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Review

Vaccine-based immunotherapy and related preclinical models for glioma

Binghao Zhao [1,5], Longping Yao[2,5], Maryam Hatami[2], Wenbin Ma [3,4], and Thomas Skutella [2,*]

Glioma, the most common primary malignant tumor in the central nervous system (CNS), lacks effective treatments, and >60% of cases are glioblastoma (GBM), the most aggressive form. Despite advances in immunotherapy, GBM remains highly resistant. Approaches that target tumor antigens expedite the development of immunotherapies, including personalized tumor-specific vaccines, patient-specific target selection, dendritic cell (DC) vaccines, and chimeric antigen receptor (CAR) and T cell receptor (TCR) T cells. Recent studies show promising results in treating GBM and lower-grade glioma (LGG), fostering hope for future immunotherapy. This review discusses tumor vaccines against glioma, preclinical models in immunological research, and the role of $CD4^+$ T cells in vaccine-induced antitumor immunity. We also summarize clinical approaches, challenges, and future research for creating more effective vaccines.

Highlights

Peptide, dendritic cell (DC), DNA/RNA, and autologous vaccines for glioma treatment are currently being investigated. The major focus is on peptide and DC vaccines.

Many immunotherapeutic strategies show efficacy in preclinical models but fail in real clinical settings, and there is a need for more accurate and precise preclinical models for evaluating glioma immunotherapies.

The combination of vaccine-based therapies with other therapeutic strategies can have potential benefits and this warrants further investigation.

The importance of preclinical models in glioma immunological research

Gliomas are the most common malignant primary brain tumors in adults and cannot be cured with usual cancer treatments. Specifically, glioma accounts for 30% of all primary brain tumors and 80% of malignant brain tumors [1]. The latest World Health Organization (WHO) CNS classification recognizes five major types of glioma: adult-type diffuse glioma, pediatric-type diffuse low-grade glioma, pediatric-type diffuse high-grade glioma, circumscribed astrocytic glioma, and ependymal tumors; of these adult-type diffuse glioma accounts for 90% [1,2]. For the purposes of this review, we refer to diffuse low-grade and intermediate-grade glioma (WHO grade 2 and 3) as lower-grade glioma (LGG), and WHO grade 3 and 4 as higher-grade glioma (HGG) [3]. WHO grade 4 wild-type isocitrate dehydrogenase (IDHwt) glioma, glioblastoma multiforme (GBM), accounts for most cases of glioma and has the worst prognosis [2]. However, before the publication of 2021 CNS tumor classification criteria, the 2016 or older versions were used: many data discussed here are based on the old classification criteria, and further studies based on the 2021 criteria are required.

A broad spectrum of novel, viable, and promising immunotherapeutic strategies have been investigated in both GBM and LGG, encompassing tumor vaccines, oncolytic viruses, immune checkpoint inhibitors (ICIs), and adoptive cell therapy (ACT) [4] (Box 1). Nevertheless, because of the lack of appropriate tumor models, there are substantial discrepancies in the treatment efficacy of various immunotherapies between preclinical and clinical studies [5]. Indeed, many immunotherapeutics show considerable efficacy in preclinical models but fail in the clinic, and it seems likely that the tumor models used thus far do not accurately reflect human conditions. The generation of models that faithfully replicate the complexity of glioma can enhance our understanding of the fundamental immunology and bridge the gap between basic and clinical research, and holds significant promise for testing novel immunotherapeutic approaches. In this review we discuss the different models that can accurately mimic the characteristics of glioma characteristics, thereby enabling cancer vaccine studies, and summarize current evidence regarding the clinical efficacy of vaccine immunotherapeutic approaches.

[1]German Consortium for Translational Cancer Research (DKTK) Clinical Cooperation Unit (CCU) for Neuroimmunology and Brain Tumor Immunology, German Cancer Research Center (DKFZ), Heidelberg, Germany
[2]Institute for Anatomy and Cell Biology, Heidelberg Medical Faculty, Heidelberg University, Heidelberg, Germany
[3]Department of Neurosurgery, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, PR China
[4]State Key Laboratory of Complex Severe and Rare Diseases, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, PR China
[5]Equal contributions

*Correspondence: thomas.skutella@uni-heidelberg.de (T. Skutella).

Box 1. Background to glioma treatment

Glioma/glioblastoma (GBM)

Glioma is the most common malignant primary tumor of the central nervous system (CNS), WHO grade 4 glioblastoma, named IDHwild-type (IDHwt) GBM, represents the majority of cases and is the most lethal. Based on the 2016–2020 statistics of the Central Brain Tumor Registry of the United States (CBTRUS), the average annual age-adjusted incidence of CNS tumors in the USA is 24.83 per 100 000 population, where GBM represents 14.2% of all CNS tumors and 50.9% of all malignant CNS tumors. However, overall survival for patients remains dismal with standard and aggressive therapies [109,110]. The median survival time for GBM is roughly 12.5–15 months, and 2 year and 5 year survival rates are 25% and 10%, respectively, with standard treatment [111]. Therefore, we urgently need more effective treatments for GBM management.

Immunotherapy

Because of the immunosuppressive tumor microenvironment (TME), immune checkpoint inhibitors (ICIs) were not effective against either newly diagnosed (ndGBM) or recurrent GBM (rGBM) [112–114]. Similarly to chimeric antigen receptor T cell (CAR-T) therapies targeting GBM-specific extracellular antigens, tumor vaccines targeting intracellular neoantigens as well as tumor-associated antigens have proved to be both safe and effective [115].

Preclinical models for glioma immunological research

Anti-glioma immunotherapeutics have shown significant efficacy in rodent models, but concerns persist regarding the ability of current models to fully replicate human immune responses owing to their inability to mimic human disease heterogeneity [6] (Figure 1). There is an urgent need for updated preclinical models to explore the anti-glioma efficacy of immunotherapy, particularly vaccines, whether administrated alone or in combination.

In vivo models

Syngeneic mouse models. **Syngeneic mouse models** (see Glossary), that are among the earliest tools for studying antitumor efficacy, involve transplanting tumor cell lines from inbred mice into genetically identical mice with intact immune systems [7]. These models offer convenience and genetic manipulation capabilities for assessing immunotherapies [8]. In studies implanting GL261 cells into C57BL/6 mice, chimeric antigen receptor T cell (CAR-T) therapy targeting epidermal growth factor receptor variant III (EGFRvIII) and CAR-natural killer (CAR-NK) cells against ERBB2 have shown promise in GBM immunotherapy [9–12]. Although the model with GL261 lacks human genomic and tumor microenvironment (TME) heterogeneity, it exhibits greater immunogenicity with more targetable **neoantigens**. Models established with other cell lines also display different immunogenicity and potential for glioma vaccine research [10].

Genetically engineered mouse models. **Genetically engineered mouse models (GEMMs)** are created through the introduction of genetic mutations specific to human cancers. For GBM, inducible GEMMs have been developed through the use of tetracycline (Tet)-inducible Cre transgenes [13]. In addition, the replication-competent avian sarcoma-leukosis virus–tumor virus receptor A (RCAS-TVA) retroviral/adenoviral gene-transfer system allows somatic cell gene transfer, and can be used to induce syngeneic tumors in outbred mice [14]. The Sleeping Beauty (SB) transposon system is powerful for cancer gene discovery and transgene insertion, and has been applied in mice for many types of cancers [15]. With this system, mouse models for astrocytoma, grade 3 glioma, and GBM have been established [16]. A novel gene-delivery system based adeno-associated virus (AAV) vectors yields higher transgene expression and cell viability. The delivery system helps to deliver CARs and transduce synthetic TCRs into lymphocytes and myeloid cells, and can boost T cell-based therapy [17]. Recently, the CRISPR-Cas9 system has emerged as a potent tool for gene manipulation across diverse cell types and organisms, and HGG GEMMs utilizing this technology demonstrate efficacy for *in vivo* studies [18]. To conclude, GEMMs provide meticulous control over molecular events, enabling oncogene

Glossary

Dendritic cell (DC) vaccines: the requirement for DC-mediated recognition and processing of tumor antigens can bypassed by preloading DCs with the specific tumor antigen of interest before vaccination. Targets for DC vaccines include TAAs and neoantigens.

