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Introduction to immunotherapy for brain tumor patients: challenges and future perspectives

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Abstract

Malignant gliomas, including glioblastoma (GBM) as the most aggressive type of adult CNS tumors, are notoriously resistant to current standard of care treatments, including surgery, systemic chemotherapy, and radiation therapy (RT). This lack of effective treatment options highlights the urgent need for novel therapies, including immunotherapies. The overarching goal of immunotherapy is to stimulate and activate the patient's immune system in a targeted manner to kill tumor cells. The success of immunotherapeutic interventions in other cancer types has led to interest in and evaluation of various experimental immunotherapies in patients with malignant gliomas. However, these primary malignant brain tumors present a challenge because they exist in a vital and sensitive organ with a unique immune environment. The challenges and current status of experimental immunotherapeutic approaches, including vaccines, immune-checkpoint blockade, chimeric antigen receptor T-cell therapy, and oncolytic viruses will be discussed, as well as the potential for combinatorial therapies.

Keywords

checkpoint | glioblastoma | glioma | immunotherapy | vaccine

The Immune Microenvironment of Glioblastoma

Although the CNS has traditionally been classified as an immune-privileged site, it has become clear that immunosurveillance is actively occurring in the brain. Egress of antigen-presenting cells from the cerebral spinal fluid (CSF) into the deep cervical lymph nodes are able to prime T and B cells against CNS antigens. However, this does not widely occur in the brain parenchyma because there are no clear drainage routes for potential antigen-presenting cells to lymph nodes.[1] Furthermore, in the CNS parenchyma of a healthy brain, the primary resident immune cells are microglia, specialized macrophages, and CNS border–associated macrophages. In this context, microglia function in immune surveillance and synapse pruning but may not be capable of functioning as antigen-presenting cells.[1,2] Furthermore, although T cells can be found in the CNS, there are extremely low levels of infiltration by CD4 + helper and CD8 + cytotoxic T cells in the healthy brain, as compared with other organs.

The immune microenvironment of the brain may undergo drastic remodeling in the presence of a tumor. For example, downregulation of sphingosine 1-phosphate receptor 1 has been shown to result in T-cell sequestration in the bone marrow of patients with brain lesions, including glioblastoma (GBM) tumors. [3]This further decreases the level of T-cell infiltration.

In addition to an overall lack of T-cell infiltration, GBMs have a high infiltration of myeloid cells. Myeloid cells comprise the majority of the innate immune response, and differentiate into monocytes, dendritic cells, macrophages, and neutrophils, etc. These cells function to protect the host by releasing inflammatory cytokines. In the setting of GBM, certain myeloid populations become polarized toward protumor function. These are myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs).[4,5] MDSCs are closely related to monocytes and neutrophils, and are not typically present in healthy tissues; infiltration of MDSCs occurs in cancer or pathological conditions associated with inflammation. The primary function of MDSCs is T-cell suppression.[4] TAMs consist both of infiltrating bone marrow–derived macrophages and brain-resident microglia. In fact, microglia and macrophages have distinct immunosuppressive signatures, and their relative levels change throughout tumor progression, with levels of TAMs increasing as the tumor grows.[6] Tumor-infiltrating TAMs that migrate from the blood show an increase in immunosuppressive markers, such as programmed death ligand 1 (PD-L1), FLT3, and TGFβ compared with CNS-resident microglia.[6] Additionally, Osteopontin (OPN) has been shown to be an important factor that mediates TAM infiltration in gliomas and promotes the M2 protumor macrophage phenotype.[7,8] Tumor-infiltrating MDSCs suppress cytotoxic T-cell activity by expressing indoleamine 2,3-dioxygenase 1 (IDO), Arginase (ARG1), and inducible nitric oxide synthase. [9]IDO depletes tryptophan and ARG1 depletes arginine, which inhibits T-cell proliferation and activation. [10]IL-10 and TGFβ, soluble factors secreted by MDSCs and TAMs, respectively, also are known to be mediators of immunosuppression.[11,12] MDSCs and TAMs constitute a large number of intratumoral immune cells in GBM patient samples, ranging from 30% to 60% of all cells composing the tumor.[13] Patient-derived MDSCs and TAMs have been shown to suppress the activation of T cells in vitro. The strong presence of immunosuppressive myeloid cells paired with a paucity of antigen-presenting cells, such as dendritic cells (DCs), renders the tumor myeloid compartment a promising target for immunotherapies.

Additionally, GBMs have fewer somatic mutations in comparison to other tumor sites, such as lung cancer and melanoma.[14] A paucity of tumor antigens may contribute to many GBM tumors being "poorly immunogenic," limiting the immune responses initiated by antigen-presenting cells.

Corticosteroid Use and Immunotherapy

Corticosteroids are commonly used both before and after tumor-resection surgery to control cerebral edema.[15,16] Dexamethasone, a synthetic glucocorticoid, is primarily used for GBM patients but has potent immunosuppressive properties. In preclinical models, dexamethasone administration led to the inhibition of naive T-cell differentiation and proliferation by upregulating cytotoxic T lymphocyte antigen 4 (CTLA-4).[17] Immunotherapy Response Assessment in Neuro-Oncology guidelines recommend that patients undergoing immunotherapy should receive as little corticosteroid treatment as possible to maximize immunotherapeutic efficacy.[18] With the development of immunotherapies for GBM, safe reduction of corticosteroids may become pertinent to the success of existing, novel, and combination therapies.

