REVIEW ARTICLE

Novel Immunotherapeutic Approaches for the Treatment of Glioblastoma

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Abstract

Glioblastoma is highly aggressive and remains difficult to treat despite being the most common malignant primary brain tumor in adults. Current standard-of-care treatment calls for maximum resection of the tumor mass followed by concurrent chemotherapy and radiotherapy and further adjuvant chemotherapy if necessary. Despite this regimen, prognosis remains grim. Immunotherapy has shown promising success in a variety of solid tumor types, but efficacy in glioblastoma is yet to be demonstrated. Barriers to the success of immunotherapy in glioblastoma include: a heterogeneous tumor cell population, a highly immunosuppressive microenvironment, and the blood–brain barrier, to name a few. Several immunotherapeutic approaches are actively being investigated and developed to overcome these limitations. In this review, we present different classes of immunotherapy targeting glioblastoma, their most recent results, and potential future directions.

1 Introduction

Isocitrate dehydrogenase (IDH) wild-type diffuse and astrocytic grade IV glioma or glioblastoma is an aggressive and ultimately lethal primary malignancy of the brain for which the current treatment paradigm has advanced little over the past 15 years. Current standard-of-care treatment protocols for newly diagnosed glioblastoma consist of maximal surgical resection followed by cycles of radiation and alkylating chemotherapy (temozolomide [TMZ]). Despite this, median survival is less than 15 months [1].

Key Points

Glioblastoma is a universally lethal brain tumor with an overall therapeutic failure.

Glioblastoma encompasses several subtypes with different immune characteristics making them differentially susceptible to available and developed therapies.

Immunotherapy is a promising therapeutic for glioblastoma and several approaches in this discipline are being developed and improved.

Combining different therapies can be an interesting route to control glioblastoma from different angles.

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Immunotherapy in the form of immune checkpoint inhibitors (ICIs) has achieved remarkable success in other aggressive cancers with previously poor prognoses such as melanoma and non-small cell lung cancer [2–4]. Unfortunately, similar results have been elusive in glioblastoma, with a multitude of failed trials [5, 6]. This reflects the multitude of challenges that limit immunotherapy efficacy, including the blood–brain barrier, intrinsic and acquired resistance to available therapies, antigen heterogeneity, and an immunosuppressive tumor microenvironment (TME). Efforts are ongoing to identify the optimal immunotherapy strategy that can overcome these challenges, using experiences from other difficult-to-treat malignancies [7]. This review outlines the current state of the glioblastoma immune landscape and immunotherapy, including each regimen's strengths and weaknesses, and describes future directions for each immunotherapeutic regimen [8].

2 Molecular Heterogeneity and Immune Landscape of Glioblastoma

Various factors have led to the central nervous system (CNS) being viewed as an immune-privileged compartment, such as the tight blood–brain barrier encircling the CNS parenchyma preventing the entry of plasma inflammatory proteins and immune cells from the periphery. Additionally, the lack of lymphatic vessels and the mild reactivity to xenografts and bacterial and viral-related proteins after non-traumatic micro-injections into the CNS parenchyma further support this notion [9–11]. Despite this immune exclusion, the CNS hosts a diverse immune cell population, including microglia (which make up approximately 80% of the CNS immune cellularity), embryonically derived microglia-like macrophages (10%), innate immune cells [monocyte-derived cells, dendritic cells (DCs), and neutrophils] (3%), and adaptive immune cells [T and B cells] (1%) [12]. These immune cells are able to elicit an immune response similar to that observed peripherally.

In 2009, Verhaak et al. attempted to classify glioblastoma into subtypes based on their molecular signature, using 200 glioblastoma samples and two normal brain tissue samples. They proposed four subtypes: neural, proneural, mesenchymal, and classical. The neural subtype was clustered with normal brain tissue, while the other three were mainly defined by aberrations in PDGFRA/IDH1, NF1, and epidermal growth factor receptor (EGFR) respectively [13]. In 2016, the World Health Organization included molecular features in the diagnosis criteria of different adult gliomas, resulting in the division of glioblastoma (IDH wild-type), oligodendroglioma (IDHmut with 1p/19q co-deletion), and astrocytoma (IDHmut without

codeletion) [14]. The recent 2021 World Health Organization classification maintained this general subdivision, with different astrocytic bifurcations grouped under a single diagnosis (astrocytoma with different grades 2, 3, or 4). Additionally, TERT promoter mutation, EGFR gene amplification, and chromosome 7/10 gain and deletion [+7/− 10] were included in the diagnosis of glioblastoma [15].

The immune landscape of glioblastoma is known to vary depending on the molecular subtype of the tumor [16, 17]. An increase in the gene signature of immune suppressive factors, chemokines, and signaling pathways was reported in mesenchymal glioblastoma compared with other subtypes (including interleukin (IL)-10, IL-23, transforming growth factor beta, programmed-death ligand 1 [PD-L1], CCL2, CCL22, and IL-6/STAT3) [16, 17]. These gene signatures are associated with an immunosuppressive phenotype of monocytes and macrophages [17]. CIBERSORT analysis reported that the mesenchymal subtype had a higher expression signature of tumor-associated macrophages, neutrophils, and CD4+ T cells, while the classical subtype exhibited an activated DC signature [16, 18]. In contrast, the proneural subset was found to be the least immunogenic, with a lower pool of B cells, CD3+ T cells, and CD163+ macrophages in the TME [19]. This heterogeneous immune landscape among the different glioblastoma subtypes should influence the choice of immunotherapy and may impact treatment outcomes. For instance, the proneural subtype is less likely to respond well to immunotherapy because of its cold TME, and has proven to be indifferent to aggressive chemotherapy and radiotherapy regimens.

3 Vaccine Therapies

3.1 Peptide Vaccine Trials

Peptide vaccines can be used to guide the immune system to neoplastic cells harboring peptide-corresponding tumor-specific antigens (TSAs) or tumor-associated antigens (TAAs) [20]. For TSA-derived peptide vaccines, epitope peptide-bearing mutations exclusively present in the tumor site are recognized by CD8+ and CD4+ T cells in a major histocompatibility complex 1 and 2 (MHC I and MHC II)-dependent mechanism. The same mechanism is applied for the TAA vaccine with a higher likelihood of systemic immune response because the antigen expression is not limited to tumor tissue [20] (Fig 1).

