

## Immunotherapy in glioblastoma treatment: Current state and future prospects

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### Abstract

Glioblastoma remains as the most common and aggressive malignant brain tumor, standing with a poor prognosis and treatment prospective. Despite the aggressive standard care, such as surgical resection and chemoradiation, median survival rates are low. In this regard, immunotherapeutic strategies aim to become more attractive for glioblastoma, considering its recent advances and approaches. In this review, we provide an overview of the current status and progress in immunotherapy for glioblastoma, going through the fundamental knowledge on immune targeting to promising strategies, such as Chimeric antigen receptor T-Cell therapy, immune checkpoint inhibitors, cytokine-based treatment, oncolytic virus and vaccine-based techniques. At last, it is discussed innovative methods to overcome diverse challenges, and future perspectives in this area.

**Key Words:** Glioblastoma; Immunotherapy; Tumor microenvironment; Chimeric antigen receptor T cell; Oncolytic viruses; Immune-checkpoint inhibitors; Brain cancer

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**Core Tip:** This study aims to review the ongoing status and improvement made in immunotherapy for glioblastoma, a malignant brain tumor. Thus, this review goes through the general concepts of the tumor microenvironment, standard treatment and its limitations and immune targeting promising methods, such as Chimeric antigen receptor T-Cell therapy, immune checkpoint inhibitors, cytokine-based treatment, oncolytic virus and vaccine-based techniques. Finally, it is explained some methods to surpass the various challenges, and future prospects in this field.

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## INTRODUCTION

Glioblastomas (GBM) are the most common type of malignant tumor affecting the central nervous system. It is more common among men and its incidence is significantly related to age, being rare among young people and more common among the elderly, especially those aged between 74 and 85 years. It has a very poor prognosis, with survival of 12 to 15 mo after diagnosis, and, when untreated, of only 3 mo[1].

Regarding clinical manifestations, the symptoms are quite diverse and common to other types of brain tumors and include manifestations associated with intracranial hypertension such as intense headache, which can be accompanied by nausea and vomiting, focal neurological deficits, memory and personality changes, and seizures[2].

GBMs are tumors that originate from glial cells and are classified according to their histological characteristics as high-grade gliomas by the WHO, and the characteristics that define this denomination include hypercellularity, nuclear atypia and dysregulation of mitotic activity, besides microvascular proliferation and tumor necrosis[3]. So, they are classified as primary if there is no pre-existing involvement or secondary if they have progressed from low-grade astrocytomas; primary GBMs represent the majority of cases and secondary GBMs correspond to only 5 to 10% and usually affect young people[4].

In addition to histopathological analysis, molecular markers are essential for the understanding of the disease, since different genetic alterations can originate this type of tumor and determine subtypes that behave differently in terms of evolution and response to treatments used, which makes the identification of these factors essential for the establishment of therapeutic strategies. In this sense, GBMs can be grouped into 4 subtypes according to their molecular characteristics: classic, neural, pro-neural and mesenchymal[3,4].

Among the mutations related to the pathogenesis of GBM, we can cite 3 main pathways: receptor tyrosine kinase signaling, inhibition of the p53 pathway, and RB, and in most cases all three types of alterations are present. These mutations are associated with activation of oncogenes that act mainly in neoplastic proliferation, apoptosis disturbances, and cell cycle checkpoint failures that promote tumor cell survival[5]. Moreover, when compared to a normal brain, GBMs present a higher expression of genes related to immune cell infiltration, especially macrophages, and angiogenesis, noticing that hypoxia, which is characteristic of necrotic tumor regions, induces a higher expression of vascular endothelial growth factor (VEGF) and, consequently, a higher vascular proliferation[6].

Due to the characteristics of its pathogenesis, there is a diversity of cells that are found in the analysis of these tumors, including non-neoplastic components of the immune system. This is related to the tumor microenvironment of glioblastoma, since it has an inflammatory and pro-angiogenic characteristic that affects the permeability of the blood-brain barrier and allows the infiltration of defense cells, especially tumor-associated macrophages (TAM). The immune system in the early stages of the disease is responsible for controlling the development of the cancer, however, as proliferation progresses, the tumor cells become able to escape this surveillance and the defense cells not only become unable to perform this control, but start contributing to the growth of the tumor[7].

The available treatment is complex and usually requires a combination of different approaches and is dependent on a number of factors. Although there are other options and studies for the development of new treatments, the therapeutic strategies are still controversial and the prognosis is unsatisfactory with a high recurrence rate[8].

In this review, we provide an analysis of the ongoing status and progress in immunotherapy for glioblastoma, going through the general information about the tumor microenvironment, fundamental knowledge on immune targeting to promising strategies like Chimeric antigen receptor (CAR) T-Cell therapy, cytokine-based treatment, oncolytic virus and vaccine-based approaches. Finally, we discuss

contemporary methods to prevail distinct challenges, and future perspectives in this field.

## CURRENT STANDARD CARE LIMITATIONS

The treatment of primary brain tumors such as GBM is still quite limited and, therefore, a major challenge in oncology. Although the treatment is difficult, expensive and subject to therapeutic failure, management protocols for patients with GBM consider multimodal therapeutic strategies that act in synergy in order to destroy the tumor. For this, such strategies must be individualized based on each patient according to their functional status, imaging exam, speed of disease progression, quality of life and clinical diagnosis. However, for new methods to be developed and current ones to be improved, it is necessary to think about the limitations of existing treatments. The [Figure 1](#) synthesizes the current GBM treatment strategies and its advantages and limitations.

### **Surgical method**

The surgical method is based on the maximum safe resection of the tumor and currently comprises the backbone of therapy for GBM[9], as in addition to reducing the volume of the neoplastic mass and the symptoms associated with parenchymal compression, the histological diagnosis and genetic study of the tumor are also possible by surgical intervention[10]. The aim of surgical treatment is to achieve a gross total resection as completely and safely as possible without risking the patient's functional status. Complete resection has been associated with a greater chance of survival and no progression than partial resection or biopsy. In this sense, some tools were developed to maximize the surgical procedure and alleviate as much as possible the neurological deficits that may be associated with the method. Among these tools, monitoring using fluorescence of tumor tissue with 5-aminolevulinic acid in conjunction with functional magnetic resonance imaging shows beneficial results[10,11].

However, GBMs are not cured with surgery alone, as almost all are recurrent and the biological pleomorphism of each tumor influences the degree of resectability of the cancer, with less malignant brain tumors being the most resectable[12]. Furthermore, the surgical method is extremely complex, delicate and expensive, because it demands a qualified neurosurgeon and sophisticated imaging equipment, in addition to the fact that the patient has the possibility of developing a neurological deficit as a result of the intervention, which may even prevent the following steps of the standard treatment, such as radiotherapy and chemotherapy[13]. Thus, it is necessary to accurately weigh the risks and benefits of the surgical technique.

### **Radiotherapy**

Radiotherapy (RT) became popular in the 1970s and 1980s and is currently a therapeutic strategy based on the use of radiation volumes focused on specific regions. This method has become standard for GBMs since 2005, as it was in that year that a phase III clinical trial solidified the role of radiotherapy and adjuvant chemotherapy in the postoperative period of GBM[14]. After the surgical diagnosis, the patient is submitted to doses of 2 Gy for 6 wk until reaching a dose of 60 Gy[13]. It is an effective method that increases patient survival in different types of doses provided, especially hypofractionated doses, which make this method viable in elderly people (over 65 years old) with glioblastoma[9].

