PAP; PSA; PSMA; PTH-rP; SART	No	1-5 mg/peptide weekly × 6	Yes	None	CTL: 9/12 (75%) Antibody response: 2/12 (17%)	PFS: 2.3 months OS: 10.6 months
Phuphanich, 2012	I	GBM, newly diagnosed 21; GBM, recurrent, 3; BSG, 1	AIM-2; MAGE1; TRP-2; gp100; HER2/neu; IL-13Rα2	Yes	1 × 10 ⁷ DC every 2 weeks × 3	No (except BSG patient)	None	Cytokine response: 5/15 (33%)	PFS: 16.9 months; OS 38.4 months (newly diagnosed GBM)

Abbreviations: AA, anaplastic astrocytoma; AG, anaplastic glioma; BSG, brainstem glioma; CTL, cytotoxic T cell; DC, dendritic cells; DTH, delayed type hypersensitivity; ELISPOT, enzyme-linked immunosorbent spot assay; GBM, glioblastoma; MRI, magnetic resonance imaging; OS, overall survival; TTP, time-to progression