Current Approaches

Vaccines

Vaccines aim to stimulate an intrinsic immune response to the tumor, with the primary goal of increasing effector cell activation and infiltration. There are a wide variety of vaccine approaches that are currently being evaluated in GBM, but they can be fundamentally categorized into 2 classes: peptide/DNA vaccines, and cell-based vaccines (Fig. 1A; Table 1). Peptide and DNA vaccines involve the injection of tumor-specific antigens or nucleic acids, often paired with immune-stimulatory molecules to strengthen the adaptive immune response to the tumor. Tumor-specific antigens are present on tumor cells but not on normal healthy cells. Cell-based therapies rely on antigen-presenting cells, such as DCs, to induce an adaptive immune response. DCs function as a mediator between the innate and adaptive arms of the immune system, and are professional antigen-presenting cells capable of activating potent T- and B-cell responses. In this arm of vaccine therapy, patient-derived DCs are isolated from patient blood, matured, and loaded with tumor antigen. Subsequently, the DCs are injected back into the patient. In addition to DC-based vaccines, tumor lysates or fixed tumor cells have been directly injected.[19] Of all current immunotherapies for GBM, vaccines have been one of the most thoroughly investigated strategies to date. Although more comprehensive reviews of vaccine approaches have been published,[20–23] a few recent approaches are discussed here.

Epidermal growth factor receptor variant III (EGFRvIII) is a GBM-specific antigen that is present in approximately 20% of GBM patients. Such specificity allows for minimal off-target toxicities, but its heterogeneous expression throughout the tumor remains a difficulty. In a phase 2 trial, a peptide vaccine against EGFRvIII, rindopepimut, was paired with temozolomide (TMZ). Although this trial reported promising results and verified safety, the phase 3 trial (NCT01480479) did not show any increase in overall survival.[24] Because the single antigen-targeting vaccine relies on stable widespread expression of the EGFRvIII epitope throughout the tumor, antigen loss and variable expression between tumor cells and patients may have contributed to the outcomes of the phase 3 trial.[25,26]

Isocitrate dehydrogenase mutation (IDH1R132H) has also become a target for peptide-based vaccines. A current investigational trial (NCT02193347) is evaluating safety and immunological activity of a vaccine (PEPIDH1M) targeted to the IDHR132H mutation seen in patients with IDHR132H + World Health Organization grade III and IV recurrent glioma.[27]

An ongoing trial (NCT02960230) is investigating an H3.3K27M epitope-specific vaccine for pediatric diffuse intrinsic pontine glioma (DIPG) and non-DIPG glioma

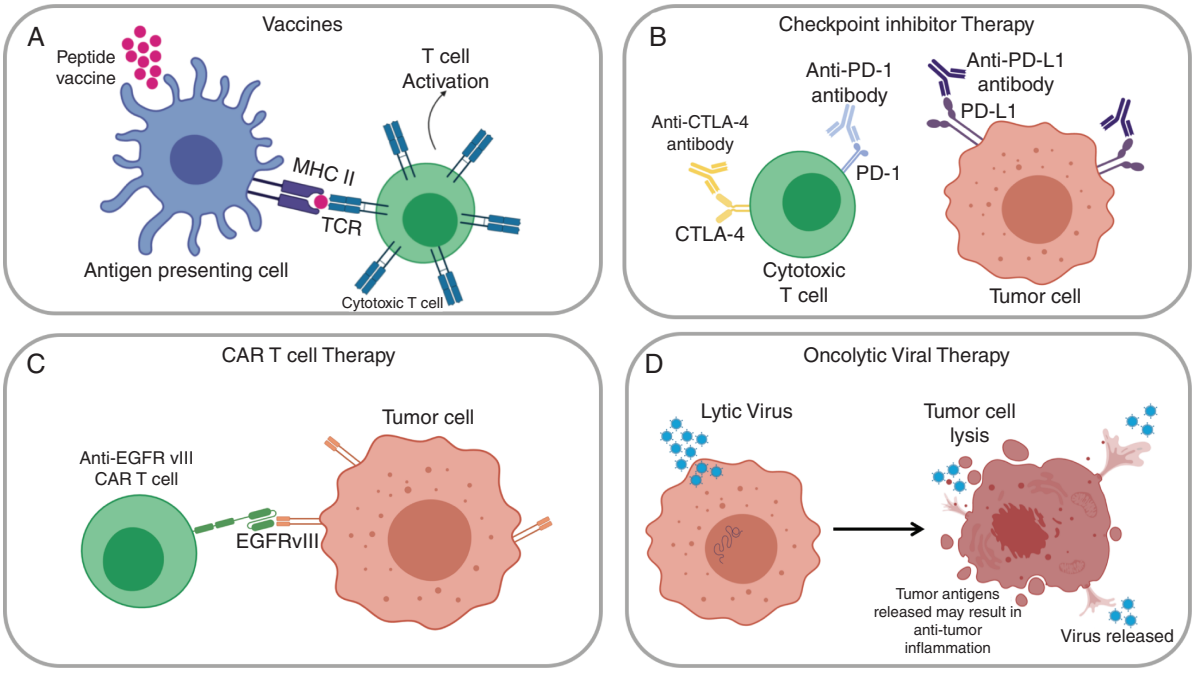

Fig. 1 Immunotherapy concepts for glioblastoma (GBM). A, Vaccines can be peptide/DNA based or cell based. Their goal is to promote antigen presentation and T-cell infiltration. Shown here is a peptide vaccine, which may encompass both broad GBM antigens and patient-specific antigens. B, Checkpoint inhibitor therapy relies on antibodies to inhibit immune-checkpoint molecules. Programmed cell death protein 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) are expressed on T cells and promote self-tolerance in healthy tissues. In GBM, this mechanism is exploited by tumor cells that express programmed death ligand 1 (PD-L1). Immune-checkpoint inhibitors bind to these molecules and limit T-cell inhibition. C, Chimeric antigen receptor (CAR) T-cell therapy uses genetically engineered T cells to target tumor-specific or associated antigens. CAR T-cell activation does not rely on MHC-dependent antigen presentation, and efficiently targets surface antigens, such as epidermal growth factor receptor variant III (EGFRvIII). D,) Viral therapies use both lytic and nonlytic viruses to kill tumor cells and induce an immune response. These viruses may directly kill tumors cells by lysis or encode enzymes that convert prodrugs into cytotoxic chemotherapies. Figures were created with BioRender.com.

