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Immunotherapy for the Treatment of Glioblastoma

Alissa A. Thomas, M.D.[2], **Marc S. Ernstoff, M.D.**[1,3]**[Professor of Medicine]**, and **Camilo E. Fadul, M.D., F.A.A.N**[1,2,3,4]**[Professor of Medicine]**

[1]Department of Medicine, Section of Hematology/Oncology, Dartmouth Medical School and Dartmouth-Hitchcock Medical Center, Lebanon NH 03756

[2]Department of Neurology, Dartmouth Medical School and Dartmouth-Hitchcock Medical Center, Lebanon NH 03756

[3]Medical Oncology Immunotherapy Program, Dartmouth Medical School and Dartmouth-Hitchcock Medical Center, Lebanon NH 03756

[4]Neuro-oncology Program, Norris Cotton Cancer Center, Dartmouth Medical School and Dartmouth-Hitchcock Medical Center, Lebanon NH 03756

Abstract

Glioblastoma, the most aggressive primary brain tumor, thrives in a microenvironment of relative immunosuppression within the relatively immune-privileged central nervous system. Despite treatments with surgery, radiation therapy, and chemotherapy, prognosis remains poor. The recent success of immunotherapy in the treatment of other cancers has renewed interest in vaccine therapy for the treatment of gliomas. In this article, we outline various immunotherapeutic strategies, review recent clinical trials data, and discuss the future of vaccine therapy for glioblastoma.

Keywords

Glioblastoma; vaccine; immunotherapy; high-grade glioma

Introduction

Glioblastoma, the most frequent and malignant primary brain tumor, stands apart from other neoplasms by its biology and location within the central nervous system (CNS). In spite of aggressive multimodal treatment including surgical resection, radiation therapy, and cytotoxic chemotherapy, the disease remains incurable with a median survival under fifteen months, and a 2 year survival of 26.5% [1]. The failure of conventional oncologic treatment to selectively eradicate glioblastoma cells has prompted investigators to look for new and more targeted therapeutic options, as well as for improved prognostic biomarkers that will help us better understand the variation of outcomes.

Immunotherapy offers a different mechanistic approach from chemotherapy, targeted therapy, radiation and surgery, and its recent success in the treatment of other cancers has fueled a resurgence of interest in this approach. Currently there are 17 FDA approved immunologic products used in treatment of human malignancies [2]. Most of the available immunologic treatments are antibody based therapies; however, in 2011 the first cell-based

Corresponding Author: Camilo E. Fadul, MD, Section of Hematology/Oncology - Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH 03756, Camilo.E.Fadul@hitchcock.org, Phone: 603-650-6312, Fax: 603-653-0610.

therapy was approved for the treatment of prostate cancer, opening the door for other immunologic approaches. In the case of gliomas, there are several tumor vaccine strategies that have been explored in clinical trials. Although the initial results are encouraging, the small non-controlled studies preclude definitive proof of improvement in survival.

In this article, we will give a brief overview of the immune system and its relation to the nervous system and cancer, as it provides the rationale for the use of immunotherapy in brain tumors. We will discuss promising immune based therapies, focusing on the outcomes and limitations of ongoing clinical trials that employ vaccines to treat patients with glioblastoma. Finally, we focus on strategies that could refine these vaccine approaches to enhance the potential benefits and become part of the conventional armamentarium to fight glioblastoma.

Overview of the immune system

The primary role of the immune system is to discriminate between self- and non-self in order to recognize foreign invaders and defend against them. The immune system can be divided into two main branches: the innate and the adaptive immune systems [3–4]. The innate immune system, the first line of defense, recognizes pathogen-associated molecular patterns, or PAMPs, via engagement of Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) which are present before an infection takes hold. The innate immune system consists of macrophages, monocytes, neutrophils, natural killer (NK) cells, basophils, eosinophils, and complement.

The adaptive immune system, by contrast, must be activated by antigens. The adaptive immune system consists of T and B lymphocytes, and antigen presenting cells. T-cells, so-named because they mature in the thymus, fall into 2 large subcategories of cytotoxic T cells and helper T cells. The cytotoxic T cells express CD8 receptor that binds to antigens presented in the context of human leukocyte antigen (HLA) major histocompatibility complex (MHC) class I molecules, and along with a second signal mediated by CD28 binding to its ligand B7, leads to cell mediated killing. The $CD4^+$ helper T cells bind to antigens presented on HLA MHC class II molecules. The $CD4^+$/MHC class II interaction leads to cytokine release and recruitment of other immune cells. By contrast, B cells mature in the bone marrow and are involved in production of antibody and antibody-dependent cell mediated cytotoxicity. Once they see antigen, B cells mature into plasmatic cells that secrete antibodies that then bind to antigens. The antigen-antibody complex signals immune detection and triggers killing by a variety of cells including NK cells.

The most active antigen-presenting cells (APCs) are dendritic cells (DCs) that reside as immature cells in almost every organ and tissue [5], sit at the interface of pathogen entry sites and continuously sample antigens. Antigen sampling results in effective antigen presentation when the DCs are triggered by other “danger signals”. “Danger signals” are signs of tissue damage or inflammation [5]. Danger-triggered DCs start to mature and become activated. Activated DCs up-regulate their chemokine-receptors, allowing trafficking to lymph nodes where they can induce T cell responses (Figure 1).

Regulation of the immune system

Though the immune system is designed to recognize between foreign invaders and self, with the constant antigen sampling, some foreign antigens, just by chance, are bound to resemble some antigens inherent in the body. The immune system has ways of regulating itself to put on a brake and prevent autoimmunity. When T cells are activated, they will upregulate membrane cytotoxic T-lymphocyte associated antigen 4 (CTLA4) and program cell death 1 (PD-1) proteins. CTLA4 competes with CD28 to bind B7 and PD-1 will bind to its ligand

PD-L1, both signals will inhibit ongoing T cell activation. Another mechanism to brake immune activation is Regulatory T cells (Tregs) which were first proposed in 1972 [6] and discovered in 1995 [7]. They are now recognized as a key regulatory pathway in tumor tolerance and have a unique cell surface signature, with expression of both CD4 and CD25 (the alpha chain of the interleukin-2 (IL-2) receptor) [7]. These cells express other cell surface markers, including CTLA4 [8] and glucocorticoid-induced tumor necrosis factor (GITR) receptor [9] and are regulated by a transcription factor called forkhead box protein 3 (FOXP3)cells [10]. T-regs actively inhibit conventional $CD4^+$ T cells, $CD8^+$ T cells, DCs, NK cells [11], thus dampening immune responses. Other immune regulatory pathways such as immune suppressive cytokines, myeloid-derived suppressor cells, regulatory B cells and natural killer cells also play important roles in generating and maintaining tumor tolerance and, along with T-regs, are considered targets of immune therapy [12–13] (Figure 1).