However, glioblastoma frequently exhibits a low tumor mutational burden, and lacks a homogenously expressed TSA that can be used as a target [21]. Peptide vaccines
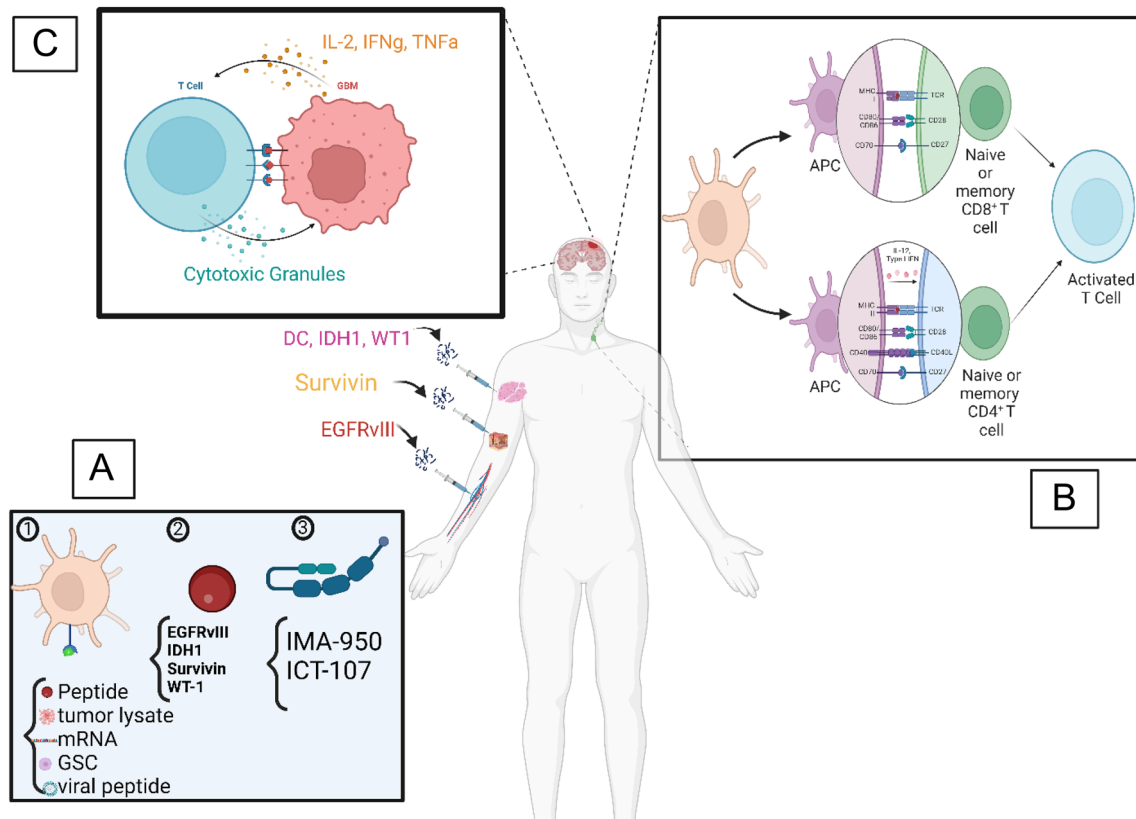
Fig. 1 Glioblastoma vaccine mechanism of action: **A** different peptide vaccine groups developed: (1) dendritic cell (DC) vaccine loaded with different tumor-specific antigens/tumor-associated antigens; (2) peptide vaccines (epidermal growth factor receptor vIII [EGFRvIII], isocitrate dehydrogenase 1 [IDH1], Survivin, William's tumor gene 1 [WT1]); (3) polypeptide vaccine. Pink-colored vaccines are injected intra-muscularly, Survivin intra-dermally, and EGFRvIII intrave- nously; **B** antigen presentation to naïve or memory T cells in the lymph nodes directly (DC vaccines) or after peptide processing (peptide vaccines); and (**C**) specific recognition of the target epitope of tumor cells by the cytotoxic CD8+ T cells and T-cell-mediated tumor killing. *APC* antigen-presenting cell, *GSC* glioma cancer stem cells, *IFN* interferon, *IL* interleukin, *mRNA* messenger RNA, *TNF* tumor necrosis factor. Figure designed on Biorender

that have been investigated in glioblastoma have variously targeted EGFRvIII (an EGFR mutant), isocitrate dehydrogenase 1 (IDH1), Survivin, and William's tumor gene 1 (WT-1). However, no peptide vaccine is currently approved for the treatment of glioblastoma although studies remain ongoing. The failure of rindopepimut, a vaccine targeting EGFRvIII in phase III trials (NCT01480479), likely reflects the limitations of a treatment approach that targets a single TSA. While specific, this target is only expressed at varying degrees in a subset of patients with glioma [22]. Indeed, targeting EGFRvIII may only result in selective targeting of antigen-positive cells, with subsequent outgrowth by antigen-negative cells [23]. Other vaccine-based approaches have explored targeting IDH1, which is much more ubiquitously expressed in high-grade gliomas that have transformed from lower grades [24]. The arginine residue mutation R132H is the most frequent IDH1 mutation; tumorigenesis derives from the generation of oncometabolite 2-hydroxyglutarate, which increases genomic hyper-methylation and malignant transformation [20].

Preclinically, Schumacher et al. showed an anti-tumoral response in a transgenic mouse model bearing a mutant peptide derived from IDH1-R132H on a humanized MHC I and MHC II [25]. In a phase I safety trial, the injection of patients with R132H-positive glioma with an IDH1 peptide vaccine resulted in an enhanced immune response in 93.3% of patients bearing variable MHC alleles, along with 3-year progression-free survival (PFS) and overall survival (OS) rates of 0.63 and 0.84, respectively. However, immune responses were restricted to MHC-II, with no CD8 immune response being elicited. This was consistent with previously published results reporting a restricted CD4 antitumoral response to IDH1-R132H [25–27]. Further mechanistic studies are needed to elucidate this MHC-II restricted response. In this trial, vaccine-related side effects were mild and limited to grade 1 [28]. Although this approach may be suitable for patients with high-grade gliomas that have transformed, this does not address those patients with IDHwt high-grade glioma.