patients.[28] More than 70% of DIPG patients harbor the H3.3K27M mutation in histone 3 variant 3; the H3.3K27M mutation serves as a negative prognostic marker because patients with the mutation have decreased survival outcomes.[29,30] The use of Poly-ICLC adjuvant along with synthetic H3.3K27M as a peptide-based vaccine may increase antigen presentation against the H3.3K27M epitope and facilitate cytotoxic T-cell infiltration. Both IDHR132H and H3.3K27M mutations are 2 very early mutations in glioma development and are reported to be present uniformly throughout the tumor tissue, making them attractive therapeutic targets with low to minimum risk of antigen-loss escape, unlike EGFRvIII-targeting vaccines discussed earlier.[27,29,31]

In addition to approaches that target a single peptide, vaccines that target multiple peptides are also being developed and tested. For example, IMA-950 is a peptide vaccine that combines 11 common GBM-derived antigens. The vaccine is composed of 9 CD8 + T-cell antigens and 2 CD4 + T-cell antigens. The 11 peptides are immunogenic and are targeted toward increased cytotoxic T-cell and helper T-cell function in GBM patients. In a phase 1 trial, IMA-950 plus granulocyte-macrophage colony stimulating factor (GM-CSF) was given with standard therapy (radiation therapy [RT] + TMZ). Ninety percent of patients were classified as responders, with 50% responding to multiple peptides. Responders produced specific peripheral CD8 + T-cell responses to the tumor-associated antigens. Interestingly, steroid administration did not affect immune response to the IMA-950 antigens.[32] Results of the phase 1/2 trial (NCT01920191) were published in 2019 and reported safety of the IMA-950 vaccine paired with the Poly-ICLC adjuvant. Patients exhibited T-cell responses both to single and multiple peptides; median survival was reported at 19 months.[33] Two current trials using the IMA-950 vaccine are ongoing: in conjunction with pembrolizumab for relapsing GBM (NCT03665545) and in conjunction with varlilumab for grade II low-grade gliomas (NCT02924038). Because of patients' relatively intact immune system, vaccine-specific responses in patients with low-grade glioma have been extremely promising.[34] Slow growth of their tumors (ie, low-grade glioma) also allows for repeated vaccinations, which may be necessary to mount high levels of vaccine-reactive T responses. As such, vaccine approaches may be particularly suitable for patients with World Health Organization grade II low-grade glioma.

Another class of GBM vaccine therapies encompass personalized vaccine approaches that target neoantigens. The GAPVAC 101 phase 1 trial (NCT02149225) exploited both unmutated antigens

Table 1 Completed and Ongoing Clinical Trials for Glioblastoma Immunotherapy

Vaccines					
Clinical Trial Name	**Description**	**No. of Participants**	**Primary Outcome Measure**	**Phase**	**Study Completed**
NCT01480479 (ACT IV)	Rindopepimut (CDX-110) +TMZ; newly diagnosed EGFRvIII GBM patients	745	Overall survival	3	Yes
NCT02193347 (RESIST)	IDH peptide vaccine (PEPIDH1M) +TMZ; recurrent grade II glioma patients	24	% patients with unacceptable (grade 3) toxicity	1	No
NCT02960230	H3.3K27M peptide vaccine; children with newly diagnosed DIPG/other gliomas	29	% patients with AEs; overall survival at 12 mo	1	No
NCT01920191 (IMA-950)	IMA950 Multipeptide vaccine + poly-ICLC; newly diagnosed GBM patients	19	Tolerability and safety	2/3	Yes
NCT02924038	Varlilumab (CDX-1127) + IMA950/poly-ICLC; low-grade glioma patients	30	AEs; CD4 + and CD8 +T-cell responses	1	No
NCT02149225 (GAPVAC)	APVAC 1 and 2 + GM-CSF + poly-iCLC +TMZ; newly diagnosed GBM patients	16	AEs	1	Yes
NCT02287428 (NeoVax)	Neoantigen cancer vaccine (NeoVax) + RT + pembrolizumab MGMT unmethylated; newly diagnosed GBM patients	46	AEs; No. of patients with actionable peptides; No. of patients able to receive post-RT vaccine therapy	1	No
NCT01280552 (ICT-107)	Randomized, double-blind, controlled study of ICT-107 with maintenance TMZ; newly diagnosed GBM following resection	124	Overall survival	2	Yes
NCT00045968 (DCVax-L)	Randomized, double-blind, controlled study of DCVax-L; newly diagnosed GBM following resection	348	PFS	3	Yes
NCT03018288 (HSPPC-96)	Randomized, double-blind study of RT +TMZ and pembrolizumab with and without HSPPC-96; newly diagnosed GBM	108	1-y overall survival	2	No
Checkpoint Inhibitors					
Clinical Trial Name	**Description**	**No. of Participants**	**Primary Outcome Measure**	**Phase**	**Study Completed**
NCT02017717 (CheckMate 143)	Nivolumab alone nivolumab + ipilimumab comparator: bevacizumab; recurrent GBM patients	626	Safety and tolerability: Overall survival	3	Yes
NCT02617589 (CheckMate 498)	Nivolumab + RT comparator: TMZ + RT MGMT unmethylated; newly diagnosed GBM patients	550	Overall survival	3	No
NCT02667587 (CheckMate 548)	Nivolumab +TMZ + RT comparator: TMZ + RT MGMT unmethylated; newly diagnosed GBM patients	693	PFS; overall survival	3	No
NCT02337491	Pembrolizumab alone pembrolizumab + bevacizumab; recurrent GBM patients	80	Pembrolizumab maximum tolerated dose; pembrolizumab dose-limiting toxicity at 6 mo. PFS	2	Yes
NCT02313272	Hypofractionated stereotactic irradiation + pemobrolizumab + bevacizumab; recurrent HGG patients	32	Pembrolizumab maximum tolerated dose	1	No
CAR T-cell therapy					
Clinical Trial Name	**Description**	**No. of Participants**	**Primary Outcome Measure**	**Phase**	**Study Completed**
NCT02208362	IL13Rα2-specific CAR T cells; refractory or recurrent malignant glioma patients	92	AEs (grade 3 or higher); dose-limiting toxicity; incidence of toxicities (including neurological);	1	No
NCT02209376	EGFRvIII CAR T cells; EGFRvIII GBM patients	11	No. of AEs (2 y)	1	Yes

