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Current Immunotherapeutic Approaches for Malignant Gliomas

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Glioblastoma is the most common malignant central nervous system (CNS) tumor (48.3%), with a median survival of only about 14.6 months. Although the CNS is an immune-privileged site, activated T cells can cross the blood-brain barrier. The recent successes of several immunotherapies for various cancers have drawn interest in immunotherapy for treatment of malignant glioma. There have been extensive attempts to evaluate the efficiency of immunotherapy against malignant glioma. Passive immunotherapy for malignant glioma includes monoclonal antibody-mediated immunotherapy, cytokine-mediated therapy, and adoptive cell transfer, also known as chimeric antigen receptor T cell treatment. On the other hand, active immunotherapy, which stimulates the patient's adaptive immune system against specific tumor-associated antigens, includes cancer vaccines that are divided into peptide vaccines and cell-based vaccines. In addition, there is immune checkpoint blockade therapy, which increases the efficiency of immunotherapy by reducing the resistance of malignant glioma to immunotherapy. Despite centuries of efforts, immunotherapeutic successes for malignant glioma remain limited. However, many clinical trials of adoptive cell transfer immunotherapy on malignant glioma are ongoing, and the outcomes are eagerly awaited. In addition, although there are still several obstacles, current clinical trials using personalized neoantigen-based dendritic cell vaccines offer new hope to glioblastoma patients. Furthermore, immune checkpoint targeted therapy is expected to decipher the mechanism of immunotherapy resistance in malignant glioma in the near future. More studies are needed to increase the efficacy of immunotherapy in malignant glioma. We hope that immunotherapy will become a new treatment of malignant glioma.

Keywords Immunotherapy; Glioblastoma; Malignant glioma; Adoptive cell transfer; Cancer vaccine; Immune checkpoint blockade.

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INTRODUCTION

The annual incidence of malignant gliomas is about 5 cases per 100,000 person-years [1]. Astrocytic tumors (pilocytic astrocytoma, diffuse astrocytoma, anaplastic astrocytoma, glioblastoma, or all other gliomas) account for 76.4% of all gliomas [2]. The most common of all malignant central nervous system (CNS) tumors is glioblastoma (48.3%), which accounts for approximately 41.8% to 57.3% of gliomas [2,3]. In Korea, glioma is the third most common (15.1%) among all brain tumors, and glioblastoma accounts for 34.6% of gliomas [4]. The standard treatment for glioblastoma consists of surgical resection followed by radiation therapy and concurrent chemotherapy [5]. Despite these treatments, the median survival of glioblastoma is only 14.6 months [5]. The 5-year survival rate for glioblastoma is 5.6% and for anaplastic astrocytoma is 30%, according to the CBTRUS report [2].

The recent significant successful results of adoptive immunotherapy and checkpoint inhibitors for various cancers have drawn interest in immune-targeted strategies for treatment of malignant glioma [6-13]. However, the CNS has been believed to be an immune-privileged site with restricted access of immune cells to the brain due to the blood-brain barrier (BBB) [14]. Therefore, people believed that immunotherapy can be limited in treating brain tumors. However, the concept of im-

mune privilege of CNS has been redefined because studies have shown that activated T cells can cross the BBB and diffusely penetrate the brain parenchyma [15-19]. Therefore, expectations were raised for the possibility of immunotherapy for malignant glioma [20]. Extensive studies related to immunotherapy for malignant glioma have been reported, and many clinical trials are underway. However, because glioblastoma induces an immunosuppressive tumor microenvironment, immunotherapy has often failed. Therefore, recently, there have been clinical trials for glioblastoma using neoadjuvant drugs to overcome immune-suppressive tumor microenvironment and increase the efficacy of immune-checkpoint inhibitors [21,22].

Herein, we will generally review immunotherapy for malignant glioma based on recently published studies. The review will be conducted by classifying therapy into passive immunotherapy, active immunotherapy, combined treatment of cytokine mediated gene therapy and virotherapy, and immunomodulatory therapy.