Neuroimmunology

It was once thought that the nervous system was an immune privileged organ, devoid of normal immunologic function [3, 14–15]. The CNS features in support of this theory included the blood brain barrier that allows for selective entry of immune cells from the peripheral blood into the brain parenchyma, the lack of lymphatic vessels and lymph nodes within the CNS, and the low numbers of circulating T cells in the CNS. Furthermore, there is less HLA presentation and absence of traditional APCs in the CNS when compared to other tissues. Nevertheless, under physiologic conditions, the brain hosts several populations of immune cells [15]. Microglia arise from hematopoietic cells and colonize CNS during embryonic development. The microglia constitutes an early line of defense for the brain. Microglial cells migrate to inflammatory zones in the CNS and become activated. Once activated they have phagocytic and antigen presenting cell properties, as well as the ability to recruit other immune cells by secreting cytokines and chemokines [16]. Macrophages and DCs both arise from monocytes. They are found in perivascular zones, choroid plexus, and meninges. Because of their role as professional APCs, DC based vaccine therapy has been the most studied approach for high grade gliomas. T cells are found in the CNS only in the activated form. Naïve T cells are not present in the CNS [17–18]. T cells are activated in cervical lymph nodes and then move into the CNS. It is unclear how antigens are transported from the brain to the cervical nodes to activate the T cells.

Cancer immunotherapy has unique challenges in the CNS due to the relative immune-privilege of the brain and the immune-suppression caused by high grade gliomas. The blood brain barrier, low numbers of T lymphocytes, and lack of a lymphatic systems make it challenging for immune cells to enter the CNS. In patients with glioblastoma, the blood brain barrier is disorganized [15, 19]. Tissue injury leads to breakdown of the tight junctions between endothelial cells that facilitate migration of leukocytes into the CNS. Though the CNS is devoid of traditional lymphatic vessels, CSF drains via Virchow-Robin spaces to the deep cervical lymphatics [17, 20]. T cells located in the cervical lymph nodes are activated and can patrol the CNS [17–18]. Activated T cells that encounter their antigen are retained in the CNS. HLA presentation occurs on astrocytes, microglia, and endothelial cells [3, 14]. The net balance is that CNS immune surveillance still occurs [3].

Patients with glioblastoma exhibit a relative systemic immune suppression compared to the general population. Adaptive immune responses are deficient [17]. The tumor microenvironment is rich with immunosuppressive factors secreted by the tumor [17], like transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF) that suppresses T cell proliferation and cytotoxic function [21–22]. VEGF inhibits the maturation of DCs. In glioma patients, there are diminished absolute counts of $CD4^+$ T cells with increased fraction of T-regs [3, 23]. This immunosuppression likely plays an important

role in tumor progression in patients with glioblastoma. In addition to being immunosuppressed from having a malignancy, older age, cytotoxic chemotherapy and exogenous administration of corticosteroids are other factors that contribute to systemic immune suppression in this population (Figure 2). It stands to reason that if the immune suppression could be reversed allowing effective immune targeting of glioma, then patients with glioma might have less tumor progression and improved outcomes.

Rationale for immunotherapy

It has been long observed that changes in the immune system relate with cancer survival. Neurologists and neurosurgeons provide anecdotal reports that glioma patients who suffer postoperative infections near the tumor bed seem to do better than the average patient similar to the observations made over a century ago by Coley [24]. Recently de Bonis and colleagues investigated the idea that postoperative infection may confer a survival advantage to patients with malignant glioma [25]. They reviewed 197 cases of newly diagnosed glioblastoma, 10 of whom had peri-operative infections. The infection group had a significant advantage in the median survival; 30 months compared to 15 months in the non-infected tumor patients. We now understand that infection can contribute to activation of immune pathways via PAMPs and activation of TLR on the innate immune system and subsequently initiate anti-tumor immunity. Indeed, a direct correlation between survival of patients with primary glioblastoma and tumor infiltration of cytotoxic and helper T cells has been observed [26]. As noted previously, glioblastoma patients are relatively immunosuppressed compared to the general population. The degree of immunosuppression also correlates with survival. Grossman and colleagues recently followed a group of 96 patients with newly diagnosed high grade gliomas through surgery, radiation therapy, and chemotherapy with temozolomide [27]. The patients had a normal $CD4^+$ count at diagnosis, but hit a nadir 2 months post-treatment. Forty percent of the study population had $CD4^+$ counts of <200, and those patients had a significantly shorter median survival at 13.1 months compared to 19.7 months in the patients with higher counts. All of these observations taken together suggest that if the immune effectors were better activated in glioblastoma, patients might have better outcomes.

Strategies for using immunotherapy in the treatment of gliomas

As with immunization for infections, immunization against tumors can theoretically occur in the form of passive or active immunotherapy [15]. In passive immunotherapy, a patient is given immune cells or antibodies capable of targeting the tumor cell. Passive immunotherapy does not require activation of the patient's own immune system, but instead immune cells are active in vitro and injected into the patient. By contrast, active immunotherapy provides a boost to the patient's native immune system (Figure 3).

In broad terms, passive immunotherapy can be further divided into 3 approaches [5] (Figure 3). The first is the direct injection of monoclonal antibodies [2–3]. In this approach antibodies that are known to interact with an antigen specific to or associated with a tumor are administered to the patient. In glioblastoma, only one monoclonal antibody has been approved for treatment. Bevacizumab is a humanized IgG1 monoclonal antibody that binds to and neutralizes the vascular endothelial growth factor (VEGF) ligand [2, 28–29]. VEGF is a tumor-associated protein, which is found on a variety of malignancies, including glioblastoma. VEGF is the central mediator of tumor angiogenesis. Although bevacizumab retains its ability to bind complement and Fc receptor, its action is through blocking the ligand that activates the VEGF receptor on tumor blood vessels thereby inhibiting angiogenesis and tumor growth. Bevacizumab may also be associated with afferent vascular

dilatation and efferent vascular constriction of tumor vessels. This may give the additional benefit of concentrating chemotherapy at tumor site.

The second approach to cancer passive immunotherapy is to stimulate the immune system with cytokines. Cytokine stimulation with IL-2 have been studied in a variety of cancers, and while it has been a successful approach in melanoma and renal cell cancer, it has not shown benefit in glioblastoma [2].