Table 1 Continued

NCT01109095	HER2 virus-specific CART cells	16	Dose-limiting toxicity after T-cell infusion	1	Yes
NCT02442297	HER2 CART cells	28	Dose-limiting toxicity after T-cell infusion	1	No
Oncolytic Viral therapy					
Clinical Trial Name	**Description**	**No. of Participants**	**Primary Outcome Measure**	**Phase**	**Study Completed**
NCT00805376 (DNX-2401)	DNX-2401 (conditionally replication-competent adenovirus) ± surgery; recurrent HGG patients	37	Maximum tolerated dose	1	Yes
NCT03896568 (Ad5-DNX-2401)	Ad5-DNX-2401 (oncolytic adenovirus) in bone marrow human mesenchymal stem cells; recurrent HGG patients	36	Maximum tolerated dose Incidence adverse events	1	No
NCT01491893 (PVSRIPO)	Recombinant nonpathogenic polio-rhinovirus chimera (PVSRIPO); recurrent GBM patients	61	Maximum tolerated dose; dose-limiting toxicities; recommended phase 2 dose	1	No
NCT02414165 (Toca 5)	Toca 511 (retroviral replicating vector encoding cytosine deaminase) +Toca FC (flucytosine) comparator: lomustine, TMZ, bevacizumab; recurrent HGG patients	403	Overall survival	2/3	Yes
NCT02457845 (G207)	HSV G207 (first-generation oncolytic HSV-1) + RT; children with recurrent supratentorial brain tumors	18	Safety and tolerability (grade 3 or higher AEs)	1	Yes
NCT03152318 (rQNestin)	rQNestin34.5v0.2 (oncolytic HSV-1) + cyclophosphamide; recurrent malignant glioma patients	108	Maximum tolerated dose	1	No
NCT00390299	MV-CEA (carcinoembryonic antigen-expressing measles virus); recurrent GBM patients	23	Maximum tolerated dose; no. and severity of all AEs; no. and severity of grade 3 + AEs; overall toxicity	1	Yes
NCT01301430 (ParvOryx01)	H-1PV; progressive primary or recurrent GBM patients	18	Safety and tolerability	1/2a	Yes
NCT03714334 (DNX-2440)	DNX-2440 conditionally replication-competent adenovirus armed with OX40 ligand (T-cell stimulator); recurrent GBM patients	24	Treatment-emergent AEs	1	No
NCT02062827 (M032-HSV-1)	M032-HSV-1 (second-generation oncolytic HSV) armed with IL-12 (immune-stimulatory); recurrent progressive/GBM patients	36	Maximum tolerated dose	1	No
Combination Therapies					
Clinical Trial Name	**Description**	**No. of Participants**	**Primary Outcome Measure**	**Phase**	**Study Completed**
NCT03726515	CART-EGFRvIII + pembrolizumab; newly diagnosed EGFRvIII MGMT-unmethylated GBM patients	7	No. of patients with treatment-related AEs	1	No
NCT03422094	NeoVax + ipilumumab/nivolumab; newly diagnosed unmethylated GBM	30	Safety and tolerability; identification of tumor-specific neoantigens	1	No
NCT02798406 (CAPTIVE)	DNX-2401 + pembrolizumab; recurrent GBM patients	49	Objective response rate (tumor size reduction)	2	No
NCT03665545 (IMA950-106)	Pembrolizumab + IMA950/poly-ICLC; relasping GBM	24	Treatment-emergent AEs	2/3	No

All information taken from www.clinicaltrials.gov. Information current as of February 9, 2020.
Abbreviations: AEs, adverse events; DIPG, diffuse intrinsic pontine glioma; EGFRvIII, epidermal growth factor receptor variant III; GBM, glioblastoma; H-1PV, H-1 parvovirus; HGG, high-grade glioma; HSPPC-96, heat-shock protein peptide complex-96; HSV, herpes simplex virus; IDH, isocitrate dehydrogenase; MGMT, O6-methylguanine-DNA methyltransferase; PFS, progression-free survival; RT, radiation therapy; TMZ, temozolomide.