Genetically engineered mouse models (GEMMs): the model indicates the mice whose genomes have been modified using genetic engineering techniques to study gene function and mimic human diseases. Cross-breeding is involved to generate stable models. Types of GEMM include 'transgenic mice', 'knockout mice', 'knock-in mice', 'conditional knockout mice', among others, with many techniques used to generate them. These models are essential tools in biomedical research because they allow scientists to explore the roles of specific genes in development, physiology, and disease.

Glioma stem cells (GSCs): functionally identified in wild-type IDH (IDHwt) glioblastoma (GBM) as cells with high expression of biomarkers such as CD133, CD44, SSEA1, L1CAM, PDGFRA, and EGFR. GSCs have tumor-propagating potential *in vivo* and sustained self-renewal potential. They can also differentiate into multiple cellular lineages.

Neoantigens: tumor-specific antigens that are derived from clonal driver mutations.

Organoids: models generated from patient-derived adult stem cells, resected tumor tissues, or induced pluripotent stem cells (iPSCs). Organoids are 3D structures that comprise various cell types and faithfully replicate the architecture and function of the original organ.

Patient-derived xenograft (PDX): involves the transplantation of patient-derived tumor cells, organoids, or tissues into immunodeficient mice. PDX models can more faithfully represent the heterogeneous nature of GBM.

Peptide vaccines: can be based on either short and long peptides: long peptide neoantigen vaccines have been used in recent clinical trials to elicit both $CD4^+$ and $CD8^+$ T cell responses. Peptide vaccines epitomize conventional vaccination methods owing to their rapid and cost-effective generation.

Syngeneic mouse models: involve transplantation of tumor cell lines from

activation at specific timepoints and facilitating new targets (e.g., neoantigens) for the evaluation of potential immunotherapeutic agents.

Humanized mouse models. **Patient-derived xenograft (PDX)** models involve transplanting patient-derived tumor cells, **organoids**, or tissues into immunodeficient mice, thereby offering a more faithful representation of GBM complexity and heterogeneity [19]. GBM PDXs recapitulate tumor morphology, invasion patterns, and molecular heterogeneity, and can reflect common or rare mutations in genes such as *TERT*, *EGFR*, *PTEN*, *TP53*, *BRAF*, and *IDH1*, but lack microvascular proliferation and necrosis [20]. Many types of immunodeficient mice are used in PDX models, such as severe combined immunodeficient (SCID) mice, non-obese diabetic (NOD) mice, NOD/SCID mice, NOG (NOD/SCID IL2Rγ^{null}), NSG™, and NOJ (NOD/SCID JAK3^{null}) mice, where NOG, NSG, and NOJ show a more immunodeficient profile with complete deficiency of NK cell function [21–24]. PDX models are widely used in studies on tumor pathogenesis and drug development, but few studies have focused on glioma vaccine research. Some potential reasons are (i) high cost and low throughput, (ii) limited T cells in thymus and lack of human leukocyte antigen (HLA) molecules in reconstructed human-like immune systems, and (iii) difficulty in generating lymph node structure and germinal centers [25].

In vitro models

Cell lines. The C6 glioma, 9L gliosarcoma, and F98 glioma cell lines are most commonly used in rat models cell lines, GL261 and CT-2A in mouse glioma models, and U87 GBM, U251 GBM, U118 GBM, and SHG-44 astrocytoma are commonly used human-derived cell lines, whereas the rat RG2, BT4C, RT-2, and CNS-1 cell lines are less frequently used [26,27]. Among the rat cell lines, C6 is extensively utilized in investigations spanning chemotherapy, antiangiogenic therapy, radiotherapy, oncolytic viral therapy, and gene therapy. However, its utility in immunotherapy studies is constrained by the allogeneic immune response [26]. The F98 cell line-derived tumor has insignificant CD3$^+$ T cell infiltration and weak immunogenicity, making it useful for immunotherapy studies [28]. Similarly to F98, the RG2 cell line has insignificant immunogenicity for MHC-I antigens, and is not commonly used for immunotherapeutic studies because of the paucity of monoclonal antibodies targeting rat antigens [26]. For murine models, the GL261 cell line demonstrates detectable expression levels of MHC-I, making it susceptible to NK cells, and MHC-II expression is inducible by stimulation with IFN-γ [29]. This cell line, characterized by a modest immunogenic profile, has proved to be advantageous for studies involving ICIs, gene therapy, and tumor vaccines [29]. CT-2A-derived tumors exhibit high intratumoral heterogeneity and a substantial tumorigenic burden, resulting in shortened median overall survival (OS) for mice harboring these tumors. Consequently, the duration of *in vivo* studies is significantly curtailed [30]. Nevertheless, this cell line facilitates the evaluation of various drugs and is particularly valuable in immunotherapy investigations [26]. For human-derived cell lines, both U87 and U251 seem to be more suitable in chemotherapeutic agent response research [31,32].

Glioma stem cells. 3D models using **glioma stem cells (GSCs)** surpass traditional 2D tumor cell lines because they incorporate cellular diversity, enhance drug response predictability, and provide a robust platform for discovering novel anticancer drug targets [33]. GSC tumorspheres, for example, originate from the symmetric and asymmetric division of patient-derived GSCs in a defined medium enriched with growth factors such as epidermal growth factor (EGF), fibroblast growth factor 2 (FGF-2), and neuronal viability supplement B27. Tumorsphere cells actively interact, fostering physical and signaling interactions that affect proliferation, invasiveness, and therapy responses *in vivo*. Importantly, they lack neighboring non-tumor cells such as astrocytes, neurons, endothelial cells, mesenchymal stem cells, brain-resident microglia, and infiltrated peripheral immune cells [34]. Coculturing tumor and non-tumor cells in heterotypic spheroids can

inbred mice into genetically identical mice with intact immune systems.
T helper cells: these undergo polarization towards distinct effector types, including type 1 T helper (Th1), Th2, Th9, Th17, and Th22 cells, as well as T follicular helper (Tfh) cells and regulatory T cells (Tregs). CD4$^+$ T helper cells can interact with B cells, other CD4$^+$ T cells, CD8$^+$ T cell, natural killer (NK) cells, and DCs to facilitate vaccine-specific immunity. T helper cells also kill tumor cells directly and are essential for tumor regression following vaccination.
Tumor-associated antigens (TAAs): are expressed both on tumors and on normal cells. Treatment efficacy is not specific when the vaccines target TAAs.

help to optimize the assessment of cancer immunotherapies. In addition, tumor antigen derived from GSCs loaded with DCs boosted GBM vaccine production and stimulated cytotoxicity T cell antitumor immunity in murine models [35,36]. It is also possible to collect mRNA from human-derived GSCs to produce personalized vaccines [36].

Organotypic tissue slices. The organotypic tissue slice model of GBM preserves the original complexity and structure of the tumor. Precision-cut tumor slices are prepared and cultured on membrane inserts in a specific medium [37]. This technique offers speed and suitability for personalized treatments without requiring selective outgrowth of tumor cells. It is particularly

