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Review

Research Progress in Immunotherapy of Gliomas

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Abstract

Although some progress has been made in tumor treatment, gliomas remain one of the tumors that can still seriously threaten human life and health. Due to the particularity of the immune microenvironment of the central nervous system and the strong invasiveness of tumors, the treatment of gliomas remains a major challenge. Currently, researchers have explored a large number of immunotherapy programs to improve the survival and prognosis of glioma patients, including tumor vaccines, immune checkpoint inhibitors, adoptive cell transfer therapy, viral vector therapy, and genetic engineering therapy. The goal of these programs is to activate or change the immunosuppressive environment and target tumor cells through drugs, combined with surgical resection, radiotherapy, chemotherapy, and anti-angiogenesis drugs, to achieve the purpose of treating glioma. This review briefly describes the immunosuppressive microenvironment of gliomas and summarizes recent immunotherapeutic strategies and their progress. The aim is to summarize the latest immunotherapies for the treatment of gliomas and provide new research directions.

Keywords: glioma; tumor immune microenvironment; immunotherapy; tumor vaccines; immune checkpoint inhibitors; adoptive cell transfer therapy; viral vector therapy

1. Current Status of Glioma Treatment

Glioma is the most common malignant tumor in the central nervous system (CNS). Traditional treatment methods include surgery, radiotherapy, and chemotherapy [1]. Glioblastoma (GBM) has a particularly heavy psychological and economic burden on patients and their families because of its highly aggressive nature, rapid growth, high recurrence rate, treatment difficulty, and short survival time. For primary GBM, most Stupp regimens are currently used as the standard of care. This includes surgical resection therapy, concurrent radiotherapy and chemotherapy, temozolomide (TMZ) for maintenance therapy, and postoperative radiotherapy or procarbazine, lomustine, and vincristine (PCV) chemotherapy for patients who are not eligible for Stupp [2]. Although the emergence of TMZ has allowed great progress in the field of chemotherapy for glioma, due to the limitation of postoperative radiotherapy dose and patient resistance to radiotherapy, the overall clinical treatment effect is diminished, and the prognosis of patients is extremely poor. For patients with GBM, the outcome is almost always progression or relapse. However, there is still no standard treatment for recurrent GBM (rGBM), and only a small number of rGBM patients with *MGMT* gene promoter methylation benefit from the use of lomustine. The European Association of Neuro-Oncology (EANO) recommends continued treatment with TMZ or bevacizumab in patients with rGBM [3]. Nevertheless, TMZ resistance often occurs in clinical applications, and bevacizumab only prolongs progression-free survival (PFS) in patients with rGBM. But when TMZ is combined with thalidomide, it appears to prolong the median survival of patients, while the toxic side effects caused by the drug are not obvious. This demonstrates the benefits of combination therapy in improving quality of life for glioma patients.

Recently, the remarkable progress of immunotherapy research in the field of tumors has begun to enable treatment of gliomas. Such treatment includes tumor vaccines, immune checkpoint inhibitors, adoptive cell transfer therapy, and viral vector therapy. Although glioma immunotherapy has been used clinically, its long-term effect is still unsatisfactory due to clear immunosuppression and immune evasion. Understanding the application of immunotherapy to the treatment of glioma and related issues will assist in the development of scientific strategies for glioma immunotherapy in clinical and basic research. This article briefly introduces the immunosuppressive microenvironment of gliomas and summarizes recent immunotherapy strategies and their progress. It further aims to summarize the latest immunotherapies for the treatment of gliomas and provide new research directions.

2. Immunosuppressive Microenvironment of Glioma

The tumor microenvironment (TME) can be described as a heterogeneous mixture of cell-rich masses contained in a modified extracellular matrix. The cellular components include non-tumor cells, tumor cells, glial stem cells, immune cells (myeloid suppressor cells, tumor-associated mi-

croglia and macrophages, CD4+ T helper cells, cytotoxic CD8+ T cells, natural killer (NK) cells, regulatory T cells, and antigen-presenting cells including dendritic cells and myeloid macrophages), and stromal cells. TME plays a very important role in the regulation of tumor physiological processes as various cellular and non-cellular components in TME interact to regulate them. The immune microenvironment refers to the immune cell components therein, and whether the immune system can effectively attack the tumor depends on the balance of the inhibitory and promotion effects of this environment on the tumor.