(APVAC1 arm) and neoantigens (APVAC2 arm) to individualized patient-specific vaccines. APVAC2 vaccines were chosen from transcriptomes and immunopeptidomes of patient tumor samples. Poly-ICLC and GM-CSF were used as adjuvants. The vaccines were injected intradermally during TMZ maintenance therapy. The study found that APVAC1 peptides were immunogenic and resulted in specific effector and memory T cells. Additionally, APVAC2-vaccinated neoepitopes resulted in a CD4 + T-cell response, but no CD8 + T-cell activation.[35] Another phase 1/1b trial (NCT02287428) used a personalized neoantigen vaccine in conjunction with RT and pembrolizumab for newly diagnosed GBM patients. In contrast, patients who did not receive dexamethasone during vaccine priming had strong immune responses against neoantigens of interest and increased CD4 + and CD8 + T-cell infiltration. In contrast, patients who received dexamethasone did not generate an IFN immune-stimulating response. Median progression-free survival was 7.6 months and median overall survival was 16.8 months.[36] Although these personalized vaccine approaches may address interpatient heterogeneity and may induce truly tumor-specific (ie, neoantigen-specific) immune responses, the magnitude of vaccine-specific T-cell responses appears relatively low[35] compared to the levels that can be achieved by adoptive-cell transfer approaches. Further engineering and combination with potent immunoadjuvants may be necessary to improve the potency of vaccine approaches.

A phase 2 (NCT 01280552), double-blind, placebo-controlled trial for the DC vaccine ICT-107 was also conducted. The study used autologous DCs that target 6 GBM tumor-associated antigens. The trial resulted in significantly increased progression-free survival in the treatment group with a marked immunological response in a subset (HLA-A2) of patients.[37] A phase 3 trial was begun but suspended in 2017 because of inadequate funding.

DCVax-L, another DC-based vaccine, was evaluated in a phase 3, randomized, double-blind, placebo-controlled trial. DCVax-L uses autologous DCs pulsed with tumor lysate. Patients received standard-of-care surgery and radiotherapy with concurrent TMZ. Following standard-of-care treatment, patients were randomly assigned to receive TMZ plus DCVax-L or TMZ plus placebo. At recurrence, all patients were eligible to receive DCVax-L without unblinding. Median overall survival was 23.1 months, with a subgroup of patients (n = 100) exhibiting a 40.5-month median overall survival.[38]

Heat-shock protein peptide complex-96 (HSPPC-96) is another vaccine-based approach. HSPPC-96 contains tumor-derived glycoprotein-96 and triggers both innate and adaptive immune responses. HSPs bind tumor-associated antigens and can be taken up by antigen presenting cells. HSPs, with HSPPC-96 most common in glioma, are a promising target for innate immune activation. Patient-derived HSP complexes are purified and directly injected as a personalized vaccine. [39]In a phase 1 trial (NCT02122822), HSPPC-96 was deemed safe for GBM patients. A randomized, double-blind, placebo-controlled phase 2 trial (NCT03018288) is currently ongoing evaluating radiotherapy plus TMZ and pembrolizumab with or without HSPPC-96.

Immune-Checkpoint Inhibitors

Immune-checkpoint inhibitors, defined as antibodies that block pathways reducing antitumor T-cell activation, have had success in multiple cancer types, including melanoma and non–small cell lung cancer.[40–42]The most successful inhibitors have been aimed at immune-checkpoint inhibitor proteins programmed cell death protein 1 (PD1), PD-L1, and CTLA-4 (Fig. 1B;Table 1). PD1 and CTLA-4 are expressed on T cells, and act as immunomodulatory checkpoints to promote self-tolerance and reduce aberrant T-cell–mediated inflammation. [42]CTLA-4 can suppress naive and memory T-cell activation by blocking signaling of costimulatory molecules. [43]In healthy brain tissue, PD-L1 is expressed by a subset of immune cells, including macrophages and microglia, and its expression is further upregulated in the GBM microenvironment.[44,45] Additionally, PD-L1 has been shown to be expressed by antigen-presenting cells, tumor cells, and parenchymal cells in GBM patients.[44,46,47] Interaction of PD1 and PD-L1 leads to reduced proliferation of antigen-specific effector T cells and reduced apoptosis of T-regulatory cells, leading to a dampened adaptive immune response.[48]

Preclinical animal models of anti-PD1 therapy showed promising results in an orthotopically implanted GL-261 GBM model.[49,50] However, the GL261 model has a higher mutational load than human GBM samples. Mutational load has been conjectured to play a large role in the success of immune-checkpoint inhibitors. Successes in melanoma and non–small cell lung cancer are strongly correlated with the high mutational loads of these tumor types.[14] SB28, another GBM model, has been shown to more accurately reflect human GBM tumors because it has fewer mutations and low MHC class I expression and CD8 + T cell infiltration.[51] A study by Genoud et al found that combination anti-PD1/anti-CTLA-4 checkpoint-inhibitor therapy was curative in more than 50% of mice with GL261 tumors but had no impact on SB28 tumors.[51] The disparity in mutational load between patient-derived tumors, as reflected by the SB28 and GL261 models, may help to explain the outcomes of immune-checkpoint inhibitors in clinical trials.