PASSIVE IMMUNOTHERAPY

Monoclonal antibody-mediated immunotherapy

Monoclonal antibodies (mAbs), which are made from a single hybridoma clone outside the body, are categorized as passive immunotherapy agents because they do not require the active role of the patient's immune system to fight the cancer [23]. The naked mAbs recognize cell surface target antigens and induce several mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) or complement-mediated cytotoxicity that leads to target cell death [24]. However, treatment targeting these antigens with naked mAbs has potential adverse effects because normal tissue cells can also express those cell surface antigens. Therefore, it is necessary to target unique tumor antigens such as epidermal growth factor receptor variant III (EGFRvIII), which is the most common genetic variation of the EGF receptor and is expressed only in cancer cells, including glioblastomas [25]. Likewise, there are other surface target antigens besides EGFRvIII that are especially overexpressed in malignant glioma and are essential for glioma proliferation, such as vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptors (PDGFR) α and β, and epidermal growth factor receptor (EGFR) [26]. However, in previous glioblastoma clinical trials investigating the efficacy of VEGFR, PDGFR, and EGFR inhibitors on glioblastoma, the treatment group showed no significant increase in overall survival compared to the control group [27].

There is also a conjugated mAb agent that increases the efficacy of cancer attack by attaching several cytotoxic agents including radioactive materials, immunotoxins, chemotherapeutic drugs, or nanoparticle-based small interfering RNA particles to a naked mAb [28]. The specificity of the mAb to a tumor antigen allows more effective delivery of these cytotoxic agents to the tumor with minimal toxicity to normal cells. Radionuclide-conjugated mAbs include tenascin-C (TN-C), an extracellular matrix hexabrachion glycoprotein expressed in pathological conditions including high-grade gliomas but not in normal brain [29]. Almost 90% of gliomas show widespread expression of TN-C compared to healthy tissues, which express it only to a minor extent [30]. It is reported that administering radioactive particles conjugated with anti-TN-C mAb (^{131}I-anti-tenascin mAb 81C6) into a surgically created resection cavity in patients with glioblastoma showed minimal systemic toxicity and encouraging survival [31]. In addition, nimotuzumab is an mAb that recognizes and binds to the extracellular domain of the EGFR [32]. The safety and efficacy of locoregional treatment of the ^{188}Re-labeled anti-EGFR mAb nimotuzumab in patients with high-grade glioma was reported previously [33,34]. There were clinical trials in patients with malignant gliomas treated with conjugated mAbs with immunotoxins such as interleukin (IL)-4 and -13−*Pseudomonas aeruginosa* exotoxins (IL4-PE and IL13-PE38), transferrin-*Corynebacterium diphtheriae* toxin (Tf-CRM107), and tumor growth factor (TGF)α-*P. aeruginosa* exotoxin (TP-38) [35,36]. Although immunotoxin therapy has shown promising results in several clinical studies, it has challenges such as vascular leak syndrome, hepatotoxicity, immunogenicity, and low penetration capabilities [37].

Adoptive cell transfer

Chimeric antigen receptor (CAR) T cells were designed originally by genetically modifying T lymphocytes to recognize and fight cancer cells [38]. When the CAR construct binds to its target antigen, T cells are activated and induced to release cytokines to kill the cancer cells [39]. CARs are composed of an extracellular domain (target and spacer domain), a transmembrane domain, and an intracellular signaling domain [40]. The targeting domain of a CAR usually consists of the single-chain variable fragment (scFv) that is derived from an antibody. Therefore, it theoretically can recognize any type of surface antigen expressed on a target cell, including proteins (e.g., HER2, PSMA, and CD19), carbohydrates (e.g., Lewis-Y), glycolipids (e.g., GD2), the extracellular portion of native receptors (e.g., natural killer group 2 member D [NKG2D], IL-4R, IL-7R, programmed death 1 [PD-1]), or ligands (e.g., IL-13) [40-44]. CAR-T cell immunotherapy has some properties of active immunity [14]. The first-generation CAR-T cells had a single CD3ζ chain signaling domain, which is the signaling domain of a T cell receptor (TCR) [45]. However, it showed poor persistence of CAR-T cells after administration and resulted in limited effects in treating patients with cancer. Thus, next-gen-

