# Glioblastoma Immunotherapy

Subjects: Immunology

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Glioblastoma multiforme (GBM) is the most aggressive type of brain tumor with dismal survival and poor response to conventional therapies. Therefore, the development of immunotherapy for GBM treatment is necessary.

Keywords: brain tumor ; glioblastoma ; immunotherapy ; adenosine ; CD73 ; CD39

## 1. Introduction

Brain tumors are heterogeneous tumors that can be classified into two general categories based on their origin. The primary brain tumors stem from the brain, while the origins of metastatic types are other organs that have metastasized to the brain [1][2]. Approximately 80% of brain malignancies originate from glial cells and are called gliomas [3]. According to the 2016 World Health Organization Classification of Tumors of the Central Nervous System, diffused gliomas are categorized into different types, including Astrocytomas, Oligoastrocytomas, Oligodendrogliomas, and Glioblastoma [4]. In this updated classification, molecular parameters are combined with the histological patterns. For instance, the mutation status of isocitrate dehydrogenase (IDH)-1/2 gene and 1p/19q codeletion status are two molecular parameters in classifications of gliomas [4][5][6]. The classification of brain tumors is thoroughly reviewed in [4][5][6]. Glioblastoma multiforme (GBM) is the most malignant and common type of brain tumor in adults. GBM can arise from astrocyte, oligodendrocyte, and even neural stem cells, and therefore, is not classified in a specific category of gliomas [7]. The word multiforme indicates the heterogeneity of this tumor in terms of molecular markers, physiopathology, clinical manifestations, and response to treatment [8].

The average survival in GBM without treatment is three months and with current treatments it is 12–19 months [9][10]. Standard treatment includes surgery, radiotherapy, and chemotherapy [9]. Temozolomide (TMZ) is the gold-standard chemotherapy used in GBM due to its high permeability to the blood–brain barrier (BBB). TMZ is usually given after surgery for six weeks with radiotherapy [11]. Despite these multiple treatments, the recurrence rate of GBM is very high, with 2-year and 5-year survivals of 26.5% and 7%, respectively [10][12]. Steroids are also used to reduce cervical edema [9]. Recently, two other treatments for GBM have been approved in the United States: (I) bevacizumab, a monoclonal antibody (mAb) against vascular endothelial growth factor (VEGF) receptor [13], and (II) tumor-treating fields [14]. However, the effectiveness of both treatments remains controversial. Accelerated approval of bevacizumab in GBM by the FDA indicates the urgent need for advanced and targeted treatment. Due to the ineffectiveness of current treatments on GBM, various types of targeted therapies, such as immunotherapy, raised hopes in the treatment of GBM.

## 2. Glioblastoma Immunotherapy

It was initially believed that the central nervous system (CNS) was an immune-privileged organ. Studies on CNS autoimmune diseases such as multiple sclerosis and encephalitis, the discovery of the CNS lymphatic system, and successful treatment of brain metastases, have shown that the CNS has an immunological activity [15]. However, some unique features of the CNS, such as the presence of the BBB, the use of corticosteroids for cerebral edema, and the immunosuppressive mechanisms of brain tumors, caused problems in immunotherapy [16]. Regarding the heterogeneous glioblastoma microenvironment (GME), severe immunosuppression, low mutational burden, and decreased antigen presentation, GBM is very poorly responsive to immunotherapy so far [16] (Table 1). Immune checkpoint inhibitors (ICIs) have become a promising immunotherapy approach in the treatment of many solid tumors (reviewed in [17]). In this method, inhibitory ICs that cause immune exhaustion are blocked, thereby restoring the immune cells' ability to induce antitumor responses [17][18]. The prerequisite of ICI treatment is the overexpression of ICs in the tumor microenvironment (TME). Overexpression of ICs has been reported only in some subtypes of GBMs [19]. Clinical trials on GBMs have demonstrated that ICIs do not have a significant advantage over other therapies such as bevacizumab, radiotherapy, and

chemotherapy. Hence, they proposed a combination of therapies or ICI applications as a neoadjuvant therapy before surgery [20][21][22]. The combined use of several ICIs, although improving the response to treatment, increases their toxicity and the likelihood of CNS autoimmunity [23][24].

