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A concise review of immunotherapy for glioblastoma

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Abstract

Glioblastoma (GB) is the most common and aggressive form of primary brain tumors in adults with a universally poor prognosis despite multimodal management including surgery, chemotherapy and radiation therapy. Among the novel therapeutic strategies, immunotherapy deserves particular attention with its potential to evoke biologic response and harness the host immune system. Considerable success achieved for other tumors has elicited great enthusiasm and prompted research on immunotherapy for GB. While the central nervous system has traditionally been thought of as an immune-privileged site, our understanding is being refined with emerging evidence. Several studies have been conducted and more are under way to establish the role of immunotherapy in management of GB. Immunotherapy of GB has yet resulted in mixed success with conflicting research findings, emphasizing the need for extensive study before its integration into routine clinical practice. Although there is a lot of room for improvement, immunotherapy for GB may be feasible and serve as a viable management strategy broadening and strengthening the therapeutic armamentarium to combat this deadly disease. Herein, we present a concise review of immunotherapy for GB.

Keywords: Glioblastoma, immunotherapy, glioma, vaccine, passive immunotherapy, active immunotherapy, cytokine therapy, central nervous system

INTRODUCTION

Glioblastoma (GB) constitutes the most common and aggressive form of primary brain tumors in adults[1]. Management of newly diagnosed GB includes maximal tumor resection followed by adjuvant chemoradiotherapy. The landmark study by European Organisation for Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada Clinical Trials Group (NCIC-CTG) in 2005 has reported

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significant median overall survival (OS) benefit with addition of temozolomide (TMZ) to conventionally fractionated radiation therapy (RT), making adjuvant chemoradiotherapy followed by adjuvant TMZ the standard of care for newly diagnosed GB patients[2]. However, disease recurrence is exceedingly common despite multimodal management. Repeat surgery, systemic agents and RT may be used in the recurrent setting as salvage therapeutic options, nevertheless, the clinical course is typically progressive with almost all patients ultimately succumbing to their disease[3-5].

Several treatment strategies are being explored to improve outcomes of patients with GB. Among these, immunotherapy deserves utmost attention with several studies assessing its role in GB management. Herein, we present a concise review of immunotherapy for GB.

MAIN IMMUNOTHERAPY APPROACHES FOR GB

Given the infiltrating nature of GB, diffuse microscopic disease may be typically present beyond the tumor bulk at initial presentation. Thus, a successful therapy should specifically address the infiltrative tumor stem cells surviving after implemented treatments such as surgery, chemotherapy and RT. Immunotherapy may conceptually take part in achieving this task, on the premise that it may facilitate combating with resistant GB cells through boosting of the host immune system. While the central nervous system (CNS) has traditionally been thought of as an immune-privileged site due to prevention of cellular and molecular diffusion by the blood-brain barrier and absence of lymphatic drainage, our understanding is being refined with emerging evidence[6-8]. The presence of a functional CNS lymphatic system has been reported recently and enhanced the focus on immunotherapy for brain tumors[9].

Herein, we review main strategies for GB immunotherapy.

Cytokine therapy

The rationale of cytokine therapy is activation of the immune system through administration of immunomodulatory cytokines. Cytokines are secreted or membrane-bound proteins and potent immunomodulators with a critical role in immune system coordination[10-13]. Anti-tumor activities of cytokines have been reported in animal studies paving the way for consequent cytokine-based cancer treatment strategies. Among the multitude of cytokines including interleukins, interferons and hematopoietic growth factors, FDA approval is currently available for interferon alpha for adjuvant treatment of melanoma and for high-dose, bolus interleukin (IL)-2 for management of metastatic melanoma and renal cell cancer[13].

An important issue in cytokine therapy is to achieve effective concentrations in the tumor without causing excessive toxicity. While local delivery by viral vectors has resulted in limited success for gliomas, intratumoral injection of IL-2 secreting allogeneic fibroblasts into GL261 tumors in mice has achieved increased survival[14-18]. Also, while IL-2 can induce over-differentiation of T cells and induce apoptosis of activated T cells, it may also activate CD4+ FoxP3 TREG regulatory cells, thereby inhibiting T cell activation and tumor killing activity. In this context, IL-7, IL-15, and IL-21 may also be used.

Liposomes and biopolymer microspheres are alternative routes utilized for intratumoral delivery of cytokines. Also, cytokines have been used for delivery of toxins in an effort to combat glioma cells with considerable success[19-21].