The CNS was once considered an immune-privileged location. Due to the lack of specialized antigen-presenting cells in the brain, as well as the lack of lymph fluid and traditional lymphatic return, it is difficult to carry out an adaptive immune response, which is one of the reasons for the formation of the inhibitory microenvironment of brain tumors. However, this immune privilege is not absolute, and there are still two types of immune cells in the CNS under physiological conditions: macrophages, which come from the blood circulation and mainly exist in areas outside the blood-brain barrier (BBB), and microglia that colonize the brain parenchyma before the barrier's development [4]. Moreover, recent studies have found the presence of lymphatic vessel structures within the CNS [5,6]. Additionally, the immune system can act on brain tumors through immune transport, mainly via three pathways: through choroidal plexus cells, which are rich in CD4+ T cells and a small number of other immune cells, allowing them to enter the cerebrospinal fluid from the blood; via blood vessels of the *pia mater* located within the subarachnoid space; and entry into the cerebrospinal fluid through the perivascular space of the cerebral vessels. Recent studies have found that cell nests may exist that accommodate a large number of dendritic cells and neutrophils in the lymphatic channels that drain from the cervical lymph nodes to the systemic lymph nodes. These nests are closely related to antigen presentation and may be potential targets for future treatments. Ferroptosis is the most important programmed death mechanism in gliomas, mediating immunosuppressive effects and regulating the glioma immune microenvironment, while neutrophils have been preliminarily shown to participate in the mechanism of hemozosis to help drive the progression of GBM, but its specific role is unclear [7].

The existence of the BBB prevents many drugs from entering brain tissue, leading to decreased drug concentration in the brain, greatly reducing the effectiveness of drugs. But it is worth exploring that not all gliomas have a complete BBB, and there is no BBB in brain metastases. An incomplete BBB not only prevents drugs from entering the brain but also induces the formation of cell nests with high drug resistance to many chemotherapy drugs at sites where the barrier remains. Additionally, tumor cells secrete a variety of cytokines that induce the formation of an immunosuppressive microenvironment and stimulate the generation of trophoblastic vessels, which together lead to immune evasion and drug resistance of tumors. For the BBB, studies on targeting pericytes (the main cells that make up the BBB), ultrasound therapy, and nanoparticles are undergoing clinical trials.

Inflammatory cells are key cells in the tumor immune microenvironment, which promote tumor proliferation and regulate immune response, among which tumor-associated macrophages (TAMs) are one of the most active types in the microenvironment and have a complex role in gliomas. M1 macrophages secrete high levels of tumor necrosis factor-α (TNF-α), interleukin-12 (IL-12), and other pro-inflammatory factors to play an anti-tumor role, while M2 macrophages secrete cytokines such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10) to inhibit the proliferation of T cells, promote tumor growth, and maintain an immunosuppressive microenvironment [8]. Myeloid-derived suppressor cells (MDSCs) have also been shown to increase in the peripheral blood of tumor patients, leading to reduced T cell function and reactive oxygen species production. Capecitabine has been shown to reduce these suppressor cells in tumors and increase the number of both CD8+ T and NK cells in TME, when administered in a low-dose, time-dependent manner [9]. Further, the microglia contained in TAMs provide important stimulation to tumors through TGF-β-dependent mechanisms. Additionally, tumor cells also induce apoptosis of CD4+ T and CD8+ T cells through Fas/FasL signaling. There are still many cellular and non-cellular components involved in the composition and regulation of the tumor microenvironment, which also lead to the more complex immune microenvironment of brain tumors. Several experiments on inhibiting the function of tumor-promoting macrophages have yielded results. For example, CCL-2 inhibitors reduce the number of tumor-promoting macrophages in TME [10] and encapsulation of the antiproliferative drug rapamycin in nanoparticles (described in more detail below) increases the concentration of antiproliferative drugs around tumor-promoting macrophages.

There are a large number of immunosuppressive factors such as TGF-β, IL10, and indoleamine 2,3-dioxygenase1 (IDO1) in the microenvironment of GBM, which is considered to be another important reason for the formation of an inhibitory tumor immune microenvironment [11]. Currently, in addition to TAMs, Tregs are considered another major cell that causes the immunosuppressive microenvironment of gliomas and many studies have shown that the higher the degree of malignant gliomas, the higher the proportion of Tregs in the microenvironment. Tregs are normally missing in healthy brain tissue but are recruited in large numbers into the GBM microenvironment, promoting disease progression.

Generally, the characteristics of the immunosuppressive microenvironment of gliomas are primarily manifested by three features. First, immune evasion—the anatomi-

cal and physiological basis of the CNS, the existence of the BBB, and the limited number of expressed tumor antigens. Second, the presence of immunosuppressive cells, such as cytotoxic T cells that lose normal function, Tregs that secrete suppressive cytokines, M2-type TAMs, and suppressor cells from other sources. Third, a large number of cytokines present in the microenvironment, for example, TGF-β, IL-10, interleukin-33 (IL-33), and vascular endothelial growth factor (VEGF), promote tumor neovascularization and hinder the activation of immune cell function.