Nivolumab, an anti-PD-1 antibody, has been used most widely in clinical trials.[52] The phase 3 clinical trial CheckMate 143 (NCT02017717) was one of the first large trials to evaluate the effectiveness of anti–PD-1 antibodies in GBM. The trial compared bevacizumab (a VEGF inhibitor) to either nivolumab monotherapy or nivolumab/ipilimumab (anti–CTLA-4 antibody) combination therapy in recurrent GBM patients.[53] As of 2017, nivolumab alone did not result in prolonged overall survival compared to bevacizumab; both arms of the trial resulted in a 42% 12-month overall survival. The trial resulted in significantly higher adverse effects in patients receiving the combination therapy. This arm of the trial (combination therapy) was discontinued and PD-1 monotherapy was continued.[53]

Standard GBM treatments, such as TMZ and RT, further compound tumor-mediated immunosuppression by causing widespread lymphopenia, with a dramatic reduction in CD4 + T cells.[54] Lymphopenia compounded with poor T-cell infiltration may also contribute to poor responses to immune-checkpoint inhibitor therapy. Further,

the efficiency of targeting biologics, such as antibodies, to the brain through the blood-brain barrier is another likely confounding factor in the failure of these therapies in GBM. Another phase 3 trial (NCT02617589), CheckMate 498, evaluated nivolumab plus RT and TMZ plus RT in O6-methylguanine-DNA methyltransferase (MGMT)-unmethylated newly diagnosed GBM patients. The trial did not meet its primary end point of overall survival.[55] A subsequent and ongoing phase 3 trial, CheckMate 548 (NCT02667587), is comparing nivolumab plus RT with TMZ plus radiotherapy in patients with MGMT-methylated tumors. The study did not show statistically significant improvement in progression-free survival and therefore missed one of its primary end points; overall survival assessment is ongoing.[56]

In a 2019 study by Zhao et al, authors investigated factors that lead to positive or negative response to anti–PD-1 therapy in recurrent GBM patients. They analyzed whether response to anti–PD-1 (pembrolizumab and nivolumab) therapy is associated with certain patient biomarkers and immune expression signatures. The authors profiled 66 patients and over time and collected DNA, RNA, and performed tissue imaging. This information was paired with clinical outcomes to determine a trend of immunotherapy response. Patients who responded to anti–PD-1 treatment had significant enrichment of MAPK pathway members, such as BRAF and PTPN11. Nonresponders had an enrichment of PTEN mutations resulting in immunosuppressive gene signatures.[57]

Another 2019 study by Cloughesy and colleagues showed that patients who received neoadjuvant pembrolizumab and continued adjuvant therapy after surgery had improved overall survival compared with patients who received adjuvant pembrolizumab alone. The authors concluded that neoadjuvant anti–PD-1 therapy results in increased infiltration of lymphocytes into the tumor, and this is a major factor contributing to improved overall survival. Interestingly, presurgical and postsurgical tumor volume and dexamethasone administration were not identified as factors affecting patient outcome.[58]

Owing to their promising results in other tumor types, checkpoint blockades remain a viable target for treating GBM. Biomarker screening of patients and selective use of checkpoint blockades may result in durable responses for a subset of patients. Combination therapies pairing checkpoint blockades with bevacizumab (NCT02337491) or laser ablation (NCT02313272) are being evaluated.

Chimeric Antigen Receptor T Cells

Chimeric antigen receptor (CAR)-based therapies rely on genetically modified T cells that express CARs engineered to recognize cancer-associated cell surface antigens[59] (Fig 1C; Table 1). They have been previously shown to be efficacious in lymphoma and acute leukemia patients.[59,60] The CAR is a hybrid of an antigen recognition domain, generally derived from a single-chain antibody, fused to the T-cell activation domain (CD3ζ); the addition of one or more costimulatory receptor intracellular domains (4-1BB, CD28, OX40) is common to increase T-cell activation and response.[61] Interestingly, CAR T cells are activated by direct binding to the target antigen but independently of antigen presentation by the MHC, which is responsible for processing and presenting internal cellular antigens. Because CAR T cells do not rely on MHC presentation, they can recognize cell surface antigens and are useful in targeting specific tumor antigens that may not be efficiently presented in the context of a particular patient's MHC repertoire.[59,62]

A promising target for CAR T therapy is IL-13Rα2. It is overexpressed in approximately 75% of GBM tumors and is linked to increased tumor invasiveness.[63] In a clinical trial (NCT02208362) of CAR T-cell therapy targeting IL-13Rα2, there was a dramatic response (~ 80% average tumor shrinkage of all 7 lesions) in 1 patient receiving intratumoral and intraventricular infusion of these CAR T cells. The patient had no detectable lesions or spine metastases for 7.5 months following CAR treatment. However, 4 new lesions became detectable at 228 days after the first CAR T-cell infusion. The emergence of new lesions was likely due to the outgrowth of cells not expressing surface IL-13Rα2.[64]

Another clinical trial (NCT02209376) employed a single intravenous infusion of autologous T cells engineered to express a CAR against EGFRvIII mutation in 10 recurrent patients with EGFRvIII + GBM.[34] The EGFRvIII mutation results in deletion of exons 2 through 7 and creates an immunogenic GBM-specific antigen.[65] The single dose of CAR T cells against EGFRvIII was delivered intravenously and did not result in cytokine release syndrome (CRS). Systemic CRS occurs when immune cells (B cells, T cells, DCs, and macrophages) become activated and release inflammatory cytokines, resulting in further immune activation. CRS may be life-threatening and is a major concern in adoptive T-cell therapies.[66,67] All patients in this study demonstrated detectable transient expansion of CART-EGFRvIII cells in peripheral blood. In 5 of 7 patients who underwent tumor resection post–CAR T-cell infusion, trafficking of CAR T cells to tumor sites and reduction of EGFRvIII expression levels were noted. As reported, CAR T-cell infiltration was associated with robust induction of inhibitory molecules, such as IDO1, PD-L1, and FoxP3, and infiltration by regulatory T cells.[68] Similar to results seen in the IL-13Rα2 CAR T study, [64]the phase 1 EGFRvIII-targeted trial revealed a significantly decreased expression of the CAR-targeted antigen expression in recurrent tumor cells. These results suggest immunoediting of the tumor and outgrowth of tumor cells without EGFRvIII expression.