Utility of cytokine immunotherapy in combination with other therapeutic modalities is being investigated to exploit the synergistic activity against GB cells[22]. Briefly, cytokine immunotherapy has achieved encouraging results despite the need for further supporting robust evidence.

Passive immunotherapy

Serotherapy and adoptive immunotherapy are passive immunotherapy strategies. Passive immunotherapy is based on delivering the patient immune cells or antibodies with the capability of targeting the tumor cells[23]. Unlike active immunotherapy in which the patient's immune system is boosted, passive immunotherapy doesn't include activation of host immunity. Infusion of LAK cells into the tumor bed was an initial attempt of passive immunotherapy in the earlier years[24,25]. Cytotoxic T lymphocytes (CTLs) were also studied for adoptive immune response[26].

The use of monoclonal antibodies as a passive immunotherapy approach may result in killing of tumor cells through different mechanisms[27]. Sparing of normal brain tissue without tumor may be achieved if the antigen targeted by the monoclonal antibody is specifically expressed by the tumor only. Vascular endothelial growth factor (VEGF) is highly expressed in GB and targeted for therapeutic exploitation with bevacizumab (BEV). As a recombinant humanized monoclonal antibody, BEV is bound to VEGF-A and exerts antitumor effect. An improvement in progression free survival and maintenance in quality-of-life and performance status has been reported with addition of BEV to RT and temozolomide[28].

Another potential target in GB is the epidermal growth factor receptor (EGFR)[29]. EGFR gene mutation typically in EGFR variant III (EGFRvIII) is very common in GB. In this context, the use of anti-EGFRvIII antibodies in combined modality GB management is an area of active investigation.

Adoptive T-cell immunotherapy

Adoptive T-cell therapy offers an alternative immunotherapeutic approach. In this treatment, tumor-specific autologous T-cells undergo *in vitro* amplification and are consequently infused to the same individual for therapeutic exploitation. Advances in genetic engineering has paved the way for adoptive T-cell immunotherapy through generation of high avidity tumor-specific T-cells[30]. Chimeric antibody receptor (CAR)-based treatments and cytomegalovirus (CMV) adoptive T-cell immunotherapy have great potential for further therapeutic exploitation[31-35].

A unique advantage of adoptive T-cell immunotherapy is the capability of expanding substantial amounts of tumor infiltrating T lymphocytes (TILs) *in vitro* without immunosuppressive environments seen *in vivo*[36].

Adverse effects of adoptive T-cell immunotherapy may include cytokine release syndrome (CRS) and tumor lysis syndrome (TLS) which underscore the importance of early detection of these syndromes through vigilant monitoring[37,38]. CRS and neurotoxicity may be triggered by the inflammatory molecule IL-1, and adding ANAKINRA, an inhibitor of IL-1, to the treatment regimen can block the molecule. Also, inserting the IL-1 inhibitor gene directly into CAR-T cells may prevent CRS.

Active immunotherapy (peptide vaccines, dendritic cell vaccines, heat shock protein vaccines)

Active immunotherapy is based on the premise that vaccination against tumor antigen stimulates an adaptive immune response against tumor cells. Target antigens include tumor-specific antigens (TSAs) expressed solely by the tumor and tumor-associated antigens (TAAs) expressed by both tumor cells and normal cells. While TSAs have a greater potential to evoke a more potent and specific immune response compared to TAAs, they are exceedingly rare. EGFRvIII, IDH-1/2 mutations (e.g., R132H), and CMV proteins are known TSAs expressed in GB and IL-13Rα2, HER-2, gp100, survivin, WT1, TRP2, EphA2, SOX2, SOX11, MAGE-A1, MAGE-A3, AIM2, SART1, and tenascin are TAAs expressed in GB[10].

Heterogeneity of GBs warrants the demand for individualized, patient-specific and non-toxic immunotherapies. Attracted by the success of vaccination against hormone-resistant metastatic prostate cancer, researchers have focused on developing vaccines against GB[39]. Herein, we review peptide vaccines, dendritic cell (DC) vaccines, and heat shock protein (HSP) vaccines.

Peptide vaccines

This strategy is a targeted approach including the direct administration of a selected protein or peptide antigen frequently used with an adjuvant such as keyhole limpet hemocyanin (KLH) to enhance the immunogenicity[10,40,41].