A third strategy to passive immunotherapy is treatment with stimulated immune effector cells. This approach has also been called adoptive immunity, or cell based therapy immunotherapy [15]. In adoptive immunity, immune cells activated *ex-vivo* are administrated to patient, either by systemic injection or directly into the tumor or tumor resection cavity. Both lymphocyte-activated killer cells (LAK) and cytotoxic T lymphocytes (CTL) have been used. LAK cells are generally obtained by cultivating autologous peripheral lymphocytes in the presence of IL-2. The culture yields both T and NK cells. The immune reaction provided by LAK cells is non-specific cytotoxicity, which is not necessarily tumor-directed. By contrast, CTLs are prepared by collecting peripheral blood mononuclear cells or tumor infiltrating lymphocytes and then stimulating them *ex vivo* with antigens. For cancer immunotherapy, autologous tumor cells are also used for the antigen stimulation, thus yielding CTLs that have been activated with specificity to the tumor.

Active immunotherapy boosts the patient's immune system by priming it with antigen exposure. There are two approaches to active immunization: peptide based therapy and cell based therapy [30] (Figure 3). In peptide based therapy, peptides are injected as a vaccine to induce immune activation. Tumors express various antigens, some of which are tumor-specific, and others which are tumor associated. Tumor antigens can be also categorized as cancer-testes antigens, tumor differentiation antigens, viral related antigens, or mutated oncogenic proteins. The peptides selected for cancer vaccines are typically small, around nine amino acids in length and are capable of binding to MHC class I molecules, which leads to activation of CTL.

Cell based active immunotherapy uses antigen presenting cells activated by tumor antigens to prime the immune system rather than the antigen itself. Since DCs are professional APCs, they are an obvious choice for active immunotherapy and have to date been the most studied cell based vaccine [5, 15, 30]. In most cases, DCs are prepared from autologous peripheral blood mononuclear cells and are cultivated in the presence of growth factors, such as granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin-4. The DCs are then matured and activated by antigens, which can be prepared from various sources, including tumor lysates, peptides eluded from activated tumor cells, defined peptides such as those used in peptide-based immunotherapy, viral antigens, mRNA derived from activated tumor cells, and whole tumor cells. The activated DCs are then given back to the patient, either injected intradermally, in the lymph nodes or locally at the tumor site.

Clinical reports of immunotherapy

Interpretation and comparison of the results of clinical trials using immune therapy against glioblastoma is extremely difficult because of heterogeneity in study design, therapeutic approach used, immune endpoints measured, and patient eligibility criteria. The classic design of cancer clinical trials does not fit the immune therapy model. For example although most vaccine trials are phase I, the "dose escalation" design does not apply since treatments usually have minimal toxicity; the dose limitation is the availability of cells and not the appearance of adverse events [17]. The few phase II clinical trials published are not randomized and use historic controls to compare outcomes. Most clinical trials included patients with recurrent glioblastoma, who may have a poor functional status, large tumor

burden and been heavily pre-treated making less likely to benefit of immune therapy. In the clinical trials there are exclusion criteria that render a highly selected population of evaluable patient [31]. Furthermore, some trials include patients with newly diagnosed and recurrent disease, and on occasions, include patients with anaplastic gliomas. Surrogate endpoints, like immunologic assays and brain imaging studies, have not been harmonized and validated in most cases.

Passive immunotherapy

The earliest attempts at immune therapy in the late 1980s into the 1990s focused on passive immunization by infusion of LAK cells directly into the tumor bed in the peri-operative and post-operative period [32–38]. Side effects from this strategy included cerebral edema, increased intracranial pressure, headaches, fever, and confusion [32, 36], but in general the treatment was well tolerated. In most instances there was no impact on survival [32, 34, 36–37]; though Hayes and colleagues and others suggested an improved median survival compared to contemporary controls in patients with recurrent glioblastoma treated with LAK cells and IL-2 [38–41].

In addition to LAK cells, CTL are the other group of cells, studied in adoptive immune responses for glioblastoma [42–50]. The first study of CTLs was by Kitahara and colleagues in 1987 [48]. Two of five patients had a reduction in tumor size by imaging following intratumoral CTL treatment. Others have also reported responses to intratumoral and systemic administration of adoptive T cell therapy [42–44, 46, 49–51].

Active Immunotherapy

In the realm of active immunity, both cell based therapies and peptide based therapies have been studied. Patients have be given individual peptides [52–54], whole tumor lysate [55], or some combination of tumor antigens and cytokines [56].

Epidermal Growth Factor Receptor variant III (*EGFRvIII*) is a tumor-specific antigen commonly expressed by glioblastoma cells but not on normal tissue [57]. PEP-3 is a 14 amino acid peptide from *EGFRvIII*, which, when coupled with a foreign “helper molecule” keyhole limpet hemocyanin (KLH) (PEP-3-KLH) has been used as a vaccine to generate *EGFRvIII* specific antibodies [57–58]. Sampson and colleagues have studied the *EGFRvIII* peptide, PEPvIII-KLH in 18 glioma patients expressing *EGFRvIII* on their tumors [53, 59]. The vaccinated patients had improved 6 month progression free survival (the primary endpoint) and improved overall survival compared to contemporary controls [52]. Of interest, sampling of the tumor in some cases at recurrence revealed loss of *EGFRvIII* expression.

Though Epidermal Growth Factor Receptor related peptides have received the most attention, there are other peptides that are tumor associated which may be useful in inducing immune responses. Yajima at colleagues created “personalized peptide vaccines” [54]. They treated 21 HLA-A2 or HLA-A24 patients with GBM with a combination of 23-25 tumor associated peptides known to bind to either HLA-A2 or HLA-A24. Among them, 5 patients had a partial response, 8 patients had stable disease, and 8 patients had progressive disease. Peptide specific IgG antibody responses were detected in the tumor bed or spinal fluid of all patients tested.

Terasaki and colleagues also explored this idea of “antigen soup” as a peptide-immunotherapy approach [60]. They enrolled 12 HLA-A24 positive patients with glioblastoma and HLA-A24. The patients were vaccinated with HLA-A24 restricted (ITK-1) peptides. ITK-1 consisted of 14 peptides known to be expressed at high levels in cancer cells and low levels in normal cells. The 14 chosen peptides were capable of inducing peptide-specific cellular immunity and humoral immunity in HLA-A24 patients with glioblastoma.

Of the 12 pts enrolled, 2 patients had a partial clinical response, 5 had stable disease, and 5 had progressive disease. All patients tolerated the vaccination without any serious adverse reactions [60].

Another approach being explored is to vaccinate with a heat shock protein in complex with autologous tumor derived peptides. Clinical trials in patients with recurrent glioblastoma have revealed both an adaptive and innate immune response with the treatment being well tolerated and suggesting an improvement in survival when compared to historic controls [61].