eration CAR constructs were developed to include CD3ζ with 1 or 2 costimulatory domains (e.g., CD28, OX40, ICOS, and 4-1BB) to enhance the persistence of CAR-T cells and antitumor effectiveness [38]. Treatment with CAR-T cells to target CD19 showed extraordinary remission in relapsed or refractory B-cell acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma, including cases that involved extensive CNS disease [6,7,15]. This treatment was approved by the US Food and Drug Administration (FDA) for pediatric and refractory adult acute ALL in 2017 [46]. Although the efficacy of treatment with CAR-T cells is proved for hematological malignancies, the effect of this treatment on gliomas has not yet been elucidated.

As described in the introduction section, although the CNS is an immune-privileged site and shows limited immune reactivity, activated T cells can cross the BBB and diffusely expand through the brain. However, the immunosuppressive glioma microenvironment suppresses T cell activity by depleting tryptophan from the microenvironment [47,48]. In addition, both microglia and myeloid cells release high level of arginase, which inhibits T cell proliferation and function [14,49]. Furthermore, unlike CD19, which is expressed uniformly on the surface of all B-cell-derived tumors, glioblastomas have inter- and intratumor cellular, genetic, and molecular heterogeneities, leading to heterogeneous expression of target antigens [50-53]. Therefore, these factors reduce the efficacy of CAR-T cell treatment for glioma. Nevertheless, there have been clinical trials for treatment of glioblastoma with CAR-T cells targeting three strong glioblastoma-restricted antigens: EGFRvIII, human epidermal growth factor receptor 2 (HER2), and IL-13 receptor α2 (IL-13Rα2) [54-57]. Since EGFRvIII, HER2, and IL-13Rα2 antigens are usually overexpressed in glioblastoma and not in normal brain tissue, they are theoretically ideal immunotherapy targets for glioblastoma treatment [58]. These trials demonstrate the potential of CAR-T cells for the treatment of glioblastoma. Although EGFRvIII-CAR T cell treatment has not yet shown significant clinical efficacy, there are currently six ongoing clinical trials, two of which combine EGFRvIII-CAR T cell treatment with chemotherapy or immune checkpoint inhibitors [58]. Unlike EGFRvIII, HER2 is expressed not only in glioblastoma, but also in breast and ovarian cancers. Because it is also expressed in some normal tissues, there are safety concerns. A previous clinical trial (NCT01109095) evaluating the safety and efficacy of HER2-specific CARs using virus-specific T cells on glioblastoma showed clinical benefits with minimal risk for 8 of 17 patients with glioblastoma [55]. Administration of IL-13Rα2-specific CAR-T cells into the postsurgical cavity of glioblastoma patients showed clinical efficacy without severe toxicity [54,56]. Clinical trials of the combined treatment of IL-13Rα2-specific CAR-T cells with immune checkpoint inhibitors, such as nivolumab and ipilimumab, in glioblastoma patients are currently ongoing (NCT04003649). However, a major limitation of single-antigen targeting CAR T-cell therapy for glioblastoma is the inherent heterogeneity of the glioblastoma tumor cells. This leads to the immune escape of tumors owing to the loss of the targeted antigen. In addition, there are other obstacles to CAR-T cell therapy, including limitations of trafficking and infiltration into tumor tissue owing to the BBB, immunosuppressive tumor microenvironment, systemic inflammatory response such as cytokine release syndrome, and limitation of CAR T cell persistence [59,60].