In the context of peptide vaccines, most extensive study has focused on targeting of EGFRvIII[42-44]. EGFRvIII is a TSA which is solely expressed by GB cells and not expressed by normal tissues[45]. This kind of targeting single tumor specific antigens has the advantage of theoretically eliminating normal tissue toxicity. The mutant form of the EGFR gene containing an in-frame deletion of exons 2-7 is found in approximately 20%-30% of patients with GB and causes tumor cell proliferation[46-49].

In the recent phase II randomized ReACT study assessing association of rindopepimut and BEV in EGFRvIII-positive relapsed GB patients reported the benefit of rindopepimut treatment with regard to multiple endpoints including the 2-year OS and PFS rates, and the authors concluded that rindopepimut administered with BEV induced a potent EGFRvIII-specific immune response leading to tumor regression and prolonged survival of recurrent GB patients[50]. Another phase II multicenter study of EGFRvIII peptide vaccination in newly diagnosed GB patients, Sampson *et al.*[45] reported significantly improved OS in vaccinated patients. A notable finding in this study was the loss of EGFRvIII antigen in most patients relapsing after vaccination, indicating a critical role for vaccine-induced immune response in tumor eradication. They concluded that a randomized phase III trial was needed to establish the role of EGFRvIII-targeted vaccination for management of GB patients[45].

However, randomized phase III trial of rindopepimut for newly diagnosed EGFRvIII-positive GB patients failed to show an OS benefit in preplanned interim analysis, leading to early closure of the study[51].

Overall, studies of Rindopepimut have demonstrated encouraging results worth further testing[52-54]. Nevertheless, GB TSAs with higher expression levels may achieve improved treatment results.

Other than EGFR related peptides, personalized peptide vaccination has been another area of investigation, resulting in encouraging therapeutic outcomes[55,56]. IDH-1 R132H mutation is a newer appealing TSA with a typically lower prevalence in primary GB compared to secondary GB and is being currently tested in clinical studies[57].

DC vaccines

DCs are efficacious antigen-presenting cells (APCs) capable of vigorously activating the T-cells to attain a durable immune response through slow processing of antigens[58-61]. These professional APCs have been judiciously utilized for GB management since they are appealing candidates for therapeutic exploitation. DCs can be categorized into myeloid DCs (mDCs) and plasmacytoid DCs (pDCs)[62,63]. In the study by Dey *et al.*[63], mice vaccinated with mDCs generated an improved antitumor T cell response compared to pDC vaccinated mice. The use of DC-based vaccines achieved impressive results for newly diagnosed GB patients[61].

In the study by Prins *et al.*[64], safety, feasibility, and immune responses were comparatively assessed for GB patients treated using DC pulsed with autologous tumor lysate or with synthetic glioma-associated antigens. The study revealed that DCs pulsed with autologous tumor lysates achieved improved anti-tumor immune response compared to DCs pulsed with synthetic glioma-associated antigens[64]. Nevertheless, DC vaccines may yet be skeptical in human clinical trials notwithstanding the promising results in animal models.

A recent study by Mitchell *et al.*[65] revealed that the efficacy of DC vaccination could be enhanced through pre-conditioning of the vaccination site with a recall antigen such as tetanus/diphtheria toxoid.

Approaches for modulation of DC migration may prove to be a viable treatment option, however, modification of autologous DCs is a difficult task with a high cost and workload.

Another appealing immunotherapy approach includes targeting of the glioma stem cells (GSCs) which are considered to take part in treatment resistance[30,66]. A survival benefit has been achieved in rodent GB models by use of GSC-antigens loaded DC vaccination[30,67,68].

Heat shock protein vaccines

Targeting of a single TSA or TAA with vaccines limit the potential antitumoral effect to the subgroup of GB patients expressing those TSAs and TAAs. Single-antigen vaccines also suffer from the heterogeneity of the GB cells expressing the antigen which may lead to their diminished activity and usefulness. In this context, an alternative strategy has been developed including vaccination with a heat shock protein (HSP) peptide complex in order to achieve targeting of multiple antigens[69]. The concentrations of HSPs may reach high levels in the presence of protein misfolding, unfolding, or aggregation and under stress-inducing environments as in GB[69-72].

The use of HSP-peptide complex in management of recurrent GB patients has been tolerated well and conferred an improvement of survival through enhanced immune response[73].