It is unclear if individual peptides or whole tumor lysate induce a better immune response, as they have never been studied head to head. Autologous tumor prepared vaccines alone or in combination with cytokines have also demonstrated isolated clinical response [55–56].

As DCs are critical in initiating antigen-specific immunity, we and others have used autologous DC vaccination as an approach to treat patients with glioblastoma. The basic strategy for DC vaccination is to give autologous DCs which have been manipulated *ex vivo* to present autologous tumor antigens [54, 62–66]. DC administration has varied by route (intradermal, subcutaneous, intranodal, intratumoral), schedule and combination with other treatment modalities. [67]. Most trials are phase I studies that included patients with recurrent high grade gliomas (variable number of glioblastomas) and in general, vaccination with DCs has been well tolerated [63–65, 68–74]. Some studies have been able to show clinical responses, either in tumor regression [62, 66, 73–74] or improved survival compared to historical or contemporary controls [70, 74–75]. Immune responses have also been demonstrated with use of surrogate endpoints. In patients who underwent reoperation after vaccination with DCs, some have infiltration of CTL within the tumor [63, 68–69].

Some studies enrolled patients with both recurrent and newly diagnosed glioblastoma, but recent series have included patients with newly diagnosed glioblastoma and in combination with other therapeutic modalities (Table 1) [31, 67–69, 75–78]. As can be seen in Table 1, of the 73 patients with newly diagnosed glioblastoma treated with DCs immune therapy as part of the first-line therapy only 2 developed severe toxicity including one report of a patient who developed a cutaneous glioblastoma at a lymph node injection site [75]. One additional high grade toxicity was seen in the 156 patients with recurrent glioblastoma treated with DC vaccines (Table 2).

We evaluated the immunologic response to cervical intranodal vaccination with autologous tumor lysate-loaded DCs in 10 patients with newly diagnosed glioblastoma after concomitant radiation therapy and chemotherapy and before starting adjuvant temozolomide [31] (Figure 4). We explored immunologic endpoints in a novel approach using hierarchical clustering analysis of the results of 5 immune assays measured before and after vaccination (Figure 5). Immune activation as determined by this methodology was associated with improved survival.

Other approaches to vaccine therapy deserve mention. In 2001, Schneider and colleges were able to deliver autologous tumor cells via a viral vaccine vector using Newcastle Disease Virus (NDV) [79]. The advantage of NDV is that it is a single stranded RNA virus that poses little health hazard to humans and has the ability to selectively kill human tumor cells. Other approaches include use of autologous tumor transfected with cytokine genes to express cytokine or DC-tumor cell fusions [80–81].

To date, all vaccine and immune therapy studies in patients with glioblastoma including ours, suffer from small sample size and thus bias induced by patient selection. There are many variables in the design of the studies without consensus in the optimal method to prepare and condition the DCs, antigen to use, site of administration, and immune assays to

monitor [82]. Careful clinical trial design that would provide information on the most favorable dose, frequency of administration, and timing to introduce DC based vaccine in the multimodality treatment scheme of patients with glioblastoma is needed. Nevertheless, the observations of induction of tumor specific immune responses, clinical response, prolonged survival in a few patients, and low toxicity is encouraging and supports the continued investigation of immune therapies in patients with glioblastoma.

Endpoints and outcomes

While primary endpoints of overall survival and progression free survival are most important in the development of a new therapy, surrogate endpoints can be very helpful in predicting clinical outcomes and fueling further research. Surrogate endpoints for vaccine therapy include a variety of immune responses [5]. Often reported are delayed-type hypersensitivity responses, interferon-gamma (IFNγ) release from peripheral blood mononuclear cells as measured by flow cytometry or IFNγ enzyme-linked immunosorbent spot (ELISPOT) assays. Delayed-type hypersensitivity reactions are used as a measure of antigen recall and correlate with peripheral blood antigen-specific T cell responses [83–84]. Nevertheless, patients with glioblastoma are frequently anergic and it is of limited use for monitoring these patients [31, 75]. IFNγ is released by Type I $CD4^+$ T cells [83]. The IFNγ ELISPOT assay is one of the common methods of assessing adaptive immune responses. Recent studies have reported a positive correlation between immune response, measured by ELISPOT assays, and clinical outcomes [83].

We have used the Dye Dilution Proliferation Assay (DDPA) in immunotherapy clinical trials to evaluate immune response by monitoring tumor-specific $CD8^+$ and $CD4^+$ precursor frequency [85]. This method also allows measurement of the proportion of $CD4^+$ and $CD8^+$ IFNγ producing cells for immune monitoring. The median values for the results of 5 immune response measurements (DDPA and ELISPOT) before and after DC based vaccination in 10 patients with glioblastoma was analyzed using hierarchical clustering analysis [31]. A measurable immune response by this composite method was associated with improved survival. There is need to standardize and prospectively validate immune monitoring assays if immunologic surrogate endpoints are to be used in larger clinical trials involving multiple sites.

Radiologic assessment by MRI has been the mainstay in the evaluation of the response to therapy in glioblastoma and used as the primary endpoint in many phase II trials. The appearance of new and more varied oncologic treatment modalities has underscored the limitations of the criteria used to assess response in clinical trials [86]. Specifically, in immune therapy trials for high grade gliomas, increased size of gadolinium-enhanced lesions on MRI studies, suggestive of recurrent tumor, have revealed inflammatory infiltration without active tumor [87]. Furthermore, the modest response on MRI does not correlate with clinical endpoints [88]. Therefore, radiologic criteria to evaluate brain tumor immunotherapy have to be refined and the use of more advanced techniques to image inflammation and immune response are needed [89].

Future Directions

The challenges of the future of immune therapies relate to enhancing antigen presentation capabilities, effectively breaking tumor-induced immune tolerance, improving activation of tumor-specific cytolytic effector cells, and the standardization and upscale production of cell based therapy. At the same time there is concern that further boosting of the immune response, albeit more effective, may result in serious adverse events secondary to brain edema and auto-immunity.

A strong and long lasting anti-tumor T cell response that confers clinical benefit is the goal on DC based immunotherapy. Enhancing the antigen presenting cell cap abilities by polarizing the cell towards a more effective (α-type) 1 DC phenotype has been reported, but there is still controversy on the culture conditions to obtain the most activated and potent DCs. A clinical trial examined using the fusion of dendritic and glioma cells combined with recombinant human interleukin 12 (rhIL-12) for the treatment of malignant glioma [66]. No serious side effects and a few responses on MRI were observed. Combination of DC vaccination with an immunoadjuvant polyinosinic-polycytidylic acid [poly (I: C)] stabilized by lysine and carboxymethylcellulose (poly-ICLC), a PAMP that activates DCs, was safe [87]. Finally the optimal route of administration of DCs for brain tumors has not been established, although animal [90] and clinical studies [91] suggest that intranodal injection is the most effective.