Other peptide vaccine approaches have targeted Survivin and WT-1. Survivin is a member of a protein family acting on apoptosis inhibition and regulation of mitosis; it has been found to be upregulated in multiple cancer types, including glioblastoma [29–31]. Early Survivin peptide vaccine trials were found to promote ex-vivo human glioblastoma cell elimination via T-cell dependent cytotoxicity [32]. Combined administration of Survivin vaccine and granulocyte-macrophage colony-stimulating factor promoted survival and decreased tumor mass and progression in GL261 glioma mice [20, 33]. However, unlike human glioblastoma, GL261 is highly immunogenic [34]. Several trials were conducted based on these preclinical findings, including a concluded phase I trial (PFS and OS of 17.6 and 86.6, respectively) [32] (ClinicalTrials.gov identifier: NCT01250470) and an actively recruiting phase II trial of SVN53-67/M57-KLH and TMZ (NCT02455557). WT-1 and its downstream molecules modulate both proliferation and migration as well as cell fate decision and death. An alteration in the WT-1 signaling pathway has been reported in multiple cancers and studies have confirmed WT-1 expression in gliomas [35–38]. A recent preclinical study demonstrated integration of high-affinity, WT-1-specific T-cell receptors using CRISPR gene editing improves antitumor T-cell function in glioblastoma both in vivo and in vitro [39]. These findings have been used to support the development of WT-1 protein as a TAA that can be targeted by peptide vaccine therapy. In a phase I study, PFS of 15.2–49.1 months was observed in seven patients with newly diagnosed glioblastoma injected with a mixture of TMZ and a WT-1-based peptide vaccine as part of a safety phase I clinical trial [40]. In another phase I/II uncontrolled study, 27 patients with World Health Organization 2016 grade III and IV gliomas were intradermally injected with a mixture of WT-1 peptide and inactivated whole cell pertussis vaccine once a week for at least 12 weeks. Progression-free survival and OS were 12.7 and 21.9 months, respectively [41]. These results may attract more attention to the inclusion of WT-1-based peptides in glioma immunotherapy vaccines.

Several clinical trials involving peptide vaccines are currently ongoing or set to begin soon. These trials are exploring various combinations of conjugated peptides, either alone or in combination with other therapies. For example, there is a phase I clinical study (NCT04808245) that aims to evaluate the safety and immunogenicity of a H3K27M peptide vaccine for H3-mutated gliomas. Another trial (NCT04280848) is conducting an immunogenicity assessment study using telomerase-derived universal cancer peptides for glioblastoma, with an expected enrollment of 56 participants. Additionally, a phase Ib trial (NCT05283109) is studying the effects of P30-linked EphA2, CMV pp65, and Survivin vaccination (P30-EPS vaccine) in HLA-A*0201-positive patients with newly diagnosed unmethylated glioblastoma. The results from these trials are highly anticipated.

3.2 DC-Based Vaccine Trials

CD4+ and CD8+ T cells typically recognize peptides presented by DCs in an MHC-dependent manner after phagocytosis and intracellular processing to generate a peptide-specific immune response. During the dual dialogue, several proinflammatory cytokines are released by DCs, improving anti-tumoral T-cell efficacy [42]. Protocols for autologous DC vaccines call for cells to be extracted from the patient, cultured, and loaded with TSA/TAA ex vivo before administration to the patient to mediate a T-cell response [20]. Success in preclinical and clinical studies motivated investigators to load DCs with different molecules to identify those with the most robust glioblastoma-specific response: peptides, tumor lysates, messenger RNA (mRNA), viruses, and cancer stem cells. We summarize the work conducted to date with these different DC-based approaches. The first class of DC vaccines are tumor peptide-loaded DCs. Dendritic cell activation can be mediated through the same epitopes used in peptide vaccines, including EGFRvIII. A phase I study injected EGFRvIII-specific peptide-KLH pulsed DCs into 12 patients with newly diagnosed glioblastoma after surgical resection and radiotherapy [43]. Median PFS and OS were 6.8 and 18.7 months, respectively, and no life-threatening adverse reactions or toxicities were observed [43]. Another phase I trial injected WT-1-loaded DCs into seven patients with high-grade gliomas. Five patients exhibited stable clinical responses with OS after the first DC vaccine of 12.3 months [44, 45]. A third phase I trial showed OS of 20.6–47.3 months and PFS of 15.4–47.3 months in patients with newly diagnosed glioblastoma treated with CMV phosphoprotein 65 RNA-specific DCs [46]. Whereas the phosphoprotein 65 RNA-DC-induced CMV-specific T-cell expansion might be of higher functionality than ex vivo, the heterogenous CMV-peptide expression on tumor cells can limit the clinical benefits (12-month OS using autologous ex-vivo expanded CMV-specific T cells) [47].

A tumor lysate-loaded DC vaccine (DC-VaxL) has been tested as well. In this technique, patient tumor cells are broken down to generate cellular fragments that are eventually presented to DCs. This allows a larger tumor-specific response owing to the presentation of usually unrecognized TAAs, but it can also generate off-target immune reactions. Yu et al. presented impressive results in a phase I safety trial of autologous tumor lysate-based DC vaccines administered to patients with recurrent glioblastoma, reporting an OS of 133 weeks and a highly specific immune response [48]. The recently published results of the phase III clinical trial for DC-VaxL found that for patients with newly diagnosed glioblastoma, the median OS was 19.3 months when treated with

DC-VaxL compared with 16.5 months for patients in the control group who received temozolomide. Similarly, for patients with recurrent glioblastoma, the median OS was 13.2 months with DC-VaxL versus 7.8 months with temozolomide. Furthermore, the survival benefit of DC-VaxL over the control population increased over time in the tails of the survival curves. At 60 months, the survival rate was 13.0% for patients with newly diagnosed glioblastoma treated with DC-VaxL and 5.7% for those treated with temozolomide. For patients with recurrent glioblastoma, the survival rate was 11.1% at 30 months with DC-VaxL and 5.1% with temozolomide. This clinically meaningful extension of the survival of patients with newly diagnosed or recurrent glioblastoma may lead to the approval of DC-VaxL as the first immunotherapy treatment for glioblastoma in the near future.