In a study by Crane *et al.*[74], the use of peptides bound to a 96 kD chaperone protein (HSP-96) for immunization of recurrent GB patients resulted in a median survival of 47 weeks after surgery and vaccination, indicating the efficacy of this approach. Another phase II trial by Bloch *et al.*[75] reported the safety of HSPPC-96 vaccine in 41 recurrent GB patients and emphasized the need for vigilance for pretreatment lymphopenia as a factor impacting outcomes of immunotherapy.

The utility of another glioma-associated antigen HSP47 was suggested to induce CTL responses with the potential of therapeutic exploitation for GB patients[76,77].

Immune checkpoint therapy

Immune checpoint inhibitors are immunomodulatory therapeutics with the capability of blocking inhibitory molecules and their receptors on effector immune cells with a resultant T-cell response against various cancers[78]. Immune checkpoint therapy offers a viable immunotherapy strategy targeting the regulatory pathways in T cells to evoke an immune response against the tumor[79]. Boosting of the antitumor immunity by immune checkpoint inhibitors mediating the T-cell response has been an appealing strategy for therapeutic exploitation[80,81].

Among the multitude of immune checkpoint molecules under current investigation and development for GB, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death 1 (PD-1) are the most popular given the favorable outcomes achieved for other tumors through their inhibition, leading to FDA approval[82-86]. Studies have suggested the ability of these immune checkpoint inhibitors to overcome the blood-brain barrier for activity within the CNS[87-90].

In the context of GB, their inhibition showed promise in preclinical trials[91-95].

Although the first phase III study of PD pathway inhibition, the Checkmate 143 trial failed to meet its primary endpoint, several studies have focused on immune checkpoint inhibition for GB patients[96-98].

As well as the enthusiasm for therapeutic exploitation of immune checkpoint blockade, there have also been important concerns about immune-related toxicity profile of immune checkpoint inhibitors, partly due to

increased amount of proinflammatory cytokines along with aberrant infiltration of stimulated T cells into normal tissues[99,100]. Nevertheless, there is a lot of room for further improvement and immune checkpoint blockade may serve as a viable immunotherapeutic strategy for patients with GB.

FUTURE DIRECTIONS

Immunotherapy for GB is being thoroughly investigated in a plethora of studies to establish the safety and efficacy for therapeutic exploitation. Results of critical studies are eagerly awaited before decision making for integration of immunotherapy into clinical practice of GB management. A few points to be considered for GB immunotherapy are as follows:

-Further investigation and understanding of the immune system evasion mechanisms may assist in improved therapeutic exploitation of personalized immunotherapeutic strategies for GB patients. Focusing on molecular subtypes of GB and identification of molecular factors affecting the interplay between the tumor and immune system may be critical for developing personalized treatments for patients suffering from this deadly disease.

-Measurement of immune response may be further optimized through the introduction of standardized and validated assays, which play a central role in therapeutic decision making for a given immunotherapeutic.

-Given the grim prognosis of GB patients, current standard management may be judiciously supported by boosting of antitumor response with immunotherapy. In this context, achieving an improved therapeutic ratio for GB patients may warrant the utilization of combination therapies with incorporation of immunotherapeutic approaches to exploit the advantage of synergistic antitumor activity of multiple treatment modalities.

Clearly, well-designed clinical trials are needed to assess efficacy and safety of combined modality GB management using immunotherapeutic agents. Improved understanding of the interactions between chemotherapy, radiotherapy and immunotherapeutic strategies will shed light on further research for optimization of more potent treatment of GB patients.

CONCLUSION

Recent years have witnessed unprecedented advances and breakthroughs in basic and translational cancer research, leading to significant improvements in therapeutic outcomes for several tumors. Utilization of immunotherapeutic strategies proved to be efficacious against several cancers, leading to their thorough investigation for management of GB patients. Immunotherapy of GB has yet resulted in mixed success with conflicting research findings, emphasizing the need for extensive study before its integration into routine clinical practice. Although there is a lot of room for improvement, immunotherapy for GB may be feasible and serve as a viable management strategy broadening and strengthening the therapeutic armamentarium to combat this deadly disease.

DECLARATIONS

Authors' contributions

Concept and design of study: Sager O, Dincoglan F, Dirican B, Beyzadeoglu M
Drafting the article: all authors
Revising the aticle critically for important intellectual content: Sager O, Dirican B, Beyzadeoglu M
Final approval of the version to be published: all authors

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All authors declared that there are no conflicts of interest.

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