Immunotherapy and radiation for high-grade glioma: a narrative review

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Abstract: Glioblastoma and other high-grade gliomas (HGGs) are the most common and deadly primary brain tumors. Due to recent advances in immunotherapy and improved clinical outcomes in other disease sites, the study of immunotherapy in HGG has increased significantly. Herein, we summarize and evaluate existing evidence and ongoing clinical trials investigating the use of immunotherapy in the treatment of HGG, including therapeutic vaccination, immune checkpoint inhibition, adoptive lymphocyte transfer, and combinatorial approaches utilizing radiation and multiple modalities of immunotherapy. Special attention is given to the mechanisms by which radiation may improve immunogenicity in HGG, why this motivates the study of radiation in combination with immunotherapy, and how to determine optimal dosing and scheduling of radiation. Though larger randomized controlled trials have not consistently shown improvements in clinical outcomes, this area of research is still in its early stages and a number of important lessons can be taken away from the studies that have been completed to date. Many studies found a subset of patients who experienced durable responses, and analysis of their immune cells and tumor cells can be used to identify biomarkers that predict therapeutic response, as well as additional glioma-specific targets that can enhance therapeutic efficacy in a challenging tumor type.

Keywords: Glioblastoma (GBM); vaccine; adoptive transfer; immune checkpoint inhibitor (ICI); radiotherapy

Introduction

Primary brain tumors are a heterogeneous group of cancers with an estimated 23,820 new cases and 17,760 deaths in the United States in 2019 (1). Glioblastoma (GBM), a type of high-grade glioma (HGG), is the most common primary brain tumor, accounting for about 80% of cases, affecting patients of all ages. Standard treatment for GBM includes maximal safe resection followed by chemoradiation [radiotherapy with daily temozolomide (TMZ)] and 6–12 cycles of adjuvant TMZ, with or without alternating electric field therapy (2-4). Despite advances in all three modalities, clinical outcomes after standard treatment remain poor with a median survival in the range of 15 months and 5-year overall survival (OS) of less than 10% (5,6). Unfortunately, with a median progression-free survival (PFS) of approximately 7 months after initial treatment, patients overwhelmingly develop recurrent disease, portending a median survival of 1–4.5 months from

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the time of progression (7). Various second-line treatments including additional surgical resection, alternating electric field therapy, bevacizumab (anti-VEGF), and chemotherapy are used, but none have consistently demonstrated prolonged survival after recurrence (8-13). In recent years, immune checkpoint inhibitors (ICIs) have demonstrated efficacy in multiple malignancies, including melanoma (14-16), renal cell carcinoma (17), non-small cell lung cancer (NSCLC) (18,19), and small cell lung cancer (20). In response to the clear need for improved treatments, and the promising results seen in other settings, the investigation of immunotherapy for the treatment of primary brain tumors has increased significantly. In this review, we will discuss the unique immunologic characteristics of the central nervous system (CNS), as well as the existing and emerging evidence regarding the use of immunotherapy in HGG. We will then focus our discussion on combinatorial therapies with an emphasis on the use of immunotherapy in conjunction with radiation in HGG. Clinical trials were identified based on the most advanced phase of research and most recent publication for all types of immunotherapy in HGG. We present the following article in accordance with the narrative review reporting checklist (available at http://dx.doi.org/10.21037/tcr-20-1933).

Immune reactions in the CNS

The CNS has traditionally been considered an immune-privileged site. This assumption was based, in part, on observations that allogeneic tissue grafts placed in the brains of animal models could actively grow (21). This phenomenon was attributed to the presence of the blood-brain barrier (BBB) and the absence of a lymphatic drainage system or specialized antigen presenting cells (APCs) (22-24). However, this assumption has been challenged by a number of studies that have instead shown an immune-specialized capacity of the CNS (25). Microglia have been identified as resident APCs in the CNS given their phenotypical and functional similarities to professional APCs, namely dendritic cells (DCs) and macrophages (26,27). Pathways of lymphatic drainage have been identified along cranial nerve sheaths (28), with up to 47% of colony-stimulating factor (CSF) draining to cervical lymph nodes observed in one study of radio-labeled albumin injected into the brains of rabbits (29), with confirmation in more modern studies (30,31). The BBB, while regulating ion concentrations and preventing the passive transport of macromolecules (32), does not uniformly prevent the passage of immune cells. Activated cytotoxic T cells (CTLs) specific to CNS antigens cross the BBB (33). These findings suggest that tumor-specific antigens (TSAs) could be presented by microglia within the CNS or by professional APCs within cervical lymph nodes and initiate a T cell-mediated response against tumor cells. A summary of immune mechanisms and therapeutic targets is shown in *Figure 1*.

The critical question now is not whether tumor-directed immune reactions occur in the CNS, but rather how the immune specializations within the brain can hinder or enhance a tumor-specific immune response. We will discuss the history and current state of clinical research of immunotherapy in HGG, as well as how the lessons learned from these studies are directing ongoing clinical trials and future research.

Therapeutic vaccination

Therapeutic vaccination is intended to generate a tumor- and patient-specific immune response (34). This is achieved by inoculating the patient with TSAs or tumor-associated antigens (TAAs). These antigens are ingested by DCs and other professional APCs and presented to naïve CD8$^+$ and CD4$^+$ T cells via major histocompatibility complex (MHC)-I and MHC-II, respectively. *In vivo* or co-administered stimulatory agents lead these T cells to mature into antigen-specific CTLs (CD8$^+$), which initiate a cell-mediated response, and helper T cells (CD4$^+$ Th1 and Th2 cells), which initiate an antibody-mediated response (35). Ideally, this cascade leads to an acquired tumor-directed immune response that correlates to clinically significant disease control. While meant to achieve the same end result, vaccines come in various forms, including peptide vaccines, heat shock protein (HSP) vaccines, and DC vaccines, each of which are forms of active immunotherapy, in which the immune system is activated to target cancer cells (36,37). On the other hand, viral, or oncolytic, vaccines are a form of gene therapy, in which a tumor-associated gene is modified to create a tumor-targeted virus (36). Completed and ongoing clinical trials investigating these therapies in adult HGG are summarized in *Table 1*.

Peptide vaccines: active immunotherapy

Peptide vaccines are non-cell-based vaccines by which tumor antigen is directly inoculated, to be ingested by APCs and presented to T cells. These antigens are often prepared
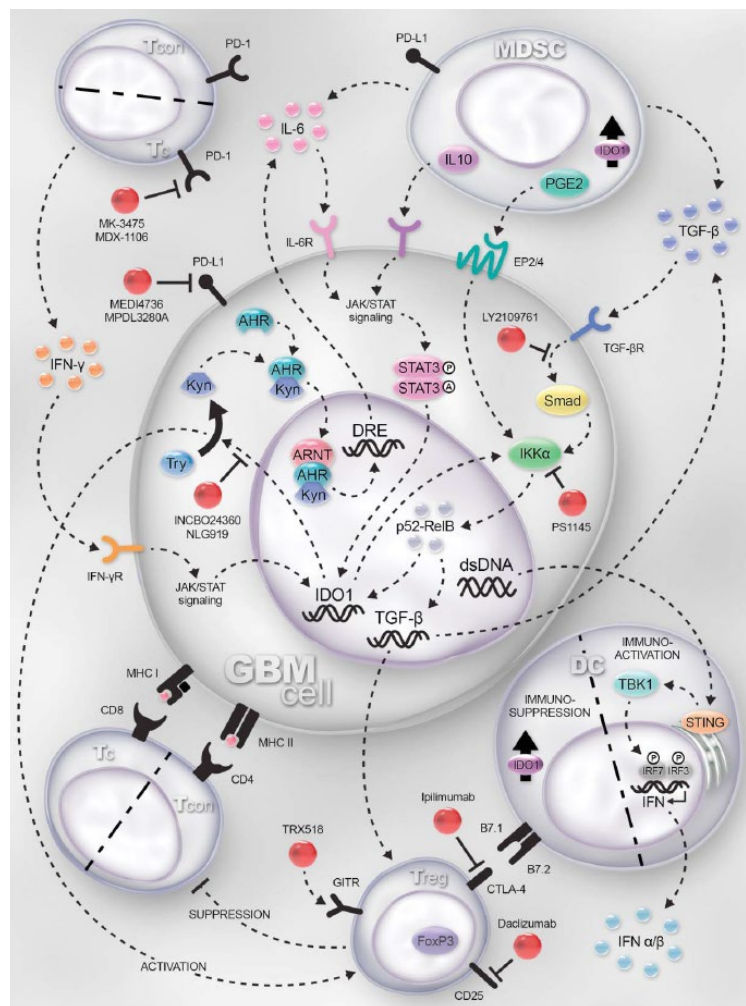
Figure 1 Mechanisms of immune reactions and therapeutic targets in glioblastoma (GBM). GBM cells, tumor-resident dendritic cells (DCs) and myeloid-derived suppressor cells (MDSC) express indoleamine-2,3-dioxygenase 1 (IDO1). IDO1 expression is regulated by the JAK/STAT and NF-κB pathways, which is induced by IFN-γ and TGF-β-receptor activation, respectively. IDO1 is a cytoplasmic enzyme that metabolizes tryptophan (Trp) to kynurenine (Kyn). Within the GBM cell, Kyn complexes with the aryl hydrocarbon receptor (Ahr), cytoplasmically, facilitating the nuclear translocation and further docking with aryl hydrocarbon receptor nuclear translocator (ARNT) to transcriptionally regulate IL-6, acting as an autocrine loop that amplifies and sustains IDO1 expression. Simultaneously, extracellular Kyn suppresses T effector responses while activating regulatory T cell (Treg; CD4+CD25+FoxP3+) function through a presumably overlapping mechanism. IDO1 directly activates NF-kB signaling which maintains and/or upregulates TGF-β expression. Increased TGF-β levels upregulate CTLA-4 and GITR expression by Treg. CTLA-4 interacts with B7.1 (CD80) and B7.2 (CD86) on DC, resulting in the induction of IDO1 (in DC) and commensurate downregulation of antigen presentation to T cells. Both GBM and MDSC express TGF-b, which synergizes with PD-L1 to suppress the T cell effector response via interaction with PD-1. Moreover, interleukin-10 (IL-10)- and prostaglandin E2 (PGE2)-expressing MDSC act on their cognate receptors expressed by GBM to ramify JAK/STAT and NK-κB-mediated signaling. DNA released by dead/dying GBM cells is phagocytized by resident DC to activate the STING pathway leading to type 1 interferon (α/β) expression, supporting increased effectiveness of anti-GBM immunity. PD-1 is highly expressed by tumor-infiltrating cytotoxic T cells and PD-L1 is upregulated on cancer/stromal cells in response to T-cell-secreted IFN-γ. Blocking the interaction of PD-1-expressing T cells with PD-L1 leads to increased effector function and enhanced GBM immunity. Targets for immunomodulation are shown in red. Note: Although IDO1 expression and signaling are shown in GBM cells, shared signaling patterns are presumed to be present in DC and MDSC as well. T_{CON}: conventional CD4+FoxP3− T cell; T_{REG}: regulatory CD4+FoxP3+ T cell; T_C: cytotoxic CD8+ T cell; INCBO24360/NLG919: inhibitors of IDO1; PS1145: inhibitor of the NF-κB pathway; TRX518: humanized monoclonal agonistic antibody for GITR; Ipilimumab: humanized monoclonal antibody for CTLA-4; LY2109761: TGF-β receptor kinase inhibitor; MK-3475/MDX-1106: humanized monoclonal antibodies to PD-1; MEDI4736/MPDL3280A: humanized monoclonal antibodies to PD-L1; Anti-Gr1: mSC-depleting antibody; Daclizumab: humanized anti-CD25 (IL-2Ra); STING: stimulator of interferon genes; TBK1: TANK-binding kinase 1; IRF3/7: interferon regulatory factor 3/7; STAT3: signal transducer and activator of transcription 3. Reprinted from Binder *et al*. (25) (https://www.tandfonline.com/doi/full/10.1080/2162402X.2015.1082027?scroll=top&needAccess=true) without changes under the terms of the Creative Commons Attribution License.