Tumor-derived mRNA is a newly emerging class of molecules that can be loaded to DCs. Interest was generated into identifying the optimal mRNA antigen to present to DCs [49–51]. Lin et al. evaluated the expression profile of glioblastoma antigens using the gene expression profiling interactive analysis to determine their influence on clinical prognosis [51]. Patient survival rate and infiltration of antigen-presenting cells were associated with six overexpressed and mutated tumor antigens (ARHGAP9, ARHGAP30, CLEC7A, MAN2B1, ARPC1B, and PLB1) in glioblastoma [51]. These promising targets will be the foundation of several clinical trials (ClinicalTrials.gov identifiers: NCT02649582, NCT02709616).

Glioma stem cells were also loaded to DCs. Glioma stem cells are a cellular subtype known for self-renewal capability, resistance to chemoradiotherapies, and tumor recurrence [52–54]. Glioma stem cell elimination can allow a greater anti-tumor response. In a recently published preclinical study, Sy Do et al. used CD133 mRNA-loaded DCs targeting humanized mouse CD133-positive glioma stem cells, leading to a robust and long-lasting immune response along with inhibition of CD133-positive glioma stem cell propagation and tumor growth [55].

Finally, viral antigen-loaded DC cytomegalovirus nucleic acids and proteins are found in both primary and recurrent glioblastoma. Thus, pp65 was incorporated into DC vaccines as a potential novel immunotherapy [56]. A phase I trial evaluating pp65-specific DCs along with preconditioning with tetanus-diphtheria toxoid in patients with newly diagnosed glioblastoma showed an encouraging PFS of 15.4–47.3 months and an OS of 20.6–47.3 months [46]. A phase II randomized clinical trial in patients with glioblastoma who underwent resection and completed standard chemoradiation with CMV pp65-loaded autologous DC-tetanus-diphtheria toxoid pre-conditioning (ClinicalTrials.gov identifier: NCT02366728) has been completed and results are pending. Another randomized phase II trial involving a CMV pp65 DC vaccine is currently recruiting (ClinicalTrials.gov identifier: NCT02465268).

3.3 Early-Phase Development of Multi-Peptide Vaccines

Vaccine strategies using multiple fusion peptides have been developed to bypass the limitations of single-peptide vaccines. An example of limitation is the restriction to the HLA-A 02 haplotype and several glioblastoma subtypes, allowing only a small subgroup of patients with glioblastoma to benefit from targeting a single tumor antigen because of the heterogeneity of antigen expression in glioblastoma [20].

3.3.1 IMA950

IMA950 was developed from the fusion of 11 TAAs found in the majority of glioblastoma subtypes to activate multiple anti-tumor TUMAP-specific T-cell clones [20]. The TUMAP-derived poly-clonality increases the probability of a highly specific T-cell response against tumor cells, owing to the limited likelihood of poly-internalization of targeted antigens [20]. In a phase I trial, patients with glioblastoma who underwent tumor resection received an intradermal injection with IMA950 before or after starting chemoradiotherapy. The majority did not have remarkable survival results (PFS at 6 months of 74% and median OS of 15.3 months) [57].

3.3.2 ICT-107

ICT-107 is a glioblastoma-specific polypeptide vaccine [20]. Dendritic cells were loaded with six peptides selected based on a gene-overexpression comparison between glioblastoma cells and nonmalignant tissues: melanoma-associated antigen 1, HER2, interferon-inducible protein AIM2, I-dopachrome tautomerase, melanocyte protein (PMEL), and IL-13 receptor subunit-α2 (IL-13Rα2) [20]. In a phase I clinical trial, ICT-107 DC vaccines were administered to 15 patients with newly diagnosed glioblastoma. Median PFS was 16.9 months and median OS was 38.4 months. Six patients did not show any evidence of tumor recurrence after 40.1 months [58]. A randomized phase II trial in 124 patients with newly diagnosed glioblastoma (77 HLA A2-positive patients) reported encouraging results in the experimental group especially for HLA-A2-positive patients including a statistically significant elevation in the PFS by 2.2 months in the ICT-107 cohort with preservation of the quality of life in addition to a considerable therapeutic benefit with ICT-107 for MGMT methylated and unmethylated HLA-A2 patients [59]. The trial was as deemed to be underpowered because of insufficient participant numbers, which disabled

weighty conclusions from subgroup analyses [59]. Financial difficulties disrupted the phase III trial of this vaccine, and progress in the ICT-107 program has stalled (ClinicalTrials.gov identifier: NCT02546102).

3.3.3 Heat Shock Proteins

Heat shock proteins (HSPs) are a member of the intracellular stress regulating chaperone family, inhibiting protein agglomeration and guiding misfolded proteins to proteasome degradation [60]. HSP70 and HSP90 have been reported to stimulate both innate and adaptive responses in addition to binding to some tumor antigens in glioblastoma [61]. A phase I clinical study vaccinated 12 patients with high-grade glioma with HSPPC-96. A tumor-specific peripheral immune response was observed in 11 patients [62]. The phase II multicenter clinical trial for patients with resectable glioblastoma treated with HSPPC-96-bind antigens reported median and 6-month OS to be 42.6 weeks and 29.3%, respectively [63]. Multiple subsequent trials are assessing combinations with radiation therapy, temozolomide, and pembrolizumab (an immune checkpoint inhibitor) with and without HSPPC-96 (NCT03018288). NCT01814813 is assessing HSPPC-96 with bevacizumab in recurrent glioblastoma.

4 ICIs

Cancer cells utilize several mechanisms to escape immune surveillance and demolition. One example is the "cold" microenvironment that promotes a state of immune tolerance and expression of immune cell inhibitory receptors ("immune checkpoints") [64, 65]. Potential therapies could target these receptors using ICIs [66]. Promising results in many solid tumors have prompted study of ICIs such as anti-programmed cell death protein 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 in glioblastoma.