**Table 1.** Advantages and disadvantages of the current immunotherapies in GBM.

| Immunotherapy | Advantage | Disadvantage | Refs. |
|---|---|---|---|
| Immune checkpoint inhibitors (PD-1, CTLA-4, LAG-3, TIM-3, IDO, CD27) | • Tolerable<br>• Reinvigorate antitumor T cells<br>• Promising results in preclinical and first phases of clinical studies<br>• Proposed as a neoadjuvant therapy | • Grade I-II toxicity in monotherapy<br>• Grade III-IV in multi ICI therapy<br>• No significant advantage (better OS and PFS) over bevacizumab or TMZ<br>• Various IC expression levels in patients<br>• Decreased effects in patients receiving TMZ | [19][20][21] [22][25][26] |
| Bevacizumab (anti-VEGF) | • FDA-approved for GBM<br>• Prevents angiogenesis<br>• Has an anti-edema effect | • Accelerated approval after phase I/II<br>• No outstanding results in extending PFS and OS | [27][28][29] |
| Cetuximab (anti-EGFR) | • Tolerable<br>• Promising results in preclinical studies | • No significant survival benefit in the phase II trial<br>• Insufficient BBB penetration due to the large size | [29][30] |
| • Immunotoxins (mAbs conjugated with bacterial toxin or anti-mitotic agents) (Depatuxizumab mafodotin, Losatuxizumab vedotin, ABBV-221, ABBV-231) | • Improved survival in combination with TMZ in the phase II trial<br>• ABBV-231 is in the phase I trial | • No significant survival benefit in the phase III trial<br>• Safety concerns<br>• Antigen-escape (downregulation of mAb target)<br>• New generations are in the evaluation process | [31][32] |
| Anti-CSF-1R mAb | • Decreases the recruitment of TAMs into the GME<br>• Under investigation in the phase I/II trial in combination with ICIs | • Might have insufficient BBB penetration due to large size | [33][34] |

| Immunotherapy | Advantage | Disadvantage | Refs. |
| --- | --- | --- | --- |
| CAR T cell against IL13Rα2, EGFRvIII, Her-2, EphA2 | • Appreciable safety profile<br>• Considerable infiltration into the GME<br>• Significant clinical response | • Relapse occurred 2–29 months after treatment<br>• Immune-escape through antigen loss<br>• Heterogeneity of GME made it difficult to use monoclonal CAR T cell for GBM (only one-third of GBM patients are EGFRvIII+)<br>• CAR T cells targeting multiple antigens are needed | [9][35][36][37] |
| BiTE (against EGFR) | • Appreciable safety profile<br>• Recruits EGFR-specific T cells in the GME<br>• Can override antigen-escape in combination with CAR T cells | • Heterogeneity of GME challenges the targeting of a specific antigen in all GBM | [38] |
| Tumor vaccines using specific peptides (Rindopepimut, survivin) or tumor lysate | • Tolerable<br>• Low off-target effects<br>• Improve OS and PFS (mOS:24 months)<br>• Synergistic effect in combination with bevacizumab | • Rindopepimut is effective only in EGFRvIII+ patients (30% of all GBM)<br>• No survival benefits due to the antigen-escape | [39][40][41][42][43] |
| DC Vaccines (ICT-107:pulsed with six peptides)(DCVax: pulsed with tumor lysate) | • ICT-107: Promising results in the phase II trial<br>• DCVax: Improves OS to 24 months<br>• Override antigen-escape<br>• Personalized medicine | • 2% serious adverse events in DC vaccines<br>• Expensive process of personalized medicine | [44][45] |
| • Viral gene therapy: (Toca-511: Metabolize prodrug (FC) to drug (5-FU))<br>• VB-111: delivers pro-apoptotic proteins<br>• Ad-RTS-hIL-12: Conditional delivering of IL-12) | • Appreciable safety profile<br>• Promising results in early trials with a 22% durable response rate<br>• Synergistic effects with ICIs | • No survival benefit in the phase III trials | [46][47][48][49][50][51] |