A third potential target for CAR T-cell therapy is human epidermal growth factor receptor 2 (HER2). HER2 is a receptor tyrosine kinase that is overexpressed in some GBM tumors and other cancer types, making it a possible CAR target. Two phase 1 trials have targeted HER2 (NCT02442297, NCT01109095). In the completed study (NCT01109095), investigators used virus-specific (CMV, Epstein-Barr, or adenovirus) T cells to express the CAR construct. This allows for enhanced immune activation by presentation of latent viral antigens. Although the trial was deemed safe, expansion of HER2-CAR T cells did not occur in the blood.[69] In the currently ongoing trial (NCT02442297), non–virus-specific autologous T cells expressing the HER2 CAR are being tested.

These data imply that development of successful CAR T therapy for GBM will require further engineering and/or integration of strategies to improve CAR T homing and persistence in the GBM tissue, overcome local immunosuppression, and address marked antigenic heterogeneity of GBM.

Oncolytic Viral Therapy

The use of viral vectors for gene therapy of GBM has been evaluated for more than 3 decades[70,71] (Fig 1D; Table 1). However, the failure of most clinical trials to show therapeutic efficacy using conventional replication-defective viral vectors has resulted in investigation into tumor-selectively replicating viruses, or oncolytic viruses.[72] Oncolytic viruses are able to selectively replicate in and kill cancer cells. Cancer cells, including CNS tumors, have defects in innate cellular immune defenses that allow for replication, production of viral proteins, and budding or lysis.[73] Viruses used as oncolytic agents are commonly attenuated or contain deletions in virulence factors, blocking their ability to infect and spread through healthy tissues.[73] Therefore, oncolytic viruses may preferentially infect tumor cells, which commonly have defects in interferon signaling, which activates antiviral defense pathways that normally block viral replication.[74]

However, replication-competent oncolytic viruses, as well as conventional replication-defective viral vectors, both can result in activation of the adaptive immune system. Through activation of toll-like receptor and pathogen-associated molecular pattern sensors, viral infection can stimulate DCs to produce type I IFNs that result in a proinflammatory immune response. [74,75]IFN upregulation causes production of the cytokines CXCL9, CXCL10, and CXCL11, which are regulators of T-cell trafficking and infiltration.[76,77] In addition to cytokine signaling, viral-induced cell lysis can create physical space for T-cell infiltration by disrupting tissue architecture and extracellular matrix, and causes the release of endogenous tumor antigens.[78]

Several viruses, including adenovirus, measles virus (MV), polio virus, parvovirus, HSV, and retroviral replicating vectors (RRVs) have been engineered to treat GBM.[79,80]

A phase 1 clinical trial (NCT00805376) using the replication-competent oncolytic adenovirus DNX-2401 was conducted in patients with recurrent glioma.[81] The DNX-2401 virus contains a deletion in the *E1A* gene, which inhibits viral replication in nonmalignant cells with a functional retinoblastoma pathway. This virus has been shown to cause glioma cell death and enhanced antitumor immunity in preclinical models.[82,83] In the clinical trial, patients received either (A) a single intratumoral injection of the virus via an implanted catheter or (B) an initial intratumoral injection followed by tumor resection and subsequent further viral injections into the walls of the resection cavity. Viral replication was observed in the tumors of group B patients. Patients in group A showed reduction in tumor size, and 20% of patients survived more than 3 years. Tumor necrosis and CD8 + T-cell infiltration were detected, but no change in the immunosuppressive molecules PD-1, PD-L1, and IDO-1H were detected. A current phase 1 clinical trial (NCT03896568) in recurrent GBM is evaluating safety of allogeneic bone marrow-derived human mesenchymal stem cells with the DNX-2401 virus.

A recent clinical trial (NCT01491893) using recombinant nonpathogenic polio-rhinovirus chimera (PVSRIPO) showed the safety and encouraging preliminary efficacy of the virus in recurrent GBM patients.[84] PVSRIPO recognizes CD155, a poliovirus receptor often expressed on GBM cells, and is attenuated by being engineered with a different internal ribosome entry site sequence from rhinovirus. Its tumor selectivity is due to the lack of innate intracellular immunity in GBM cells. The trial resulted in increased survival in patients at 24 and 36 months compared to historical controls.[59]

Another approach, combining virotherapy and gene therapy, uses a nonlytic RRV. The virus, Toca 511 (vocimagene amiretrorepvec), integrates into the host genome and spreads efficiently through rapidly proliferating cells, and encodes yeast cytosine deaminase as a prodrug activator, which converts the prodrug 5-fluorocytosine (Toca FC) into the potent chemotherapeutic drug 5-fluorouracil.[83,85] As the virus integrates permanently into the cancer cell genome, oral administration of the prodrug can achieve tumor-cell killing over multiple cycles. Preclinical studies showed prolonged survival, intratumoral infiltration of T cells, and a decrease in immunosuppressive myeloid cells after prodrug conversion.[86,87] Phase 1 clinical trials evaluating Toca 511 in recurrent high-grade glioma patients established the safety of the virus and persistence of RRV in the tumor, and promising evidence of therapeutic benefit.[88] In 2019, a phase 3 trial was completed in recurrent GBM patients undergoing resection. The trial missed its primary end point, but a patient subset analysis has yet to be published.[89]