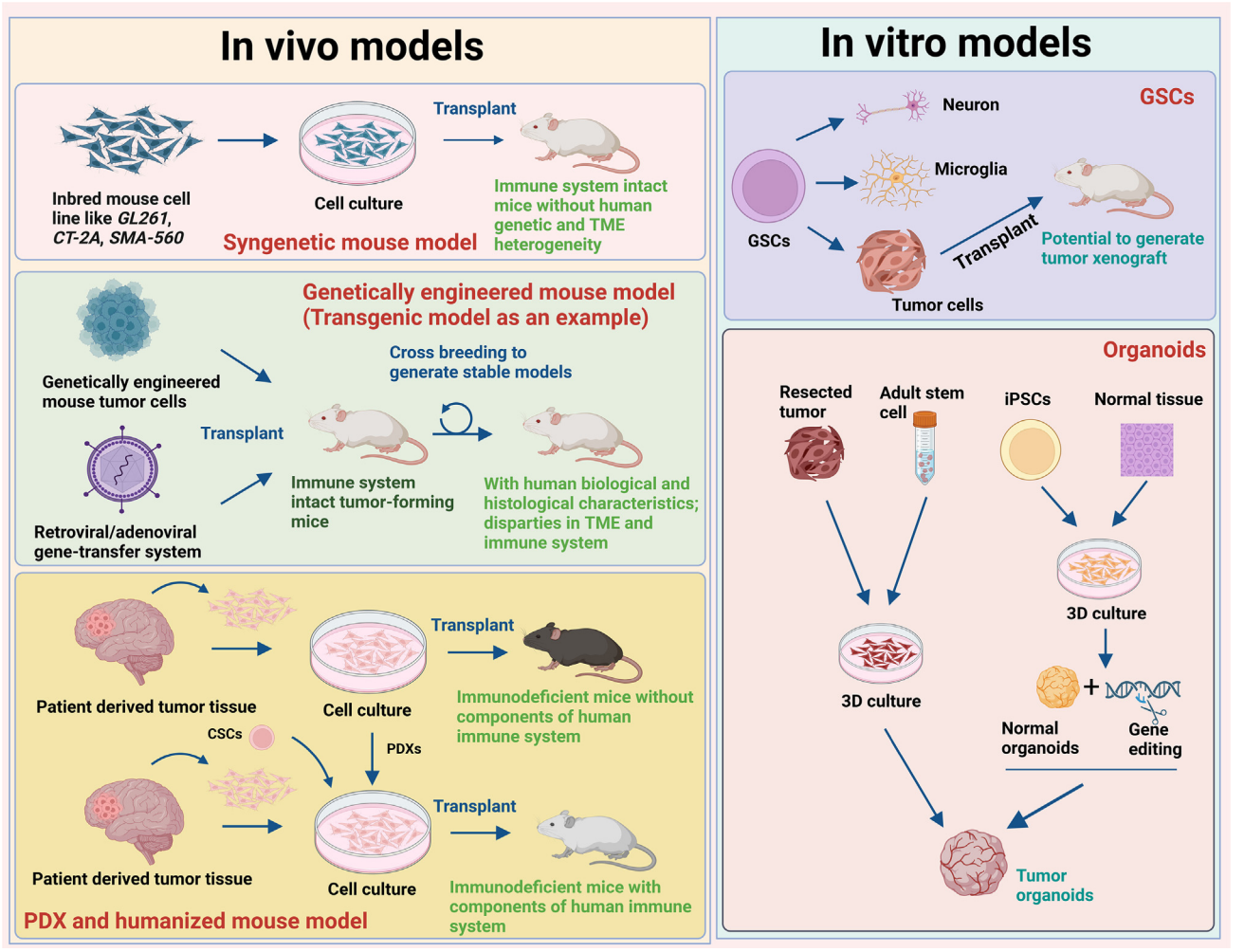

Figure 1. Commonly used preclinical models for glioma immunological research. The preclinical models can be categorized into *in vivo* and *in vitro* subsets. For glioma immunological research, *in vivo* models include syngeneic mouse models, GEMMs, patient-derived xenografting (PDX), and humanized mouse models. Syngeneic mouse models involve the transplantation of glioma cell lines such as GL261, CT-2A, SMA-560 into mice with an intact immune system. In GEMMs, genetically engineered mouse tumor cells are transplanted into immunocompetent mice via retroviral/adenoviral gene-transfer platform, then robust models are generated by cross-breeding the immune system intact mice. Both PDX and humanized mouse models are similar in that the tumor tissues are human-derived. A major discrepancy is that PDX and humanized models involve immunodeficient mice that do not express all components of the human immune system. Organoids can be derived from resected tumor tissues, adult stem cell, iPSCs, or normal tissues. Detailed features of the preclinical models can be found in Table 1. Abbreviations: CSCs, cancer stem cells; GEMM, genetically engineered mouse model; GSCs, glioma stem cells; iPSCs, induced pluripotent stem cells; TME, tumor microenvironment. Figure created with BioRender.

useful for studying GBM invasiveness and the patient-specific effects of anti-invasive drugs [37]. However, it has limited throughput and demands specialized analysis tools, making it a labor-intensive approach.

Spheroids and organoids. GBM spheroids represent aggregates comprising GBM and TME cells, either suspended or embedded in a 3D matrix. Specific culture conditions can facilitate sphere formation and maintain a phenotype and genotype similar to that of human GBM. Organoids – sophisticated 3D structures comprising various cell types – faithfully replicate the architecture and function of the original organ [38,39]. They can be generated from patient-derived adult stem cells, resected tumor tissues, or pluripotent stem cells such as embryonic or induced pluripotent stem cells (iPSCs) [40]. Compared to other traditional models, brain organoids offer valuable tools for cancer research, individualized drug screening, and personalized medicine, and may bridge the gap between *in vitro* and *in vivo* cancer models [41]. In a recent study, researchers innovatively established patient-derived GBM organoids that perfectly recapitulate the molecular, genetic, and cell-type heterogeneity of parental tumors [42,43]. In contrast to previous approaches, the authors dissected tumor tissues into 1 mm fragments, omitting extracellular matrix and growth factors. These fragments were cultured on an orbital shaker for 1–2 weeks, forming 3D structures. Through histopathology, molecular profiling, and single-cell RNA sequencing (scRNA-seq), diverse cellular subtypes were confirmed that faithfully replicated tumor phenotypes. Importantly, these organoids mimicked GBM features, including a hypoxic gradient, preserved vasculature, and an intact TME [42]. A protocol for generating organoids from LGG primary tissues by utilizing physiologic (5%) oxygenation conditions was recently established. The *in vitro* model showed a 91% (20/22) success rate for the creation of grade 1–4 glioma and 87% (13/15) rate for grade 1–3 glioma [44]. The model recapitulated stemness, proliferation, stromal composition profiles, mutational traits, and metabolic characteristics of the respective parental tumor specimens [44]. Moreover, the integrity of these organoids could be maintained for weeks to months, thus facilitating prolonged study periods. Immunocompetent organoids have also emerged that allow interactions between tumor and immune cells, particularly in the context of immunotherapy. These models can be achieved by incorporating autologous or allogeneic peripheral blood mononuclear cells (PBMCs) or specific immune cell populations such as tumor-associated macrophages (TAMs) and tumor-infiltrating lymphocytes [19,42].

In addition, organoids retain the neoantigen features of the parental tumors. Neoantigen-reactive T cells could be generated by coculturing matched PBMCs with candidate peptides for targeting tumor-derived organoids, and the immunogenicity of neoantigen epitopes can also be rapidly evaluated in organoids [45]. However, none of the current *in vitro* models can fully replicate the complex interactions of malignant cells within the glioma TME. Therefore, the selection of the most appropriate *in vitro* model is of the utmost importance. The principal characteristics of these preclinical models are summarized in Figure 1 and Table 1.

Mutant IDH (IDHmt) gliomas need more accurate models

IDH mutations seem to promote gliomagenesis, and inhibitors of mutated IDH could be an effective therapy. Testing such inhibitors requires accurate *in vivo* models, and the establishment of accurate mouse models of IDHmt glioma is imperative. There are some established *in vivo* and *in vitro* models for IDHmt glioma, such as PDX and GEMM [46–48], but relatively few studies on IDHmt glioma models have been published despite the urgent need to fast-track IDH inhibitors to clinical trials [49]. Notably, a group utilized a humanized murine model to establish a **peptide vaccine** targeting the IDH1 mutation in IDHmt glioma that induced IFN-γ release from T cells [50]. Superiorities such as a safe profile, strong immunogenicity, and marked efficacy of IDH1 vaccines and combined therapies have been confirmed or are being investigated in clinical trials [51,52].