| Immunotherapy | Advantage | Disadvantage | Refs. |
| --- | --- | --- | --- |
| Oncolytic virotherapy<br>(Adenovirus, polio-rhinovirus chimera, herpes simplex virus) | • Safe intratumoral administration, induces innate and adaptive immune responses<br>• Turns immunosuppressive to immune-active TME<br>• Promising survival results in early trials | • Evaluation in phase II trials as a monotherapy or with ICIs | [52][53][54] |
| Adenosinergic pathway (ARs, CD39, CD73, ADA) | • High expression in all types of GME<br>• No antigen escape<br>• Turns immunosuppressive GME into immune-active GME<br>• Reduces angiogenesis<br>• Potentiates other immunotherapies such as ICIs, CAR T cell, and NK cell therapy<br>• Synergistic effects with conventional therapies<br>• Overrides chemoresistance | • Not entered in clinical trials yet<br>• mAbs might have insufficient BBB penetration<br>• All pathway components should be targeted to get maximum results<br>• Not effective as monotherapy and should be used as combination therapy | [55][56][57][58][59][60][61][62][63][64][65][66][67] |

PD-1. Programmed cell-death protein-1; CTLA-4. cytotoxic T-lymphocyte-associated protein-4; LAG-3. Lymphocyte activation gene-3; TIM-3. T-cell immunoglobulin and mucin domain-containing protein-3; IDO. Indoleamine-2,3-dioxygenase; ICI. Immune checkpoint inhibitor; OS. Overall survival; TMZ. Temozolomide; VEGF. Vascular endothelial growth factor; GBM. Glioblastoma multiforme; PFS. Progression-free survival; EGFR. Endothelial growth factor receptor; BBB. Blood-brain barrier; mAb. Monoclonal antibody; CSF-1R. Colony stimulating factor-1 receptor; GME. GBM microenvironment; CAR. Chimeric antigen receptor; IL13Rα2. Interleukin-13 receptor α2; Her-2. Human epidermal growth factor receptor-2; BiTE. Bispecific T cell engager; mOS. Mean OS; DC. Dendritic cell; FC. Fluorocytosine; 5-FU.5-Flurouracil; ARs. Adenosine receptors; ADA. Adenosine deaminase.

In addition to ICIs, the use of mAbs and their derivatives such as nanobodies, single-chain variable fragment (scFv), bispecific T-cell engager (BiTE), and immunotoxins is also a routine method in immunotherapy [29][68]. Bevacizumab was the first mAb to be accelerated and approved in GBM [13]. This anti-VEGF mAb prevents angiogenesis in the TME [13]. Application of mAbs against endothelial growth factor receptor (EGFR) also yielded promising results in initial studies but was discontinued in clinical trials due to a lack of significant increase in patient survival and rising safety concerns [30][31][32]. The EGFR variants, especially EGFR class III variant (EGFRvIII), are overexpressed in a considerable part of GBM patients, making them an ideal target for immunotherapy [69]. However, the association of EGFR overexpression and mutations with the overall survival of patients is still controversial [70]. Moreover, the results of trials showed EGFRvIII downregulation following targeted therapy against EGFRvIII [35][71]. This has raised the question of whether EGFRvIII mutation represents a driver mutation, or maybe it is only a passenger mutation with no considerable impact on the survival of glioma cells. Currently, other generations of conjugated mAb are being studied in trials. The greatest challenge of mAb therapy in brain tumors is the large size of mAbs and the lack of proper penetration into the TME due to the BBB. The smaller derivatives of mAb or making the BBB permeable to these factors could enhance the treatment responses [29].