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Table 1 Therapeutic vaccination clinical trials

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
Peptide vaccines											
NCT00643097	ACTIVATE	EGFRvIII	Phase II, single arm	Adults, new GBM s/p GTR and chemoRT	Rindopepimut vaccine every 2 weeks ×3 then monthly concomitant with standard adjuvant TMZ, until progression or death	Matched cohort (more KPS 80)	Experimental: n=18; Matched cohort: n=17	PFS: 14.2 (9.9–17.6) vs. 6.3 (4.1–9.0); OS: 26.0 (21.0–47.7) vs. 15.0 (11.4–19.8)	–	Published	(38)
NCT00643097	ACT II	EGFRvIII	Phase II, 2 arms	Adults, new GBM s/p GTR and chemoRT	Rindopepimut vaccine every 2 weeks ×3 then monthly until progression or death and after the 3rd vaccine: Arm I: standard dose TMZ (200 mg/m^2 on days 1–5); Arm II: dose intensified TMZ (100 mg/m^2 on days 1–21)	Matched cohort (same as above)	Arm I: 12; Arm II: 10; matched cohort: same as above	PFS (Arms I and II): 15.2 (11.0–18.5); OS (Arms I and II): 23.6 (18.5–33.1)	–	Published	(39)
NCT00458601	ACT III	EGFRvIII	Phase II, single arm	Adults, new GBM s/p GTR and chemoRT	Rindopepimut vaccine every 2 weeks ×3 then monthly, concomitant with standard adjuvant TMZ until intolerance or progression	None	65	PFS: 9.2 (7.4–11.3); OS: 21.8 (17.9, 26.5)	–	Published	(40)
NCT01480479	ACT IV	EGFRvIII	Phase III, randomized, double-blind, placebo-controlled	Adults, new GBM s/p GTR and chemoRT	Rindopepimut vaccine every 2 weeks ×2 then monthly, concomitant with standard adjuvant TMZ until intolerance or progression	Placebo vaccine	405	PFS: 8.0 (7.1–8.5) vs. 7.4 (6.0–8.7); OS: 20.1 (18.5–22.1) vs. 20.0 (18.1–21.9)	–	Published	(41)
NCT01498328	ReACT	EGFRvIII	Phase II, randomized, double-blind, placebo-controlled	Adults, relapsed GBM, bevacizumab-naïve	Rindopepimut vaccine every 2 weeks ×3 then monthly with bevacizumab given every 2 weeks	Placebo vaccine	73	PFS6: 28% vs. 16% (P=0.12); PFS: HR 0.72 (0.43–1.21, P=0.22); OS: HR 0.53 (0.32–0.88, P=0.01)	–	Published	(42)
NCT02454634	NOA-16	IDH1R132H	Phase I, single arm	Adults, new HGG s/p chemoRT	IDH1R132H peptide vaccine every 4 weeks ×8 with topical imiquimod, concomitant with standard adjuvant TMZ	None	33	32-week PFS: 87.5%	2 serious AEs, 1 probably related; 93.3% CTL and/or humoral response	Completed, abstract only	(43)
NCT02193347	RESIST	IDH1R132H	Phase I, single arm	Adults, recurrent grade II gliomas	IDH1R132H peptide vaccine every 2 weeks ×3, followed by re-resection, followed by maintenance vaccine with TMZ	None	24 enrolled	N/A	Primary: toxicity; secondary: immunogenicity	Active, not recruiting	N/A
NCT01250470	SurVaxM Peptide Vaccine	Survivin	Phase I, single arm	Adults, recurrent HGG, HLA-A*02(+) or HLA-A*03(+)	SurVaxM vaccine every 2 weeks ×4	None	9	PFS: 4.1; OS: 20.2	1 G3 AE, not related to vaccine	Completed	(44)
Multi-peptide vaccines											
NCT01222221	Cancer Research UK IMA950-101	11 GBM TAAs	Phase I, 2 arms	Adults, new GBM, HLA-A*02(+)	IMA950/GM-CSF vaccine injected 11 times over 24 weeks: Arm I: started 7–14 days prior to chemoRT; Arm II: started 7 days after chemoRT, concomitant with standard adjuvant TMZ	None	45: Arm I: 22; Arm II: 23	PFS6: 74.4%; PFS9: 30.8%; OS: 15.3 months	2 dose-limiting grade 3 AEs; OS for ISR vs. no ISR: 26.7 vs. 13.2 (HR 0.33, P=0.0001)	Published	(45)
NCT01920191	IMA950 Multi-peptide Vaccine with Poly-ICLC	11 GBM TAAs	Phase I/II, single arm	Adults, new HGG, HLA-A*02(+)	IMA950/poly-ICLC vaccine injected 9 or 11 (protocol revision) times over 24 weeks starting 7 days concomitant with standard adjuvant TMZ	None	GBM: 16; Grade III astrocytoma: 3	For GBM pts: PFS6: 81%; PFS9: 63%; OS: 19 months (17.3–27.9)	–	Published	(46)
UMIN000001243	ITK-1 Personalized Peptide Vaccine	4 of 14 GBM TAAs based on 4 highest IgG titers	Phase I, single arm	Adults, recurrent GBM, HLA-A*24(+)	ITK-1 (14 peptide candidates, 4 chosen per highest IgG titers for each) vaccine every week ×6	None	12	PFS6 16.7%; PFS: 2.3 (1.7–3.5); OS: 10.6 (8.0–12.5)	–	Published	(47)
N/A	ITK-1 Personalized Peptide Vaccine vs. Placebo	4 of 14 GBM TAAs based on 4 highest IgG titers	Phase III, randomized, double-blind, placebo-controlled	Adults, recurrent GBM, HLA-A*24(+)	ITK-1 (12 peptide candidates, 4 chosen per highest IgG titers for each) vaccine every week ×12	Placebo vaccine	88: Experimental: 58; Control: 30	OS: 8.4 (6.6–10.6) vs. 8.0 (4.8–12.9)	Unfavorable OS in experimental group associated with SART2-93 peptide selection, ≥70 years old, >70 kg body weight, and PS 3; OS for pts without SART2-93 and age <70 years old: 9.6 (7.3–12.0) vs. 4.7 (3.7 vs. 6.8), HR 0.49, P=0.031	Published	(48)

Table 1 (continued)