3. Tumor Vaccine Therapy

As a new research field, tumor vaccines have a wide range of areas to be explored. The current tumor vaccines are mainly peptide and cell vaccines.

Among the cell vaccines under study, the dendritic cell vaccine (DCVax) is a prominent representative. It uses tumor-associated antigens (TAAs) from different sources (such as autologous tumor lysates, antigenic peptides, and RNA) to shock the allogeneic dendritic cells (DCs), thus generating DCs with strong antigen-presenting abilities to activate the immune response. The safety of DCVax in glioma patients has been verified through multiple clinical trials. Although the effect is uneven, such vaccines are able to achieve results in combination with anti-PD-1 therapy; however, this means that high-grade gliomas cannot be cured by DCVax alone. DCVax has been shown to have different clinical response outcomes in patients with different molecular expression patterns. For example, in glioma patients with low levels of expression of B7-H4, a co-inhibitory molecule expressed on tumors and tumor-associated macrophages/microglia, overall survival can be noticeably improved [12]. This result suggests that for highly heterogeneous tumors, it may be more appropriate to compare the mutation characteristics of individual tumors by sequencing and select specific targets to produce vaccines, such as the gliomas described here. A common emerging target for dendritic cell vaccine therapy is the cytomegalovirus (CMV)-derived antigen Pp65. CMV DNA has been shown to be present in a variety of cancers and is related to tumorigenesis and tumor regulation. Pp65 and other CMV antigens have been shown to be expressed in approximately 90% of GBM samples but not in normal brain tissue [13]. It is an attractive target for immunotherapy. DCs can also be used to synthesize peptide vaccines, for example, ICT-107 is a hexapeptide DC vaccine that contains six peptides, such as human epidermal growth factor receptor 2 (HER2), and has been shown to improve median progression free survival (PFS) in patients.

The most studied peptide vaccine is Rindopepimut, which mainly targets the epidermal growth factor receptor variant III deletion (*EGFRvIII*) mutant and initiates an immune response against the EGFRvIII protein, showing impressive efficacy in preclinical models. For example, for brain melanoma expressing EGFRvIII in C3H mice, median survival in the treatment group improved by 600%. In a multicenter clinical trial of Rindopepimut and adjuvant chemotherapy given to EGFRvIII-positive patients with newly diagnosed GBM, a significant improvement of 21.8 months [14] in median survival was found. However, subsequent studies found that the EGFRvIII target vaccine did not improve the survival rate of GBM patients, and GBM patients who received this vaccine lost EGFRvIII expression after relapse, suggesting that relapsed tumors may develop resistance to EGFRvIII-targeted memory T cells [15]. Additionally, the use of heat shock protein peptide complex-96 (HSPPC-96) to make peptide vaccines has been shown to prolong the median overall survival of patients. Mutation of isocitrate dehydrogenase 1 (IDH1) leads to the formation of a unique group of glioma subtypes, among which codon 132 deficiency is the most common IDH1 mutation type (R132H). R132H mainly appears on major histocompatibility complex class II (MHCII) like molecules. Studies have found that in oligodendroglioma, astrocycloma, and secondary glioblastoma, the frequency of mutations in the *IDH1* gene is as high as 60–80% [16]. Michael Platten *et al*. [17] found that an IDH1 (R132H)-specific peptide vaccine (IDH1-vac) induced a specific T helper cell response, which has performed well in clinical trials where a vaccine-induced immune response was observed in 93.3 % of patients with multiple *MHC* alleles, with a 3-year progression-free rate of 0.63 and a 3-year no-mortality rate of 0.84. AG-881, a dual inhibitor of IDH1 and IDH2, is currently in Phase I clinical trials [18]. The drug has shown efficacy against IDH1 R132H mutations, and due to its high brain penetration, there is promise for the treatment of brain gliomas in the future. In diffuse midline glioma (DMG), a driver mutation in the histone H3 gene H3F3A results in amino acid exchange between lysine and methionine at position 27 (H3K27M), which occurs in histone 3.1 (H3.1K27M) and histone 3.3 (H3.3K27M). A Phase I clinical trial of H3.3K27M specific peptide vaccine in combination with the adjuvant polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose (Poly ICLC) is currently underway in children with diffuse midline glioma (DMG) with K27M mutations or other gliomas. Another related vaccine trial is a peptide vaccine consisting of ephrin type-A receptor 2 (EphA2), IL-13Rα2, and survival peptide, which demonstrated its safety and tolerability in children newly diagnosed with DMG [19]. While both peptide vaccine approaches are promising, they are currently limited to children carrying the *HLA-A2* allele, highlighting the critical importance of identifying new epitopes for vaccine research in the future. SurVaxM is a peptide-vaccine conjugate that has been shown to activate the immune system against its target molecular survival, which is highly expressed in glioblastoma cells. Manmeet S. *et al*. [20] conducted a phase IIa multicenter open trial, which demonstrated the

safety and tolerability of SurVaxM, and SurVaxM in combination with TMZ for GBM is a promising treatment option.