Table 1. Commonly used preclinical models for glioma immunological research[a]

Model name	Description	Tumor source	Animal immune status	Strengths	Limitations
Syngeneic mouse models	Transplantation of tumor cell lines from inbred mice into immunocompetent mice	Mouse (GL261, CT-2A, SMA-560 cell lines)	+	Well established and widely used in preclinical research; mimic GBM pathological features	High immunogenicity; high mutation load
GEMMs	the model indicates the mice whose genomes have been modified using genetic engineering techniques to study gene function and mimic human diseases	Mouse	+	Can be used to study pathogenic mechanisms for specific genes; partially mimic GBM histological and biological features	Disparities in the TME and immune system; expensive, less scalable
Humanized mouse models	Transplantation of patient-derived tumor cells, tissues, organoids, or stem cells into humanized mice	Human	Immunodeficient	Mimic GBM histological and biological features; low graft-versus-host rejection risk; preserve components of the human immune system; more suitable for immunotherapy efficacy evaluation	Do not fully replicate GBM clinical features
PDX	Transplantation of patient-derived tumor tissue or organoids into immunodeficient mice	Human	Immunodeficient	Mimics the histological and biological features of GBM; low graft-versus-host rejection risk	Dependence on an immunodeficient model that lacks essential components of human immune system; cannot fully assess the efficacy of various immunotherapies
GSCs	Unlike 2D tumor cell lines, 3D GSCs incorporate cellular diversity and provide robust platform for finding novel anticancer drug targets	Human	NA	Incorporate cellular diversity and enhance drug response predictability; maintain self-renewal, proliferation and genetic traits of human samples	Lack tissue-like organization and extracellular matrix; lack neighboring non-tumor cells; hinder studies on interactions between different cell types
Organoids	Sophisticated 3D structures comprising various cell types from primary tissue	Human	NA	Replicate the function, composition, and microenvironment of the original organ; closely simulate tumor sensitivity; bridge the gap between *in vivo* and *in vitro* models; allow interactions across different cell types	Unclear whether can be adapted for organoids from non-epithelial tumors; require more molecular and genetic characterization

[a]Abbreviations: GBM, glioblastoma; GEMM, genetically engineered mouse model; GSC, glioma stem cell; NA, not applicable; PDX, patient-derived xenograft; TME, tumor microenvironment.

Vaccine therapies in clinical trials

Various forms of single or combined vaccination therapies, such as peptide vaccines [51,53–69], **dendritic cell (DC) vaccines** [70–76], RNA/DNA vaccines [77], and other autologous vaccines [78,79], are currently under scrutiny in clinical trials (Table 2). However, our discourse focuses on peptide and DC vaccines.

Peptide vaccines

Peptide vaccines are typically categorized into two groups: long peptides, ranging from 15 to 31 amino acids in length, and short peptides, spanning 8–10 amino acids. Short peptides were commonly used in earlier clinical trials, whereas more recent trials have used long peptide neoantigen vaccines that elicit both $CD4^+$ and $CD8^+$ T cell responses [80]. Overall, peptide vaccines

Table 2. Key clinical trials investigating therapeutic vaccination against glioma[a]

Targets	Vaccine name	Clinical trial[b]	Phase	Populations	Main findings	Refs
Peptide vaccines						
EGFRvIII	Rindopepimut	ACTIVATE	Single arm, Phase 2, with a matched cohort	18 ndGBM EGFRvIII$^+$	mPFS, 14.2 months (95% CI 9.9–17.6); 6 months PFS, 67% (95% CI 40–83%); mOS, 26.0 months (95% CI 21–47.7)	[53]
	Rindopepimut	ACT II	Single arm, Phase 2, with a matched cohort	22 ndGBM EGFRvIII$^+$	mPFS, 11.8 months (95% CI 8.1–15.6); 6 months PFS, 81.8% (95% CI 58.5–92.8%); mOS, 19.3 months (95% CI 15.6–30.7); 12 months OS, 86.4% (95% CI 63.4–95.4%)	[54]
	Rindopepimut	ACT III	Single arm, Phase 2	65 ndGBM EGFRvIII$^+$	mPFS, 9.2 months (95% CI 7.4–11.3); mOS, 21.8 months (95% CI 17.9–26.5); mPFS for MGMT methylated, 14.7 months (95% CI 7.4–20.5); mOS for MGMT methylated, 21.8 months (95% CI 17.9–26.5)	[55]
	Rindopepimut	ACT IV	Randomized, Phase 3	745 ndGBM EGFRvIII$^+$; 371 in the rindopepimut + TMZ group versus 374 in the TMZ group	mOS, 20.1 months (95% CI 18.5–22.1) versus 20.0 months (95% CI 18.1–21.9) (HR 1.01, 95% CI 0.79–1.30); mPFS, 8.0 months (95% CI 7.1–8.5) versus 7.4 months (95% CI 6.0–8.7) (HR 1.01, 95% CI 0.80–1.29)	[56]
	Rindopepimut	ReACT (NCT01498328)	Randomized, Phase 2	73 rGBM EGFRvIII$^+$; 36 in rindopepimut + BEV group versus 37 in BEV group	mPFS, HR 0.72 (95% CI 0.43–1.21); mOS, HR 0.53 (95% CI 0.32–0.88)	[57]
IDH1-R132H	IDH1-vac	NOA-16 (NCT02454634)	Single arm, Phase 1	33 newly diagnosed IDHmt, grade 3–4 glioma	3 year PFS, 63% (95% CI 44–77%); 3 year OS, 84% (95% CI 67–93%)	[51]
HSP	HSPPC-96	NCT00293423	Single arm, Phase 2	41 rGBM	mOS, 10.7 months (95% CI 8.7–12.6); 6 months OS, 90.2% (95% CI 75.9–96.8%); 1 year OS, 29.3% (95% CI 16.6–45.7%)	[58]
	HSPPC-97	NCT00905060	Single arm, Phase 2	46 ndGBM	mOS, 23.8 months (95% CI 19.8–30.2); mPFS, 18.0 months (95% CI 12.4–21.8); 2 year OS, 50.0% (95% CI 35.1–64.9%); 3 year OS, 32.6% (95% CI 20.0–48.1%)	[59]
Survivin	SurVaxM	NCT01250470	Single arm, Phase 1	Nine recurrent glioma	mOS, 21.7 months; mPFS, 4.4 months	[60]
	SurVaxM	NCT02455557	Single arm, Phase 2a	64 ndGBM	mPFS, 11.4 months (95% CI 9.9–12.7); mOS, 25.9 months (95% CI 22.5–29.0); 6 months PFS, 69.8% (95% CI 56.8–79.5%); 1 year PFS, 47.6% (95% CI 34.9–59.3%); 1 year OS, 87.2% (95% CI 76.1–93.4%)	[61]
Personalized antigen/TAAs	APVAC1/APVAC2	GAPVAC-101 (NCT02149225)	Single arm, Phase 1	15 nGBM; 15 received APVAC1 before SOP, 11 received APVAC2 after SOP	mOS, 29.0 months; mPFS, 14.2 months; APVAC1 mainly induced a CD8$^+$ T response; APVAC2 mainly induced a CD4$^+$ T helper cell response	[62]

(continued on next page)

Table 2. (continued)