One of the challenges with vaccine therapy is the self-imposed “brake” on the immune system in glioma patients, which may limit the immune system’s response to a vaccine. There are a few potential targets that could remove the brake of the immune system. One strategy might be to deplete the regulatory T cells. To that end, an antibody against CD-4 or CD25 could be used to target Tregs, or more general immunotoxins could be used. A study suggests that administration of daclizumab, an antibody against IL-2Rα, when patients are lymphopenic after administration of temozolomide enhances the antitumor immunity of vaccination by depleting Treg. Once T cells are activated, they up regulate molecules such as CTLA4 and PD-1 to limit their activity (Figure 6). Use of blocking humanized monoclonal antibodies such as ipilimumab (anti-CTLA4) to these check point molecules appear very promising and have already made it to the clinic in treating patients with metastatic melanoma [92]. A phase I clinical trial using a vaccine comprising of autologous tumor cells genetically modified by a transforming growth factor–β2 (TGF-β2) antisense vector in 6 patients with recurrent glioblastoma was well tolerated with indication of anti-tumor induced immunity [93]. Use of additional inflammatory cytokines such as IL-12, IL-7, and IL-15, activating antibodies to costimulatory molecules such as CD40, or blocking antibodies to immune inhibitory cytokines such as IL-10 or TGFβ in combination with DC vaccination can potentially enhance clinical activity [94]. Which of these strategies in combination with vaccination will yield the best therapeutic ratio (most effective and less toxic) is to be determined.

Treatment for high grade glioma involves a multi-disciplinary approach using surgery, radiation therapy, and chemotherapy. Clarifying when and how immune therapy should be given with these other modalities and the role of steroid use in this population of patients will require well designed and appropriately powered clinical trial. In small studies, we and others have demonstrated that DC vaccines can be given safely to patient with glioblastoma undergoing temozolomide chemotherapy alone or in combination with radiotherapy [31]. The studies confirm that tumor-specific immune responses in these patients can be induced [31,58, 69,75]. The rational to give chemotherapy with immunotherapy may relate to the chemotherapy effects on tumor release of relevant antigens, on inhibiting the regulatory compartment, and on the ability to change the tumor vasculature providing better access for effector cells [69, 95–97]. Another possibility is that vaccination sensitizes the tumor to chemotherapy [75, 95, 97].

Conclusion

Research over the last 10 years has demonstrated that immune therapy for glioblastoma triggers a measurable immune response in spite of poor tumor antigenicity and considerable immune suppression. If that antitumor effect is enough to translate in improvement in survival is still to be proven. The limited number of patients with glioblastoma, the lack of a

cooperative group that can do large clinical trials for the study of brain tumor immunotherapy, and the variability in approaches and immune monitoring assays used are the major barriers to determine if immune therapy could be part of the standard of care. Furthermore, the challenge of immunotherapy is to understand the various regulatory and co-stimulatory factors in the patient and the tumor microenvironment and being able to manipulate these forces effectively to enhance anti-tumor immune response and clinical benefit. As immunotherapy evolves, prognostic and predictive biomarkers will be important to determine which patients will make the best candidates for vaccine therapies. There is need for harmonization and validation of immunologic endpoints as well as imaging techniques that allow adequate monitoring of patients with brain tumors receiving immune base therapies. With our expanded knowledge of immune pathways and the effects tumors have on immune function we will be better able to develop vaccine strategies for the future.

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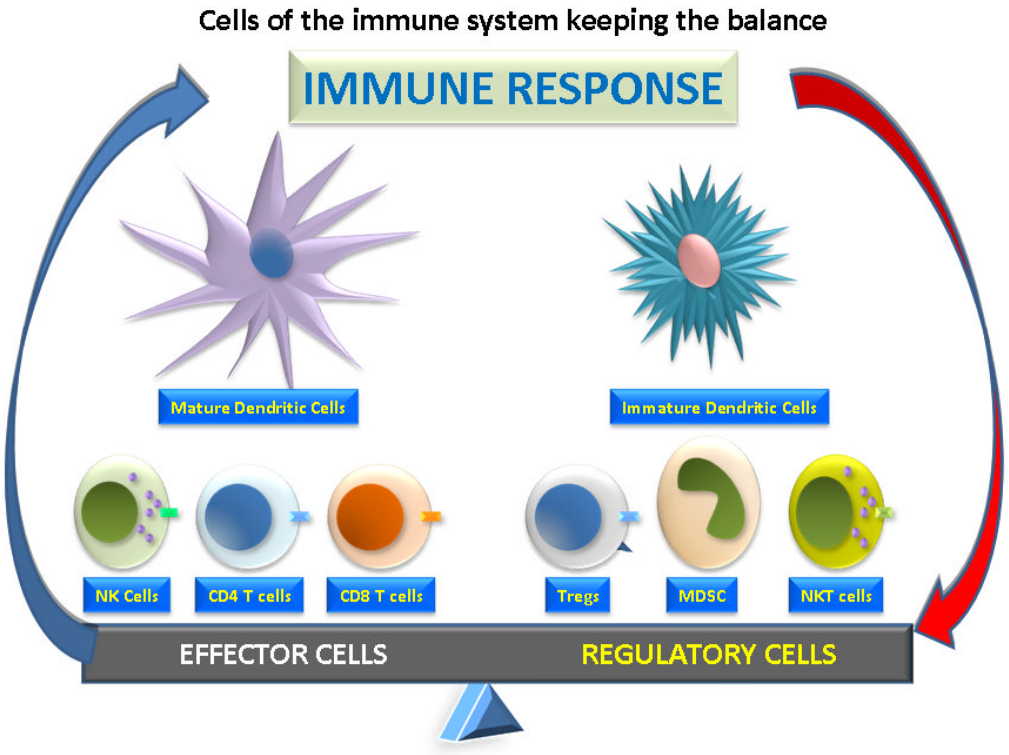

Figure 1.

Cells of the immune system include effector cells and regulatory cells. Among the effector cells are $CD4^+$ helper T cells, $CD8^+$ cytotoxic T lymphocytes (CTL), natural killer cells (NK) and dendritic cells (DC), which are the antigen presenting cells. The regulatory cells include regulatory T cells (Treg), which express CD4, CD25 and CTLA4, and myeloid-derived suppressor cells (MDSC).