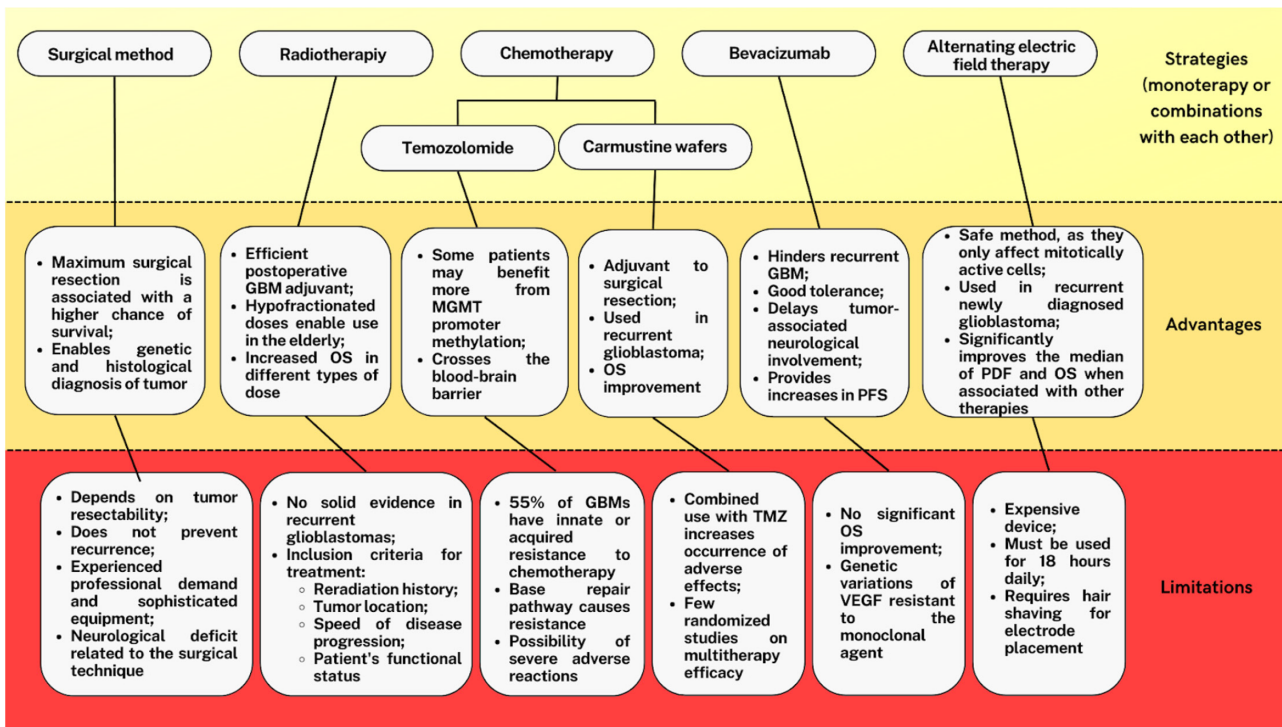
The combination of radiotherapy for 6 wk and chemotherapy with adjuvant Temozolomide 75 mg/m<sup>2</sup> for 6 wk and 150-200 mg/m<sup>2</sup> every 28 d for 6 mo is the gold standard treatment for young patients with glioblastoma. This combination of strategies significantly improved the survival of younger patients between 2 and 5 years[14].

RT has an important limitation in the sense that its use does not have much favorable evidence in recurrent gliomas, although it is extremely useful as a palliative therapy for small recurrent tumors[15]. In addition, it is necessary to be wise in the use of radiation, since the treatment protocol requires the patient's history of previous radiation, as well as the location of the tumor and the maximum dose for the structure in which it is allocated[16]. Finally, the therapeutic algorithm assesses the speed of disease progression and the patient's functional status. Thus, the use of chemoradiotherapy is not indicated for individuals over 70 years of age who do not have a good functional status, which is measured by the Functional Status Score for the Intensive Care Unit scale[15].

### **Chemotherapy**

**Temozolomide:** Temozolomide (TMZ) is an alkylating agent that is cell cycle independent and is the most effective chemotherapy for GBM to current date. This efficiency is due to the ability to cross the blood-brain barrier and transportable cytosolic transformation to the cell nucleus[17]. The current standard of care in newly diagnosed GBM includes administration of 75 mg/m<sup>2</sup> of TMZ daily during the 6 wk of radiotherapy. Then, 150-200 mg/m<sup>2</sup> are maintained for 5 d at each 28-d cycle with 6 cycles of the drug[13].

However, this therapeutic strategy is variable based on the age of the patient, performance status according to the Karnofsky performance score, the promoter methylation status of the repair enzyme O(6)-Methylguanine-DNA-methyltransferase (MGMT) and the tumor recurrence[14], since TMZ does



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**Figure 1** Scheme about current glioblastomas treatment strategies and its advantages and limitations. GBM: Glioblastomas; OS: Overall survival; PFS: Progression-free survival; TMZ: Temozolomide; VEGF: Vascular endothelial growth factor;

not prevent this event. This enzyme can cause patient resistance to TMZ, and some patients who have MGMT gene promoter methylation in the tumor may benefit from reduced drug resistance.

About 55% GBMs[12] have innate or acquired resistance to chemotherapy due to non-methylation of the MGMT promoter. In this way, the alkyl groups are removed from the O6 position of the guanine, reducing the pharmacological efficacy of the alkylating agents[18]. Another important mechanism of resistance to chemotherapy is the reduction of TMZ cytotoxicity by the base excision repair pathway. This pathway, mainly composed of poly (ADP-ribose) polymerase-1, is capable of repairing the bases methylated by the alkylating agent in the DNA and, therefore, reducing the occurrence of apoptotic events in tumor cells[19,20]. Thus, the use of iniparib and velparib is promising, either alone or in combination with TMZ, to reduce drug resistance[20,21].

It is noteworthy that the MGMT promoter methylation status is not routinely evaluated for all patients with the discussed disease and, if evaluated, the result may not be taken into account for TMZ treatment decision making in some clinics, as there may be lower availability of treatment agents, presence of severe adverse reactions to chemotherapy, associated comorbidities and preference for treatment by the patient.

**Carmustine wafers:** Carmustine wafers are biodegradable chemotherapy intratumoral implants[22] used as an adjunct to surgical resection since 1995 in patients with recurrent GBM, since there is an improvement in overall survival (OS) of 7.2 mo in the carmustine group *vs* 5.4 mo in the placebo group [23]. However, its combined use with TMZ still divides authors, since some scientists believe that concomitant use is associated with an increase in the occurrence of adverse effects[24]. Therefore, it is necessary to have a randomized controlled clinical trial to support or refute the safety and efficacy of simultaneous use of carmustine wafer with TMZ.

**Biological agent:** Bevacizumab, a drug containing antiangiogenic monoclonal antibodies that has been in use since 2009 against the progressive form of the disease, binds to the VEGF making it difficult for recurrent GBM and rapid neurological involvement associated with the tumor, being a well-tolerated drug and capable of reducing cerebral edema, which allows a reduction in the use of corticosteroids and associated adverse effects[25].

The aforementioned drug is recommended as monotherapy or in association with other chemotherapy drugs, such as irinotecan, carmustine, lomustine, carboplatin or temozolomide[26,27], in newly diagnosed or recurrent glioblastoma. Several clinical trials over the past decade in patients with newly diagnosed GBM have shown improvements in progression-free survival (PFS), although they have not shown significant improvement in overall survival (OS). A recent study evaluated the combination of lomustine and bevacizumab in recurrent GBM and concluded with a survival of 5.1 mo [28].



However, there are genetic variations of VEGF that can determine the success or failure of bevacizumab therapy, requiring great care in the administration of this biological agent. Moreover, as the anti-VEGF method did not convincingly show improvement in OS as a monotherapy, it is necessary to evaluate the combination of this type of drug with other known therapeutic options used in neuro oncology.

### **Alternating electric field therapy**

Tumor treatment fields (TTFs) are a therapeutic method that uses alternating currents of low intensity (1-2 V/cm) and intermediate frequency through electrodes placed on the skin around the region of a malignant tumor to stop growth and to induce apoptosis of mitotically active cells[29,30], which is considered a safe method, as it does not affect non-dividing cells.

A 2015 study revealed that the combination of TTFs and TMZ significantly improves median PFS and OS compared to TMZ monotherapy during maintenance therapy with less occurrence of electrical device-related adverse effects[31]. Current treatment guidelines incorporate TTF into the therapeutic regimen of patients with newly diagnosed and recurrent GBM[13].

However, the device is expensive, must be used at least 18 h a day and requires hair shaving of users for proper application of electrodes[32]. This can affect the patient's self-esteem and quality of life, in addition to causing a possible low adherence to treatment.

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## **PIVOTAL ROLE OF THE TUMOR MICROENVIRONMENT**

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### ***The central nervous system as an immune-distinct site***

The role of the tumor microenvironment in the modulation of antitumor immune responses is becoming clearer[33]. The central nervous system (CNS) is usually described as an immune-privileged site, which means that it shows attenuated responses to alloantigen challenges[34]. Classically, the property of CNS immune privilege has been attributed to two mechanisms: (1) The blood-brain barrier (BBB); and (2) the absence of classical lymphatic drainage of CNS antigens[35]. The BBB is a semi-permeable cellular barrier composed of specialized endo-thelial cells (non-fenestrated, firmly attached by tight junctions), astrocyte end-feet, and pericytes. Its main function is to tightly regulate the movement of ions, molecules, and cells (*e.g.*, immune cells) between the blood and the brain[36,37]. The ability to block the entry of possibly neurotoxic molecules, primarily through ATP-binding cassette transporter-mediated efflux, is one of the main challenges posed to immunotherapy[38]. On the other hand, the lack of professional antigen-presenting cells in the CNS parenchyma, low expression of MHC class I and II, and the first apparent absence of classic CNS lymphatic drainage also limit the ability of an immune response to CNS-derived antigens[39,40]. Given that efficient anti-tumor responses require not only that cancer-specific T cells be generated, but also that these T cells come into direct contact with the tumor cells, it becomes evident that the CNS provides an immune-privileged microenvironment for tumor growth and proliferation.