Targets	Vaccine name	Clinical trial[b]	Phase	Populations	Main findings	Refs
	PPV poly-ICLC	NCT02287428	Single arm, Phase 1/1b	Eight ndGBM with MGMT unmethylated	mOS, 16.8 months; mPFS, 7.6 months; tumor-infiltrating T cell migration	[63]
	PPV HLA-24	NA	Randomized, Phase 3	88 rGBM; 58 in PPV group versus 30 in the placebo group	mOS, 8.4 months (95% CI 6.6–10.6) versus 8.0 months (95% CI 4.8–12.9) (HR 1.13, 95% CI 0.60–1.90)	[64]
	IMA950 poly-ICLC	NCT01920191	Single arm, Phase 1/2	16 ndGBM and three grade 3 astrocytoma	mOS, 19.0 months; 6 months PFS, 68%; 9 months PFS, 58%	[65]
	IMA951 poly-ICLC	NCT03665545	Single arm, Phase 1/2, *post hoc* study	56 rHGG; 16 in IMA950 group versus 40 in the non-vaccinated group	mOS, 7.8 months (95% CI 6.9–8.7) versus 10.0 months (95% CI 7.4–12.6; $P = 0.69$); mPFS, 2.6 months (95% CI 0.4–2.9) versus 4.2 months (95% CI 2.8–5.5; $P = 0.50$)	[66]
	IMA952	NCT01222221	Single arm, Phase 1	45 ndGBM receiving IMA950 plus GM-CSF	mOS, 15.3 months; 6 months PFS, 74%; 9 month PFS, 31%	[67]
WT1	WT2725	NCT01621542	Single arm, Phase 1	44 advanced solid tumors including GBM	mPFS, 1.97 months (95% CI 0.9–11.0); mOS, 10.2 months (95% CI 4.3–22.5)	[68]
H3K27M	H3-vac	NCT04808245	Single arm, Phase 1	Eight H3K27M-mt DMG	mPFS, 6.2 months; mOS, 12.8 months; H3K27M-vac mainly induced a $CD4^+$ T helper cell response	[69]
DC vaccines						
TAAs/neoantigens (HER2, TRP2, gp100, MAGE1, IL13RA2, AIM2, etc.)	DCVax	NCT00068510	Single arm, Phase 1	23 GBM: 15 ndGBM, 8 rGBM	TTP, 15.9 months; mOS, 31.4 months; 1 year OS, 91%; 2 year OS, 55%; 3 year OS, 47%; mOS for nGBM, 35.9 months; 1 year OS for nGBM, 93%; 2 year OS for nGBM, 77%; 3 year OS for nGBM, 58%; mOS for rGBM, 17.9 months	[70]
	DCVax-L	NCT00045968	Non-randomized, Phase 3, with a matched cohort	331 GBM: 267 ndGBM, 64 rGBM; 232 in the DCVax-L group, 99 in the placebo group	For nGBM, mOS was 19.3 months (95% CI 17.5–21.3) versus 16.5 months (95% CI 16.0–17.5) (HR 0.80, 98% CI 0.00–0.94); for rGBM, mOS 13.2 months (95% CI 9.7–16.8) versus 7.8 months (95% CI 7.2–8.2) (HR 0.58, 98% CI 0.00–0.76)	[71]
	ICT-107	NA	Single arm, Phase 1	21 GBM: 17 ndGBM, three rGBM, one brainstem glioma	mPFS, 16.9 months (95% CI 8.9–49.8); 6 months PFS, 100%; 1 year PFS, 62.5% (95% CI 34.9–81.1%); mOS, 38.4 months (95% CI 25.9–40.7); 6 months OS, 100%; 1 year OS, 100%	[72]
		NCT01280552	Randomized, Phase 2	124 ndGBM; 81 in the ICT-107 group, 42 in the control group	mOS, 17.0 months (95% CI 13.7–20.6) versus 15.0 months (95% CI 12.3–23.1) (HR 0.87, $P = 0.58$); mPFS, 11.2 months (95% CI 8.2–13.1) versus 9.0 months (95% CI 5.5–10.3) (HR 0.57, $P = 0.011$)	[73]

Table 2. (continued)

Targets	Vaccine name	Clinical trial[b]	Phase	Populations	Main findings	Refs
	AV-GB-1	NCT03400917	Single arm, Phase 2	57 ndGBM	mOS, 14.0 months (95% CI 10.1–18.3); 6 months OS, 87.5%; 1 year OS, 55.4%; 18 month OS, 38.5%; mPFS, 8.5 months (95% CI 6.5–9.1); 6 months PFS, 69.7%; 1 year PFS, 26.8%; 18 months OS, 16.1%	[74]
	pp65-DC	ATTAC	Single arm, Phase 2	12 ndGBM	mPFS, 10.8 months; mOS, 18.5 months	[75]
		ATTAC-GM (NCT00639639)	Single arm, Phase 2	11 ndGBM	mPFS, 25.3 months (95% CI 11.0–NA); mOS, 41.1 months (95% CI 21.6–NA)	[76]
DNA vaccine						
IGF-1R	IGV-001	NCT02507583	Single arm, Phase 1b with a matched cohort	33 ndGBM	mPFS, 9.8 months; 6 months PFS, 85%; mOS, 17.3 months	[77]
Other autologous vaccines						
TAAs	AFTV	NA	Single arm, Phase 1	12 ndGBM	mOS, 10.7 months; mOS for responders, 20.3 months; mOS for non-responders, 5.0 months	[78]
		NA	Single arm, Phase 1/2a	24 ndGBM	mPFS, 8.2 months; mOS, 22.2 months; 2 year OS, 47%; 2 year PFS, 33%; 3 year OS, 38%	[79]

[a]Abbreviations: AIM2, absent in melanoma 2; BEV, bevacizumab; CI, confidence interval; DCs, dendritic cells; DMG, diffuse mideline glioma; EGFRvIII, epidermal growth factor receptor variant III; GBM, glioblastoma; GM-CSF, granulocyte-macrophage colony stimulating factor; H3K27M, lysine to methionine substitution at position 27 in histone H3; HER2, human epidermal growth factor receptor 2; HGG, high-grade glioma; HLA, human leukocyte antigen; HR, hazard ratio; HSP, heat-shock protein; IDH, isocitrate dehydrogenase; IGF-1R, insulin-like growth factor 1 receptor; IL13RA2, interleukin 13 receptor α2; MAGE1, melanoma-associated antigen 1; MGMT, O^6-methylguanine-DNA methyltransferase; mOS, median overall survival; mPFS, median progression-free survival; nGBM, newly diagnosed GBM; poly-ICLC, polyinosinic–polycytidylic acid, stabilized with poly-L-lysine and carboxymethylcellulose; PPV, personalized peptide vaccine; rGBM, recurrent GBM; rHGG, recurrent HGG; SOP, standard of care; TAA, tumor-associated antigen; TERT, telomerase reverse transcriptase; TMZ, temozolomide; TRP2, tyrosinase-related protein 2; TTP, time to progression.
[b]Clinical trials registered at https://clinicaltrials.gov/.

epitomize conventional vaccination methods owing to their rapid and cost-effective generation. IDH stands out as a prominent illustration of shared driver mutations in neuro-oncology that exhibit a remarkably high prevalence in gliomas. The majority of IDH mutations, particularly replacement of arginine 132 by histidine (IDH1R132H), generate an immunogenic neoepitope that can be presented on MHC-II within glioma tissue, prompting spontaneous $CD4^+$ T cell responses, and can be targeted through peptide vaccination in MHC-humanized preclinical models [50,81]. The pioneering NOA-16 Phase 1 clinical trial assessed a 20 amino acid vaccine in newly diagnosed grade 3–4 IDHmt astrocytoma patients. Vaccine-induced immune responses were observed in 93.3% (30/32) of patients, with a 3 year progression-free survival (PFS) of 64% and a 3 year OS rate of 84% [51]. No significant adverse events were observed [51]. The encouraging outcomes from NOA-16 offer justification for advancing to Phase 2 trials and exploring combination strategies to amplify the benefits of the vaccine. Intriguingly, in NOA-16, patients with both sustained and transient peripheral immune responses demonstrated satisfactory clinical responses. It is plausible to hypothesize that a robust peripheral T cell response preceding T cell invasion into the brain may predict the response to the tumor vaccine. Another Phase 1 trial is currently investigating combined therapy with an IDH vaccine (IDH-vac) together with ICIs in a randomized, three-arm design [52]. Similar to the IDH mutation, the histone H3 lysine-

to-methionine mutation at position 27 (H3K27M) is both clonal and tumor-specific [82]. Although the majority of H3K27M-mutant (H3K27Mmt) gliomas afflict pediatric patients, the mutation is also a recurrent event in midline and infratentorial gliomas in young adults, and these are generally defined as H3K27Mmt diffuse midline glioma (DMG) which is associated with a poor prognosis. Earlier studies demonstrated that an H3K27M-specific long peptide vaccine (H3K27M-vac) induces mutation-specific immune responses against H3K27Mmt HGG in MHC-humanized mouse models [83]. The outcomes from the inaugural H3K27M-vac treatment trial have now been disclosed, encompassing eight patients with H3K27Mmt DMG. Among these, five patients received H3K27M-vac in conjunction with anti-PD-1 treatment [69]. The median PFS was 6.2 months, and median OS reached 12.8 months for all participants. Importantly, the vaccine demonstrated a favorable safety profile [69]. Through TCR sequencing and various *in vitro* experiments, researchers additionally observed that H3K27M-vac predominantly induces mutation-specific $CD4^+$ T cell responses, akin to the findings in the NOA-16 trial. Notably, T cell clones were identified in both peripheral blood and cerebrospinal fluid (CSF) [69]. These findings not only affirm the efficacy of H3K27M-vac but also contribute essential insights into the fundamental mechanisms underlying vaccine immunotherapy. Several Phase 1 trials evaluating H3K27M-vac as a monotherapy or in combination with other treatments are currently ongoing.