Recently, NK cells have received much attention as alternative CAR-engineered effectors for the treatment of glioblastoma [61]. The important role of NK cells in cancer therapy has been reported [62,63]. NK cells are not only involved in antitumor immunity by eliminating malignant cells, but also regulate tumor-specific adaptive immune responses through crosstalk with dendritic cells (DCs) [64]. There are several advantages of CAR-NK cells for cancer treatment compared to CAR-T cells. First, NK cells show superior safety due to a shorter life span and limited *in vivo* expansion relative to T cells. CAR-NK immunotherapy has shown reduced risk for graft versus host disease (GVHD), cytokine release syndrome, and neurotoxicity [65-67]. This difference might be partly due to a different spectrum of the secreted cytokines between CAR-NK and CAR-T cells [68]. Activated NK cells usually release IFN-γ and GM-CSF, whereas CAR-T cells predominantly produce cytokines, such as IL-1a, IL-1Ra, IL-2, IL-2Ra, IL-6, TNF-a, MCP-1, IL-8, IL-10, and IL-15 [68-70]. Second, CAR-NK cells can potentially eradicate cancer cells in not only a CAR-dependent, but also a CAR-independent manner [68]. CAR-NK cells still have natural cytotoxic activity against cancer cells through a CAR-dependent mechanism in which the antibody is bound to the target cells, leading to ADCC-like activity [71], which can be activated via a CAR-independent mechanism, including natural cytotoxicity receptors, NKG2D, costimulatory receptor DNAM-1 (CD226), and killer cell immunoglobulin-like receptors [72,73]. Therefore, CAR-NK cells can eliminate efficiently a heterogeneous tumor such as glioblastoma through both CAR-dependent and NK cell receptor-dependent mechanisms [68]. Third, because CAR-NK cell treatment showed reduced risk for alloreactivity and GVHD, it is possible to produce CAR-NK cells from multiple sources, including NK92 cell lines, peripheral blood mononuclear cells (PBMCs), umbilical cord blood (UCB), and induced pluripotent stem cells (iPSCs) [74]. This could eliminate the need for the personalized and patient-specific product that currently is required for CAR-T cell treatment and allow CAR-NK cells to be provided as an "off-the-shelf" product [75].

Clinical studies on the treatment of malignant glioma using

CAR-T cells and CAR-NK cells are in progress. The ongoing work with CAR-NK-92 cells, which are allogeneic off-the-shelf therapeutics based on PBMCs, UCB, and iPSCs, can be evaluated for their effectiveness against malignant glioma in the near future [61]. However, the immunosuppressive microenvironment and immune escape due to highly heterogeneous expression of CAR target tumor-associated antigens (TAAs) in glioblastoma remain significant obstacles for treatment of malignant glioma with CAR-T and CAR-NK cells. Nevertheless, CAR-NK cells naturally exhibit cytotoxic activity through CAR-independent receptors that are expressed by tumor cells, which may help to eradicate glioblastoma cells with low or heterogeneous expression of the CAR target TAA [61]. It was reported that NK cell cytotoxicity against stem cell-like glioblastoma cells was more significant compared to differentiated glioblastoma cells [76]. The higher cytotoxic effect of NK cells on stem cell-like glioblastoma cells, which have more heterogeneous TAAs than differentiated glioblastoma [77], shows the potential for treatment with CAR-NK cells against malignant glioma with heterogeneous features. In addition, although they provide increased safety when compared with CAR-T cells, the shorter life span of NK cells will likely limit their long-term effectiveness and require repeated treatment [61]. However, a recent study showed that a human IL15/IL15Rα complex secreted from oncolytic viral (OV)-IL15C-infected glioblastoma cells prolonged survival and activated both NK and CD8+ T cells *in vitro* [78]. Consistent with this, combination therapy of OV-IL15C and off-the-shelf EGFR-CAR-NK cells significantly improves therapeutic outcomes in glioblastoma mouse models. In addition, there was a study on CAR-NK cells transduced with bispecific CAR constructs as a solution to antigen loss in EGFRvIII-directed CAR-NK cell therapy for glioblastoma, targeting both mutated and wild-type EGFR [79]. Intratumoral injections of dual-specific EGFR- and EGFRvIII-directed CD28.CD3ζ.CAR-NK-92 prolonged the survival of glioblastoma xenograft mouse models without antigen escape [80,81].