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Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
NCT02149225	GAPVAC-101	5–10 unmutated TAAs and 1–2 mutated TAAs based on tumor mutation/transcriptome analysis	Phase I, single arm	Adults, new GBM, HLA-A*02(+) or HLA-A*24(+)	APVAC1 (5–10 synthetic unmutated antigens), followed by APVAC2 (1–2 synthetic neoantigens), concomitant with standard adjuvant TMZ. Both vaccines personalized based patient's tumor mutation/transcriptome analyses	None	15	PFS: 14.2; OS: 29.0	–	Published	(49)
UMIN000000002	An Autologous Tumor Vaccine with RT	Autologous tumor peptides	Phase I/IIa, single arm	Adults, new GBM	Autologous formalin-fixed tumor vaccine every week ×3 starting on week 4 of radiation without no TMZ	None	22	PFS: 7.6 (4.3–13.6); OS: 19.8 (13.8–31.3)	–	Published	(50)
UMIN000001426	An Autologous Tumor Vaccine with Adjuvant TMZ	Autologous tumor peptides	Phase I/IIa, single arm	Adults, new GBM	Autologous formalin-fixed tumor vaccine every week ×3 starting on first day of standard adjuvant TMZ	None	24	PFS: 8.2 (CI N/R); 33% progression free at 24 months, associated with diameter of DTH (delayed-type hypersensitivity) response; OS: 22.2 (2.7–41.7)	–	Published	(51)
NCT01903330	Bevacizumab +/– Gliovac (ERC1671)	Autologous tumor peptides and pooled allogeneic peptides	Phase II, randomized, double-blind, placebo-controlled	Adults, recurrent GBM, bevacizumab-naïve	Gliovac (ERC1671, autologous and pooled allogenic tumor peptides), GM-CSF, cyclophosphamide, and bevacizumab in 4-week cycles until intolerance or progression	Placebo and bevacizumab	84 expected	Interim analysis (n=9): PFS: 7.3 vs. 5.4; OS: 12.1 vs. 7.6	Primary: toxicity; secondary: PFS, OS, immunogenicity	Recruiting, interim results published	(52)
Heat shock protein vaccines											
NCT00293423	HSPPC-96 Vaccine (phase I)	HSPPC-96	Phase I, single arm	Adults, recurrent GBM	Re-resection followed by autologous HSPPC-96 vaccine every 2 weeks (n=6) or every week ×4 then every 2 weeks (n=6) until intolerance or progression	None	12	OS: 10.1	No severe AEs	Published	(53)
NCT00293423	HSPPC-96 Vaccine (phase II)	HSPPC-96	Phase II, single arm	Adults, recurrent GBM	Re-resection followed by autologous HSPPC-96 vaccine every 2 weeks (n=6) or every week ×4 then every 2 weeks (n=6) until intolerance or progression (median 6 doses)	None	41	PFS: 4.4 (3.2–5.5); OS: 9.8 (8.0–11.6)	1 serious AE related to vaccine	Published	(54)
NCT01814813	Bevacizumab +/– HSPPC-96 Vaccine	HSPPC-96	Phase II, randomized	Adults, recurrent GBM	Re-resection followed by: Arm I: HSPPC-96 vaccine and concomitant bevacizumab for 12 2-week cycles or until progression; Arm II: HSPPC-96 vaccine, bevacizumab added if progression, for 12 2-week cycles or until 2nd progression	Arm III: bevacizumab alone until progression	90	PFS (P<0.01), OS (P=0.16): Arm I: 3.7 (2.9–5.4), 6.6 (5.4–10.4); Arm II: 2.5 (2.0–3.5), 9.2 (5.7–11.6); Arm III: 5.3 (3.7–8.0), 10.7 (8.8–17.2)	–	Active, not recruiting, unpublished data	(55)
NCT00905060	HeatShock	HSPPC-96	Phase II, single arm	Adults, new GBM s/p ≥90% resection and chemoRT	HSPPC-96 vaccine weekly ×4, then monthly concomitant with standard adjuvant TMZ, until depletion of vaccine or progression	None	46	PFS: 17.8 (11.3–21.6); OS: 23.8 (19.8–30.2)	–	Completed, abstract only	(56)
NCT03650257	Adjuvant TMZ +/– HSPPC-96 Vaccine	HSPPC-96	Phase II, randomized	Adults, new GBM s/p ≥80% resection and chemoRT	Starting 2 weeks after chemoRT, HSPPC-96 vaccine weekly ×4, then in 2 weeks, then in 3 weeks concomitant with standard adjuvant TMZ	SOC treatment	150 expected	N/A	Primary: OS; secondary: PFS, immunogenicity, AEs	Recruiting	N/A
Dendritic cell vaccines—peptide loaded											
N/A	EGFRvIII-targeted DC Vaccine	EGFRvIII	Phase I, single arm, 3+3	Adults, new GBM s/p resection and chemoRT	After standard chemoRT, DC vaccine every 2 weeks ×3, dose escalated in groups of 3 patients	Historical control	12	OS: 22.8 (17.5–29.0) vs. 15.6 (historical)	–	Published	(57)

Table 1 (continued)

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Table 1 (continued)

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
N/A	ICT-107	6 GBM TAAs	Phase I, single arm	Adults, new or recurrent GBM or brainstem glioma, HLA-A*01(+) and/or HLA-A*02(+)	ICT-107 DC vaccine every 2 weeks ×3 after standard chemoRT (new) or re-resection (recurrent) and prior to standard adjuvant TMZ (new)	None	New GBM: 16; Recurrent GBM: 3; Brainstem: 1	New GBM (n=16); PFS: 16.9 (8.9–49.8); OS: 38.4 (25.9–40.7)	–	Published	(58)
NCT01280552	Adjuvant TMZ +/– ICT-107	6 GBM TAAs	Phase II, randomized, double-blind, placebo-controlled	Adults, new GBM, HLA-A*01(+) and/or HLA-A*02(+)	ICT-107 DC vaccine weekly ×4 prior to standard adjuvant TMZ, then concomitant with TMZ at month 1, 3, 6, and every 6 months thereafter	Placebo control (unpulsed DCs)	Experimental: 81; Control: 43	PFS: 11.2 (8.2–13.1) *vs.* 9.0 (5.5–10.3), HR=0.57, P=0.011; OS: 17.0 (13.7–20.6) *vs.* 15.0 (12.3–23.1), HR=0.87, P=0.58	–	Published	(59)

Dendritic cell vaccines—tumor lysate loaded

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
NCT00068510	Autologous Tumor Lysate DC Vaccine (phase I)	Tumor lysate (autologous)	Phase I, single arm	Adults, new HGG s/p resection and chemoRT	Before standard adjuvant TMZ, DC vaccine every 2 weeks ×3, then concomitant with TMZ every 3 months until depletion of vaccine or progression	Phase I cohort of peptide-loaded DC vaccine (NCT00612001)	28	PFS: 18.1 *vs.* 9.6; OS: 34.4 *vs.* 14.5	–	Published	(60)
EY-DOH-MD #0910072504	Autologous Tumor Lysate DC Vaccine (phase I/II)	Tumor lysate (autologous)	Phase I/II, single arm	Adults, new or recurrent HGG	After resection and standard RT (new, use of TMZ not reported) or re-resection (recurrent), DC vaccine given weekly ×4, every 2 weeks ×2, every month ×4	Matched cohort	New GBM: 8; Recurrent GBM: 8; Recurrent WHO III: 1	New and recurrent GBM (n=16): OS: 17.1 *vs.* 12.5	–	Published	(61)
N/A	ChemoRT +/– Autologous Tumor Lysate DC Vaccine	Tumor lysate (autologous)	Phase II, randomized	Adults, new GBM s/p resection and chemoRT	Concomitant with chemoRT, DC vaccine given 10 times over 6 months	SOC treatment	Experimental: n=18; SOC control: n=16	PFS: 8.5 *vs.* 8.0 (P=0.075); OS: 31.9 *vs.* 15.0 (P<0.002)	–	Published	(62)
NCT01006044	Autologous Tumor Lysate DC Vaccine (phase II)	Tumor lysate (autologous)	Phase II	Adults, new GBM s/p resection with <1 cc residual	DC vaccine given prior to chemoRT ×1, then concomitant with standard adjuvant TMZ, monthly ×3, bimonthly ×4, quarterly until depletion of vaccine	None	31	PFS: 12.7 (7–16); OS: 23.4 (16–33.1)	–	Published	(63)
NCT00576446	Resection with Gliadel Wafers followed by Autologous Tumor Lysate DC Vaccine	Tumor lysate (autologous)	Phase I, single arm	Adults, new or recurrent HGG	Resection with Gliadel Wafer placement, then DC vaccine given every 2 weeks ×3 (sequencing with standard therapies N/R)	None	New GBM: 8; New WHO III: 3; Recurrent GBM: 15; Recurrent WHO III: 2	PFS, OS: New GBM: 4.8 (1.2–25.5), 27.7 (10.5–39.1); recurrent GBM: 1.9 (0.6–3.6), 10.9 (6.3–21.3)	–	Published	(64)
NCT01213407	Adjuvant TMZ +/– Audencel	Tumor lysate (autologous)	Phase II, randomized	Adults, new GBM, s/p resection (≥ 70%) and chemoRT	After chemoRT, Audencel (DC vaccine) given weekly ×4, then monthly concomitant with standard adjuvant TMZ	SOC treatment	Experimental: 34; SOC control: 42	PFS: 6.6 (4.5–9.1) *vs.* 6.9 (5.8–9.3), P=0.83; OS: 18.3 (14.2–21.8) *vs.* 18.4 (11.3–22.1), HR=0.99, P=0.89	–	Published	(65)
NCT00045968	Adjuvant TMZ +/– DCVax®-L	Tumor lysate (autologous)	Phase III, randomized, double-blind, placebo-controlled	Adults, new GBM s/p resection and chemoRT	Concomitant with standard adjuvant TMZ, DCVax-L every 10 days ×3, then months 2, 4, 8, then every 6 months thereafter, crossover permitted (90% received DCVax-L)	Placebo vaccine	Experimental: 232; Control: 99	Entire cohort: OS: 23.1 (21.2–25.4)	–	Active, not recruiting, interim results published	(66)
NCT01808820	Autologous Tumor Lysate DC Vaccine with Imiquimod	Tumor lysate (autologous)	Phase I, single arm	Subjects 13 y or older, recurrent HGG, s/p re-resection (≤2 cc residual)	DC vaccine/imiquimod given weekly ×4	None	20 expected	N/A	Primary: toxicity; secondary: PFS, OS, immunogenicity	Active, not recruiting	N/A
NCT01204684	Autologous Tumor Lysate DC Vaccine +/– Imiquimod or Poly-ICLC	Tumor lysate (autologous)	Phase II, randomized	Adults, new or recurrent HGG	Arm I: DC vaccine with placebo cream or injection Arm II: DC vaccine with imiquimod cream Arm III: DC vaccine with poly-ICLC (sequencing with standard therapies N/R)	Arm I	60 expected	N/A	Primary: most effective combination; secondary: PFS, OS	Active, not recruiting	N/A

Table 1 (continued)

Table 1 (continued)