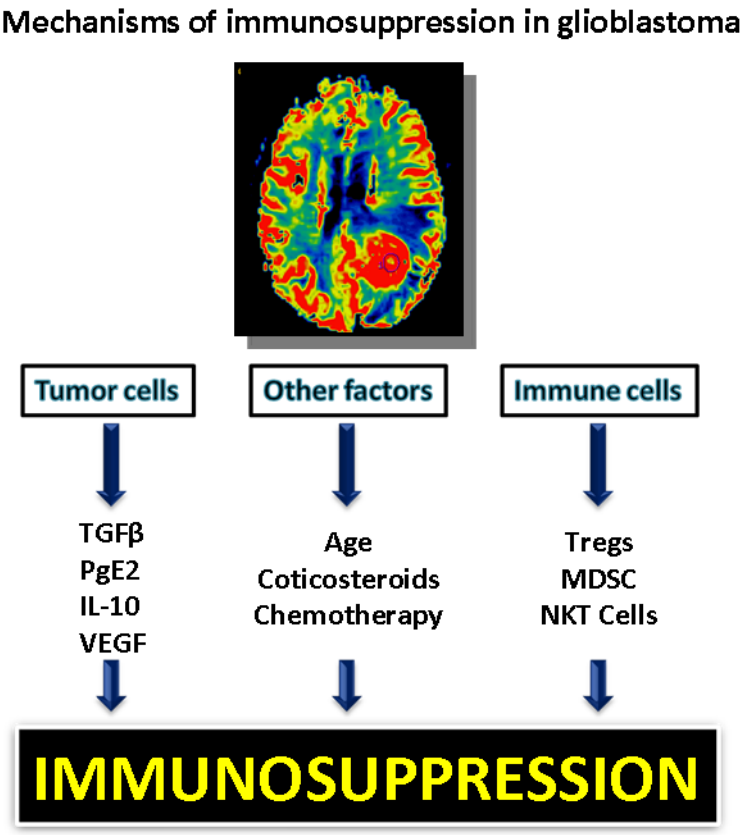

Figure 2.

Factors contributing to immunosuppression in glioblastoma include tumor factors, exogenous factors, and immune factors. The glioma cells secrete immunosuppressive cytokines, such as TGFβ, prostaglandin E2, IL-10, and VEGF. Age, exogenous steroids, and chemotherapy all contribute to exogenous immune suppression; while regulatory immune cells such as regulatory T cells (Treg) and myeloid derived suppressor cells (MDSC), also dampen the immune response.

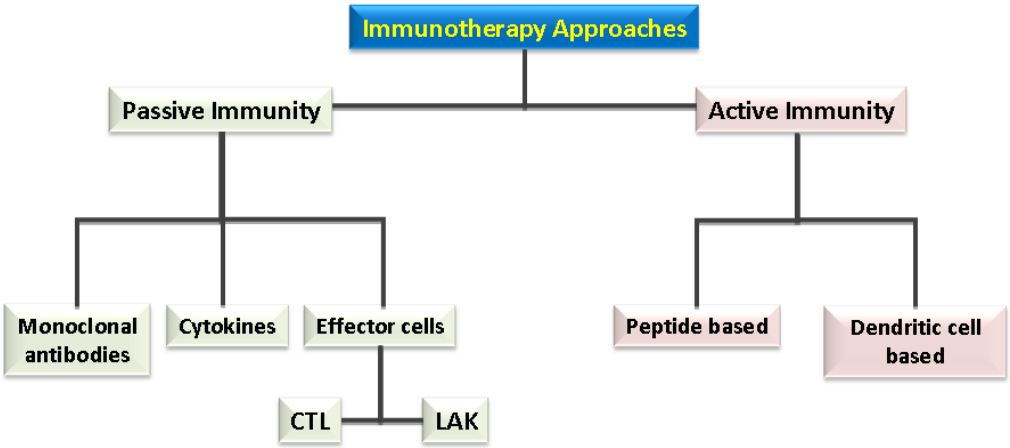

Figure 3.

Approaches to immunotherapy can be divided into passive immunity and active immunity. Passive immunotherapy approaches include direct administration of monoclonal antibodies or cytokines or adoptive immunity with cytotoxic T lymphocytes (CTL) or lymphocyte activated killer (LAK) cells. Active immunity includes peptide based immunotherapy and dendritic cell therapy.

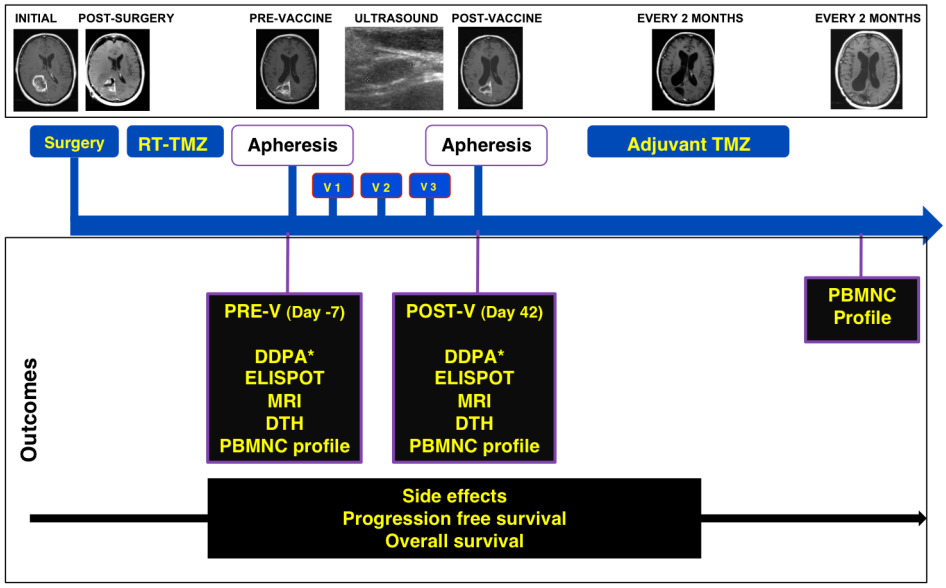

Figure 4.

Four weeks after complete combined RT-TMZ, patients had a prevaccination (V) aphesis, DTH panel placement, and MRI. One week later, the first vaccination (V1) was administered, and 2 additional vaccinations were given 2 weeks apart. Two weeks after the third vaccine patients had a post-V apheresis, DTH panel placement, and MRI, followed by 12 cycles of adjuvant TMZ. DDPA indicates dye dilution proliferation assay; DTH, delayed-type hypersensitivity reaction; ELISPOT, enzyme-liked immunosorbent spot assay; PBMNC, peripheral blood mononuclear cells; POST-V, postvaccination; PRE-V, prevaccination; RT, radiation therapy; TMZ, temozolomide; v, vaccination. Printed with permission from Fadul et al, 2011 [31].