Because the antigens within GBM are highly specific and have almost no contraindications to drug combination, vaccine therapy meets the requirements of customized, safe and effective vaccines for patients. It is not only necessary to find reliable biomarkers to predict vaccine response, but also a future major challenge to apply the best vaccine in association with conventional treatment.

4. Immune Checkpoint Blocking Therapy

Immune checkpoints are regulatory molecules that play an inhibitory role in the immune system and are a mechanism by which the body prevents damage from autoimmune responses. At the same time, it also inhibits the function of immune cells, leading to immune escape of tumors. PD-1 (programmed cell death protein 1) and PD-L1 (programmed cell death protein ligand 1) are important negative factors that regulate T cell immune function and are key immune checkpoints. Immune checkpoint inhibitors (ICIs) have achieved great success in the treatment of advanced melanoma and non-small cell lung cancer, by blocking immune checkpoints such as PD-1, PD-L1, Cytotoxic Lymphocyte-Associated Antigen-4 (CLAT-4), and so on, restoring the immune function and anti-tumor activity of T cells, and reducing T cell failure [21]. Anti-PD-1/PD-L1 can enhance CD4+ and CD8+ T cell activity and increase T cell secretion of interferon (IFN)-γ, IL-2 and IL-10. Anti-CLAT-4 can mediate Treg depletion or functional blockade, thereby enhancing the immune activity of T cells against tumors [22]. However, researchers are exploring whether ICIs are actually effective in primary brain tumors. Laboratory studies have found that anti-PD-1 therapy can promote the transformation of M2 macrophages to the M1 phenotype, which is very promising. However, due to the obvious heterogeneity of PD-1 and PD-L1 expression on the surface of brain tumor cells, the therapeutic effect of ICIs in brain tumor patients may be limited. Moreover, because GBM tumor cells are prone to mutation and have a highly immunosuppressive microenvironment, chemotherapy and radiotherapy often lead to tumor mutation and resistance, so it is easy to generate therapeutic resistance. Studies have shown that chemotherapy may lead to hypermutation of gliomas, thus losing response to PD-1 inhibitor [23]. ICIs can act on the promoters of Erap1 and Tap1 molecules in the endogenous antigen presentation pathway, upregulating the expression of the two, so that more MHC class I molecules reach the surface of the cell membrane and increase T cell infiltration, but researchers have found that the loss of p53 function in mice with p53 gene mutation made ICIs lose this effect, eventually leading to reduced T cell infiltration [24]. Many patients with GBM must use dexamethasone to treat edema caused by tumor invasion and radiotherapy, but dexamethasone combined with ICIs can lead to worsening of patient outcomes. Researchers have found that the inhibitory effect of steroids on ICIs treatment can be overcome by enhancing CD28 stimulation or blocking CTLA-4. In general, due to the existence of immunotherapy resistance, the current use of immune checkpoint inhibitors alone has not been shown to have significant benefits for GBM, but the combination of other treatment modalities and ICIs show good synergistic effects and clinical application prospects.

CD47 is a protein molecule found on the surface of a variety of solid tumors and is involved in immune escape of tumors. CD47 is beneficial for reducing the sensitivity of the immune system to non-malignant cells under physiological conditions, but tumor cells use its overexpression to evade macrophage phagocytosis. Studies in mouse models have found that the anti-CD47 antibody magrolimab (Hu5F9-G4) effectively kills tumor cells and is a potentially effective therapeutic drug [25].