ACTIVE IMMUNOTHERAPY

A cancer vaccine is a type of active immunotherapy that involves exogenous administration of activated DCs presenting TAAs or selected TAAs combined with adjuvants that activate DCs or even DCs themselves [82]. The main purpose of therapeutic cancer vaccines is to stimulate the patient's adaptive immune system against specific TAAs. The stimulated adaptive immune system induces tumor regression and gains long-lasting memory responses to prevent tumor resurgence. Successful therapeutic vaccination against cancer requires delivery of large amounts of a high-quality antigen to DCs, optimal DC activation, induction of strong and sustained CD4+ T helper cell and CD8+ cytotoxic T lymphocyte responses, and efficient infiltration of immune cells to the tumor microenvironment while maintaining a durable response [82]. The two main categories currently used for treatment of glioblastoma are peptide vaccines and cell-based vaccines [83].

Peptide vaccines

A peptide vaccine refers to exogenous administration of specific tumor antigens to induce stimulation of an adaptive immune system against specific TAAs. To maximize specificity against tumors, peptide vaccines against malignant glioma require specific TAAs that are highly expressed in glioma but not in normal tissue. Antigens expressed only in cancer cells but not in normal cells often are called tumor-specific antigens (TSAs) to distinguish them from TAAs [83]. TSAs that have not been previously reported are usually termed "neoantigens."

EGFRvIII was the most widely used TSA for the peptide vaccine against glioblastoma. An anti-EGFRvIII peptide vaccine, rindopepimut (CDX-110), showed promising results for treatment of newly diagnosed glioblastoma in an early-phase clinical study [84]. However, a multicenter, double-blind, phase III clinical trial (ACT IV) that enrolled 745 patients with newly diagnosed glioblastoma showed no significant improvement of median overall survival in the rindopepimut group compared to the control group [85]. Unlike DC-based vaccines, peptide vaccines are inherently related to a relatively weak immune response and are usually required in combination with immunostimulatory adjuvants such as Toll-like receptor agonists or DCs to enhance the immune response [86]. In addition, although there are possible benefits of peptide vaccine that contains a tumor-specific epitope, the heterogeneity of glioblastoma may limit the efficacy of vaccinations that target only one TSA [87]. Therefore, future directions with peptide vaccinations may require targeting multiple epitopes to overcome the inherent heterogeneity of glioblastoma tumor cells [86].

A cytosolic enzyme, isocitrate dehydrogenase (IDH), is mutated frequently in gliomas but not in normal cells [88]. Therefore, mutations in IDH1 can be a TSA for gliomas. Approximately 80% of low-grade gliomas have IDH mutations, the most common of which is the IDH1 R132H mutation (70% of all IDH mutations). The IDH1 R132H mutation is expressed in approximately 5%−12% of glioblastomas and typically is associated with secondary glioblastomas [88,89]. A previous study found that an IDH1 R132H vaccine showed a specific antitumor immune response against IDH1 R132H-mutated tumors in a major histocompatibility complex (MHC)-humanized animal model [90]. Conceptually, the results raised expectations about the effectiveness of the IDH1 R132H vaccine in patients with IDH1 R132H-mutated low- and high-grade gliomas. A

phase I clinical trial investigating the efficacy of IDH1 R132H peptide vaccines in grade III and IV gliomas with the IDH1 R132H mutation (registration no. NCT02454634, clinicaltrials.gov) has recently been completed [91]. The study reported that the three-year progression-free and death-free rates were 0.63 and 0.84, respectively. In patients with immune responses, the two-year progression-free survival rate was 0.82. In addition, there is a high frequency of pseudoprogression, indicating intratumoral inflammatory responses [91]. Currently, the phase I clinical trial investigating the efficacy of IDH1 R132H peptide vaccines in IDH1 R132H-mutated recurrent grade II gliomas (registration no. NCT02193347, clinicaltrials.gov) is ongoing.