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
NCT01957956	Allogenic Tumor Lysate DC Vaccine (New GBM)	Tumor lysate (allogeneic)	Early Phase I	Adults, new GBM	DC Vaccine starting on second cycle of standard adjuvant TMZ, every 4 weeks ×11 or until intolerance or progression	None	21 expected	N/A	Primary: toxicity; secondary: OS, PFS, ORR, time to response, duration of response	Active, not recruiting	N/A
NCT03360708	Allogenic Tumor Lysate DC Vaccine (recurrent GBM)	Tumor lysate (allogeneic)	Early phase I	Adults, recurrent GBM	DC vaccine every 3 weeks ×13 or until intolerance or progression	None	20 expected	N/A	Primary: toxicity; secondary: OS, PFS, ORR, time to response, duration of response	Recruiting	N/A
NCT02010606	Allogenic Tumor Lysate DC Vaccine from a GBM Stem-like Cell Line	Tumor lysate (allogeneic)	Phase I, single arm	Adults, new or recurrent GBM	Starting after chemoRT (new), DC vaccine given weekly ×4, then every 8 weeks concomitant with standard adjuvant TMZ, until depletion of vaccine or progression	None	39 enrolled	N/A	Primary: toxicity; secondary: OS, PFS, QoL, ORR, immunogenicity	Active, not recruiting	N/A
Dendritic cell vaccines—mRNA loaded											
NCT00846456	GSC Antigen mRNA DC Vaccine	Autologous GSC antigens	Phase I/II, single arm	Adults, new GBM s/p resection (≤5 cc residual)	After chemoRT, DC vaccine twice in first week, weekly ×3, then every 2 weeks concomitant with standard adjuvant TMZ	Matched cohort (n=10)	7	PFS: 22.8 vs. 7.8, P=0.0018; OS: 25.0 vs. 19.2, P=0.11	–	Completed, interim results published	(67)
NCT02649582	ADDIT-GLIO	WT1	Phase I/II, single arm	Adults, new GBM	One week after chemoRT, DC vaccine given weekly ×3, then monthly concomitant with standard adjuvant TMZ, for up to 12 cycles	None	20 expected	N/A	Primary: OS; secondary: feasibility, toxicity, immunogenicity, ORR, QoL	Recruiting	N/A
NCT02709616	PERCELLVAC	Autologous TAAs	Phase I, single arm	Adults, new GBM	After chemoRT, DC vaccine every 2 weeks concomitant with standard adjuvant TMZ	None	10 expected	N/A	Primary: toxicity; secondary: OS, PFS, immunogenicity	Active, not recruiting	N/A
Dendritic cell vaccines—glioma stem cell loaded											
NCT01567202	ChemoRT +/− Autologous GSC DC Vaccine	Autologous GSC antigens	Phase II, randomized, double-blind, placebo-controlled	Adults, new or recurrent GBM s/p ≥95% resection	2 weeks after resection, DC vaccine weekly ×3, prior to and/or concomitant with standard chemoRT (new GBM)	Placebo vaccine	Experimental: 22; Placebo control: 21	PFS: 7.7 vs. 6.9, P=0.75; OS: 13.7 vs. 10.7, P=0.05	–	Published	(68)
NCT01171469	Allogeneic GSC DC Vaccine	Allogeneic GSC antigens	Phase I, single arm	All ages, recurrent HGG or medulloblastoma	DC vaccine/imiquimod every 2 weeks ×4, then every 4 weeks ×10	None	8 enrolled	N/A	Primary: MTD; secondary: time to progression	Completed, not published	N/A
Dendritic cell vaccines—viral antigen loaded											
NCT00639639	CMV pp65 DC vaccine	pp65	Phase I, single arm	Adults, new GBM s/p >90% resection	DC vaccine given every 2 weeks ×3, then every 4 weeks ×3–9 or until progression, concomitant with adjuvant dose intensified TMZ	Matched cohort (n=23)	11	PFS: 25.3 (11.0–not reached) vs. 8.0 (6.2–10.8), P=0.0001; OS: 41.1 (21.6–not reached) vs. 19.2 (14.3–21.3), P=0.0001	–	Published	(69)
NCT03927222	I-ATTAC	pp65	Phase II, single arm	Adults, new GBM, MGMT unmethylated, CMV(+)	DC vaccine every 2 weeks ×3, then monthly ×7, concomitant with adjuvant dose intensified TMZ	None	48 expected	N/A	Primary: OS; secondary: PFS, toxicity, immunogenicity	Recruiting	N/A
NCT02465268	ATTAC-II	pp65	Phase II, randomized, double-blind, placebo-controlled	Adults, new GBM	DC vaccine every 2 weeks ×3, then monthly ×7, concomitant with adjuvant TMZ	Placebo vaccine	120 expected	N/A	Primary: OS; secondary: PFS, immunogenicity	Recruiting	N/A
Viral (oncolytic) vaccines											
NCT01491893	Recombinant Poliovirus (PVSRIPO, phase I)	CD155	Phase I, single arm	Adults, recurrent GBM	PVSRIPO vaccine intratumoral injection ×1	Matched cohort (n=104)	61	OS: 12.5 (9.9–15.2) vs. 11.3 (9.8–12.5)	1 DLT: grade 4 intracranial hemorrhage; 1 grade 5 seizure attributed to PVSRIPO; 19% had grade 3+ AEs	Published	(70)

Table 1 (continued)

Table 1 *(continued)*

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
NCT02986178	PVSRIPO (phase II)	CD155	Phase II, single arm	Adults, recurrent GBM	PVSRIPO vaccine intratumoral injection ×1	None	122 expected	N/A	Primary: ORR; secondary: OS, PFS, safety	Recruiting	N/A
NCT00805376	DNX-2401 Oncolytic Adenovirus	E1A mutant	Phase I, 2 arms	Adults, recurrent HGG	Arm I: DNX-2401 vaccine intratumoral injection ×1 Arm II: DNX-2401 vaccine intratumoral injection ×1, then resection 14 days later with resection cavity vaccine injection	None	Arm I: 25; Arm II: 12	OS: 13.0	No DLTs; no serious AEs	Published	(71)
NCT01470794	Toca 511 (phase I)	Glioma-selective	Phase I, single arm	Adults, recurrent HGG	Re-resection with resection cavity injection of Toca 511 vaccine and oral 5-FC	None	53 (23 in phase III eligible subgroup, based on population and dose received)	OS: 11.9 (10.7–15.1)	–	Published	(72)
NCT02414165	Toca 5 Trial (phase II/III)	Glioma-selective	Phase II/III, randomized	Adults, recurrent anaplastic astrocytoma or GBM	Re-resection with resection cavity injection of Toca 511 vaccine and oral 5-FC	SOC treatment (lomustine, TMZ, or bevacizumab)	403	OS: 11.1 *vs.* 12.2, HR 1.06, P=0.6154	–	Completed, abstract only	(73)

EGFRvIII, epidermal growth factor variant III; GBM, glioblastoma; s/p, status post; GTR, gross total resection; chemoRT, chemoradiation; KPS, Karnofsky performance status; TMZ, temozolomide; n, number of patients; PFS, progression free survival; OS, overall survival; PFS6, 6 month progression free survival; HGG, high grade glioma (i.e., WHO Grade III or IV glioma); AE, adverse event; CTL, cytotoxic T lymphocyte; G3, grade 3; TAA, tumor-associated antigen; GM-CSF, granulocyte-macrophage colony-stimulating factor; PFS9, 9 month progression free survival; ISR, injection site reaction; Poly-ICLC, polyinosinic-polycytidylic acid-poly-l-lysine carboxymethylcellulose; RT, radiation; HSPPC-96, heat shock protein peptide complex 96; SOC, standard of care; DC, dendritic cell; N/R, not reported; ORR, objective response rate; QoL, quality of life; GSC, glioma stem cell; WT1, Wilms tumor 1; MTD, maximum tolerated dose; CMV, cytomegalovirus; pp65, phosphoprotein 65; MGMT, O⁶-methylguanine DNA methyltransferase; DLT, dose limiting toxicity; 5-FC, 5-fluorocytosine.

in combination with carrier proteins and immunostimulatory adjuvants, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and polyinosinic-polycytidylic acid-poly-L-lysine carboxymethylcellulose (poly-ICLC) (74). The ideal peptide is one that has a high affinity for MHC-I, thereby increasing the probability of being cross-presented to stimulate CD8$^+$ T cells. A number of such glioma TAAs have been identified, including gp100, AIM-2, TRP-2, and MAGE-1 (58). However, the most studied peptide in HGG is epidermal growth factor receptor variant III (EGFRvIII), a cell surface protein with a tumor-specific epitope expressed by approximately one third of GBMs (75,76). This protein is seen in several other epithelial tumors, but not on normal tissue (77). Its immunogenicity and efficacy as a therapeutic vaccine has been studied in a series of phase II and III trials. In the ACTIVATE phase II, multicenter trial, 18 adults with newly diagnosed EGFRvIII positive GBM who underwent a gross total resection and chemoradiation were given rindopepimut (also known as CDX-110), an EGFRvIII-targeted peptide vaccine, with standard adjuvant TMZ and found to have significantly better outcomes compared to a matched cohort (38). They reported a median PFS of 14.2 months (95% CI, 9.9–17.6) vs. 6.3 months (95% CI, 4.1–9.0) in the matched cohort and median OS of 26.0 months (95% CI, 21.0–47.7) vs. 15.0 months (95% CI, 11.4–19.8). Interestingly, for recurrent tumors that were re-resected (n=11), 82% no longer exhibited EGFRvIII expression, suggesting selective eradication of EGFRvIII positive cells versus downregulation of EGFRvIII as an adaptive immune escape mechanism.