Toll-like receptors (TLRs), the first line of defense in the immune system, are receptors associated with detecting bacteria, viruses, and other dangerous signals. In the glioma microenvironment, TLR is expressed on both immune cells and tumor cells, playing a dual role by eliciting anti-tumor (innate immunity and adaptive immunity) and proto-tumor (tumor cell proliferation, migration, invasion, and glioma stem cell maintenance) responses. To date, researchers have developed several TLR-targeted therapies, targeting glioma bodies, stem cells, immune cells, and immune checkpoint axes. Some TLR agonists have shown survival benefits in clinical trials, but the prospect of their combination with radiotherapy, chemotherapy, vaccination, and ICIs for gliomas is of even greater interest. TLR agonists can be used as immunomodulators to enhance the effects of other treatments, avoid dose accumulation, and more importantly, they can release a powerful anti-tumor response when bound to immune checkpoint inhibitors by upregulating PD-1/PD-L1 overexpression, delaying the generation of immune checkpoint resistance to PD-1/PL-L1 blockade [26].

Glioblastoma stem cells (GSCs) are genetically and epigenetically driven, contributing to tumor growth, evasion of immune surveillance and development of drug resistance, so they are one of the key targets of GBM therapy. Yin Yang 1 (YY1) is a zinc finger transcription factor, a structural regulator on the chromatin interaction loop that controls gene expression. YY1 interacts with other chromatin regulators, participates in chromatin interactions, especially those that connect active regulatory elements, and is essential for the maintenance of GSCs. Studies have shown that YY1 acts as a checkpoint to maintain the transcriptional status of GSCs by controlling RNA Pol II transcription and RNA processing. YY1 regulates the transcriptional process by inhibiting interferon signaling through m6A modification regulators, while YY1-associated transcriptional CDK9 inhibitors have shown efficacy in inhibiting GSCs [27–30]. Targeting the YY1-CDK9 transcrip-

tional extension complex can enhance the anti-PD-1 response by modulating the immunosuppressive microenvironment in GBM, which provides a new combination strategy for immunotherapy in GBM.

More than 90% of GBM expresses the tryptophan (Trp) metabolizing enzyme, lactam 2,3-dioxygenase 1 (IDO1). IDO1 is a promoter of immune resistance in GBM cells, and it is often expressed in wild-type isocitrate dehydrogenase (IDH) GBM. It has been found in tumor cells that IDO1 increases the expression of complement factor H (CFH) and its subtype H factor like protein 1 (FHL-1) without dependence on tryptophan metabolism. Human tumor cells utilize the non-enzymatic activity of IDO1 to enhance the expression levels of CFH and its truncated isotype, FHL-1. Moreover, tumor cell FHL-1 enhances macrophage maturation, enhances the expression of macrophage ARG1, CCL2, and IL-6, and reduces the survival rate of brain tumor laboratory mice by inhibiting the immune response of anti-GBM T cells and NK cells [31,32]. The expression of IDO1 and CFH was positively correlated in GBM tissue resected by patients, and elevated levels of CFH/FHL-1 in tumors were associated with reduced survival in GBM patients. This may also explain the fact that monotherapy with IDO1 enzyme inhibitors has not achieved good efficacy in previous drug treatment regimens, precisely because of the non-enzymatic function of IDO1 in human tumors. Future studies should focus on the non-enzymatic function of IDO1 to reverse its immunosuppressive effects in brain tumors.

Tregs in glioblastoma binds to CD80 or CD86 through CTLA-4, which significantly inhibits effector T cell activation [33]. Additionally, the main regulator in Tregs, rat forkhead box protein P3 (FoxP3), induces the expression of Heme Oxygenase-1 (HO-1), while HO-1 inhibits T cell proliferation [34]. Therefore, the inhibitory effect of Tregs on immune cells, on the one hand, inhibits the secretion of pro-inflammatory factors and, on the other hand, also promotes the secretion of immunosuppressive factors. Glucocorticoid-induced tumor necrosis factor-related protein (GITR) is an immune checkpoint that is constitutively expressed in Treg cells, and GITR activation accelerates cell proliferation, enhances cellular effector function, and is considered an important marker of Tregs. It has been reported that activating GITR not only stimulates the proliferation of Tregs, but also inhibits its immunosuppressive function, promotes both the resistance of effector T cells to the inhibitory effect of Tregs and the conversion of Tregs to Th9 [35]. Th9 cells are a newly discovered class of CD4+ helper T cells, which are expressed differently in different tumor tissues and even tumor tissues in different sites, which can inhibit tumor growth and mainly exert antitumor effects by secreting cytokines such as interleukin-9 (IL-9). That is, stimulating GITR causes Treg cell instability and accelerates Treg cell depletion. The study found that anti-GITR agonist antibody therapy preferentially targets GBM Treg cells, converts Treg cells in an immunosuppressed state into anti-tumor Th1-like CD4+ T cells, and also reduces the resistance of GBM to anti-PD-1 therapy. Simultaneously, because Treg cells infiltrated into the GBM microenvironment have their own specific phenotype, they have tumor specificity, which reduces the incidence of adverse events during immunotherapy.