Fortunately, increasing evidence has pointed to the CNS, not as an immune-privileged site, but rather as an immune-distinct site that remains accessible to the onset of antitumor immune responses and immunotherapy[35]. Recent studies suggest the existence of a functional meningeal lymphatic system that drains cerebrospinal fluid (CSF), macromolecules, and immune cells from the CNS into the deep cervical lymph nodes[41]. Investigating these antigenic presentation routes will be an important step in understanding the immune-distinct properties of the GBM microenvironment.

### ***Immunosuppressive mechanisms in GBM***

Although revolutionary in the treatment of cancer patients, immunotherapy is critically dependent on the availability of preexisting anti-tumor immunity[42,43]. GBM is widely recognized to induce local and systemic immunosuppression, which is a hindrance to the use of immune-modulating therapies [44].

GBM cells can evade immune surveillance through the release of various soluble mediators that exert a variety of immunosuppressive effects[45]. The best-characterized GBM-derived immunomodulatory factors are the transforming growth factor  $\beta$  (TGF- $\beta$ ), interleukin 10 (IL-10), and prostaglandin E2 (PGE-2)[45-48]. In the presence of TGF- $\beta$ , CD4+ T cells upregulate FoxP3 and differentiate into Treg cells with potent immunosuppressive potential. These converted suppressor cells not only do not respond to TCR stimulation and produce neither Th-1 nor Th-2 cytokines, but also express TGF- $\beta$  and inhibit normal T cell proliferation *in vitro*[49,50]. It has also been shown that this cytokine inhibits the expression of five cytolytic gene products - specifically, perforin, granzyme A, granzyme B, Fas ligand, and interferon (IFN)- $\gamma$  - which are co-responsible for CD8+ T cell-mediated tumor cytotoxicity[51]. Additionally, there is a TGF- $\beta$ 1-mediated downregulation of activating receptor NKG2D on the surface of CD8+ T cells and natural killer (NK) cells, thereby precluding cytotoxicity against GBM cells[52]. On the other hand, TGF- $\beta$ 2 can prevent neoantigen presentation and facilitate immune escape from T lymphocytes through the down-regulation of HLA-DR antigen expression on tumor cells[53]. Altogether, these immunosuppressive stimuli of T or NK cell activity prevent the effective immune-mediated clearance of tumor cells

[54,55].

IL-10 also plays a pivotal role in modulating the activity of resident and infiltrating immune cells and tumor cells in GBM, predominantly inducing an immunosuppressive phenotype[47]. Upon activation by GBM cell-derived IL-10, tumor-microglia and macrophages are then elicited to produce most of the IL-10 in the tumor microenvironment[56]. Increased secretion of IL-10 was associated with enhanced expression of other anti-inflammatory cytokines, such as IL-4, CCL2, and TGF- $\beta$ [57]. In the presence of IL-10, TAMs downregulate the expression of antigen-presenting molecules, thereby impairing CD4+ T cell activation[58]. Along with TGF- $\beta$ , IL-10 is also able to exert FOXP3-expressing naive T cells differentiation into Treg cells, hence leading to Treg-driven immunosuppression[59-61]. Conversely, recent data have shown that a subset of IL-10-releasing HMOX1+ myeloid cells, spatially localizing to mesenchymal-like tumor regions, also induce T-cell exhaustion and thus contribute to the tumor microenvironment[62].

In turn, PGE-2 has been shown as a key mediator of immunosuppressive activity through the expansion of myeloid-derived suppressor cells (MDSCs)[48,63]. VEGF, on the other hand, is the most important mediator of angiogenesis in glioblastoma, which has made it one of the main therapeutic targets in GBM treatment[64]. Finally, through the activation of hypoxia-inducible factor 1- $\alpha$ , hypoxia regulates the expression levels of the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed death-ligand 1 (PDL-1), and other immunomodulatory surface ligands, which hinder effective anti-tumor immune responses[65].

GBM cells can attenuate anti-tumor responses through the expression of a plethora of cell surface immunosuppressive factors, including the so-called immune Checkpoint molecules (ICs). Coupled with programmed cell death-1 (PD-1) located on the surface of activated T-cells, GBM and immunosuppressive (*e.g.*, Treg) cells membrane-bound PDL-1 can exert T-cell exhaustion and anergy[66,67]. Hence, PDL-1 upregulation in the tumor microenvironment propitiates resistance against T cell-mediated killing, in a protective process termed a “molecular shield”[68]. Conversely, the expression of the CD95 (Fas) ligand by GBM cells can also attenuate immune attack through the induction of CD95-Dependent apoptosis in infiltrating lymphocytes[69]. In turn, CTLA-4 is also an important ICs due to its capacity to compete with CD28 for binding to costimulatory molecules (CD80 and CD86) on antigen-presenting cells, thereby precluding the activation of T cells[67,68,70,71]. Lastly, indoleamine 2,3-dioxygenase 1 (IDO) and Lectin-like transcript-1 (LLT-1), are known to increase intratumoral Treg and myeloid-derived suppressor cells, and to repress NK cell activity, respectively[72,73].

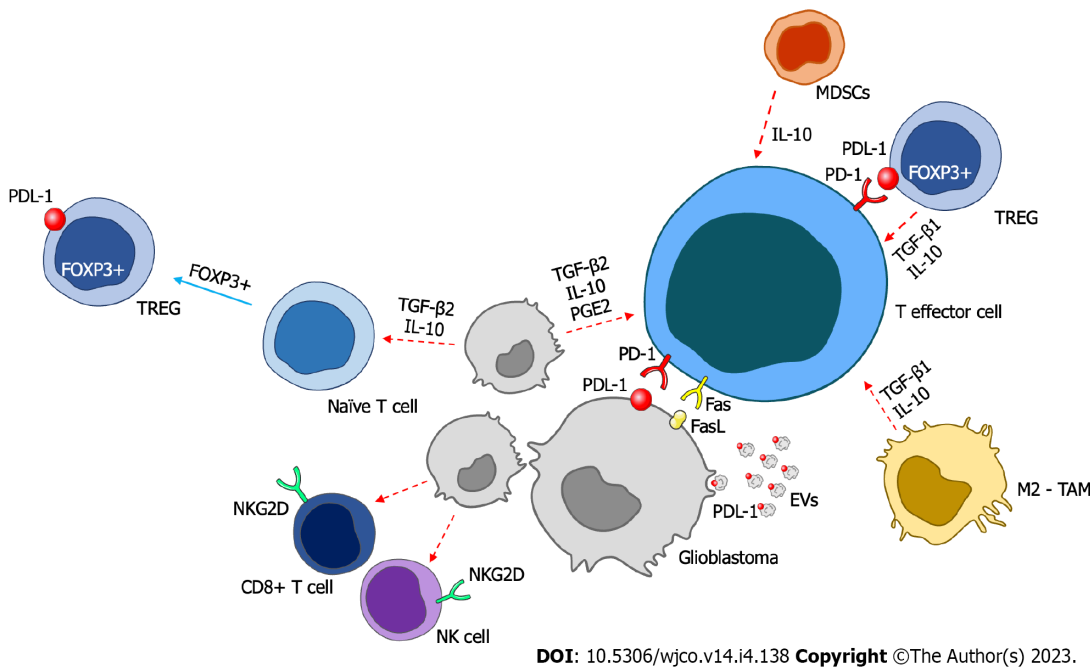
Increasing evidence has reaffirmed the pivotal role of immunosuppressive monocytes, including MDSCs, and tumor-derived extracellular vesicles (EVs) in GBM-induced local and systemic immunosuppression[74]. EVs are defined as biologically active particles that carry both GBM-derived soluble factors and membrane-bound receptors that can be functionally delivered to target cells[74]. In combination with the tumor milieu, these particles can induce the conversion of monocytes to an immunosuppressive phenotype[75]. The role of EVs in direct T-cell inhibition has also been demonstrated. Ricklefs *et al*[76] recently showed that glioblastoma EVs block T cell activation and proliferation in response to T cell receptor stimulation. This mechanism of immunosuppression and its local and systemic effects have great potential for exploration in the context of immunotherapy. The **Figure 2** synthesizes the GBM-induced immunosuppressive microenvironment.