In the follow-up phase II trial, ACT II, patients meeting the same inclusion criteria were either given standard dose TMZ (n=12) or dose-intensified TMZ (n=10) (39). Despite more severe and sustained lymphopenia in the dose-intensified group, these patients paradoxically exhibited greater magnitudes of antibody- and cell-mediated immune responses. Again, the median PFS and OS for the entire cohort were favorable (15.2 and 23.6 months, respectively) compared to the same matched cohort described above. The ACT III study (also single-arm, phase II, multicenter) was designed to confirm these results and did so, with a cohort of 65 patients achieving a median OS of 21.8 months (40). However, in the anticipated randomized, double-blind, placebo-controlled, phase III trial, ACT IV, interim analysis showed no OS benefit (median 20.1 vs. 20.0 months, P=0.93), and the trial was closed early (41). The use of rindopepimut was also investigated in the recurrent

setting in the ReACT randomized, phase II trial, where bevacizumab was given with or without rindopepimut to 73 patients. The investigators found a trend toward improved PFS at 6 months (28% vs. 16%, P=0.12), the same median PFS of 3.7 months between groups, and, notably, a significant improvement in OS [hazard ratio (HR) 0.53, 95% confidence interval (CI), 0.32–0.88] (42). The authors concluded that efforts to validate the potential benefits of rindopepimut in larger trials were warranted.

Early clinical investigations of a vaccine targeting an IDH type 1 mutant peptide, IDH1R132H, are underway. This point mutation in IDH-1 is a common driver in glioma tumor development (78-80), and an immunogenic MHC-II-binding epitope has recently been identified and confirmed in murine models (81). The clinical trials utilizing an IDH1R132H peptide vaccine [(43), NCT03893903, NCT02193347], as well as the survivin-targeted vaccine, SurVaxM (44), are summarized in *Table 1*.

To optimize the likelihood of immunogenicity and tumor-directed response, multi-peptide vaccines target a number of tumor antigens commonly seen in HGG. Utilized in several clinical trials, IMA950 is a multi-peptide vaccine containing 11 TAAs, which are expressed on the majority of GBMs via human leukocyte antigen (HLA) receptors (82). Cancer Research UK IMA950-101, a phase I, two-arm, first-in-human study of IMA950 included 45 adults with newly diagnosed GBM who were HLA-A*02 positive (45). Eleven injections were given over 24 weeks starting either before (arm I) or after (arm II) standard chemoradiation. Overall, there were two grade 3 dose-limiting toxicities, 90% developed a TAA-specific response, and 50% developed a response to multiple TAAs. PFS was 74.4% at 6 months and 30.8% at 9 months. Median OS was 15.3 months, but, interestingly, patients who had an injection-site reaction (58% overall) had prolonged OS (26.7 vs. 13.2 months, P=0.0001). In subsequent studies, investigators attempted to optimize the immunogenicity of the vaccine by use of the adjuvant poly-ICLC, which had previously shown efficacy in a murine model (83), and human studies with other peptide vaccines (84,85). In one phase I/II study of newly diagnosed WHO grade III (n=3) and IV (n=16) glioma patients, the first 6 patients received IMA950 intradermally and poly-ICLC intramuscularly (IM) (46). Then the protocol was changed to optimize vaccine formulation by mixing IMA950 and poly-ICLC, which was injected subcutaneously (SC, n=7) or IM (n=6). The initial formulation only led to single peptide CD8$^+$ T cell responses, while the mixed formulation elicited

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multipeptide CD8^{+} and CD4^{+} T cell responses. Median OS for the GBM patients was 19 months. IMA950/poly-ICLC mixed and administered SC is being further investigated in a phase I/II, randomized trial with or without pembrolizumab (NCT03665545).

While the prior mentioned studies used the same synthetic or allogeneic peptide(s) for all patients, several studies have investigated the use of personalized multi-peptide vaccines that include antigens for which the patient has the highest IgG titers (47-49). Others have created personalized vaccines by using antigen collected from the patient's resected tumor (50,51). These, and other ongoing studies, are summarized in *Table 1*. This approach has been used more frequently in DC vaccination, which is discussed in further detail below.

HSP vaccines: active immunotherapy

HSPs normally regulate chaperone proteins and facilitate proper protein folding (86), but in the setting of GBM, they bind multiple TAAs, forming heat shock protein peptide complexes (HSPPCs) (87). Like other molecules, they are ingested, processed, and presented by APCs, making them a potential vector for multi-peptide TAA recognition (88). Furthermore, HSPPCs bind macrophages and stimulate the production of proinflammatory cytokines (89), and also bind immature DCs prompting them to mature (90), thereby contributing to both innate and adaptive immune responses. Thus, HSPPCs are attractive for use in therapeutic vaccinations due to their immunostimulatory capacities and the range of antigens they carry. They also provide a personalized therapy as they are harvested from the patient's tumor. The most commonly used HSPPC in clinical trials is HSPPC-96. In a phase I, single arm trial, an autologous HSPPC-96 vaccine was given to 12 patients with recurrent GBM, and 11 exhibited an immune response with a median OS of 10.8 months, compared to 3.7 months in the non-responder, with no severe adverse events (53). In a follow-up phase II trial in 41 patients, survival results were similar with a median OS of 9.8 months (95% CI, 8.0–11.6) and PFS of 4.4 months (95% CI, 3.2–5.5) (54). The same group initiated a phase II trial of 90 patients with recurrent GBM randomized to HSPPC-96 vaccine with concomitant bevacizumab, HSPPC-96 with addition of bevacizumab and continued vaccine administration at progression, or bevacizumab alone. Though the study is still active, the investigators have released data showing no PFS or OS benefit [Parney I, 2019, unpublished data (55)]. The

HSPPC-96 vaccine is also being studied in the treatment of newly diagnosed GBM. One phase II, single arm trial of 46 patients, published in abstract only, achieved an impressive median PFS of 17.9 months (95% CI, 11.3–21.6) and OS of 23.8 months (95% CI, 19.8–30.2) (56). A larger randomized phase II study is currently underway (NCT03650257).

DC vaccines: active immunotherapy

Unlike peptide and HSP vaccines, DC vaccines are cell-based vaccines produced by harvesting the patient's DCs, culturing and priming the cells *ex vivo*, then reinoculating the patient with them. DCs are the most powerful stimulators of cell-mediated immune response, so priming them *ex vivo* should optimize the likelihood of obtaining such a response. DCs can be "pulsed" or "loaded" with tumor peptides, products of lysed autologous tumor cells, tumor-derived mRNA, glioma stem cells (GSCs), and viral antigens that have been associated with HGG (36). Numerous early stage clinical trials to assess the safety and efficacy of DC vaccines in HGG have been published since the 1990's, and are summarized in other reviews (91,92). Notably, the rate of adverse events, and most significantly the rate of adverse autoimmune reactions, has been much lower than those seen with ICIs (91,93), so only pertinent reports of severe toxicities are mentioned here. We will focus our review of DC vaccines on trials that have made OS comparisons, as well as recently published and ongoing studies, all of which are summarized in *Table 1*.

Overall, the summarized studies of DC vaccines show promising survival results, with median OS for newly diagnosed GBM ranging from 17.0 to 41.1 months and PFS from 6.6 up to 25.3 months. While survival times are expectedly shorter for studies in recurrent GBM, with median OS ranging from 10.9 to 15.3 months and PFS from 1.9 to 6.3 months, these compare very favorably to historical controls. Unfortunately, improved survival has not been uniformly seen in the subset of these studies that are randomized. For example, in single arm, phase I trial, ICT-107, a DC vaccine loaded with 6 GBM TAAs, was used to treat 20 adults with newly diagnosed or recurrent GBM or brainstem gliomas that were HLA-A*01 and/or HLA-A*02 positive (58). Among the 16 newly diagnosed GBM patients, median OS was an impressive 40.1 months. A follow-up study was designed as a phase II, randomized, double-blind, placebo-controlled trial, which included only new diagnoses of GBM that were, again, HLA-A*01 and/or HLA-A*02 positive (59). Patients were randomized to

receive either the ICT-107 DC vaccine (n=81), or a placebo unpulsed DC vaccine (n=43), prior to and concomitant with standard adjuvant TMZ. Unfortunately, there was no significant difference between OS (17.0 *vs.* 15.0 months, HR 0.87, P=0.58), with values resembling historical controls. PFS was, however, significantly improved in the experimental arm (11.2 *vs.* 9.0 months, HR 0.57, P=0.011) but the absolute benefit was modest. In another larger randomized, phase II trial, Audencel, a tumor lysate-pulsed DC vaccine, was given after completion of standard chemoradiation (n=34), but there was no significant improvement in OS (18.3 *vs.* 18.4 months, HR 0.99, P=0.89) or PFS (6.6 *vs.* 6.9 months, P=0.83) compared to standard adjuvant TMZ (n=42) (65). However, the randomized studies are not uniformly negative. In another randomized, phase II trial of a tumor lysate DC vaccine, patients in the experimental arm received the vaccine 10 times over the course of 6 months following standard chemoradiation and experienced a markedly improved OS of 31.9 months as compared to 15.0 months in the standard of care (SOC) control arm (P<0.002) (62). A modest but significant OS benefit was also seen in a study of a GSC loaded DC vaccine (68). This phase II, randomized, double-blind, placebo-controlled study included both newly diagnosed and recurrent GBM patients who underwent a near total resection and received either 3 injections of the GSC vaccine (n=22) or a placebo vaccine (n=21). Median OS was 13.7 months in the experimental arm and 10.7 months in the placebo control arm (P=0.05). When controlling for IDH1 and TERT promoter status, B7-H4 expression, and new or recurrent disease, the experimental arm had more significantly prolonged OS (HR 2.5, P=0.02) and a nonsignificant trend toward improved PFS (HR 1.37, P=0.37).