Anti-PD-1/PD-L1 monoclonal antibodies are difficult to manipulate into an effective role; the reasons are complex, but they must be related to the existence of the BBB. The BBB makes it impossible for drugs to be efficiently concentrated around a tumor and efficacy is naturally poor. Currently, commonly used clinical anti-PD-1 monoclonal antibodies include nivolumab and pembrolizumab and commonly used anti-PD-L1 monoclonal antibodies include atezolizumab, avelumab, and durvalumab [36]. To solve this problem, researchers have tried to use nanoparticles to host monoclonal antibodies and specific ligands (such as peptides) to increase the targeting effect of nanocarriers and, by binding to specific receptors, induce the antibody to cross the BBB, increasing the blood concentration around the tumor in the brain. Commonly used nanomaterials include polymeric nanoparticles, liposomes, and micelles. For example, nanoparticles modified with rabies virus glycoprotein (RVG) peptides can specifically bind to nicotinic ACh receptor (N-AChR) and effectively mediate the passage of anti-PD-1 drugs through the BBB [37]. Additionally, Hua *et al*. [38] have also developed a RVG-29 modified docetaxel-loaded nanoparticle (RVG29-DTX-NP) for targeted treatment of gliomas.

5. Adoptive Cell Transfer Therapy

Adoptive cell transfer therapy (ACT) specifically optimizes the selection of tumor antigens, or is employed to introduce genetically engineered cells specific for tumor antigens and then provide appropriate stimulation to promote proliferation, amplification, and maintain effective function, so as to achieve therapeutic results. Before cell metastasis, the immune environment in the host can first be modified, such as by dissecting the lymph or removing Tregs, to provide a favorable environment for anti-tumor cell proliferation.

The study of chimeric antigen receptor T-cell immunotherapy (CAR-T) cells is at the forefront of the ACT research field. CAR-T cells are modified T cells that specifically recognize tumor-associated antigens, which have been approved for specific hematologic malignancies and are being explored for use with various solid tumors. CAR-T cell therapy has proven effective in preclinical studies of GBM [39]. CAR (chimeric antigen receptor) is equivalent to the navigation of T cells to locate tumor cells; its target is CD19 on the surface of tumor cells. It has been possible to create CARs that simultaneously induce T cell activation, proliferation, and cytokine release. CAR-T cell therapy is not effective for solid tumors because cells accumulate into

clumps, the number of CD19 molecules exposed to the surface is small, and because of the immunosuppressive environment. According to current research on GBM, several tumor antigens suited as targets for CAR include IL-13 receptor α2, EGFRvIII, and HER2. However, in clinical applications, it has been found that CAR-T cell therapy is seriously limited by antigen escape. For example, in the case reports of CAR-T cell therapy targeting the IL-13 receptor α2 in GBM, the expression of this antigen was significantly reduced when the tumor relapsed. A significant proportion of glioma patients have partial or complete loss of target antigen expression on the surface of tumor cells. Bielamowicz and his partners [40] designed a trivalent CAR-T cell targeting HER2, IL-13Rα2, and EphA2, which captures almost all tumor cells in the GBM patient-derived xenograft model, improves anti-tumor activity, and reduces antigen escape.

There is growing evidence that radiation therapy acts as a sensitizer for immunotherapy, with tumor cells having higher radiosensitivity than normal cells in terms of DNA repair and cell cycle regulation. For a long time, radiation therapy has been considered an immunologically inert treatment, but in fact, it upregulates the expression of MHCI on the surface of tumor cells, better induces tumor antigen presentation, and radiation-induced DNA damage causes damaged DNA to leak into the cytoplasm, activating both innate and adaptive immunity, thereby turning the immune environment from "cold" to "hot" [41], thus improving the effectiveness of immunotherapy for brain tumors, Radiation therapy before CAR-T therapy improves the ability of CAR-T cells to cross the BBB, but the exact mechanism is still unclear.

DCs are essential for adaptive immunity, so if the function of the DCs does not express normally, does this lead to an inactive tumor immune microenvironment? For this reason, sufficiently activating the mediated presentation function of DCs has become one of the directions for solving this problem. Recently, researchers have found that Lin-CCR2+ subsets of hematopoietic stem cells (HSCs) can be directed into brain tumors by intravenous injection, differentiate into DCs, and exert antigen presentation, which shows a great synergistic effect between anti-PD-1 therapy and adoptive T cell therapy in GBM [42].