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## CYTOKINE THERAPY

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Cytokine therapy in the treatment of GBM is based on the use of pro-inflammatory cytokines, in order to promote reversal of the immunosuppressive microenvironment triggered by this tumor and subsequent activation of the immune response[76,77]. Mainly, IFN- $\alpha$ , TNF- $\alpha$  and IL-12 have been assessed as possible therapeutic options for glioblastoma[78,79]. In this sense, IFN- $\alpha$  is related to increased activity and reduced exhaustion of T cells and macrophages, besides inhibiting tumor angiogenesis and immune suppression-related gene expression[79]. On the other hand, TNF- $\alpha$  promotes dendritic cells maturation and, consequently, T cell stimulation, while IL-12 is related to enhanced CAR-T cell efficacy, increased infiltration of CD4+ T cells and decreased frequency of T-regulatory cells in the tumor microenvironment[80,81]. Nevertheless, the therapy with IFN- $\alpha$  presents high toxic systemic potential and low efficiency in maximum tolerated doses[82]. The possibility of collateral effects implies a damage to the user, clinical trials reveal hyperthermia, shivering, headaches, gastrointestinal symptoms, decline in systolic and diastolic blood pressure and associated orthostatic hypotension[83]. This means that the therapy is a resource with limited use at least at this moment. It is expected that, in the future, this route will be used in conjunction with other therapeutic forms, such as inhibitors of anti-apoptotic proteins, to increase efficacy and tolerability[84]. In another perspective, glioma cells infected by a vector capable of transducing TNF- $\alpha$  decreased tumor growth rate in a mouse animal model, which constitutes a different therapeutic strategy for the treatment[82]. Additionally, the administration of TNF- $\alpha$  is also a problem to solve because the intravenous administration is known for the capacity to induce toxicities for the patients[76]. Recently, the discover of an interleukin-7 agonist had shown the ability to repair the lymphopenia caused by the standard treatment for GBM and also improved the



**Figure 2 Simplified scheme of glioblastomas-induced immunosuppressive microenvironment.** MDSCs: myeloid-derived suppressor cells; NK: Natural killer. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

immune system by elevating the CD8 serial lymphocytes in murine models, but this discover needs more studies to be apply for patients with this primary glioma[85].

## IMMUNE CHECKPOINT INHIBITORS

Immune checkpoints are molecular receptors that perform an inhibitory function in order to control exacerbated immune activity and prevent uncontrolled activity of this system[86]. These receptors are found on T cells (CD4 and CD8), dendritic cells (DC), NK cells and B cells[87].

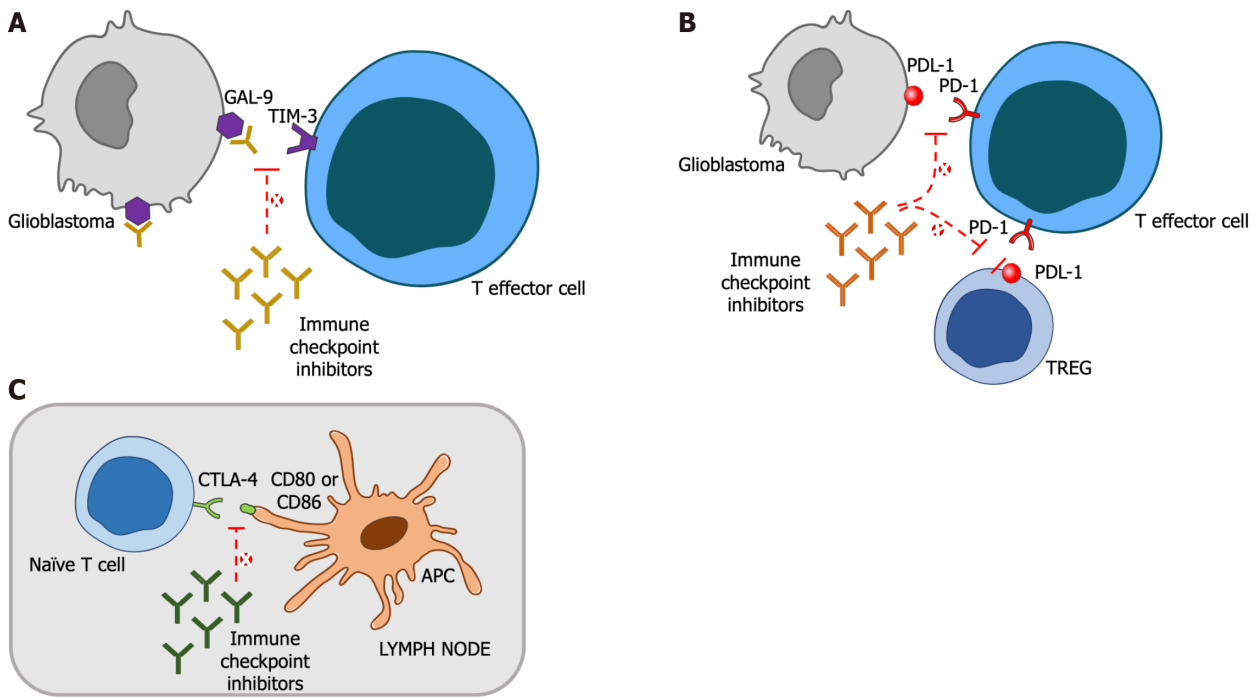
Cancer cells have some mechanisms that allow them to reduce the effectiveness of the immune system during the attack on mutated cells[88]. One of these mechanisms is the expression of molecules that interact directly with the immune checkpoint receptors resulting in reduced immune activity from the inhibition of essential cells of the protection system. Thus, immune checkpoint inhibitors have emerged as a therapeutic alternative, in order to prevent the occurrence of inhibition of immune cells from the interaction of receptors of these cells and molecules produced by glioblastoma cancer cells[87].

In this regard, studies have identified the main receptors of immune checkpoints and that have physiological importance in glioblastoma. PD-1, T cell immunoglobulin and mucin domain 3 (TIM3), CTLA4, lymphocyte activation gene 3 (LAG3), T-cell immunoglobulin and ITIM domain (TIGIT) and CD96 are inhibitory receptors expressed on immune system cells, such as lymphocytes (T and B) and NK, and have corresponding ligands produced by cancer cells[87].

Thus, studies aimed at blocking the immune checkpoint in glioblastoma have been initiated[89,90]. A study conducted in murines, associated anti-PD-1 and temozolomide (chemotherapeutic agent used in the treatment of GBM) in the treatment of glioblastoma and obtained a good antitumor efficacy[89]. However, the response in humans did not show the same efficacy, as evidenced by the randomized phase III clinical trial of 369 patients diagnosed with GBM who were treated with nivolumab (anti-PD-1) and did not show improved survival compared to the control group[90]. However, the preclinical trials are promising and the therapeutic model is still recent. This means that therapy based on blocking ICIs may yet yield an important efficiency in the lives of patients diagnosed with GBM. In Figure 3, there is a representation of immune checkpoint inhibition targets: TIM-3/Galactin 9 (GAL-9), PD-1/PDL-1, and CTL-4/CD80 or CD86.

### PD-1/PD-L1

The PD-1 receptor is expressed on T cells, B cells, TAMs, MDSCs and NK cells[91]. For inhibition of these cells to occur the PD-1 receptor interacts with PD-L1, which is expressed on GBM tumor cells. This interaction results in T-cell apoptosis, inhibition of T-cell cytotoxicity, and blockage of inflammatory mediator production. Thus, immunotherapy aims to target the PD-1/PD-L1 pathway and generate an antitumor response[87].



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**Figure 3** Immune checkpoint inhibition targets: T cell immunoglobulin and mucin domain 3/ Galactin 9, programmed cell death-1/programmed death-ligand 1, and cytotoxic T-lymphocyte-associated protein 4/CD80 or CD86. A: T cell immunoglobulin and mucin domain 3/ Galactin 9; B: programmed cell death-1/programmed death-ligand 1; C: cytotoxic T-lymphocyte-associated protein 4/CD80 or CD86. TIM-3: T cell immunoglobulin and mucin domain 3; GAL-9: Galactin 9; PD-1: Programmed cell death-1; PDL-1: Programmed death-ligand 1; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

The anti-PD-1/PD-L1 class is a category that includes pembrolizumab, nivolumab, durvalumab and atezo-lizumab[92]. These ICIs have shown good results in some types of cancer, such as melanoma and non-small cell lung cancer[93,94], but for GBM, the overall efficacy is not yet optimal, especially in monotherapy, since GBM is a disease with unique peculiarities. However, studies using combination therapy with other ICIs are ongoing and have brought positive preliminary results, despite difficulties that still need to be overcome[92]. One of these challenges is the need for these ICIs to cross the blood brain barrier, which is very peculiar to brain tumors and makes chemical therapy of this type of cancer difficult[95].

### TIM3/GAL9

TIM3 is a membrane protein, normally found on CD4+ and CD8+ T lymphocytes, and is also an inhibitory receptor for antitumor T cell activity[11]. GAL9 is a binding protein to TIM3. This binding results in the activation of the TIM3/GAL9 pathway, which induces T cell apoptosis, a fact that directly impacts antitumor immune activity[96,97].