EGFRvIII represents a prominent shared neoepitope target that is expressed in 20–30% of GBM. In preliminary trials the EGFRvIII-specific peptide vaccine, rindopepimut, elicited a robust, durable, and promising immune response. However, it is noteworthy that the poor function of peripheral T cells make it difficult to evaluate real T cell-mediated activity, and a significant proportion of tumors lose EGFRvIII expression after recurrence [55]. The ACT-IV trial, a Phase 3 randomized study, is currently comparing rindopepimut to chemotherapy with temozolomide in newly diagnosed GBM (ndGBM) patients harboring EGFRvIII mutations [56]. Unfortunately, rindopepimut did not yield a significant improvement in OS, which was the primary outcome in the ACT-IV trial. Furthermore, there was no evidence of vaccine-driven antigen loss at tumor recurrence, which serves as a surrogate for vaccine-induced biological activity [56]. Valuable lessons, such as the importance of carefully interpreting the results of early-stage clinical trials, gaining a deeper understanding of the underlying mechanisms, and refining trial design, can be gleaned from the ACT-IV trial. Notably, findings from the Phase 2 ReACT trial indicate that the combination of rindopepimut and bevacizumab prolonged OS for recurrent GBM (rGBM) patients with EGFRvIII mutation [57]. Bevacizumab appears to augment the T cell response of rindopepimut, although this observation requires further validation.

Another noteworthy approach involves individualized peptide vaccines that can broaden potential patient populations and enhance clinical benefits [84]. In the Phase 1 GAPVAC trial, an individualized peptide vaccine was integrated into the standard of care for GBM patients. This trial used two treatment phases to extend the therapeutic window. Patients were randomly allocated to two cohorts: in one cohort, patients received the APVAC1 vaccine before standard of care, whereas in the other cohort patients received the APVAC2 vaccine at least 1 week after completing the standard-of-care cycle [62]. The trial encompassed 15 patients, and the median OS and PFS were 29.0 and 14.2 months, respectively [62]. Through *in vitro* and sequencing experiments, APVAC1 induced durable $CD8^+$ T cell immunity lasting for several months. APVAC2 was found to elicit multifunctional $CD4^+$ T cell responses, some of which were accompanied by $CD8^+$ T cell activity [62]. The GAPVAC trial highlights two issues: the necessity to explore HLA class I epitopes to enhance the success of glioma vaccination, and the potential exploitability of HLA class II epitopes that exhibit higher immunogenicity than class I epitopes.

DC vaccines

DC-mediated recognition and processing of tumor antigens can be bypassed by preloading DCs with the specific tumor antigen of interest before vaccination [85]. Autologous DCs, generated *ex vivo*, are loaded with **tumor-associated antigens (TAAs)** through methods such as whole-tumor lysates, coculture with antigenic peptides, viral transfection, or mRNA electroporation [85]. These matured, antigen-loaded DCs are then reintroduced into patients as a vaccine with the aim of reaching draining lymph nodes to transfer antigens and prime T cells. DC vaccines, through targeting TAAs, can stimulate the immune system to recognize, target, and eliminate tumor cells.

To date, various DC vaccines have been developed and explored in clinical trials. These have targeted TAAs such as WT1, HER2, IL13Rα2, survivin, and MAGE-A3, as well as neoantigens such as EGFRvIII and IDH1R132H [86–88]. The majority of these trials are in Phase 1/2. In a notable Phase 3 trial, an autologous DC vaccine (DCVax-L) was investigated in combination with standard of care for both ndGBM and rGBM compared to a contemporaneous matched external cohort [71]. Initially, 331 ndGBM were enrolled after standard of care, and 232 received DCVax-L