Human cytomegalovirus (hCMV) is a well-known herpes virus. Although their roles remain controversial, several hCMV proteins including IE1, US28, pp65, gB, HCMV IL-10, and pp28 have been found in glioblastoma cells but not in normal tissues [92,93]. The clinical trial PERFORMANCE (registration no. NCT02864368, clinicaltrials.gov), investigating the effect of a CMV peptide vaccine (PEP-CMV) against glioblastoma, is currently ongoing [94].

Cell-based vaccines

DCs, which are antigen-presenting cells, are crucial for the adaptive immune system and immunosurveillance. However, the uptake and process of DCs often fail after receiving the peptide vaccine, even in conjunction with immunostimulatory adjuvants [95,96]. DC vaccination aims to address this failure by reversing the ignorance of the immune system to TAA or TSA cells [97]. To achieve this, the DCs are stimulated to mature and loaded with tumor-associated peptide antigens on their MHC molecules *ex vivo*. Generation of a monocyte-derived DC vaccine for cancer therapy is performed in the following way: 1) isolating CD14+ monocytes from patient PBMCs; 2) these monocytes are cultured on granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4 for 5−7 days to differentiate into immature DCs; 3) for differentiation of immature DCs into mature DCs, immature DCs are incubated for 16−20 hours in a cytokine cocktail with GM-CSF, IL-4, tumor necrosis factor alpha, IL-1β, and IL-6; 4) the DCs are then loaded with TAAs or TSAs; 5) DC uptake and process these antigens and present epitopes on their MHC molecules at the cell surface; and 6) these mature antigen-loaded DCs are then injected back into the patient [98,99].

DCs express MHC class I, which presents antigen to CD8+ cytotoxic T cells, and MHC class II, which presents antigen to CD4+ helper T cells. During uptake and processing of foreign antigens, immature DCs start to undergo maturation and migrate to the spleen or adjacent lymph nodes [100]. Upon maturation, DCs induce naïve T cells to differentiate into T cells capable of secreting anti- or pro-inflammatory immune responses [100,101]. Given these adaptive immunity characteristics, DCs are the strongest and most efficient endogenous stimulus of new T- and B-cell responses [98].

Theoretically, DC vaccination can induce those adaptive immunity systems, leading to long-lasting immunological protection against glioma [102]. Therefore, there have been extensive clinical trials of DC immunotherapy for glioblastomas and other high-grade gliomas [103-109]. In 2012, Ardon et al. [108] reported a phase I/II clinical trial that enrolled 77 patients with newly diagnosed glioblastoma. In that study, four weekly induction autologous glioblastoma lysate-loaded DC vaccines were administered intradermally to glioblastoma patients after radiotherapy, but before maintenance chemotherapy with temozolomide. Subsequently, maintenance chemotherapy was initiated and boost injections were administered four times throughout the course. The results showed a median overall survival of 18.3 months in the intention-to-treat (ITT) group without major toxicity. A recent phase III clinical trial of an autologous tumor lysate-pulsed DC vaccine in patients with newly diagnosed glioblastomas showed extended patient survival [104]. The authors reported that the median overall survival of the ITT group (n=331) was 23.1 months from the time of surgery, with 2- and 3-year survival rates of 46.2% and 25.4%, respectively.