HSV, MV, and parvovirus have also been used in oncolytic viral therapies. HSV-based therapies include HSV1716, G207 (NCT02457845), rQNestin 34.5 (NCT03152318), and G47Δ viruses.[90,91] Recently it has been reported that G47Δ was able to achieve a 1-year survival rate of 92% in recurrent GBM patients after repeated stereotactic intratumoral injections on rerecurrence.[92] An ongoing phase 1 trial using oncolytic MV (Carcinoembryonic Antigen-Expressing Measles Virus) is being assessed in recurrent GBM patients (NCT00390299). A phase 1/2a clinical trial using an oncolytic parvovirus (ParvOryx01) (NCT01301430) resulted in a proinflammatory response and tumor infiltration with activated CD8 + cytotoxic T cells.[93,94]

Encouraging survival data (ie, long tail in survival curves) suggest that oncolytic viruses may be truly effective in subpopulations of patients. Therefore, it is critically important to find solid biomarkers for selecting patient populations who are more likely to respond.

Future Directions and Combination Therapies

With the lack of success in immune monotherapies, it has become clear that combination therapies are a promising path forward. Because most of the current

immunotherapies target only one immunological mechanism, there is a clear need to develop coordinated approaches to attack the comprehensive aspects of immune mechanisms Table 1.

Vaccine-based immunotherapies may be a useful and promising treatment for gliomas, especially in combination with other therapies, such as adjuvant and/or checkpoint blockade therapy. The flexibility of a vaccine approach allows for the development both of patient-specific and broad, common-tumor, antigen-targeted therapies.

As a monotherapy, immune-checkpoint inhibitors have not resulted in improved survival outcomes in malignant gliomas, unlike some other tumor types. However, they remain an extremely viable target for combination therapies. For example, cytokine therapies to increase T-cell infiltration or myeloid-based approaches may result in increased efficacy of immune-checkpoint inhibitors. Further, it has been reported that EGFRvIII CAR T-cell therapy resulted in marked upregulation of PD-L1 in the tumor microenvironment; accordingly, combining adoptive T-cell therapy with an anti–PD-L1 antibody may show promise as a combination therapy.[68] This is currently being evaluated in a combination EGFRvIII CAR T and pembrolizmuab phase 1 clinical trial in newly diagnosed GBM patients (NCT03726515). Another ongoing pilot study is evaluating the safety and immunogenicity of NeoVax (a personalized neoantigen-based vaccine) plus ipilimumab or nivolumab (NCT03422094).

In this context, although CAR T cells present a promising opportunity for tumor-specific targeted therapy, antigen escape and intratumoral heterogeneity remain a hindrance. As with other monotherapies, CAR T-cell therapy would likely be enhanced by combinatory approaches to increase T-cell homing, persistence, and function as well as antigen spreading in the tumor.

Another promising strategy in the developing field of oncolytic virotherapy is arming these viruses with immunoregulatory genes to enhance their cytotoxic potency and immunostimulatory effects. For example, a phase 1 clinical trial is investigating the DNX-2440 virus, which is the DNX-2401 adenovirus armed with OX40 Ligand (NCT03714334).[95] As another example, an HSV-based virus, M032-HSV-1, is armed with IL-12 to further activate antitumor immunity and limit angiogenesis (NCT02062827).[96]

Combination therapies using immune-checkpoint inhibitors along with oncolytic viruses are also being investigated. For example, a current phase 2 trial is combining DNX-2401 and pembrolizumab (an anti–PD-1 checkpoint inhibitor) in recurrent GBM patients. Furthermore, oncolytic viruses are also being used to deliver checkpoint inhibitor agents for expression directly within the tumor, thereby mitigating the potential for systemic autoimmune adverse effects. Mitchell et al described an RRV that carries a single-chain variable fragment (scFv)–PD-L1. On infection with the virus, target cells release the scFv–PD-L1 that blocks PD-1 binding, resulting in a robust antitumor immune response in preclinical models.[97]

For the success of any immunotherapeutic strategy, a major goal is to increase antitumor T-cell activity and antigen presentation within the tumor, although the microheterogeneity of GBM presents a challenge because this predisposes to immunoediting and tumor recurrence. The profoundly immunosuppressive nature of these tumors must also be overcome or altered to evoke a sustained and targeted antitumor immunity.[98,99] Combination therapies to activate T cells and simultaneously eliminate or reprogram suppressive myeloid cells is a promising avenue of investigation. Inducing a coordinated antitumor response in T cells, DC, and macrophages is also being investigated.[100]

Conclusions

Many immunotherapeutic approaches have been evaluated in patients with GBM, including checkpoint inhibitors, CAR T cells, oncolytic viral therapy, and vaccines. The limited success of these therapies, in comparison with results obtained in other tumor types, presents a challenge for the field of cancer immunotherapy. The microheterogeneity of glioma cells even within the same tumor, the strong presence of immunosuppressive cells in the tumor microenvironment, and the paucity of infiltrating T cells may limit effective and durable antitumor responses. In addition, better homing and function of antitumor effector cells, as well as increased epitope spreading, would likely contribute to the effectiveness both of monotherapies and combination therapies for GBM tumors. Combinatorial approaches are being pursued and are likely to show improved therapeutic results in clinical trials.

It is important to recognize that some promising outcomes in early-phase, single-arm trials failed in subsequent pivotal trials with randomized design (eg, rhindopepimut, Toca511 as discussed earlier). Comparison with historical data for single-arm studies of immunotherapy has been known to have typical pitfalls, such as selection of eligible patients with low tumor burden and no or low corticosteroid use. Investigators should be aware of these limitations and incidences in the past single-arm trials, and should conduct appropriate comparative trials before declaring any treatment as anything more than promising.

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Conflict of interest statement.

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