The reasons for the varied results of these studies are not clear but may be related to appropriate patient selection. For example, in the study reported by Wen *et al.*, patients positive for either HLA-A*01 or HLA-A*02 were included, but the HLA-A*02 positive patients exhibited a higher rate of immune response which correlated to a higher rate of OS compared to non-responders (59). Furthermore, in this study, 33% of the control arm patients had a detectable immune response to one or more TAAs contained in the experimental vaccine, perhaps suggesting that the placebo unpulsed DC vaccine was stimulating an immune response to *in vivo* TAAs. A follow-up phase III trial is being conducted now, including only HLA-A*02 positive patients with a less active placebo, though it is currently suspended due to financial issues (NCT02546102). Another notable ongoing study is the phase III trial investigating DCVax-L, a tumor lysate DC vaccine, given to patients with newly diagnosed GBM concomitant with standard adjuvant TMZ, with crossover allowed following recurrence (NCT00045968). An interim analysis reported an impressive median OS of 23.1 months (95% CI, 21.2–25.4) for the entire cohort (n=331), but the primary endpoint of PFS has not yet been assessed (66).

Viral vaccines: gene therapy

Viral, or oncolytic, vaccines are used in the treatment of malignant disease when a specific virus or viral gene product has been associated with the tumor. In GBM, the cytomegalovirus (CMV) antigen pp65 has been associated with GBM (94) and studied in a number of viral antigen-loaded DC vaccine studies (see *Table 1*). Several studies have also investigated the direct inoculation of various modified nonpathogenic viruses, including PVSRIPO [recombinant polio-rhinovirus (70)], DNX-2401 [a tumor-selective oncolytic adenovirus (71)], and Toca 511 [a gamma-retrovirus (72)], with interesting results. In each of these studies, the virus is injected directly into the tumor and/or resection cavity. In the case of PVSRIPO, the virus selectively infects tumor cells due to their overexpression of CD155. APCs are also infected but chronically and sublethally, leading to constitutive activation with a proinflammatory response that supports a T cell mediated tumor response. In a single arm, phase I trial published in the *New England Journal of Medicine*, 61 patients with recurrent GBM received an intratumoral injection of PVSRIPO with one dose-limiting toxicity (grade 4 intracranial hemorrhage), one grade 5 toxicity (seizure), and 19% rate of grade 3 or higher adverse events (70). Median OS was 12.5 months (95% CI, 9.9–15.2), which was not significantly different from a matched cohort, 11.3 months (95% CI, 9.8–12.5). However, OS in the experimental group reached a plateau of 21% (95% CI, 11–33%) at 24 months, and this was sustained at 36 months, leading the investigators to open a larger phase II trial (NCT02986178). Both of the other studies also had a subset of patients who achieved a sustained response. In the DNX-2401 trial, 20% of patients (5 of 25) survived more than 3 years after the injection, and 3 of the 5 patients had more than a 95% reduction in their tumor volume (71). In the Toca 511 trial, patients with recurrent HGG underwent re-resection with injection of the vector followed by oral

5-fluorocytosine (5-FC). The vector encodes cytosine deaminase, which converts 5-FC to 5-fluorouracil (5-FU) within tumor cells. Within the study of 53 patients, 23 were deemed the "phase III eligible subgroup" based on their uniformity of patient and treatment characteristics. Of the subgroup, 21.7% (5 of 23) had a complete response and remain alive and in response 33.9 to 52.2 months after Toca 511 administration (72). The follow-up phase III trial enrolled 403 patients, and, unfortunately, interim analysis recently showed that the primary endpoint of OS was not met (11.1 *vs.* 12.2 months, HR 1.06, P=0.6154) (73). The investigators did note that pre-planned subgroup analyses showed compelling results, but these data are not yet published, and as with preceding studies, may show subsets of patients with durable responses. Interestingly, a similar rate of about 20% of patients in all three trials had a sustained response. This may suggest a common immunologic feature of the tumor or microenvironment that potentiates the therapy. Comparison of biomarkers may elucidate this phenomenon.

Immune checkpoint inhibition: passive immunotherapy

In the normally functioning immune system, immune cells express checkpoint proteins, such as CTLA-4 and PD-1, which, when bound by their ligands, such as PD-L1, attenuate immune cells to prevent normal tissue damage. In the setting of malignant disease, tumor cells escape the immune system in part by overexpressing inhibitory proteins like PD-L1 (95,96). ICIs are monoclonal antibodies that block CTLA-4, PD-1, or PD-L1 to prevent interactions between these proteins, thereby circumventing T cell attenuation and tumor cell immune escape. As opposed to active immunotherapy, ICIs are a type of passive immunotherapy, in which an immune effector molecule is introduced to the patient's body (37). These therapies have significantly improved survival in a number of solid tumors (14-20), which has generated clinical research in a broad range of disease sites, including primary brain tumors (see *Table 2*). The most commonly used ICIs in clinical practice for other solid tumors, and under investigation for use in HGG, are ipilimumab (anti-CTLA-4), nivolumab (anti-PD-1), pembrolizumab (anti-PD-1), atezolizumab (anti-PD-L1), and durvalumab (anti-PD-L1).

Early data on the safety and efficacy of ICIs for use in recurrent GBM comes from several basket trials. The phase Ia PCD4989g study included 16 adults with recurrent GBM who received atezolizumab every 3 weeks until disease progression or intolerance (97). Median PFS (1.2 months, 95% CI, 0.7–10.7) and OS (4.2 months, 95% CI, 1.2–18.8+) were comparable to historical controls, but 3 patients (18.8%) experienced long-term survival of 16.0+ months. The investigators reported three grade 3 toxicities, with one serious adverse event leading to a treatment interruption. Recurrent bevacizumab-naïve GBM patients were also included in the Keynote-028 basket trial of pembrolizumab (NCT02054806). The analysis of 26 patients at a median follow-up of 14 months, published in abstract form only, showed a more favorable PFS of 2.8 months (95% CI, 1.9–9.1) and OS of 14.4 months (95% CI, 10.3–not reached) (98). Furthermore, while one patient had a partial response (PR), 12 patients (46.2%) had stable disease (SD) with a median duration of 9.1 months (range, 1.6+–9.8+). Safety data was comparable to the PCD4989g study, with report of four grade 3–4 toxicities, none of which required treatment interruption.

The presence of durable responders and acceptable safety outcomes led to the development of additional glioma-specific clinical trials, the results of which have been mixed but generally underwhelming. A recent meta-analysis was done on all studies of GBM that have been published in full manuscript form and report on efficacy and survival outcomes after treatment with ICIs (112). This included four retrospective studies (113-116), two phase I studies (97,110), and two phase II studies (100,101), encompassing 203 patients (predominantly with recurrent GBM) treated with atezolizumab, nivolumab, ipilimumab, and pembrolizumab as monotherapy or in combination with each other or bevacizumab. The median PFS and OS were 2.1 and 7.3 months, respectively, which is favorable compared to historical controls, but not as impressive as the results of vaccine therapy trials, which report median OS on the range of about 10–11 months in patients with recurrent GBM (47,53,54,70).

Moreover, three large randomized trials have reported disappointing results on interim analyses. In the CheckMate-143 trial, adults with recurrent GBM received either nivolumab (n=184) or SOC bevacizumab (n=185) with no significant differences in PFS (1.5 *vs.* 3.5 months) or OS (9.8 *vs.* 10.0 months) (102). In the CheckMate-498 trial, adults with newly diagnosed O-6-methylguanine-DNA methyltransferase (MGMT) unmethylated GBM were randomized to receive nivolumab every 2 weeks concomitant with radiation (without TMZ) and adjuvant nivolumab every 4 weeks, or SOC chemoradiation with TMZ, with an expected 550 patients (NCT02617589). In a press release on their website, Bristol-Myers Squibb