The application of autologous T cells genetically engineered with a chimeric antigen receptor (CAR) demonstrates remarkable efficiencies in many blood cancers and solid tumors [72]. These cells are against a tumor-specific antigen (TSA) and can sustain antitumor activity with the help of various costimulatory molecules [72]. The CAR T cells used in GBM were against EGFRvIII, interleukin 13 receptor-α2 (IL13Rα2), human epidermal growth factor receptor-2 (HER2), and Eph receptor-A2 (EphA2) [35][36][37][72]. The results of the trials indicate a relative response to this treatment. Given the heterogeneity and high plasticity in the GME, the use of a specific CAR T cell reduces the expression of the target antigen, and the tumor escapes the CAR T cell response [9]. Therefore, studies on the application of bivalent and trivalent CAR T cells are ongoing [37]. Another way to overcome antigen escape is to use BiTEs along with CAR T cells. Choi et al. developed an anti-EGFRvIII CAR T cell, which also expresses anti-EGFR BiTEs [38]. It initially targets positive EGFRvIII cells and then recruits T cells specific for wild-type EGFR to the TME. The initial results against heterogeneous GBMs were promising [38].

Tumor vaccines containing TSAs are another cancer immunotherapy method aiming to stimulate the patient's adaptive immunity against TSAs [29]. Peptide vaccines containing EGFRvIII and survivin peptides in patients who were positive for these antigens raised proper responses, although the issue of antigen escape in this method is also challenging [40][41][42]. Ex vivo pulsing the patient's autologous dendritic cells (DCs) with specific peptides (in ICT-107) or tumor lysate (in DCVax) in DC vaccines stimulates a better immune response than peptide vaccines [44][45]. This type of treatment is a personalized treatment that can overcome the high heterogeneity of GBM in patients. However, immunosuppressive GME causes pulsed DCs to become inefficient in antigen presentation. Initial clinical trials of tumor vaccines alone or in combination with bevacizumab or chemotherapy and surgery have yielded encouraging results [9][40][41].

According to initial observations of tumor regression in viral infections, viral therapy is currently used in various cancers, mostly solid tumors [73]. Viruses can be used in gene therapy, delivering the desired genes to the TME. These genes mainly produce pro-apoptotic proteins (in VB-111 vaccine), inflammatory cytokines (in Ad-RTS-hIL-12 vaccine that encodes IL12 conditionally), or enzymes that convert prodrugs to anticancer drugs (in Toca-511) [46][50][51]. Another type of virus therapy involves oncolytic viruses that selectively infect and lyse cancer cells in which antiviral responses are impaired [73]. Adenovirus, herpes simplex virus, and poliovirus are being studied in GBM and have shown a relative response in combination with other treatments [9]. Viral therapy can also stimulate innate and adaptive immune systems that enhance antitumor responses [9].

As can be seen, most of the immunotherapy methods used in GBM have been effective in the preclinical and early clinical stages but have not been very successful in the higher stages of the clinical trials (Table 1). There are several reasons for such an inadequate response in GBM patients. High heterogeneity of GBM between patients and high plasticity, even in one patient at different times, makes GBM resistant to immunotherapy [16]. Evaluation of tumor markers before treatment and development of personalized medicine can lead to overcoming GBM heterogeneity and plasticity. The severe immunosuppressive GME appears to be another barrier to immunotherapy. Immunosuppression in GME undergoes numerous and complex mechanisms so that single-arm immunotherapy cannot break this tolerance. Besides local immune suppression, GBM can suppress systemic immunity in the patient [16][74][75][76]. The GME-infiltrated T cells are mainly differentiated to regulatory T cells (Tregs) due to the high levels of tumor growth factor (TGF)-β and indoleamine-2,3-dioxygenase (IDO) in the GME [77][78]. IDO metabolizes tryptophan to kynurenine, leading to a change in the phenotype of microglial cells (CNS-resident macrophages) or tumor-associated macrophages (TAMs) to an M2 phenotype [67]. M2-TAMs promote tumor progression by further suppressing immune responses and expressing ICs [67].

On the other hand, the use of corticosteroids in GBM to reduce cerebral edema increases immunosuppression and reduces immunotherapy effects [79]. Interestingly, studies have shown that radiotherapy and chemotherapy, such as TMZ in some cases of GBM, can increase immunosuppression and decrease the effects of ICI, which challenges combination therapy [80][81]. Furthermore, the low mutational burden in GBM limits neoantigen production and presentation to the adaptive immune system [82]. All of the mentioned mechanisms make GBM an immunologically cold tumor. Knowing the different aspects of immunosuppression in GBM makes it possible to achieve a successful strategy in GBM immunotherapy by targeting several pathways simultaneously.

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