Challenges of tumor vaccines for treatment of malignant glioma

Although tumor vaccines are reported to be safe and effective in treating malignant gliomas, they do not show curative treatment results. Because glioblastomas induce an immunosuppressive glioma microenvironment, the effectiveness of cancer vaccines can be reduced [93]. Glioblastoma cancer stem cells downregulate expression of MHC molecules to escape from the tumor antigen-cognate T lymphocytes recognizing tumor cells in an MHC-dependent manner [110]. In addition, glioblastoma cancer stem cells induce immunosuppressive molecules in the glioblastoma tumor microenvironment such as programmed death ligand 1 (PD-L1) and regulatory T cells inducing cytokine TGF-β and cytotoxic T-lymphocyte−associated protein 4 (CTLA-4) [111]. Furthermore, radiotherapy, chemotherapy, and steroids, which are important parts of standard glioblastoma treatments, are associated with depletion of leukocytes, leading to immunosuppressive status [112]. Both significant antigenic heterogeneity and few mutations that could be targeted immunotherapeutically in glioblastoma are problems in tumor vaccine immunotherapy for glioblastoma [14, 113]. Therefore, it has been reported the possible efficacy of bispecific or tri-specific antibody immunotherapy for glioblastoma [114,115]. In addition, against these limitations, personalized

neoantigen-based DC vaccines have raised new expectations for glioblastoma immunotherapy. Early therapeutic vaccination strategies focused on TAA, and were largely unsuccessful in generating clinically effective antitumor immune responses owing to the lack of tumor specificity and poor immunogenicity [116]. Mutations occurring in tumors can generate novel epitopes of self-antigens called neoantigens. Whole-exome sequencing technology has made it possible to identify personalized neoantigens more reliably and efficiently [117]. In addition, the development of algorithms for predicting MHC class I-binding epitopes has significantly contributed to the identification of potentially immunogenic neoepitopes [116]. Together, these scientific advances have made it possible to produce personalized therapeutic cancer vaccines tailored to each patients' tumor. Therefore, neoantigen-based DC vaccines are highly specific to individuals, and targeting neoantigens, which are classified as TSA, can effectively stimulate T cells to generate a strong immune response highly specific to cancer cells [95]. Several phase I clinical trials of personalized neoantigen-loaded DC vaccines against various cancers, including glioblastoma, are ongoing [95]. However, there are still many obstacles such as limitations with DC maturation and efficiency of DC migration, the need for highly specialized facilities and personnel, the time required for *ex vivo* culture and complex processes for screening individual neoantigens, and the high cost of use in actual clinical practice [95,96].

COMBINED TREATMENT OF CYTOKINE MEDIATED GENE THERAPY AND VIROTHERAPY

Cytokines such as IL2, IL4, IL12, and IFN-γ can induce robust immune responses to glioma cells, and virus-mediated cytokine therapy has been shown to be effective in treating gliomas [118,119]. Intratumoral injection of a combination of adenoviral (Ad) vector expressing Flt3L (Ad-Flt3L) and an Ad vector expressing herpes simplex virus type 1−thymidine kinase (Ad-TK) showed tumor regression and long-term survival in animal models of glioblastoma [120,121]. Flt3L increases the migration and infiltration of DCs into the tumor microenvironment [122]. Activation of ganciclovir induced by Ad-TK kills glioma cells, and damage-associated molecular patterns (DAMPs) are released from the dying glioma cells. DAMPs trigger an immune response against self-antigens, and glioma-infiltrating DCs are able to phagocytose these DAMPs and tumor antigens [119]. The DCs loaded with tumor antigens on MHC are presented to naïve T cells in cervical drainage of lymph nodes, and cytotoxic glioma-killing T cells are generated. The tumor-specific effector T cells migrate to the tumor microenvironment and kill residual glioma cells through production of granzyme B, perforin, and cytokine IFN-γ [119].

IMMUNOMODULATORY THERAPY (IMMUNE CHECKPOINT TARGETED THERAPY)

Immune checkpoint molecules on the surface of activated T cells act as gatekeepers of immune responses that prevent excessive inflammatory response [123]. The most well-known immune checkpoints, PD-1 and CTLA-4, inactivate activated T cells and induce apoptosis of T cells [83]. Binding of PD-1 on the T cell surface to its ligand PD-L1 on the surface of cancer cells leads to inhibition of T cell activation through decreased TCR signaling and reduced induction of crucial transcription factors such as activator protein 1 and nuclear factor of activated T cells [83,124]. Similar to PD-1, CTLA-4 weakens T cell activation by competing with the co-stimulatory molecule CD28 for binding of ligands CD80 and CD86 (B7) expressed on antigen-presenting cells [125]. Therefore, inhibition of these immune checkpoint molecules (PD-1 and CTLA-4) can induce sustained T cell activation and enhance the effectiveness of immunotherapy against cancer.