The expression of GAL9 is higher in tissues from glioma patients and the TIM3/GAL9 interaction is involved with a higher malignancy of this type of CNS tumor. Thus, TIM3 has also become a potential target of immune checkpoint inhibitors in an attempt to boost immune activity against tumor invasion and result in a better prognosis for the patient[97].

### CTLA4

CTLA4 is an inhibitory receptor expressed on T cells and has relevance when dealing with GBM and a worse prognosis of this disease from the activation of this receptor[70]. The process is based on the interaction of T cells with antigen-presenting cells in the peripheral lymphatic tissue through co-stimulatory and coinhibitory receptors, such as CTLA4[98]. CTLA4 binds to CD80/CD86 receptors on antigen-presenting cells. Thus, this receptor is involved with the initial process (antigen presentation) of immune activity and its activation reduces the activation and proliferation of antigen-specific T cells that will act directly on the CNS and tumor cells[87].

CTLA4 has a higher expression in more serious gliomas and is related to a worse disease prognosis, as it is related to reduced antitumor immune activity[71].

Based on this, in 2011, the Food and Drug Administration approved the use of ipilimumab in the therapy of some tumors. Ipilimumab is a monoclonal antibody that binds to CTLA4 receptors and blocks the inhibition of T cells that occurs through this molecule[87].



**LAG3**

LAG3 is a regulatory protein expressed on the membrane of T cells and when activated by specific ligands, it generates an inhibitory effect on immunity. It is believed that one of these ligands is FGL1 and that it is expressed by cancer cells and induces a decrease in antitumor activity, but this mechanism is still not well known, especially in relation to gliomas[99].

In addition, it is possible that LAG3 generates immunosuppression by acting in conjunction with other immune checkpoints, such as PD-1[99]. A process that has already been reported in breast cancer studies, which identified a co-expression of LAG3 and PD-1 in the tumor process, generating T-cell inhibition[100].

**TIGIT/CD96**

TIGIT and CD96 are co-inhibitory receptors[87]. TIGIT is expressed on various immune cells such as T cells, regulatory T cells (Tregs) and natural killer (NK) cells[101]. CD96, on the other hand, has been found mainly on conventional T cells, NK cells and NKT cells[87].

High expression of PD-1 and TIGIT was found in CNS infiltrating lymphocytes, acting at the site of GBM[101]. Thus, a combined blockade therapy for PD-1 and TIGIT has shown improved efficacy and survival for patients with GBM[101].

CD96 is directly linked to the inflammatory response in GBM and additionally, a direct and synergistic correlation of this receptor with other immune checkpoints such as PD-1, CTLA-4, TIGIT and TIM-3 has been described[102]. With this, it was found that a simultaneous blockade of CD96 and other ICIs results in enhanced antitumor immunity and better prognosis[102].

**CAR T-CELL THERAPY**

Chimeric antigen receptors are synthetic receptors capable of redirecting the immune functions of T lymphocytes to a specific target antigen and thus, T cells exert short and long-term effects by triggering complex antitumor responses[103]. CAR-Ts have an extracellular domain with a tumor binding site as the single-chain variable fragment (scFv), a flexible hinge, a transmembrane region, and an intracellular signaling domain of T cells. In addition, CARs can be subdivided, according to the amount of CD3 $\zeta$  stimulatory domains, into first, second and third generation, and the most modern CARs have two costimulatory domains linked to CD3 $\zeta$  in order to potentiate its ability of signaling activation[104]. Since CAR-Ts has been used effectively against hematological tumors, the objective is to adapt the method for solid tumors such as GBM so that the activation of T cells in the tumor microenvironment promotes targeted immunological mechanisms of cell death to specific targets in the tumor, achieving the same success as the treatment in non-solid tumors, regardless of the presentation of the peptide by histocompatibility complexes[105]. The most promising studies addressing T cell therapy against GBM have explored CAR-T cells targeting human epidermal growth factor receptor 2 (HER2), variant epidermal growth factor receptor III (EGFRvIII) and alpha receptor 2 of IL-13 (IL-13 R $\alpha$ 2) mainly, as well as evaluating the different forms of therapy administration (local or systemic)[106-108].

EGFRvIII consists of an oncogenic mutation pattern existing in human tumors that allows the identification of specific tumor antigens by the immune system. EGFRvIII is relatively common, especially when it comes to GBM, in which the mutation is present in approximately 30% of scenarios[109]. EGFRvIII expression in patients with GBM is considered a marker of poor prognosis probably because the receptor enhances tumor oncogenic signaling[110]. In this sense, the first clinical study that investigated CAR-Ts therapy directed at EGFRvIII was conducted by O'Rourke *et al*[107] and evaluated 10 patients with recurrent EGFRvIII + GBM. The results demonstrated that the administration of CAR-Ts Cells by infusion is a safe route to be used, as there was no evidence of toxicity outside the tumor microenvironment or cytokine release syndrome. Although the study did not have the objective of evaluating the effectiveness of the therapy, it was observed that no patient had GBM regression and one patient remained in stable disease for more than 18 mo. Therefore, the assay also revealed a consistent response with immunological checkpoints and immunosuppressive molecules such as IDO 1, PD-L1, TGF- $\beta$  and IL-10 and this indicated that EGFRvIII+ led to an antitumor response[107]. Complementarily, a recent study evaluated apheresis and infusion products from the previous study to explore EGFRvIII as a therapeutic target for GBM and concluded that PD1 is a predictive marker of peripheral graft and progression-free survival in transduction products of patients with targeted CAR-Ts to EGFRvIII. Furthermore, it was also observed that PD1 was expressed concomitantly with ICIs (CTLA4, TIM3, LAG3) and activation markers (GRZB, HLA-DR) suggesting that PD1 is the protagonist of these correlations with the clinical response surrogates in the study. However, the aforementioned correlations were not present before the generation of CAR-Ts. Therefore, it has been proposed that the PD1 marker may predict better response to therapy against recurrent GBM and that the preparation of the infusion product is responsible for the differences in therapeutic results found in the study[111].

HER2 is also a tumor-associated antigen that is expressed by about 80% of GBM, however, the receptor is also expressed in physiological host cells and this gives HER2 the potential to generate autoimmunity when used as a specific target antigen[112]. An early trial involving HER2 CAR T Cells in

cancer patients did not produce positive effects. The study was associated with acute toxicity with fatal outcome in one patient[113]. However, a subsequent preclinical study yielded a more favorable outcome as CD28-costimulated HER2-CER T cells were tolerated by 17 patients with GBM without dose-associated toxic effects. Trial findings showed that one patient had a partial response to therapy for 9 mo, 7 remained with stable disease for 8 wk to 29 mo, and 8 had tumor progression. Additionally, patients had an overall survival of about 11 mo from T cell infusion (95% CI: 4.1–27.2 mo) and HER2 CAR T cells were present in blood at up to one year of follow-up[106]. IL-13R $\alpha$ 2 is another tumor-associated antigen that is expressed in up to 50% of GBM and despite being expressed in normal tissue, it is not expressed at significant levels in normal brain tissue[114,115]. Interestingly, the first trial that evaluated the safety and feasibility of CAR-T-s targeting IL-13R $\alpha$ 2 for the treatment of recurrent GBM was done by Brown *et al*[116] and included three patients with the malignancy. Among the three patients included, one had reduced global expression of IL-13R $\alpha$ 2 in the tumor after treatment and another patient showed an increase in the necrotic portion of the tumor where IL-13-zetacin + T cells had been administered. Despite the small sample, the findings of the work were favorable and were fundamental for the advancement in knowledge about the therapeutic method[116]. In this regard, new initial studies, albeit promising, have emerged with the aim of improving the CAR-Ts. Some works, for example, such as that of Muhammad *et al*[117], validated a new TanCAR [IL-13 (4MS) and EphA2 scFv] that proved effective in destroying GBM cancer cells recognizing IL-13R $\alpha$ 2 or EphA2 receptors and did not damage normal IL-13R $\alpha$ 1/ IL-4R $\alpha$ . Therefore, it proved to be an option with the potential to remedy difficulties in current therapy by preventing antigen escape and reducing extra tumor toxicity[117]. In addition, another initial work constructed an IL-13R $\alpha$ 2 directed to humanized third-generation CAR and evaluated its efficacy against GBM *in vitro* and reported that the receptor achieved satisfactory results that support its use in clinical trials[118].