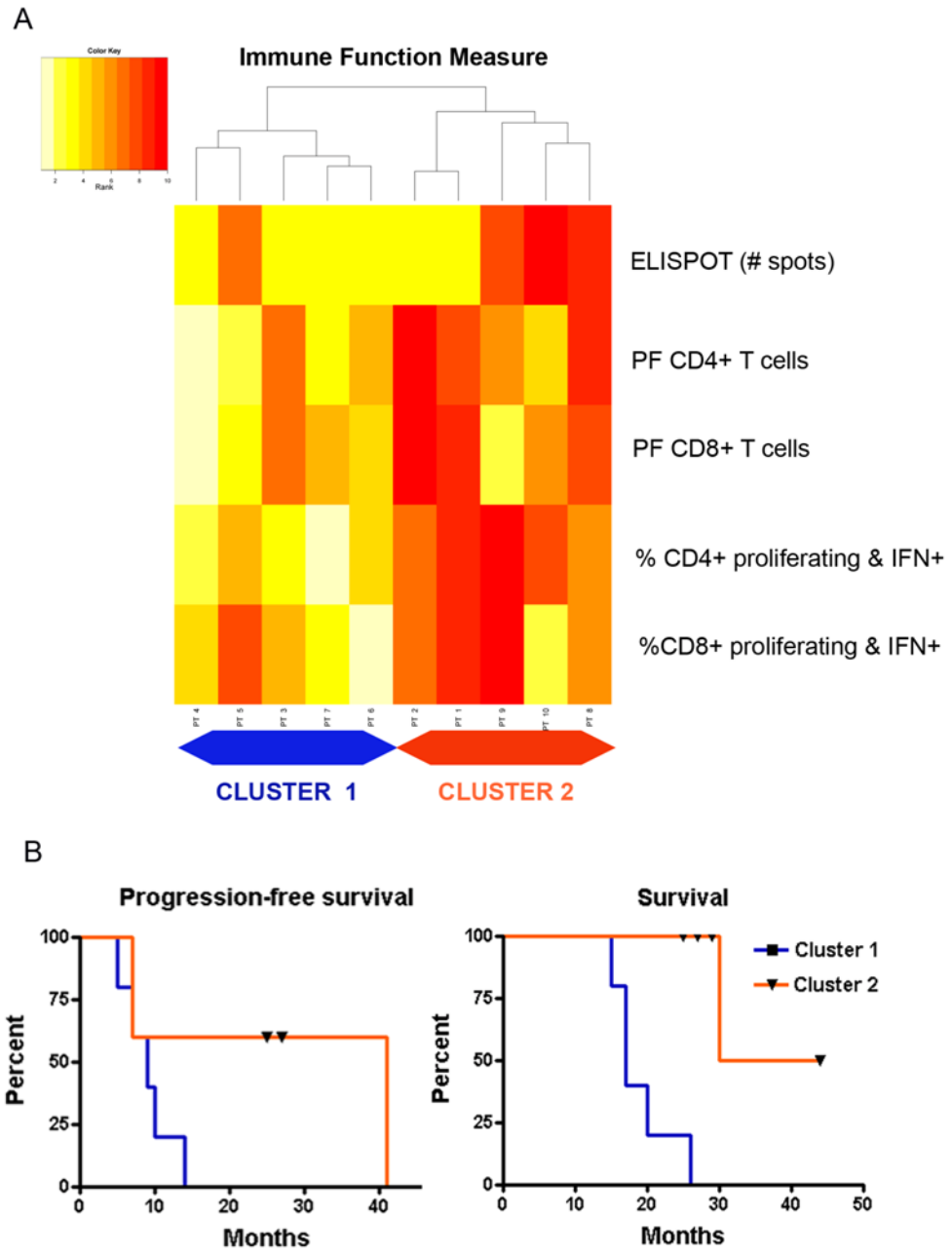

Figure 5.

(5A) Heatmap of hierarchical clustering analysis of the postvaccination immune responses. Five patients with generally low ranks in immune function (pale yellow colors) formed cluster 1 on the left. Five other patients with higher ranks in immune function measures (dark red colors) formed cluster 2 on the right. (5B) Kaplan-Meier curve of overall survival for the 2 clusters. The overall survival was significantly different between cluster 1 (median = 17 mo) and cluster 2 (median = not achieved) ($P = 0.002$). ELISPOT indicates enzyme-linked immunosorbent spot assay; IFN, interferon; PF, precursor frequency. Printed with permission from Fadul et al, 2011 [31].