Table 2 Immune checkpoint inhibition clinical trials

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/Arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
Basket trials of immune checkpoint inhibitors											
NCT01375842	PCD4989g	PD-L1	Phase Ia, single arm	Adults, recurrent GBM	Atezolizumab given every 3 weeks until progression or intolerance	None	16	PFS: 1.2 (0.7–10.7); OS: 4.2 (range, 1.2–18.8+); 3 pts with long-term survival (range, 16.0–18.8+)	PR: 1 (6.3%); SD: 3 (18.8%); AEs: 3 G3, 1 serious AE leading to interruption	Published	(97)
NCT02054806	Keynote-028	PD-1	Phase I, single arm	Adults, recurrent GBM, bevacizumab-naïve	Pembrolizumab every 2 weeks until progression, intolerance, or 24 mo	None	26	PFS: 2.8 (1.9–9.1); OS: 14.4 (10.3–not reached)	PR: 1 (3.8%); SD: 12 (46.2%), median duration 39.4 weeks (range, 7.1+–85.9+); G3–4 AEs: 4	Active, not recruiting, abstract only	(98)
NCT02335918	Varlilumab (anti-CD27) and nivolumab	PD-1, CD27	Phase II	Adults, recurrent GBM, bevacizumab-naïve	Varlilumab and nivolumab every 2 weeks ×16	None	22	OS: 9.7 (6.7–14.8), 8 pts survived >12 mo (range: 13.7–23+); PR: 2; SD: 9	Serious AEs: 2; DLT: 0	Completed, abstract only	(99)
NCT02526017	Cabiralizumab (anti-CSF-1R) and nivolumab	PD-1, CSF-1R	Phase 1a/1b, 2 arms	Adults, recurrent GBM	Arm I: Cabiralizumab; Arm II: Cabiralizumab and nivolumab	None	295 expected (entire cohort)	N/A	Primary: toxicity, ORR; secondary: OS, PFS, immunogenicity, biomarkers	Active, not recruiting	N/A
Single agent immune checkpoint inhibitors											
NCT02550249	Neoadjuvant and adjuvant nivolumab	PD-1	Phase II, single arm	Adults, new or recurrent GBM	Nivolumab 2 weeks prior to resection ×1, then every 2 weeks (starting after chemoRT, concomitant with adjuvant TMZ for newly diagnosed) until progression or intolerance	None	Newly diagnosed: 3; recurrent: 27	PFS: 4.1 (2.8–5.5); OS: 7.3 (5.4–7.9)	G3–4 AE: 1	Published	(100)
NCT02852655	Adjuvant +/− neoadjuvant pembrolizumab	PD-1	Phase II, randomized	Adults, recurrent GBM	Arm I: Pembrolizumab 2 weeks prior to resection ×1, then every 3 weeks until progression or intolerance; Arm II: After resection, every 3 weeks until progression or intolerance	None	Arm I: 16; Arm II: 19	PFS: 3.3 vs. 2.4 (HR 0.43, P=0.03); OS: 13.7 vs. 7.5 (HR 0.39, P=0.04)	G3–4 AE: 10 (67%) vs. 7 (47%), P=0.46; AEs leading to treatment discontinuation: 2 vs. 0	Published	(101)
NCT02017717	CheckMate-143	PD-1	Phase III, randomized	Adults, recurrent GBM	Nivolumab every 2 weeks until progression or intolerance	Bevacizumab every 2 weeks until progression or intolerance	Experimental: 184; Control: 185	PFS: 1.5 vs. 3.5; OS: 9.8 vs. 10.0	G3–4 AE: 18% vs. 15%; AEs leading to treatment discontinuation: 10% vs. 15%	Active, not recruiting, abstract only	(102)
NCT02617589	CheckMate-498	PD-1	Phase III, randomized	Adults, new GBM, MGMT unmethylated	Nivolumab every 2 weeks concomitant with standard radiation without TMZ, then every 4 weeks after radiation until progression or intolerance	SOC treatment	550 expected	N/A	Primary: OS; secondary: PFS	Active, not recruiting, unpublished data	(103)
NCT02667587	CheckMate-548	PD-1	Phase III, randomized, double-blind, placebo-controlled	Adults, new GBM, MGMT methylated	Nivolumab concomitant with standard chemoRT	Placebo infusion	693 expected	N/A	Primary: OS, PFS	Active, not recruiting, unpublished data	(104)
NCT02337686	Pembrolizumab and re-resection	PD-1	Phase II, single arm	Adults, recurrent GBM	Pembrolizumab ×1–2 prior to resection, then afterwards until progression or intolerance	Matched SOC cohort	Experimental: 15; matched cohort: 10	PFS: 7 [4–16]; OS: not reached	–	Active, not recruiting, abstract only	(105)
NCT02530502	Pembrolizumab with chemoRT	PD-1	Phase I, single arm	Adults, new GBM, s/p resection	Pembrolizumab every 3 weeks, concomitant with standard chemoRT	None	4 enrolled	N/A	Primary: DLT; secondary: toxicity, immunogenicity, biomarkers	Active, not recruiting	N/A
NCT04047706	Nivolumab and IDO1 inhibition (BMS-986205) with RT or ChemoRT	PD-1, IDO1	Phase I, 2 arms	Adults, new GBM	Arm I: nivolumab every 2 weeks ×3 and IDO1 inhibitor daily concomitant with standard chemoRT and with adjuvant TMZ; Arm II: nivolumab and IDO1 inhibitor with radiation alone and adjuvantly	None	30 expected	N/A	Primary: toxicity; secondary: OS, PFS, ORR	Recruiting	N/A

Table 2 (continued)

Table 2 *(continued)*

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/Arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
NCT02336165	Durvalumab with RT (new) or with/without bevacizumab (recurrent)	PD-L1	Phase II, 5 arms	Adults, new MGMT unmethylated, or recurrent GBM	Durvalumab (MEDI4736) every 2 weeks and: Arm I (newly diagnosed): concomitant radiation without TMZ; Arm II–IV (bevacizumab-naïve): 3 dose levels of bevacizumab including none; Arm V (bevacizumab-refractory): continued bevacizumab	None	159 enrolled; Arm V: 22	Arm V: PFS: range, 0.2–5.6, 11 pts (50%) with PFS ≥1.8 mo; OS: range, 0.2–11.9, 8 pts (36%) with OS ≥5.1 mo	Primary: OS, PFS; secondary: toxicity, QoL, biomarkers	Active, not recruiting, abstract only (re: Arm V)	(106)
NCT02337491	Pembrolizumab +/− bevacizumab	PD-1	Phase II, randomized	Adults, recurrent GBM	Arm I: pembrolizumab every 3 weeks and bevacizumab every 2 weeks until progression or intolerance; Arm II: pembrolizumab every 3 weeks until progression or intolerance	None	Arm I: 50; Arm II: 30	PFS: 4.1 (2.8–5.5) *vs.* 1.4 (1.4–2.7); OS: 8.8 (7.7–14.2) *vs.* 10.3 (8.5–12.5)	–	Completed, abstract and unpublished data	(107,108)
NCT02311582	Pembrolizumab +/− MRI-guided laser ablation	PD-1	Phase I/II, 2 arms	Adults, recurrent GBM	Phase II: Arm I: MLA, then pembrolizumab every 3 weeks; Arm II: pembrolizumab every 3 weeks	None	58 expected	N/A	Primary: PFS; secondary: OS, immunogenicity, biomarkers	Recruiting	N/A
NCT03718767	Nivolumab for IDH-mutated glioma with hypermutator phenotype	PD-1	Phase II, single arm	Adults, recurrent glioma, IDH-mutated with tumor specific mutational load	Nivolumab every 2 weeks ×8, then every 4 weeks ×12	None	95 expected	N/A	Primary: PFS; secondary: OS, QoL	Recruiting	N/A
NCT03047473	The SEJ Study	PD-L1	Phase II, single arm	Adults, new GBM s/p chemoRT	Avelumab every 2 weeks with standard adjuvant TMZ	None	30 expected	Interim analysis (n=8): PFS: 11.9; CR: 2 (25%); PR: 1 (12.5%); SD: 1 (12.5%); AEs requiring treatment break: 2	Primary: toxicity; secondary: OS, PFS, ORR, biomarkers	Active, not recruiting, abstract only	(109)
Dual immune checkpoint inhibitors											
NCT02017717	CheckMate-143	PD-1, CTLA-4	Phase I, randomized	Adults, recurrent GBM	Arm I: nivolumab 3 mg/kg every 2 weeks; Arm II: nivolumab 1 mg/kg and ipilimumab 3 mg/kg every 3 weeks ×4, then nivolumab 3 mg/kg every 2 weeks; Arm III: nivolumab 3 mg/kg every and ipilimumab 1 mg/kg every 3 weeks ×4, then nivolumab 3 mg/kg every 2 weeks (nonrandomized)	None	Arm I: 10; Arm II: 10; Arm III: 20	PFS, OS: Arm I: 1.9 (1.3–4.6), 10.4 (4.1–22.8); Arm II: 1.5 (0.5–2.8), 9.2 (3.9–12.7); Arm III: 2.1 (1.4–2.8), 7.3 (4.7–12.9)	PR: 1 in Arm I, 2 in Arm III; SD (≥12 weeks): 2 in Arm I, 2 in Arm II, 4 in Arm III; AEs leading to discontinuation: 1 in Arm I, 3 in Arm II, 4 in Arm III	Published	(110)
NCT02311920	Ipilimumab and/or Nivolumab	PD-1, CTLA-4	Phase I, randomized	Adults, new GBM s/p chemoRT	Adjuvant TMZ and: Arm I: ipilimumab every 4 weeks ×4, then every 3 months ×4; Arm II: nivolumab every 2 weeks ×8, then every 2 weeks ×24; Arm III: ipilimumab every 4 weeks ×4 and nivolumab every 2 weeks ×32	None	32 enrolled	N/A	Primary: toxicity; secondary: OS, biomarkers	Active, not recruiting	N/A
NCT02658981	Adult Brain Tumor Consortium 1501	PD-1, LAG-3, CD137	Phase I, 4 arms	Adults, recurrent GBM	Arm I: anti-LAG-3 (BMS-986016) every 2 weeks; Arm II: anti-CD137 (urelumab, BMS-663513) every 3 weeks; Arm III: anti-LAG-3 and nivolumab every 2 weeks; Arm IV: anti-CD137 every 4 weeks and nivolumab every 2 weeks (arm closed 10/16/18)	None	100 expected	Interim analysis (n=44): OS: Arm I: 8; Arm II: 14; Arm III: 7; Arm IV: N/R	Primary: toxicity; secondary: OS, PFS, ORR	Recruiting, interim analysis published (abstract only)	(111)
NCT02794883	Tremelimumab (anti-CTLA-4) and/or Durvalumab	PD-L1, CTLA-4	Phase II, 3 arms	Adults, recurrent HGG	Re-resection after first cycle of: Arm I: tremelimumab every 4 weeks; Arm II: durvalumab every 2 weeks; Arm III: tremelimumab every 4 weeks and durvalumab every 2 weeks	None	36 enrolled	N/A	Primary: immunogenicity; secondary: toxicity, OS, PFS, biomarkers	Active, not recruiting	N/A
NCT03707457	Nivolumab and anti-GITR, IDO1 Inhibitor, or Ipilimumab	PD-1, GITR, IDO1, CTLA-4	Phase I, 3 arms	Adults, recurrent GBM	Arm I: nivolumab and anti-GITR every 4 weeks; Arm II: nivolumab every 4 weeks and IDO1 inhibitor daily; Arm III: nivolumab and ipilimumab every 3 weeks ×4, then nivolumab every 4 weeks	None	30 expected	N/A	Primary: toxicity; secondary: OS, PFS, immunogenicity, biomarkers	Active, not recruiting	N/A
NCT03233152	GlitIpNi	PD-1, CTLA-4	Phase I, single arm	Adults, recurrent GBM	Nivolumab ×1 24 hours prior to re-resection with ipilimumab injection into resection cavity, then nivolumab every 2 weeks ×5	None	6 expected	N/A	Primary: toxicity, OS, PFS	Recruiting	N/A

PD-L1, programmed death ligand 1; GBM, glioblastoma; PFS, progression-free survival; OS, overall survival; PR, partial response; SD, stable disease; AE, adverse event; PD-1, programmed death-1; G3–4, grade 3–4; DLT, dose limiting toxicity; CSF-1R, colony-stimulating factor 1 receptor; ORR, objective response rate; chemoRT, chemoradiation; TMZ, temozolomide; HR, hazard ratio; MGMT, O^6-methylguanine DNA methyltransferase; SOC, standard of care; RT, radiation; IDO1, indoleamine-2,3-dioxygenase 1; QoL, quality of life; MLA, MRI-guided laser ablation; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; LAG-3, lymphocyte-activation gene 3; n, number of patients; HGG, high grade glioma (i.e., WHO Grade III or IV glioma); GITR, glucocorticoid-induced TNFR family related gene.

stated that the experimental treatment "failed to prolong overall survival" compared to the control arm [Bristol-Myers Squibb, 2019, unpublished data (103)]. The CheckMate-548 phase III, randomized, double-blind, placebo-controlled study of standard chemoradiation with TMZ, with or without concomitant nivolumab, for an expected 693 patients with newly diagnosed MGMT-methylated GBM, has also failed to meet its primary endpoint of PFS, while the OS endpoint has yet to mature [Bristol-Myers Squibb, 2019, unpublished data (104)].