4.1 Inhibitors Targeting PD-1, PD-L1, and/or Cytotoxic T-Lymphocyte-Associated Protein 4

Downstream effectors of the PD-1/PD-L1 pathway have a suppressive role on tumoral T cells [67]. Programmed-death ligand 1 is upregulated in multiple types of solid tumors. Usually, higher expression of PD-L1 predicts better clinical efficacy of PD-1/PD-L1 checkpoint blockades [68]. Nduom et al. reported that tumor cells with a PD-L1 expression of 1% or more were observed in 61% of patients with glioblastoma and 38% had at least 5% or greater PD-L1 expression [69]. The results of a phase I clinical trial called KEYNOTE-001, which investigated the effectiveness of pembrolizumab (anti-PD1) in treating advanced stage non-small cell lung carcinoma, showed that patients with non-small cell lung carcinoma who had PD-L1 expression on more than 50% of cells had a response rate of 45.2%. Patients with lower levels of PD-L1 expression had lower response rates, with 16.5% for those with 1–49% expression and 16.5% for those with less than 1% expression [70, 71]. These findings led to the accelerated Food and Drug Administration approval of pembrolizumab for patients with non-small cell lung carcinoma with PD-L1 expression greater than 1% [72]. Nevertheless, despite high levels of expression of PD-L1 on both tumor cell and immunosuppressive immune cells in the TME, a good infiltration of proinflammatory immune cells is required to obtain a clinical response with pembrolizumab [73]. The first clinical trial utilizing nivolumab (anti-PD1) therapy administered with or without ipilimumab (cytotoxic T-lymphocyte-associated protein 4) was CheckMate 143. Results from different trial phases demonstrated a higher clinical tolerance in the nivolumab-alone arm, and no significant survival difference between the two groups was reported [74, 75]. Two large phase III clinical trials were subsequently conducted (ClinicalTrials.gov identifier: NCT02617589 and NCT02667587). No survival benefit was observed for nivolumab when added to standard treatment in patients with glioblastoma and methylated MGMT or when compared to TMZ. Emphasizing the clinical and immunological relevance of the timing of anti-PD1 treatment, Cloughesy et al. reported that the time of initiation of the anti-PD1 treatment can be of considerable importance. According to their findings, neoadjuvant pembrolizumab can lead to longer patient survival in addition to a higher focal expression of PD-L1, compared with adjuvant pembrolizumab treatment [76].

In summary, three large phase III trials demonstrated that nivolumab does not produce a survival advantage when given to unselected patients with glioblastoma [6]. However, clinical trials of other PD-1 and PD-L1 inhibitors, combinations with other immunotherapies, and evaluating predictive biomarkers are ongoing (Table 1).

5 Oncolytic Viral Therapies

Oncolytic virus therapy is a promising new immunotherapy for cancer treatment. An oncolytic virus is a genetically engineered or naturally existing virus that can preferentially target and destabilize cancer cells without affecting normal tissues [77].

5.1 Recombinant Oncolytic Poliovirus, PVSRIPO: Food and Drug Administration Breakthrough Therapy Designation

PVSRIPO is a live, weakened, poliovirus type 1 vaccine currently recognized as a promising cancer therapy (Fig. 2).

Table 1 Examples of ongoing clinical trials using immune checkpoint inhibitors for patients with recurrent glioblastoma

ClinicalTrials. gov identifier	Trial phase	Experimental treatment	Control or comparator treatment	Estimated enrollment	Primary endpoint or outcomes	Estimated completion date
NCT04888611	II	Neoadjuvant camrelizumab (PD-1 inhibitor) plus glioblastoma stem-like cell antigen-primed DC vaccines	Neoadjuvant camrelizumab plus placebo	40	OS and PFS	1 May, 2024
NCT04977375	Ib/II	Pembrolizumab with stereotactic radiation therapy and surgical resection	None	42	OS and safety and tolerability measured by the incidence of adverse events	December 2024
NCT05053880	Ib/IIa	Ib: IV ACT001 (anti-PDL1) dose exploration with pembrolizumab (anti-PD1)	None	18	Several including incidence of TEAEs, DLTs	November 2023
		IIa: ACT001 plus pembrolizumab		30	PFS	
NCT05540275	II	Sintilimab[a] plus bevacizumab for patients with bevacizumab-refractory recurrent glioma with PTEN or TERT gene mutations	Sintilimab plus bevacizumab for patients with bevacizumab-refractory recurrent glioma without PTEN or TERT gene mutations	30	Overall response rate, duration of response and incidence of TEAEs	December 2026
NCT04656535	0/I	Intravenous AB154[b] combined with AB122[c]	Surgical cohort investigating the effects of AB154 + AB122 in the tumor and tumor microenvironment	46	Incidence of TEAEs	July 2023

DC dendritic cells, *DLTs* dose-limiting toxicities, *OS* overall survival, *PD-1* programmed cell death protein 1, *PDL1* programmed-death ligand 1, *PFS* progression-free survival, *TEAEs* treatment-emergent adverse events, *NB* not a comprehensive table

[a]One anti-PD-1 antibody same as nivolumab approved in China

[b]Humanized immunoglobulin G1 monoclonal antibody that targets the TIGIT immune checkpoint

[c]Fully human immunoglobulin G4 monoclonal antibody that targets the PD-1 immune checkpoint

It targets cells expressing the CD155/Necl5 poliovirus receptor, which is an onco-fetal cell adhesion molecule frequently expressed in glioblastoma. In the PVSRIPO vaccine, the adjacent internal ribosome entry site was substituted with human rhinovirus type 2 to prohibit neurovirulence and limit viral affinity to CNS cellularity [78].

Desjardins et al. evaluated seven PVSRIPO doses, with a range between 10^7 and 10^{10} viral particles = 50% tissue-culture infectious doses injected intratumorally to 61 adult patients who had recurrent supratentorial glioblastoma. Survival rate reached a plateau of 21% at 24 months in the experimental group compared to historical controls and remained stable through 36 months [79]. According to a combined analysis of recent virotherapy trials for recurrent glioblastoma, the percentage of patients who survived for 24 months overall is estimated to be 15%, while the proportion who survived for 36 months is 9% [80], which makes the aforementioned results clinically significant. These results from a completed phase I clinical trial (ClinicalTrials.gov identifier: NCT01491893) motivated the Food and Drug Administration to approve a breakthrough therapy designation to the recombinant oncolytic poliovirus PVSRIPO in May 2016.