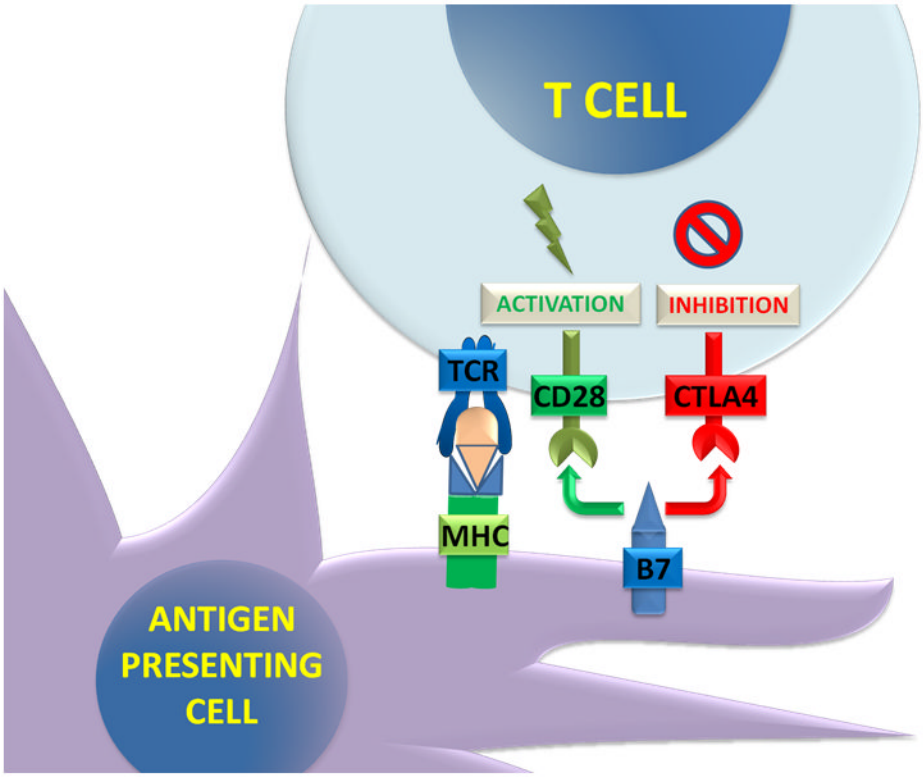

Figure 6a

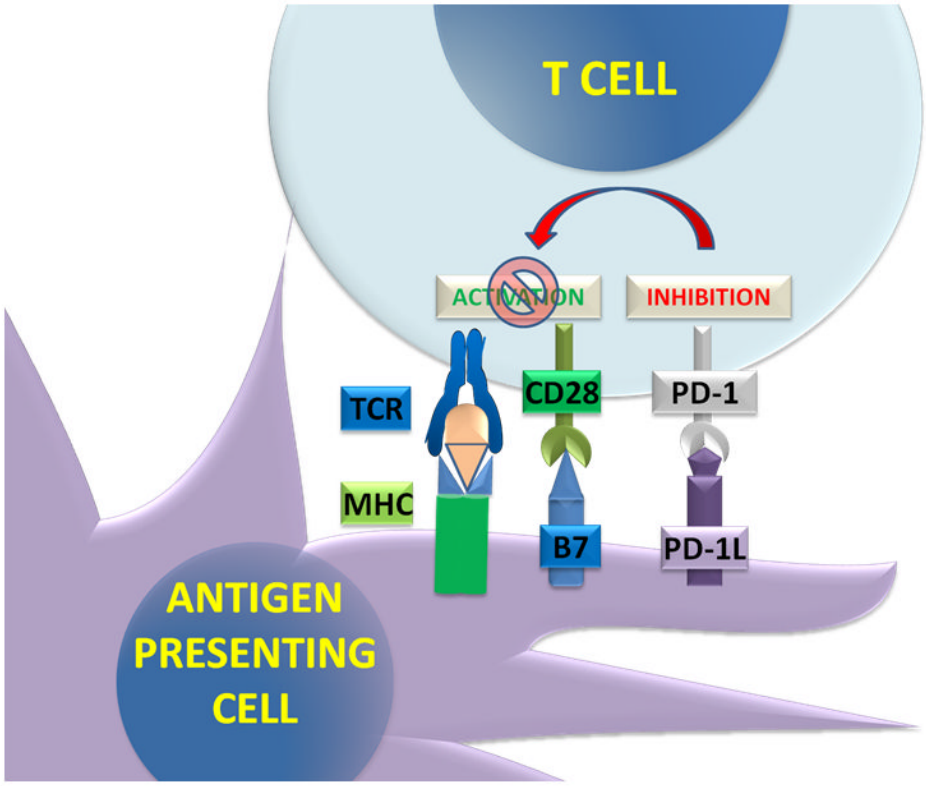

Figure 6b

Figure 6.

T cell regulation. (6A) The cytotoxic T cells express CD8 receptor that binds to the MHC receptor on the APC, along with a second signal mediated by CD28 binding to its ligand B7. The binding of the T cell receptor and CD28 lead to activation and cell mediated killing. Activated T cells upregulate CTLA4 and PD-1 proteins. CTLA4 competes with CD28 to bind B7. (6B) When PD-1 binds to its ligand PD-L1, it inhibits T cell activation.

Table 1

Published clinical trials using DC vaccine for newly diagnosed GBM; studies that included more than 3 patients. In total 73 patients have been given DC vaccines and 2 had grade III+ toxicities.

Author	Year	Total	Pre-vaccine therapy	Vaccine	Route	Age (Median)	Overall survival (months)	Toxicity Grade III+
Yu[68]	2001	7	RT	DC/Eluted peptides	ID Deltoid	50	14	No
Liau[69]	2005	7	RT	DC/Eluted peptides	ID Deltoid	33	23	No
Walker[78]	2008	7	No RT 1 RT/TMZ	DC/Lysate	ID Abdomen	53	11	No
Wheeler[75]	2008	11	?	DC/Lysate	ID?	?	?	1(*)
Ardon[72]	2010	8	RT/TMZ	DC/Lysate	ID Deltoid	50	24	1(edema)
Chang[70]	2011	8	RT	DC/Lysate	SC Axilla	60	24	No
Prins[76]	2011	15	RT/TMZ	DC/Lysate	ID Axilla	48	36	No
Fadul[31]	2011	10	RT/TMZ	DC/Lysate	Cervical Lymph Node	60	28	No

*Cutaneous glioblastoma at the site of irradiated tumor cell inoculation for DTH testing. Same as in Table 2. RT indicates radiation therapy; TMZ, Temozolomide; DC, Dendritic cells; ID, Intradermal; SC, Subcutaneous

Table 2

Published clinical trials using DC vaccines for recurrent glioblastoma. 159 patients have been treated with only 2 grade III/IV toxicities.

Author	Year	Total	Pre-vaccine therapy	Vaccine	Route	Age (Median)	Overall survival (months)	Toxicity Grade III+
Kikuchi[62]	2001	5	RT/Chemo	DC/Glioma fusion	ID	42	?	No
Yamanaka[73]	2003	8	RT	DC/Lysate	ID/Tumor	46	?	No
Yu[63]	2004	9	Chemo	DC/Lysate	Deltoid	45	33	No
Kikuchi[66]	2004	6	RT/Chemo	DC/Glioma fusion	ID	45	10	No
Liau[69]	2005	5	RT/TMZ	DC/Eluted Peptides	ID Deltoid	40	23	No
Yamanaka[74]	2005	24	RT/Chemo	DC/Lysate	ID/Tumor	49	16	No
Rutkowski[96]	2005	7	RT/Chemo	DC/Lysate	ID	55	?	1 (edema)
Walker[78]	2008	2	RT/TMZ	DC/Lysate	ID Abdomen	?	5	No
Wheeler[75]	2008	23	RT/TMZ	DC/Lysate	ID?	?	?	1(*)
Vleeschouwer[64]	2008	56	RT/TMZ	DC/Lysate	ID	45	9	No
Chang[70]	2011	6	RT	DC/Lysate	SC	37	39	No
Prins[76]	2011	8	RT/TMZ	DC/Lysate	ID Axilla	48	21	No

*Cutaneous glioblastoma at the site of irradiated tumor cell inoculation for DTH testing. Same as in Table 1. RT indicates radiation therapy; TMZ, Temozolomide; DC, Dendritic cells; ID, Intradermal; SC, Subcutaneous; Chemo, Chemotherapy