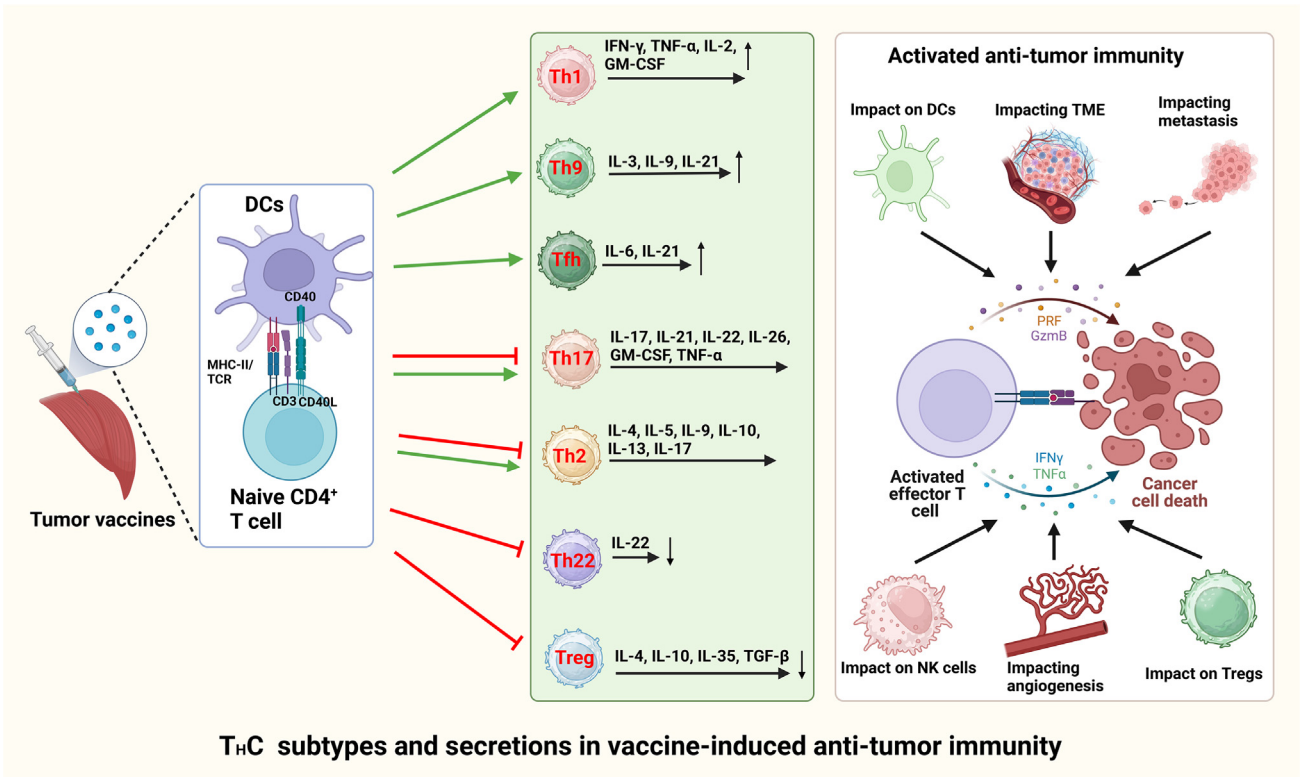

Figure 2. T helper cell subtypes and secretions in vaccine-induced antitumor immune responses. Activation of T cell receptor (TCR) signaling, costimulation, and the presence of specific cytokines have been demonstrated to stimulate the polarization of naive $CD4^+$ T cells and their differentiation into various T helper subtypes, including type 1 T helper (Th1), Th9, Th17, Th2, and Th22 cells, as well as T follicular helper (Tfh) cells and regulatory T cells (Tregs). After the injection of tumor vaccine, Th1, Th9, and Tfh cells that mainly display antitumor immune activity and stimulate the immune response are potentially activated, whereas Th22 and Treg cells are thought to predominantly induce an immunosuppressive tumor microenvironment (TME) and support tumorigenesis. Th17 and Th2 cells have both antitumor and pro-tumor functions. Th1 cells potentially inhibit metastasis, angiogenesis, and Treg activity while enhancing the activity of M1-like macrophages, cytotoxic T lymphocytes (CTLs), and B cells through the secretion of IFN-γ, TNF-α, IL-2, and GM-CSF. Th9 cells activate $CD8^+$ T cells, dendritic cells (DCs), and Tregs in the TME via IL-3, IL-9, and IL-21. Tfh cells predominantly promote B cell antitumor activity and inflammation in the TME via IL-6 and IL-21. On the other hand, Th22 cells and Tregs mainly regulate stromal function, inhibit immune cell infiltration, and induce tumor stemness to facilitate immune escape, thereby promoting tumor proliferation and migration, via the secretion of IL-22, IL-4, IL-10, IL-35, and TGF-β. Abbreviations: GM-CSF, granulocyte-macrophage colony stimulating factor; GZMB, granzyme B; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; NK cells, natural killer cells; EMT, epithelial–mesenchymal transition; PRF, perforin; TNF, tumor necrosis factor. Figure created with BioRender.

and 99 received a placebo. However, 64 patients in the placebo group experienced recurrence and subsequently crossed over to receive DCVax-L, while 120 patients in the DCVax-L group continued with DCVax-L treatment [71]. The external cohort consisted of patients receiving standard of care or approved therapies. Among the 232 ndGBM and 64 rGBM patients, the median OS was notably longer than the matched external cohort (22.4 months vs 16.5 months for ndGBM; 13.2 months vs 7.8 months for rGBM) [71]. Despite the observed survival benefit with DCVax-L in both ndGBM and rGBM, the crossover methodology and limitations of the historical control group raise concerns about the generalizability of these results. Future studies in this field should prioritize well-designed control groups to enhance the robustness of the findings.

RNA vaccines

mRNA emerges as a preferable option for tumor vaccines for several reasons. Patient-derived mRNA can be efficiently amplified *in vitro*, thus requiring only a small fraction of cells for amplification, and mRNA-based vaccines can be developed even with limited tissue samples [89]; in addition, mRNA has a safer profile than DNA because there is no risk of integration into the human genome [89–91]. One of the earliest platforms for investigating mRNA vaccines in human GBM is the DC-pulsed mRNA vaccine. Several small clinical trials have demonstrated that this type of mRNA vaccine induces significant $CD8^+$ cytolytic T cell activity, thus potentially reducing recurrence rates and prolonging OS in GBM patients. Moreover, long-term survivors exhibited a durable phosphoprotein 65 (pp65)-specific immune response for several months,

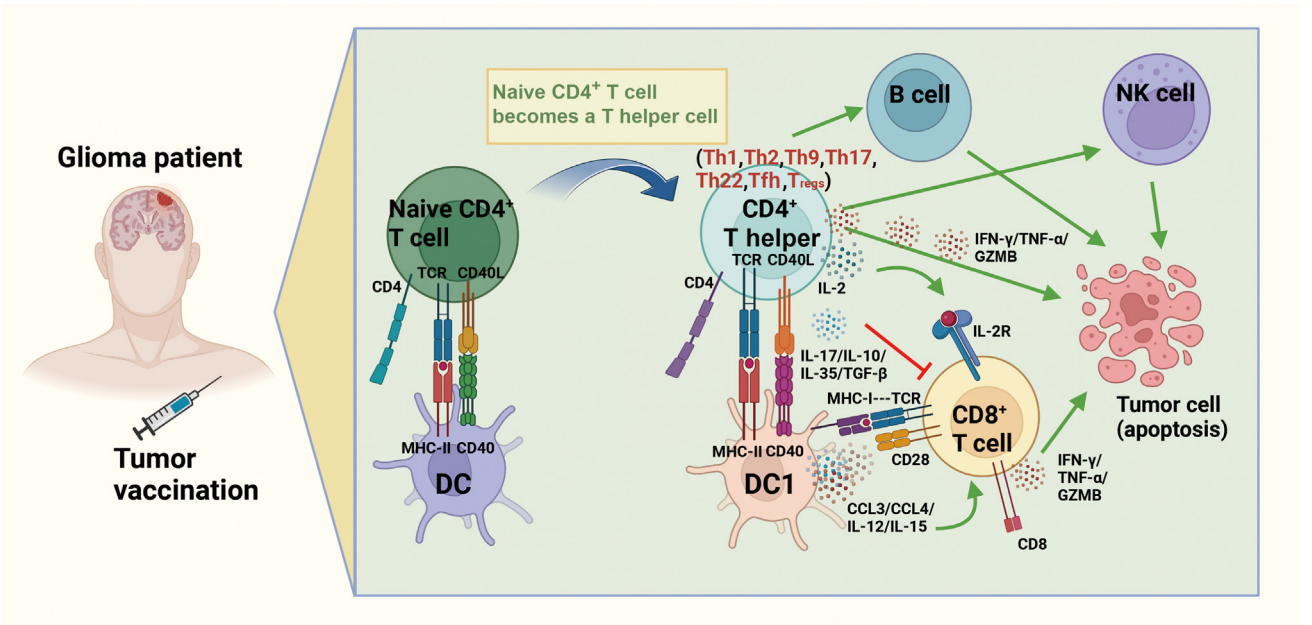

Figure 3. Biological activity of T helper cells in promoting tumor microenvironment (TME) inflammation after vaccination. Through interactions with dendritic cells (DCs), naive $CD4^+$ T cells can develop into T helper cell subsets. T helper cells license DCs for cross-presentation by CD40–CD40L interaction, which leads to upregulation of MHC-II on DCs. Licensed DCs secrete CCL3/4/5, as well as IL-12 and IL-15, thereby recruiting and promoting the proliferation of $CD8^+$ T cells via CD25 (IL-2Rα) induction on $CD8^+$ T cells in response to IL-2 from T helper cells. On the other hand, IL-17, IL-10, IL-35, and TGF-β may inhibit $CD8^+$ T cell cytotoxicity and reduce the release of IFN-γ, TNF-α, and GZMB for killing tumors. Via tumoral MHC-II, which can be induced by T helper cell-derived IFN-γ, T helper cells may directly kill tumor cells by secreting GZMB. In addition, T helper cells can promote the role of B cells in antitumor activity and induce natural killer (NK) cell responses to promote tumor cell apoptosis. Abbreviations: CCL3/4/5, C-C motif chemokine ligands 3, 4, and 5; CD40L, CD40 ligand; GZMB, granzyme B; IFN-γ, interferon γ; IL, interleukin; IL-2Rα, IL2 receptor α; MHC, major histocompatibility complex; TCR, T cell receptor; Tfh, T follicular helper cell; TGF-β, transforming growth factor β; Th1/2/9/17/22, type 1/2/9/17/22 T helper cells; TNF-α, tumor necrosis factor α; Tregs, regulatory T cells. Figure created with BioRender.

and enhanced pp65–specific INF-γ levels correlated positively with longer OS. Several ongoing Phase 1 and 2 trials (NCT02465268, NCT03688178, NCT04573140) are evaluating DC-pulsed mRNA vaccines encoding cytomegalovirus (CMV) LAMP-pp65 tumor antigens, although their results have not yet been presented [91,92]. These findings underscore the effectiveness of mRNA vaccines in GBM treatment, and emphasize the need for further research to expand large-scale good manufacturing practices, optimize administration and adjuvant selection, and precisely identify antigen candidates.