NK cells have a variety of cytotoxic mechanisms and regulate the immune response by producing cytokines, playing a key role in anti-cancer immunity. NK cells are generally considered to have anti-GSC activity and are able to reduce the systemic tendency of GBM cells to metastasize. Through sequencing, it was found that NK cell infiltration in tumor cells is even higher than that of T cells. Traditional CAR-T therapies have been applied to generate CAR-NK cells that exhibit effective specific tumor targeting while providing an ideal safety profile. For example, DNAX-activating protein 12 (DAP12) and DAP10, which have been used in some studies to replace CD3 in traditional CAR-T therapies, induce powerful destructive activity in NK cells once phosphorylated [43]. When stimulated by IL-15, CAR-NK cells preferentially attack GSCs. CAR-NK cells that are specifically directed to the tumor site, while binding to the target antigen on the tumor cell, also trigger the activation receptor on the NK cell, thereby causing a strong anti-tumor response. This method, known as NK cell engagement, effectively enhances the tumor-killing activity mediated by NK cells. Recent studies have shown that this method exhibits enhanced killing of CD30+ tumor cells when used in Hodgkin lymphoma and leukemia, resulting in CAR-like responses [44]. Although the role of CAR-NK cell therapy in GBM is not yet certain, this study does provide a direction for the treatment of GBM. Other researchers have found that KLRB1 (the NK cell gene encoding CD161) [45] has an inhibitory effect when expressed on CD4+ T and CD8+ T cells and the expression silencing of this gene and other NK cell genes leads to improved anti-tumor response.

6. Viral Vector Therapy

Since 1991, when the journal *Science* first reported a genetically modified variant virus for the treatment of human glioma *in vitro* [46], oncolytic viruses have been developed rapidly in the field of glioma treatment. Some less virulent viruses are selected in nature and genetically modified to target tumor cells because of their own inhibition oncogenes are inactivated or defective and they can be selectively infected by the modified virus. The principle of an oncolytic virus (OV) exerting anti-tumor effects lies in the generation of *in situ* vaccines and the activation of immunosuppressive microenvironments. The virus proliferates in large numbers in tumor cells until the cancer cells are lysed, while the lysate also stimulates the body's immune response, which is equivalent to producing an *in situ* vaccine capable of reversing tumor-induced immunosuppression. Adenovirus, herpesvirus, and pox virus are by far the most commonly used types of modification. Recent studies have even reported the use of the Zika virus to target GBM.

The trial that best illustrates the promise and challenges of immune-viral therapy is T-Vec and the anti-PD-1 ICI-pembrolizumab [47]. T-Vec is a genetically modified herpes simplex virus type I that lacks the *viral ICP34.5 and ICP47* genes to enhance tumor tendencies, reduce neurotoxicity, and encode GM-CSF. T-Vec has been clinically approved for the treatment of advanced melanoma, based on the widespread use of ICIs and BRAF inhibitors in melanoma. It remains to be studied whether T-Vec can play a role in glioma treatment. A randomized study using T-Vec with ipilimumab (an anti-CTLA-4 ICI) conducted by Chesney J *et al.* [48] and the clinical analysis results in the phase 1b pilot phase of the randomized pembrolizumab $\pm$ T-Vec study of Ribas A *et al.* [49] were encouraging. In the early stages of the MASTERKEY-265 trial, detailed patient sample analysis showed that OV injection turned the

tumor microenvironment from cold to hot. Although ineffectiveness was shown in subsequent Phase 3 clinical trials, encouraging early data suggests that this may be due to certain unpredictable variables that future studies should be committed to solving.

Several projects using engineered adenovirus against tumors are ongoing. DNX-2401 is an OV modified by selectively replicating adenovirus deletion of the Rb pathway, which can preferentially infect cells expressing GRD-binding integrin. At present, DNX-2401 combined with TMZ, IFN-γ, and pembrolizumab in the treatment of GBM clinical trials are being carried out, all showing clinical safety, with the median survival of patients in the trial reaching up to 11 months, while three patients showed complete long-term remission [50]. VB-111 (replication-deficient adenovirus), which has an antiangiogenic effect, performed well in early clinical trials of VB-111 in combination with bevacizumab in the treatment of rGBM, but failed in Phase 3 clinical trials because bevacizumab may inhibit the antitumor activity of VB-111. Others have reported promising test results by using adenovirus gene AdV-tk-mediated cytotoxic immunotherapy combined with acyclovir to induce cell death [51]. Additionally, the attenuated strain of recombinant mumps virus (rMuV-S79) has been found to kill glioma cells and inhibit tumor cell proliferation [52].