Therefore, CAR-T-s therapy targeting specific antigens is very promising and has the potential to become a therapeutic option for solid malignancies with poor prognosis such as GBM. However, the evidence is still limited, which creates a series of challenges to be overcome by the therapeutic method. The main obstacles to a safe and effective CAR-Ts therapy are the access of immune cells to the CNS and the heterogeneity of the tumor microenvironment. The first is mainly due to the existence of the endothelial blood-brain barrier and the epithelial blood-brain barrier[119]. The second occurs because GBM is characterized by a complex and active tumor microenvironment capable of evading the functionality of CAR-T-s, as well as hindering the recognition of a single specific target antigen[120]. In this regard, one way to improve access to the CNS would be to add property to CAR-T cells through gene editing. The development of innovative CAR-Ts that can target different tumor-associated antigens or program different CAR-Ts to recognize a single tumor-associated antigen is a possible solution to immune escape or target antigen escape. A recent study targeted 3 antigens using a single universal tricistronic (U) transgene product of CAR-T-s specific for HER2, IL-13R $\alpha$ 2 and EphA2 showing an effective alternative to the interpatient variability that is one of the obstacles to therapy. The *in vitro* test of the study showed an improvement in the survival of the animals, corroborating the initial hypothesis [121]. The work by Muhammad *et al*[117], cited above, starts from the same premise that the new TanCAR destroyed tumor cells by recognizing both IL-13R $\alpha$ 2 and EphA2 alone or together, also corroborating for a more effective therapy by avoiding immune escape and recognition of non-target antigens. Another possibility to deal with difficulties in therapy with CAR-Ts cells is the remodeling of immune cells in the tumor microenvironment. This technique is based on the use of CAR-T cells with the objective of recruiting pro-inflammatory cytokines, mainly IL-7, IL-8 and IL-12, enhancing the death of GBM cells[122-124]. In addition, the blocking of immune suppression signals through chimeric decoy and switch receptors has also been explored. For example, Liu *et al*[125] added genetically modified switch receptors including the extracellular domain of PD1 and the transmembrane and cytoplasmic signaling domains of CD28 in order to stimulate the performance of CAR-T cells in solid tumors and the study data revealed a strategy potentially efficient therapy. Finally, the expansion of the use of bispecific T cell couplers (BiTE) in combination with CAR-T cells as a new artifice for the recognition of multiple antigens has also been discussed[126]. Bearing in mind that EGFRvIII-specific CAR-T cells may not be satisfactorily efficient in view of the heterogeneity of the GBM tumor microenvironment, Choi *et al*[127] proposed the use of CARBiTE cells capable of secreting wild-type EGFR-specific BiTEs. The results of the initial study were positive and showed that BiTE cells annihilated heterogeneous GBM tumors in mice and did not promote toxicity against human skin grafts *in vivo*.

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## ONCOLYTIC VIRUSES

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Over the last few years, oncolytic viruses (OVs) have gained prominence in tumor treatment, including GBM. OVs are particularly suitable for GBM therapy due to its privileges, such as lack of distant metastasis and tumor's limitations, allowing the use of viruses at this site as a promising form of immunotherapy[128]. They are administered intravenously or intratumorally to achieve its neutralizing effects.

OVs can be defined as weakly pathogenic viruses that can selectively infect, replicate in, and kill cancer cells without damaging normal cells and leading to tumor cells apoptosis[129]. This occurs through antitumor reactions of tumor-specific cell killing and the induction of the host's systemic antitumor and/or antiviral immunity. Thus, OVs activate the innate immune system *via* pattern recognition receptors and pathogen-associated molecular patterns, leading to a physiological response of immune cells recruitment, such as neutrophils, natural killer cells, macrophages, Th1 cells and its associated cytokines that promotes cell lysis[128,130]. Moreover, this response induces an adaptive immune reaction to new cancer antigens and may possibly develop a long-term immunotherapy repercussion[131]. Besides this, OVs can also be used as non-replicating viral vectors to deliver therapeutic genes, serving as vehicles to efficiently achieve tumor cells[104]. In Figure 4, there is a graphical representation of how OV therapy for GBM works.

Currently, OVs are being tested for their effectiveness against GBM in leading clinical trials using over 20 distinct viral strains like herpes simplex virus[132], adenovirus[133], measles virus[134], parvovirus[135], Newcastle disease virus[136], reovirus[137], poliovirus[138] and zika virus[139]. In Table 1, the clinical trials using virotherapy for GBM are summarized.

As aforementioned, the cooperation of the innate and adaptive immune systems is crucial in oncolytic virotherapy response, and matching it with other immunotherapy strategies such as checkpoint inhibitors increases the immunological response and tumor regression[140-142].

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## VACCINE-BASED THERAPY

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In recent years, it has been discussed the great possibility of combating and stabilizing oncological conditions through immunotherapy, and the proposal of vaccine therapies is a remarkable point. In this sense, when thinking about GBM, the proposal of an alternative therapy that generates a more positive prognosis for patients, through vaccination, is a matter of much research and debate.

Many vaccines with a variety of immunological bases have been developed and tested in the treatment of GBM. There are four commonly used approaches to base GBM vaccines on: Peptide and DNA vaccines, which use genetic information from the tumor itself, and are more specific in their use. Cellular vaccines, based on dendritic cells prepared also with tumor antigens, and mRNA-based ones, with viral vectors[143]. In general, the principle behind this bet is on the immune response, thinking about the ability of the tumor to evade the individual immune response.

Thus, one of the ways found to "combat" this disease is to use the immune system itself, more specifically, a response coordinated by T lymphocytes capable of recognizing tumor antigens and reacting against them. In this sense, the initial proposal aims to use specific tumor antigens (TSAs) to obtain an immune response, having as a basis for this process peptides based on the tumor characteristics that trigger an anti-tumor immune response by mimicking neoantigens in glioblastoma cells[144, 145].

Personalized neoantigen vaccines are a different approach to anti-tumor vaccine development, with trials already showing increased survival in patients with a recent diagnosis of GBM, demonstrating a potential to alter the immune environment in GBM[85].

However, there are some points of conflict within this vaccine therapy, since the tumor heterogeneity, with factors expressed differently among individuals, which would generate a high specificity in the manufacture of the vaccine, a need for customization, not being extremely effective on a large scale, hindering the inclusion of patients[146]. This treatment also has a limitation, generated by antigenic escape in the face of tumors that do not express this antigen. In addition, the collection of peptides for the vaccine base, meets a barrier, since the association of a disparate tumor profile, with possible formations of nonspecific epitopes - a tumor formation not from mutations, but from exacerbated expressions of factors that are expressed in normal tissues - raises a predisposition to responses beyond the tumor affection, such as autoimmune responses and inflammatory processes in other regions[146].

Another point of study that has been gaining prominence are DC vaccines, being considered one of the most promising at the moment. This is due to the role they play in immune regulation and in the GBM picture. Thus, they are extremely important for the induction of acquired immunity, also influencing the lymphocytic response, its differentiation, and antigen presentation. With this in mind, within GBM pictures, DCs are found with reduced function, being in an inhibited or immature state, which can be related to the severe tumor microenvironment, DCs are kept with low function due to the inhibitory effect of the immune microenvironment, and this status is problematic for body function, but reversed by DC vaccines[147]. This is due to the fact that the advantages of DCs vaccines are based on *in vitro* matured dendritic cells, usually from the affected individual himself, which can activate previously inhibited Ts lymphocytes, increasing the patient's adaptive response, increasing the expression of MHCs, cytokines and chemokines, and promoting an intense migration of immune cells to the immunosuppressive microenvironment found in GBM[147].

Currently, some studies have shown that DC vaccines can improve the picture of GBM, with some age-related factors seeing a better prognosis in younger patients. Another study, in phase II clinical trial, showed that the use of the vaccine after tumor resection, obtained a median overall survival of 23.4 mo,