5.2 HSV-1, Adenovirus, Reovirus

Herpes virus simplex 1 (HVS-1) is the most commonly used and sole multi-generational engineered virus in oncolytic virus therapy. Different HSV-1 generations only differ in the number of added genes to the original HSV-1 genome. Todo et al. engineered G47Δ, a triple-mutated third-generation oncolytic HSV-1 expressing an additional deletion mutation to the second-generation HSV-1, G207 [81]. For human application, G47Δ is currently the only third-generation HSV-1 available [82]. Following the successful conclusion of a Japanese phase I–IIa trial in patients with recurrent glioblastoma, a phase II single-arm safety study (UMIN-CTR Clinical Trial Registry UMIN000015995) focused on the efficacy of G47Δ in 19 adult patients with residual or recurrent supratentorial glioblastoma was conducted, and recently published its results [83]. The 1-year survival rate after G47Δ injection was 84.2%, median OS after G47Δ injection reached 20.2 and 28.8 months, respectively. In addition to fever, vomiting, nausea, lymphocytopenia, and leukopenia as G47Δ-related common adverse events, increasing numbers of tumor-infiltrating CD4+/CD8+ lymphocytes and persistent low numbers of Foxp3+ cells were
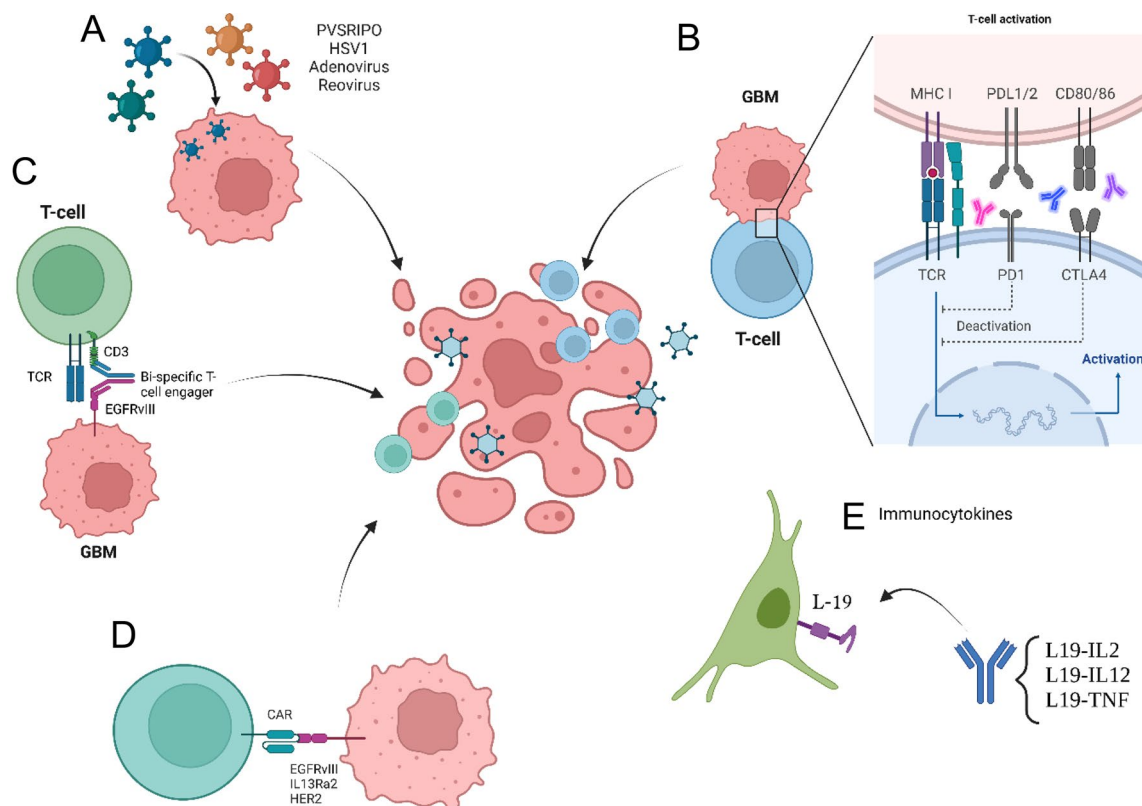
Fig. 2 Anti-glioblastoma immunotherapy approaches; **A** oncolytic virus vaccine; **B** immune checkpoint inhibitors; **C** bi-specific T-cell engagers; **D** chimeric antigen receptor (CAR) T-cell therapy; and **E** immunocytokines. *CTLA4* cytotoxic T-lymphocyte-associated protein 4, *EGFR* epidermal growth factor receptor, *GBM* glioblastoma, *IL* interleukin, *MHC* major histocompatibility complex, *PD1* programmed cell death protein 1, *TCR* T-cell receptor, *TNF* tumor necrosis factor. Figure designed on Biorenders

found upon biopsy [83]. In another recent study, the same group confirmed that G47Δ is safe for patients with progressive glioblastoma when administered intratumorally at doses of up to 1×10^9 plaque-forming units per dose for two doses within a 14-day period. Japan's Ministry of Health, Labour and Welfare then approved Delytact® (commercial name for G47Δ) for the treatment of malignant glioma. Efficacy findings were less exciting with a median OS of 7.3 months and a 1-year survival rate of 38.5%, both from the last G47Δ administration. According to the investigators, these results justified the foundation of the phase II clinical trial in patients with progressive glioblastoma [84].

Recombinant adenovirus is another promising oncolytic viral therapy. Adenovirus has a double-stranded DNA genome and infects human cells using the coxsackie and adenovirus receptor [77]. After endocytosis into tumor cells, adenoviruses remain episomal in the cytosol while expressing its genome. The genetically modified adenovirus ONYX-015 was the first to undergo a phase I clinical trial, where it resulted in no serious side effects being reported [85]. Adenovirus Delta-24 was designed to selectively replicate in cells deficient in the Rb/p16 tumor suppressor pathway [77]. Lang et al. reported objective and long-lasting biological and clinical responses from a phase I dose-escalation clinical trial in 37 patients with recurrent glioblastoma. For patients with a long-term response (> 3 years), evidence demonstrated immune infiltration (T cells and macrophages) into the TME with minimal toxicity especially when administrated with convection-enhanced delivery [86, 87]. Results from a phase II clinical trial evaluating the anti-tumor response of DNX-2401, a genetically modified oncolytic adenovirus, and intravenous pembrolizumab are eagerly awaited (NCT02798406).