$CD4^+$ T helper cells in tumor vaccination

Neoantigens mainly induce specific CD4 T cell response

Historically, tumor vaccines have predominantly focused on eliciting a cytotoxic $CD8^+$ T cell response against neoepitopes presented on MHC-I. However, it is noteworthy that the majority of neoantigens are presented on MHC-II molecules, thereby eliciting a response from $CD4^+$ **T helper cells** [93]. In the context of ICI therapy, efficacy in experimental glioma treatment relies not solely on cytotoxic $CD8^+$ T cells but also on antigen-specific $CD4^+$ T cells, particularly in tumors expressing MHC-II-restricted neoantigens [94]. Moreover, clinical investigations have indicated that personalized neoepitope-specific vaccines predominantly provoke a $CD4^+$ T cell response rather than a $CD8^+$ T cell response in the realm of cancer treatment [95]. The efficacy of neoepitope-specific vaccines hinges not only on the quantity and antigenicity of the neoepitopes but also on their clonality. For this reason, driver mutations emerge as an attractive target for immunotherapies because neoantigens stemming from such mutations predominantly manifest clonality [96].

Types and roles of CD4 T helper cells

T helper cells undergo polarization towards distinct effector types, including type 1 T helper (Th1), Th2, Th9, Th17, and Th22 cells, as well as T follicular helper (Tfh) cells and regulatory T cells (Tregs) [97,98]. Their profiles and secretions, that are pivotal in promoting antitumor immunity, are delineated in Figure 2. Specifically, Th1 and Th2 cells represent two highly prominent subclasses that are characterized by the early production of IFN-γ and interleukin 4 (IL-4), respectively. Acknowledging the potential antineoplastic attributes of Th1 cells, some investigations have utilized Th1 cells to augment the efficacy of T cell immunotherapy in oncology. In a murine model of GBM, $CD4^+$ CAR-T cells expressing the Th1 phenotype demonstrated protracted persistence and sustained effector potency compared to the corresponding $CD8^+$ CAR-T cells [99]. Similarly, TCR-engineered T cells targeting human telomerase reverse transcriptase (TERT) exhibited attributes aligning with the Th1 subset and displayed a robust antitumoral response in a xenograft mouse model [100].

Through differentiation, Th2 cells that release interleukins IL-4, IL-5, IL-10, IL-13, and IL-17 have dichotomous roles that encompass both antitumor and pro-tumor functions [101,102]. In a murine lymphoma model, Th2 cells demonstrated comparable *in vivo* antitumor activity to Th1 cells; nevertheless, the mechanisms of action diverged significantly between these two subsets. Specifically, Th1 cells stimulated cellular immunity and lymphocyte infiltration, whereas Th2 cells fostered inflammatory responses and contributed to tumor necrosis [103]. Tfh cells may also contribute. Their primarily function is to facilitate B cell proliferation, somatic hypermutation, and class-switch recombination [104]. In addition, through their participation in B cell function, Tfh cells may also instigate antitumoral antibody responses.

Mechanisms of T helper cells in inducing antitumor immunity

In the sphere of immunotherapy, $CD4^+$ T helper cells assume a pivotal role in orchestrating an optimal $CD8^+$ T cell-mediated antitumor immune response [105]. In an MHC-II-deficient model of squamous cell carcinoma there was considerable diversity among neoantigen-specific TCRs

Clinician's corner

The immunosuppressive tumor microenvironment, lymphopenia after radiotherapy and chemotherapy, and sequestration of T cells in the bone marrow contribute to the failure of immune checkpoint inhibitors (ICIs) in glioma. Therefore, novel strategies are necessary.

In vivo models in glioma immunological research include syngeneic mouse models, genetically engineered mouse models, patient-derived xenotransplantation, and humanized mouse models; *in vitro* models include glioma stem cells and organoids. However, no model is perfect in glioma research.

IDH mutant gliomas have a different biological activity from glioblastoma (GBM), and it is imperative to establish accurate models for this type of glioma.

$CD4^+$ T helper cells actively interact with dendritic cells (DCs), other $CD4^+$ T cells, $CD8^+$ T cells, B cells, and natural killer cells in vaccine-specific immunity; T helper cells can kill tumor cells directly via secreting IFN-γ, TNF-α, and GZMB.

Vaccines being investigated in clinical trials include peptide vaccines, DC vaccines, RNA/DNA vaccines, and autologous vaccines, of which peptide and DC vaccines show the most clinical benefit.

Given that many vaccine trials are at the Phase 1/2 stage, clinicians should prepare for larger-scale studies in the future.

under adoptive immunotherapy. $CD4^+$ T cells expressing high- or moderate-avidity TCRs undergo *in vivo* proliferation to cross-present antigens, and neoantigen-specific $CD4^+$ T cells exhibit enhanced efficacy when exposed to IL-7/IL-15. $CD8^+$ T cells expressing low levels of PD-1 are observed in the TME, whereas $PD\text{-}1^+$ $CD8^+$ T cells are principally found in tumor-draining lymph nodes [105]. Interactions between T helper cells and DCs, as well as with tumor-infiltrating myeloid cells, play a pivotal role in transmitting essential multimodal signals that augment the function of cytotoxic T lymphocytes (CTLs) [106]. In the initial stages, $CD4^+$ and $CD8^+$ T cells independently encounter antigens, a process facilitated by distinct subsets of conventional DCs (cDCs) subsequent to immunization [107]. Interactions between CD40 on cDCs and its cognate ligand CD40L on T helper cells constitute a pivotal step in the licensing process. This interaction augments antigen cross-presentation by DCs and facilitates direct engagement with $CD8^+$ T cells [107]. Subsequently, T helper cells convey advantageous signals which are then transmitted to $CD8^+$ T cells, thereby promoting antitumor efficacy. Finally, T helper cells can directly induce apoptosis in tumor cells by releasing factors such as IFN-γ, tumor necrosis factor α (TNF-α), and granzyme B (GZMB). The principal biological activities of T helper cells in the antitumor process are delineated in Figure 3.

Concluding remarks and future directions

This review has addressed the preclinical models used to study glioma immunotherapy, and has underscored the central role of T helper cells in driving immune responses triggered by tumor vaccines and summarized current clinical settings for glioma vaccines. Although some immunotherapeutic strategies demonstrate substantial benefits in preclinical models, they generally fail in real clinical settings. Therefore, we require advanced and accurate preclinical models to fill the gap between the two study types (see Outstanding questions). In addition, many basic and clinical studies have been conducted based on earlier CNS tumor classifications, and this complicates interpretation of the findings.

Glioma vaccines hold substantial promise, and future research will need to address several key areas. First, there is a need for meticulous selection of tumor-specific neoepitopes/neoantigens, with a focus on their association with tumor stemness and antigen persistence, to catalyze the development of novel vaccine types. Moreover, the development of precise individual-mimic preclinical models should be advanced, and multi-omics and sequencing should be leveraged for multimodal biomarkers to predict immunotherapy responses, and spatial transcriptomics can aid in characterizing distinct spatial positions and cell–cell interactions. Other key areas include the development of DNA vaccine vectors for the delivery of antigen-specific CARs or TCRs for heightened therapeutic potential, and investigations into vaccine monotherapy or combined therapy with ACT or ICIs through larger-scale clinical trials because novel CAR-T therapies have shown promising efficacy in small-scale clinical trials [12,108]. Lastly, it is crucial to refine the studies based on the new glioma classification criteria. Overall, despite the need for refinement, tumor vaccines emerge as burgeoning immunotherapies that are poised to bestow clinical benefits upon glioma patients.

Outstanding questions

What are the commonly used preclinical models in glioma immunological research? What are the advantages and disadvantages of each?

What should we consider as preclinical models for IDHmt glioma, and what are the difficulties in generating the specific models?

What is the role of T helper cells in tumor vaccination treatment? How do they interact with other immune cell types to promote an antitumor immune response?

Why are antigen-presenting cells and myeloid cells so essential for tumor vaccination, and what is the detailed mechanism in each case?

Which vaccines are in clinical trials for glioma? What are the differences between vaccines targeting neoantigens and TAAs?

What are the future directions for fundamental and clinical studies on tumor vaccines?

Author contributions

All authors contributed to the concept and design of this work, the acquisition, analysis, and interpretation of data, and drafting the manuscript. M.H. and T.S critical revised the manuscript for important intellectual content, and L.Y. and T.S supervised the project. All authors read and approved the final manuscript.

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Declaration of interests

The authors declare no conflicts of interests.

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