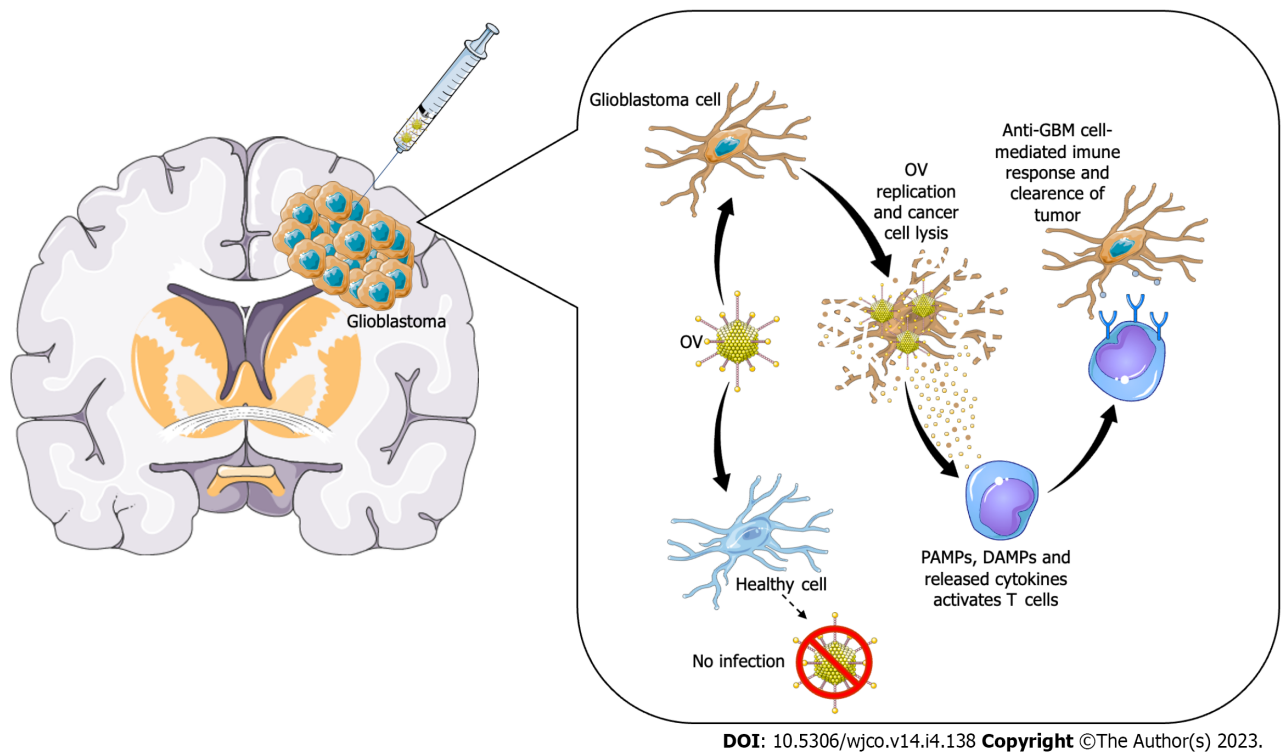
**Table 1 Ongoing and completed clinical trials of oncolytic virus therapy in glioblastoma**

NCT Number	Title	Status	Enrolled patients	Interventions	Country	Phase
NCT03714334	DNX-2440 Oncolytic Adenovirus for Recurrent Glioblastoma	Unknown status	24	Drug: DNX-2440 injection	Spain	Phase 1
NCT03294486	Safety and Efficacy of the oncolytic virus Armed for Local Chemotherapy, TG6002/5-FC, in Recurrent Glioblastoma Patients	Unknown status	78	Drug: Combination of TG6002 and 5-flucytosine (5-FC, Ancotil®)	France	Phase 1 and 2
NCT02197169	DNX-2401 With Interferon Gamma (IFN-#) for Recurrent Glioblastoma or Gliosarcoma Brain Tumors	Completed	37	Drug: Single intratumoral injection of DNX-2401; Drug: Interferon-gamma	United States	Phase 1
NCT01956734	Virus DNX2401 and Temozolomide in Recurrent Glioblastoma	Completed	31	Procedure: DNX2401 and Temozolomide	Spain	Phase 1
NCT05095441	A Clinical Study of Intratumoral MVR-C5252 (C5252) in Patients With Recurrent or Progressive Glioblastoma	Not yet recruiting	51	Biological: C5252	United States	Phase 1
NCT01174537	New Castle Disease Virus (NDV) in Glioblastoma Multiforme (GBM), Sarcoma and Neuroblastoma	Withdrawn	0	Biological: New castle disease virus	Israel	Phase 1 and 2
NCT01491893	PVSRIPO for Recurrent Glioblastoma (GBM)	Completed	61	Biological: Recombinant nonpathogenic polio-rhinovirus chimera (PVSRIPO)	United States	Phase 1
NCT00028158	Safety and Effectiveness Study of G207, a Tumor-Killing Virus, in Patients With Recurrent Brain Cancer	Completed	65	Drug: G207, an oncolytic virus	Not provided	Phase 1 and 2
NCT03896568	MSC-DNX-2401 in Treating Patients With Recurrent High Grade Glioma	Recruiting	36	Biological: Oncolytic Adenovirus Ad5- DNX-2401; Procedure: Therapeutic conventional surgery	United States	Phase 1
NCT01582516	Safety Study of Replication competent Adenovirus (Delta-24-rgd) in Patients With Recurrent Glioblastoma	Completed	20	Biological: Delta-24- RGD adenovirus	Netherlands	Phase 1 and 2
NCT03072134	Neural Stem Cell Based Virotherapy of Newly Diagnosed Malignant Glioma	Completed	13	Biological: Neural stem cells loaded with an oncolytic adenovirus	United States	Phase 1
NCT01301430	0 Parvovirus H-1 (ParvOryx) in Patients With Progressive Primary or Recurrent Glioblastoma Multiforme.	Completed	18	Drug: H-1PV	Germany	Phase 1 and 2
NCT05084430	Study of Pembrolizumab and M032 (NSC 733972)	Active, not recruiting	28	Drug: M032; Drug: Pembrolizumab	United States	Phase 1 and 2
NCT02031965	Oncolytic HSV-1716 in Treating Younger Patients With Refractory or Recurrent High Grade Glioma That Can Be Removed By Surgery	Terminated	2	Biological: Oncolytic HSV-1716; Drug: Dexamethasone; Procedure: Therapeutic conventional surgery	United States	Phase 1
NCT02798406	Combination Adenovirus + Pembrolizumab to Trigger Immune Virus Effects	Completed	49	Biological: DNX-2401; Biological: Pembrolizumab	United States	Phase 2
NCT03657576	Trial of C134 in Patients With Recurrent GBM	Active, not recruiting	24	Biological: C134	United States	Phase 1
NCT03152318	A Study of the Treatment of Recurrent Malignant Glioma With rQNestin34.5v.2	Recruiting	62	Drug: rQNestin; Drug: Cyclophosphamide Procedure: Stereotactic biopsy	United States	Phase 1
NCT03043391	Phase 1b Study PVSRIPO for Recurrent Malignant Glioma in Children	Active, not recruiting	12	Biological: Polio/ Rhinovirus Recombinant (PVSRIPO)	United States	Phase 1
NCT05139056	Multiple Doses of Neural Stem Cell Virotherapy (NSC-CRAAdS-pk7) for the Treatment of Recurrent High-Grade Gliomas	Withdrawn	0	Biological: Neural Stem Cells expressing CRAAdS-pk7; Procedure: Resection	Not provided	Phase 1
NCT02062827	Genetically Engineered HSV-1 Phase 1 Study for the Treatment of Recurrent Malignant Glioma	Active, not recruiting	24	Biological: M032 (NSC 733972)	United States	Phase 1
NCT04482933	HSV G207 With a Single Radiation Dose in Children With Recurrent High-Grade	Not yet recruiting	40	Drug: Biological G207	United States	Phase 2



Glioma						
NCT02986178	PVSRiPO in Recurrent Malignant Glioma	Active, not recruiting	122	Biological: PVSRiPO	United States	Phase 2
NCT03911388	HSV G207 in Children With Recurrent or Refractory Cerebellar Brain Tumors	Recruiting	15	Biological: G207	United States	Phase 1
NCT02457845	HSV G207 Alone or With a Single Radiation Dose in Children With Progressive or Recurrent Supratentorial Brain Tumors	Active, not recruiting	13	Biological: G207	United States	Phase 1
NCT00528684	Safety and Efficacy Study of REOLYSIN® in the Treatment of Recurrent Malignant Gliomas	Completed	18	Biological: REOLYSIN®	United States	Phase 1
NCT03973879	Combination of PVSRiPO and Atezolizumab for Adults With Recurrent Malignant Glioma	Withdrawn	0	Biological: PVSRiPO; Drug: Atezolizumab	Not provided	Phase 1 and 2
NCT00314925	Safety Study of Seneca Valley Virus in Patients With Solid Tumors With Neuroendocrine Features	Unknown status	60	Drug: Seneca Valley virus (biological agent)	United States	Phase 1

Most data were obtained from findings from [www.clinicaltrials.gov](http://www.clinicaltrials.gov) using the search terms “glioblastoma” and “oncolytic” filter.



**Figure 4 Simplified scheme of oncolytic virotherapy for glioblastomas.** GBM: Glioblastoma; OV: Oncolytic virus; PAMPs: Pathogen-associated molecular patterns; DAMPs: Damage-associated molecular patterns. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

among some patients[85]. However, a meta-analysis of randomized controlled trials on the efficacy of DC vaccines demonstrated that the use of the vaccine in newly diagnosed glioblastoma patients did not show a substantial effect on overall patient survival[148]. Thus, it is still an area that needs more studies and trials with more advanced phases, and the ability to inhibit glioma is still a point to be better tested in future studies.