A phase Ib study in 2018 conducted by Samson et al. demonstrated that intravenous administration of fnoncolytic human orthoreovirus (reovirus) lead to infection of both glioblastoma and brain metastases tumor cells, and increased cytotoxic T-cell tumor infiltration in comparison to patients not treated with the virus. They also found an upregulation of interferon-regulated gene expression and the PD-1/PD-L1 pathway in tumors via an interferon-related mechanism, and that supplementation of anti-PD-1 to the reovirus improves systemic efficiency of the therapy in a preclinical glioblastoma model [88]. These findings are worth further clinical investigation.

6 CAR T-Cell Therapy

Chimeric antigen receptor (CAR) T-cell therapy is another extensively investigated potential treatment, especially after the success reported with hematological malignancies [2–4].

A small number of patients with glioblastoma achieved complete response to CAR T cells [89]. Potential roadblocks challenging the therapeutic success of CAR T cells in solid tumors include: limited number of targetable antigens with a heterogenous antigen expression, serious off-target side effects due to the expression of the targeted antigen by non-malignant tissues, limited activation and expansion of injected T cells before reaching tumor sites, short-lasting local immune response, and the immunosuppressive TME [89]. Several techniques to overcome these challenges are currently in clinical and preclinical studies. This section focuses on completed clinical trials.

6.1 EGFRvIII

As EGFRvIII is expressed in around 30% of glioblastoma tumors, it was among the first targets of CAR T-cell therapy. In a phase I/II trial (ClinicalTrials.gov identifier: NCT01454596), patients with EGFRvIII-expressing glioblastoma were infused with EGFRvIII-targeting CAR T cells. No adverse events associated with the administration of up to 1×10^{10} CAR T cells were observed. Serious adverse events occurring in one patient receiving 3×10^{10} CAR T cells included dyspnea and hypoxia. Another patient who received an administration of 6×10^{10} CAR T cells experienced severe adverse effects. The patient developed acute dyspnea and oxygen desaturation 1 h after the CAR infusion, and these symptoms were not successfully managed by bilevel positive airway pressure and intubation. Tragically, the patient died 4 h post-infusion because of severe pulmonary edema [90]. The gravity of adverse events along with a low clinical success owing to the selection pressure and outgrowth of antigen-negative cells reduced the excitement for the use of this tumor antigen as a target for CAR T cells.

6.2 IL-13Rα2

IL-13Rα2 is an IL-13 receptor frequently overexpressed in adult and pediatric glioblastoma tumors, but under-expressed in non-malignant tissues, except the testes [91]. Chimeric antigen receptor T cells carrying a mutated form of IL-13 have been engineered to target IL-13Rα2-expressing tumors. Feasibility and safety of this CAR construct were confirmed in up to 12 intracranial injections (maximum dose 1×10^{18} CAR T cells) into three patients with recurrent glioblastoma [92]. Two phase I clinical trials are currently recruiting patients to investigate the anti-tumoral activity of IL-13Rα2 CAR T cells, the first with or without nivolumab and ipilimumab (ClinicalTrials.gov identifier:

NCT04003649) and the other in different leptomeningeal disease-causing tumors including glioblastoma (ClinicalTrials.gov identifier: NCT04661384).

6.3 HER2

HER2 is an EGFR overexpressed in multiple cancer types (including approximately 80% of glioblastoma tumors) but normally expressed in non-malignant epidermal cells [93]. Work by Shen et al. has shown that third-generation HER2-specific CAR T cells can efficiently eradicate glioblastoma cells in vitro and can have enhanced immune activity if combined with PD-1 blockades [91, 94]. Additionally, no dose-limiting toxic effects were observed in patients intravenously injected with up to 1×10^8 HER2-specific CAR T cells, engineered with a CD28.ζ endodomain [95].

Several studies reported an increased anti-tumoral efficacy of CAR T cells boosted by cytokines including IL-12 and IL-23, against several systemic tumor models [96–98]. This motivated Agliardi et al. to intratumorally inject IL-12 alongside CAR T cells in a glioblastoma mouse model to test its enhancing effects [99]; results included enhanced cytotoxicity of CAR-T cells, modulation of TME, and increased infiltration of CD4+ T cells. Further incorporation of cytokines into the CAR T-cell construct for glioblastoma immunotherapy should be explored [99].

Several other tumor targets are currently in preclinical investigation including CAIX, CSPG4, CD70, EphA2, and TROP2 [91]. Nevertheless, the intensive investigation on CAR T cells encompasses other angles other than antigen specificity to surmount the abovementioned roadblocks. We believe the most challenging is the antigen dilemma; very few tumor-specific antigens are available as targets with only a small proportion of tumor cells expressing them, making high-efficiency CAR treatment targeting a single antigen of low likelihood. Neoantigens seem like a potential solution to this dilemma; however, most of them are derived from intracellular proteins and are patient specific, causing feasibility issues [89, 100]. An ideal route of delivery of CAR T cells would be systemically, if side effects could be prevented. Unfortunately, this is not the case, systemic and off-target toxicities were reported in several studies [101]. Localized administration of CARs [102, 103] and the inducible suicide genes developed [104] and continuously improved [105] are being used to enhance safety. Studies have shown that the CAR T-cell generation protocol, including initial activation with anti-CD3/anti-CD8 activation beads instead of traditional concavaline A and culture with hIL7/hIL15-supplemented media, plays a crucial role in the longevity and anti-tumor efficiency of the generated CARs [106, 107]. Therefore, small adjustments to the CAR generation protocol can be remarkably effective. Finally, despite the immunosuppressive TME being a significant obstacle to the anti-tumoral

efficacy of CAR T cells, potential solutions are in the near future. For instance, cytokine-secreting CARs, such as IL-15-secreting CARs, can mitigate the immunosuppression of the regulatory T cells in the TME [108] and the use of cytokine switch receptors that convert inhibitory cytokine signals into survival-promoting signals [109].