Previously, both anti-PD-1 and anti-PD-L1 antibodies have achieved marked responses in a variety of cancers [8-13,126]. Glioblastoma cells are also known to show high expression of PD-L1 leading to an immunosuppressive tumor microenvironment [127]. A recent large open-label phase III CheckMate-498 trial (NCT02617589) for newly diagnosed glioblastoma patients with unmethylated O6-methylguanine-DNA methyltransferase (MGMT) patients compared the treatment outcomes between the standard Stupp regimen (radiotherapy plus temozolomide) and anti-PD-1 antibody plus radiotherapy. However, the anti-PD-1 antibody plus radiotherapy group showed no benefit in overall survival compared to the control group [128]. More recently, a randomized, triple-blind, phase III CheckMate-548 trial (NCT02667587) showed similarly disappointing results. The study compared anti-PD-1 antibody + temozolomide + radiotherapy versus placebo + temozolomide + radiotherapy in newly diagnosed glioblastoma patients with a methylated MGMT promoter. The patients did not show benefits with anti-PD-1 treatment in either overall survival or progression-free survival [129]. However, a combination of anti-CTLA-4 and anti-PD-1 therapy showed a significantly increased cure rate in a glioblastoma animal model [130]. A phase III clinical trial is underway to determine whether the combined treatment of CTLA-4 blockade and anti-PD-1 treatment is effective in recurrent glioblastoma (NCT02017717).

However, treatment with immune check inhibitors alone often fails to produce an immune response to the tumor. This is because glioblastomas tend to be "cold" tumors that contain

no or few immune cells and are not sensitive to immune check inhibitors [131]. Therefore, to reduce the immunosuppressive tumor microenvironment with immune check inhibitors and maximize antitumor efficacy with personalized vaccines, the combined therapy of personalized vaccines and immune check inhibitors has been attempted. A recent preclinical study reported that neoantigen vaccination showed a significant survival benefit when combined with αPD-L1 treatment in a glioblastoma mouse model [132]. In addition, clinical trials of the combined treatment of personalized neoantigen vaccine (NeoVax) with the immune checkpoint inhibitor anti-PD-1 (pembrolizumab) in newly diagnosed glioblastoma patients are currently ongoing (NCT02287428).

CONCLUSIONS

We reviewed several well-established immunotherapies currently used for treatment of malignant glioma. However, as described above, the malignant glioma and its CNS are challenging to treat with immunotherapy due to the limitations of immune access to the CNS, immunosuppressive glioma microenvironment, heterogeneous expression of antigens, and low tumor mutation burden. In addition, standard treatments for glioblastoma such as chemoradiotherapy and steroids are associated with immunosuppressive status. Therefore, despite centuries of efforts, immunotherapeutic successes for malignant glioma remain limited. However, many clinical trials of CAR-T cells and CAR-NK cells for treatment of malignant glioma are ongoing [20], and the outcomes are eagerly awaited. In addition, although there are several obstacles such as limitations of techniques, high cost, and specialized facilities and personnel, ongoing clinical trials using personalized neoantigen-based DC vaccines offer new hope to glioblastoma patients. Furthermore, checkpoint blockade is expected to help decipher the mechanism of immunotherapy resistance in malignant glioma in the near future. Overall, more studies are needed to lower the resistance to immunotherapy and increase the efficacy of immunotherapy in malignant glioma. We hope that immunotherapy will become a new successful treatment of malignant glioma.

Ethics Statement

Not applicable

Availability of Data and Material

Data sharing not applicable to this article as no datasets were generated or analyzed during the study.

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Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

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