Some other vaccine ideas have already been proposed, such as using isocitrate dehydrogenase as the basis for the vaccine, since mutation in this enzyme occurs purely in tumor cells, making it an interesting tumor-specific antigen to use[146]. In addition, vaccines that inactivate tumors are also an attraction for research, given their success in other pathologies, not only in treatment but also in prevention, but there is still a low efficiency for the treatment of neoplasms, requiring more research for the development and application in GBM. More advanced research is needed for the use of these other

vaccine approaches.

Another alternative attempt for the treatment of GBM, are oncolytic virotherapies, using previously known viruses, which would be injected intratumorally, enabling an inflammatory reaction and an immune response against the tumor-virus unit. Many researches and vaccines have already been approved with this type of technology, and it is a promising therapy that acts both by selectively infecting tumor cells, replicating and leading to tumor death, and by being used to transport factors for gene therapy, through viruses with alterations in their replication[104]. Regarding GBM, some vaccines, such as DNX-2401, have already gone through initial testing phases and showed positive results. However, updates of the studies are needed to better understand the spectrum and efficiency of the action of this vaccine. In addition, other vaccines are under study such as ParvOryx, Toca 511, Reovirus, and HSV type 1, being tested in patients with GBM, but still in early stages of testing[86].

Furthermore, vaccination focused on eliminating EGFRvIII is also an important resource against GBM, as it is an important TSA in this pathology[146]. Thus, the EGFRvIII anti-tumor vaccine is another interesting therapy. Some late stage studies were able to observe a good humoral induction and cytotoxic T response with the use of this TSA, after good conduct in animal studies. However, the results were not as significant as expected in survival and remission rate, in human trials[146]. Besides that many adverse effects have been found such as seizures, edema, thrombocytopenia and pulmonary embolism, and these complications when coupled with the fact that not all GBM patients express EGFRvIII, become a limitation for this therapy, since not all patients could use this vaccine[140].

The benefits of vaccination are already found in some studies, demonstrating an increase in patient survival when compared to other measures used, including the surgical approach, demonstrating the advances in this research[148,149]. However, only 3 vaccination agents have reached phase III clinical trial: Rindopepimut, DCvax and PPV[143].

Thus, the key point for vaccine therapy is the choice of the appropriate immune target with a reduction of vaccine toxicity. The search for TSA and possible alternatives must take into account the immune alterations caused by the tumor microenvironment, the immune status of the affected individual and possible adverse effects, which need to be reduced to their maximum. Moreover, there is a very important factor, even with the momentary trend towards personalized vaccines, the questioning of how to make this new reality feasible, generates a need to search for a combination of antigens of greater spectrum, having in mind also, how the vaccine process will reverberate in the organism, thinking about a long-term immune response, and what are the predictions for the future, which makes the development of studies with more solid results indispensable[143]. In addition, the possibility of combining vaccines with other immunotherapies has shown considerable benefit when compared to the use of some vaccines alone, and needs to be further investigated as an approach to be considered in patient management[86,104].

## IMMUNOTHERAPY LIMITATIONS AND CHALLENGES

Immunotherapy options currently available for the treatment of GBM are vast. These include vaccines, oncolytic viruses, immune checkpoint inhibitors, and genetically modified T cells[85]. In this sense, the various ongoing studies and clinical trials may provide favorable outcomes in expanding the use of these therapies in the near future, and, given the potential to manipulate or enhance the immune system apparatus to attack and kill tumor cells, immunotherapy has enlightened and generated a lot of excitement in the treatment of GBM. However, so far, there are some limiting factors that hinder the applicability of immunotherapy in the treatment of glioblastoma, whether related to individual anatomical and immunological factors or to routes of administration and adverse effects[140-142].

The blood-brain barrier is one of the major limitations to GBM immunotherapy. These specialized endothelial cells attached to astrocytes and pericytes hinder drug delivery, leading to inefficient therapeutic action[104,150]. Also, GBM is able to induce alterations in the BBB, forming a structurally different barrier (i.e., brain tumor barrier) that also contributes to poor penetration of therapeutic agents [77]. Furthermore, intratumoral heterogeneity plays a pivotal role in immunotherapy resistance, given the rapid growth of resistant clones after the selective destruction of susceptible ones[151]. The immunosuppressive microenvironment of this tumor also poses a challenge in the immunotherapeutic approach[152]. Treg cell upregulation leads to inhibition of effector T cells, thus impairing the use of CAR-T cells[145]. Regarding cytokine therapy, despite its ability to modulate the microenvironment of GBM, leading to increased DC cells maturation, T cell infiltration and reduced exhaustion[81], its systemic use presents severe toxicity and poor absorption, which greatly hampers the use of this therapy[78]. In this regard, future studies on the topic might provide further options for these limitations to be overcome in the near future.

In order to increase the therapeutic effectiveness of the current immunotherapy approaches, various strategies have been developed to increase drug penetration and decrease the occurrence of adverse effects. Of note, we highlight (1) the use of combined therapies, for synergistic action[153]; (2) targeted drug delivery, which increases pharmacokinetic properties and reduces toxicity[79]; and (3) intrathecal administration, to overcome the blood-brain barrier[140-142].

Furthermore, given the intrinsic heterogeneous nature of GBM and its ability to evade and resist single treatments, it is crucial that future interventions should explore the combination of biological (immunomodulators and cell based delivery systems), physical (ultrasound, 3D printed implants, heat) and chemical (delivery technologies, radiation, chemotherapy) approaches to not only treat GBM more adequately but also improve the patient's prognosis, selecting ideal combination strategies to overcome the limiting barriers. In this regard, techniques using anti-PD-1/PD-L1 antibodies combined with antibodies targeting CTLA-4, TIM-3, LAG-3, 4-1BB, or OX-40 are under study[154]. Furthermore, anti-PD-1/PD-L1 therapy combined with tumor-specific peptide vaccination or CAR-T cell therapy is also worth exploring, and can provide a harmonious combination approach to surpass the obstacles[155, 156].

Finally, exploring effective predictive biomarkers of clinical efficacy, combined with other therapeutic strategies, is a critical issue to avoid treatment delay and early mortality[157,158]. In this sense, there is a demanding need to incorporate the status of known biomarkers into daily clinical practice, which may assist not only in patient selection, but also in the adjustment of treatment schedule based on the patient-specific diagnosis.

With various ongoing clinical trials for new molecular targeted therapies, cancer vaccines and immune-modulators, it can be expected that in the near future more compelling interventions against GBM will become available.

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## CONCLUSION

In this way, it is possible to see that the treatment for GBM is advancing and discoveries are being made. However, the immunosuppressive nature of this primary glioma and the pleomorphism presented by the constitutional cells represents important challenges to implant a successful therapy with less harm for the patient. The need for resolutions to prevent the collateral damage caused by the current standard treatment and for the alternative immunotherapies, which are being developed, demonstrates potential to be the next stage in this field alongside the increase of searching for other approaches. The main objective is to better manage this aggressive malignant brain tumor to modify the current prognostic perspective. This review shows an overview of this reality and it is stated that, based on particular pathogenesis of GBM, it is necessary an individualized treatment according to the tumor progress follow-up.

The potential of the immunotherapy presented by previous and current clinical trials reveals a hopeful perspective for patients with GBM. It is expected that a combination of therapies would be used to avoid collateral damages and improve the recovery. Risks and costs of the surgical method, radiotherapy and chemotherapy suggest several issues that alternative approaches do not have and it is more favorable as a palliative therapy than as a healing mechanism, and still usage problems must be solved for them to be applied. Biological agents and Tumor treatment fields also have benefits, even though they are, respectively, susceptible to genetic variabilities and need expensive devices to put into practice as the **Figure 1** illustrates. The intervention with cytokine therapy and agonists are a recently explored field and demonstrates the ability to use different inflammatory cytokines to remodel the immune response, nevertheless there are also problems with the form of administration and the doses due to systemic toxicity. Immune checkpoints inhibitors reveal the ability to curb the immunosuppressive strategies of GBM, but the response in humans has not shown yet the same efficacy demonstrated in animal models. Chimeric antigen receptor T cell therapy is also a hopeful route of treatment due to its potential to redirect the immune response for specific targets, however the difficult to transpass the BBB and the microenvironment possessed by the active tumor, which enables evasion and difficult to recognize, are also challenges to be solved for highly functional deployment. Vaccine-based therapy is also being developed and four approaches are more currently discussed. In summary, the immunotherapy options display advantages and limitations. Thus, more advancements in ways to prevent toxic activity or/and ineffectiveness of the hopeful new recently discovered immunotherapies are fundamental to increase life expectancy and reduce suffering for the patients.

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## FOOTNOTES

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