7 Recent Immunotherapies

7.1 Immunocytokines

Cytokine administration is an emerging technique to convert the immunologically suppressive glioma microenvironment into a friendly environment, allowing for effective antitumor immunity. Multiple proinflammatory cytokine candidates (e.g., IL-2, IL-6, IL-12, tumor necrosis factor [TNF]-α) are currently being investigated for this type of therapy [110]. Different approaches are used to deliver cytokines to the TME including local administration, protein fusion, and inducible expression with different constructs (especially CARs) [110]. In an interesting study, Weiss et al. used three murine L19 targeting cytokine-fused antibodies (L19-mIL-2, L19-mIL-12, and L19-mTNF) to assess the efficacy of these cytokines in modeling the TME [110]. They discovered that L19 intravenous administration of L19-mIL-12 or L19-mTNF but not L19-mIL-2 cured a proportion of tumor-bearing mice before depletion of CD4 or CD8 T cells, suggesting adoptive immunity dependency of these cytokines [110]. A clinical trial evaluating the safety and efficacy of the L19-hTNF in patients with IDH-1$_{wt}$ glioblastoma is currently underway (ClinicalTrials.gov identifier: NCT03779230.)

7.2 BiTEs

Bi-specific T-cell engagers (BiTEs) are an innovative form of immunotherapy that hold promising potential for treating several types of tumors, including glioblastoma [111, 112]. Bi-specific T-cell engagers allow for the co-localization of cytotoxic T cells with heterogeneous T-cell receptors and tumor cells by utilizing two linked antigen-recognizing variable fragments without the Fc region of antibodies. This unique feature allows the BiTEs to penetrate the blood–brain barrier following intravenous administration [113, 114]. The bispecific fragment recognizes the CD3 protein on T cells and tumor-specific antigens present on tumor cells. EGFRvIII was the first target of BiTEs for glioblastoma. Choi et al. designed the first murine BiTES recognizing CD3 and EGFRvIII on glioblastoma, and reported a potent elimination of EGFRvIII-expressing tumor cells with a low dose of the drug (10 ng/mL) [112]. Subsequently, Gedeon

et al. proposed fully humanized BiTEs that have proven high functionality [115]. Amgen conducted the first phase I clinical trial on humans using CD3-EGFRvIII BiTES (AMG 596). While this trial was completed in December 2021 (NCT03296696), official results are not yet published. However the interim analysis indicates that AMG 596 was tolerated and efficient for patients with recurrent glioblastoma [116]. Another phase I clinical trial was initiated (NCT04903795), this time targeting EGFRvIII and CD3 using a single-chain variable fragment 139 and 28F11, respectively. These two fragments are linked and were previously used in clinical settings [117, 118]. A more comprehensive review of BiTEs use in glioblastoma was previously published by our team [119].

8 Treatment Sequencing and Combination Approaches

As previously indicated, the current standard of care for glioblastoma proposes a maximum possible resection of the tumor, with concomitant radiotherapy and oral adjuvant chemotherapy (TMZ) without an official inclusion of any immunotherapy. Several preclinical and clinical studies explored the combinatorial benefits of multiple immunotherapies or the combination of classical and immunotherapy against glioblastoma [120]. Temozolomide with ICIs is the most studied combination for glioblastoma, TMZ is an alkylating agent that deposits methyl groups on the guanine base of the DNA, leading to a DNA double-stranded break and cell apoptosis of rapidly proliferating cells. The tumor lysates resulting from this mechanism and from the DNA double-stranded break induced by radiotherapy are phagocytized by the antigen-presenting cell and presented to the T cell, which, subsequently, promotes a polyclonal infiltration of T cells [121], and this made the rational foundation to investigate the combination of TMZ and ICIs.

Despite that the standard-dose TMZ might downregulate PD-L1 from the tumor surface and mitigate the efficacy of anti-PD1/PD-L1 [122], a metronomic or low dose of TMZ has been shown to maintain or increase the survival benefits of anti-PD1 therapy and increase the count of TILs in preclinical studies [123, 124]. The combination of radiotherapy alone or radio-chemotherapy with nivolumab (anti-PD1) was not successful in phase III clinical trials of CheckMate 498 and 548 [125, 126].

The combination of multiple checkpoint blockades was also assessed; anti-PD1 and anti-LAG3 together significantly increased survival in a cohort of mice in comparison to the no-treatment arm. However, the survival benefits and immune profile were similar to the mono-therapy groups (anti-PD-1 or anti-LAG3 alone) [127].

Anti-vascular endothelial growth factor inhibitors effect on glioblastoma is increasingly being investigated, Soubéran et al. [128] have reported increased innate immune cell infiltration between days 21 and 28 post-treatment with anti-vascular endothelial growth factor in GL261 glioma-bearing mice [128], which made it a rational combination therapy with sintilimab (anti-PD-1 approved in China) in a newly opened clinical trial (NCT05540275).

9 Conclusions/Future Research

Despite promising results of different immunotherapies in other cancer types, therapeutic success with immunotherapy in glioblastoma has been limited. Roadblocks include a limited number of targetable antigens with a heterogenous antigen expression, serious off-target side effects, limited activation and expansion of injected T cells before reaching tumor sites, and the immunosuppressive TME [89]. Additionally, the best murine models currently available are still insufficient in providing an accurate representation of human tumors because of poor recapitulation, which includes in vitro genetic deviation, changes in tumor cells, and poor recapitulation of the human TME. Furthermore, most syngeneic mouse models carry a higher mutational burden than human glioblastomas, making them less effective in mimicking the human condition. Further efforts dedicated to overcoming the challenges limiting the therapeutic success of immunotherapies in solid tumors are warranted, especially regarding the generation of more accurate preclinical mouse models. Preclinical efforts with emerging new treatment pathways allow a better understanding of immunobiological dynamics in glioblastoma and ultimately have the potential to have a meaningful impact on patient outcomes [7, 129]. Evidence suggests that mono-immunotherapy may not be sufficient to overcome potent immunosuppression present in glioblastoma, and attempts are being made to develop combinatorial approaches [130].

Personalized treatment is another emerging therapeutic pathway [79]. The heterogenicity of glioblastoma makes it very difficult to develop a monotherapy that provides broad meaningful benefits. The Ivy Brain Tumor Center at the Barrow Neurological Institute tests new combination therapies for glioblastoma and other aggressive brain tumors and adjusts through a phase 0 clinical trial that matches new combination drug regimens to an individual's unique tumor after advanced genetic testing within 10 days from surgery [131].

Immunotherapy restores hope in improving clinical outcomes for patients with glioblastoma, a devastating highly lethal disease. Despite the disappointing results of most of the concluded clinical trials, they have further developed our understanding of glioblastoma physiopathology. Exiting ongoing preclinical and clinical studies are relying on and building upon what was learned from previous experiences.

Declarations

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