Recently, researchers in the United States have developed a new tumor-attacking virus, which kills tumor cells in the brain while blocking the formation of tumor trophoblastic vessels. There are also other studies that used E-cadherin (CDHI) with the *HSV-ITK* gene to construct the oncolytic herpesvirus OV-CDHI, which significantly enhances the treatment effect in GBM patients, and its safety is also guaranteed [53]. A study of GBM and brain metastases found that OV virus could be found at the tumor lesion site by establishing an intravenous pathway to inject edited OV virus, while the expression of IFN-α, -β, and -γ in tumor tissues was also increased, indicating that this edited OV virus not only crossed the BBB, but also indirectly promoted the expression of PD-L1, while activating the tumor immune microenvironment and forming a good synergistic therapeutic effect [54]. PVSRIPO, a recombinant poliovirus vaccine, recognizes CD155 receptors that are highly expressed in GBM cells. According to Duke University's research results [55], engineered oncolytic poliovirus (PVSRIPO) can effectively prolong the survival of rGBM patients, showing obvious survival advantages.

OV therapy for brain tumors has only been effective in a small number of patients, and many clinical trials have failed to achieve the endpoint of effectively improving their median survival. However, since OV is similar to the role of *in situ* vaccines, it is proposed that its combination with ICIs can improve the immunosuppressive microenvironment. At present, the fastest research is the combination of OV and the PD1/PD-L1 antibody, the combination of OV and the CTLA-4 antibody is also being evaluated. Additionally, oncolytic viruses armed with a variety of cytokines have also been used in combination with CAR-T cell therapy, and these combination strategies have shown enhanced anti-tumor activity in transplanted tumor models [53].

7. Other Treatments

It is worth looking forward to studies that explore the use of genetic material to treat GBM. Because some of the genetic material in tumor cells, such as nucleotides, is different from natural cells, genetic engineering can be used to make highly targeted drugs. An siRNA antisense oligonucleotide that targets the insulin-like growth factor-1 (IGF-1) receptor (IMV-001), for example, is a drug that targets insulin-like growth factor-1 (IGF-1). The IGF-1 receptor is a constitutive overexpressed oncogenic receptor in GBM, which is beneficial for tumor cell resistance to apoptosis and radiation. IMV-001 significantly prolongs median survival in patients eligible for Stupp [56]. Recently, the antitumor effects of chlorogenic acid, a phenolic compound, have attracted attention and demonstrated good safety and tolerability in a phase I clinical study of advanced malignant glioma, and patients with rGBM also benefit from chlorogenic acid treatment [57].

Based on credible preclinical data, it has been found that the combination therapy of radiotherapy and immunotherapy is very promising. Radiation generates new peptide sequences, upregulates the expression of MHCI molecules on the surface of tumor cells, activates the tumor suppressor microenvironment, especially high-dose low-grade irradiation, and in addition to reducing the number of tumor cells and inducing immunogenic cell death, it also eliminates immunosuppressive cells in tumors, such as regulatory T cells (Treg) [58]. A single dose of local irradiation of 2 Gy leads to inflammation, inhibits tumor neovascularization, and recruits specific T cells to tumor sites, which together have been validated in mouse models. However, due to the narrow time window available for patients to produce a large number of immune cells after radiotherapy, it is necessary to closely monitor the patient's immune status following radiotherapy so as to select the appropriate time to add immunotherapy [59]. Recently, some investigators have studied the combination of radiotherapy and CAR-T/T cells to recruit bispecific antibodies for tumor treatment, as an additive effect has been observed in mouse models, and more research is required to better understand its joint mechanism of action [60]. Additionally, the combination of oncology vaccine and radiotherapy is another combination regimen worthy of consideration.

8. Summary and Outlook

In summary, the balance between immune system promotion and suppression is essential for the treatment of tumors; no matter which therapy is started, it is inseparable from the core of immune system activation. Future stud-

ies should focus on solving the problems of glioma immune evasion and immunosuppression, and activation of the tumor-suppressive immune environment. For vaccine therapeutics, the high specificity of antigens within GBM enables the need to personalize safe and effective vaccines for patients, and there are few contraindications for drug combination. In the future, not only will the search for reliable biomarkers predict vaccine response, but it will also be a major challenge to apply the best vaccine to conventional treatment. Currently, there are many immunotherapy options for gliomas, but good efficacy cannot be achieved through a single treatment. It is more likely that a combination of a variety of means, combine different immunotherapy methods with surgery, radiotherapy and chemotherapy, is expected to prolong the survival of glioma patients and improve their quality of life.

Author Contributions

ZW has determined the research direction. ZD and ZW have conducted literature search, reading, and analysis. ZD and ZW have written the review. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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