Various hypotheses have been proposed to explain the lackluster outcomes of these trials, including low mutational burden of gliomas, low prevalence of PD-1 expressing CD8$^+$ T cells coupled with low PD-L1 expression of glioma tumor cells, and immunosuppressive characteristics of the glioma tumor microenvironment (TME). Tumor mutational "burden" or "load" (TML) is a quantification of the rate of nonsynonymous mutations (i.e., those tumor DNA mutations causing an alteration to the transcribed amino acid sequence). TML is correlated to the number of neoantigens transcribed, which is in turn correlated to the likelihood of a tumor-specific immune response and response to ICIs (117-119). High mutational burden is seen often in some solid tumor histologies, such as melanoma and NSCLC, but this level of burden is seen in <10% of GBM (120,121). A subset of patients with GBM have been identified as having a hypermutator phenotype, which is associated with mismatch repair (MMR) deficiency (121,122). While the prevalence of MMR deficiency in GBM is low (<5%) (121,123), a phase II trial is currently open to investigate the efficacy of nivolumab in patients with recurrent gliomas with the hypermutator phenotype (NCT03718767). Regarding checkpoint proteins, higher PD-1 and PD-L1 expression has been shown to predict response to ICIs in other cancers (124,125). Several studies have found low rates of PD-1 expression on tumor infiltrating leukocytes (TILs) in glioma specimens (~34%) with even lower rates of PD-L1 expression on tumor cells (~7%) (121,126), suggesting that these inhibitory pathways may play a less significant role in glioma immune escape compared to other cancers. Several ongoing studies are investigating the use of novel ICIs targeting checkpoint proteins that may be more pertinent to immune escape in gliomas, such as CD137 and LAG-3 [NCT02658981, (111)], GITR (NCT03707457), and CD27 [NCT02335918, (99)]. Many ongoing studies are also evaluating for more reliable biomarkers to predict a response to therapy. For example, in the Adult Brain Tumor Consortium 1501 trial, one patient

had a complete response and, prior to immunotherapy, was found to have higher T cell receptor (TCR) clonality and peripheral blood with more CD137$^+$CD8$^+$ T cells and fewer Foxp3$^+$CD137$^+$CD4$^+$ T cells than the other 5 patients analyzed (111). The glioma TME also seems to play a large role in suppressing tumor-specific immune responses. These immune modulating mechanisms are varied and complex, including cytokine expression that favors regulatory T cells (Tregs, which are CTLA-4$^+$ and secrete immunosuppressive cytokines TGF-β and IL-10) and recruits immunosuppressive macrophages (via CSF-1), as well as expression of pro-apoptotic cell surface proteins (e.g., CD95 and CD70) (127). Indoleamine-2,3-dioxygenase 1 (IDO1), expressed in high levels in GBM specimens, causes inhibition and apoptosis of CTLs as well as amplification of Tregs (128). Several ongoing studies are utilizing nivolumab in combination with inhibitors of immune suppressors, including an IDO1 inhibitor (NCT03707457), cabiralizumab (a monoclonal anti-CSF-1R antibody, NCT02526017), and galunisertib (a TGF-β receptor I inhibitor, NCT02423343).

Another approach being studied in HGG is the use of dual immune checkpoint blockade, based on its efficacy in other tumor types (129-131). In the phase I exploratory study of CheckMate-143, patients were randomized to receive nivolumab or combined nivolumab/ipilimumab at various doses (110). The investigators found similar efficacy between arms, but in the dual ICI arms there were more adverse events leading to treatment discontinuation, which was the basis for using nivolumab alone in the phase III study. However, toxicity was acceptable overall, and a number of studies are open to further investigate the efficacy and safety of dual ICIs in newly diagnosed GBM and recurrent HGG (see *Table 2*).

Of note, the safety of ICIs has generally been considered acceptable in the published trials of their use in gliomas. However, in comparison to vaccine therapies, which have very rare adverse events and dose limiting toxicities, ICIs can cause severe immune-related toxicities in some patients. Thus, their relative benefit must be considered in relation to the potential for toxicity. While these toxicities are felt to be acceptable in the treatment of other malignancies, these disease sites have a more clearly defined benefit that has yet to be shown in HGG.

Adoptive lymphocyte transfer (ALT): passive immunotherapy

As another form of passive immunotherapy, ALT aims to

provide an exogenous tumor directed T cell population. This population may be developed from tumor-specific T cells, either CTLs harvested from peripheral blood or TILs from tumor specimens, which are expanded *ex vivo* before being (re)introduced into the patient following chemotherapy preconditioning (132). Alternatively, normal T cells can be harvested from peripheral blood and modified to express tumor-specific TCRs or chimeric antigen receptors (CARs) (133,134). TCR T cells are developed by identifying TCR genes in tumor-reactive T cells, then isolating and transferring these genes to normal T cells to activate them against a TAA. On the other hand, CAR T cells are developed by engineering a receptor composed of the variable regions of an antibody specific for a TAA, linked to intracellular signaling proteins and co-stimulatory molecules. TCR T cells can only recognize intracellular antigens by processes of MHC expression and co-stimulation, both of which are often downregulated by tumor cells. On the other hand, CAR T cells can recognize peptides or cell surface components (including carbohydrates and glycolipids) in an MHC-independent manner without a need for co-stimulation, giving them a broader range than TCR T cells, more tumor infiltrative capacity than monoclonal antibodies, and fewer barriers to activating an anti-tumor immune response than normal T cells (135). There are adoptive cell therapies that utilize other cellular populations, such as natural killer cells, lymphokine-activated killer cells, and gamma-delta cells, but these are used less often in current clinical research. Because of the distinct advantages of CAR T cells, the majority of adoptive cell research utilizes this approach and will be the focus of this section, with additional studies summarized in *Table 3*.

The majority of CAR T cell preclinical and clinical trials utilize EGFRvIII, HER2, IL13Rα2, or EphA2 as targets. Two phase I studies using EGFRvIII CAR T cells in adults with recurrent EGFRvIII positive GBM have been published to date. In a first-in-human trial of 10 patients who received a single infusion of CAR T cells without preconditioning chemotherapy, the authors reported 3 neurological adverse events that were possibly related to treatment, but may have been related to the disease itself (136). The median OS for this cohort was 8.2 months. Notably, one patient had SD 18 months later at the time of publication. Interestingly, the investigators obtained post-treatment tissue from 7 patients, five of which exhibited less TAA expression with increased expression of inhibitory cell surface proteins and Tregs compared to pre-treatment specimens, indicating adaptive

changes in the local TME. The second published phase I trial included 18 patients who received preconditioning chemotherapy followed by EGFRvIII CAR T cell infusion with IL-2 in a dose-escalated design (137). There were 2 treatment-related adverse events, including one death at the highest CAR T cell dose level. Similar to the prior study, median survival outcomes were unimpressive with a PFS of 1.3 months (IQR, 1.1–1.9) and OS of 6.9 months (IQR, 2.8–10.0), but one patient achieved a progression-free interval of 12.5 months, two patients lived more than 12 months, and a third was alive at the time of analysis 59 months later. An additional phase I EGFRvIII CAR T cell trial in recurrent GBM is open (NCT03170141), and another, in newly diagnosed GBM that infused CAR T cells during standard maintenance TMZ, was terminated after enrolling 3 patients, results not yet reported (NCT02664363).

HER2 is expressed on up to 80% of GBM tumors, and, like EGFRvIII, is not expressed in normal neurons or glia (143). In the phase I, single arm HERT-GBM study, 17 patients (10 adults and 7 children) with recurrent GBM were treated with HER2-targeted CAR T cells infused at least once, and up to 6 times if an objective response was achieved (138). Eight patients experienced clinical benefit with one PR lasting 9 months and 7 with SD lasting 2–29 months. Three patients were alive without disease at 24–29 months. Median OS was 11.1 months (95% CI, 4.1–27.2) from the time of progression and 24.5 months (95% CI, 17.2–34.6) from the time of initial diagnosis. The iCAR trial is a phase I follow-up study from the same group of investigators that separates patients into "high risk" and "standard risk" groups based on the degree of HER2 positivity of the tumor (51–100% or 1–50%, respectively, NCT02442297).

Another recently published first-in-human phase I CAR T cell trial investigated the use of IL13Rα2 as a target for adults with recurrent GBM (139). IL-13 receptor α2 is overexpressed by 45–75% of GBM tumors, not significantly expressed on normal brain tissue, and, when bound to its ligand IL-13, releases the immunosuppressive TGF-β, making it an attractive therapeutic target (144-146). In this trial, 3 patients underwent resection of recurrent tumor with placement of a catheter, followed by intracavitary or intratumoral CAR T cell infusion up to 12 times. Two patients had grade 3 adverse events, one headache and another with transient neurologic symptoms. One patient's tumor was analyzed before and after CAR T cell therapy and was noted to have less IL13Rα2 expression, presenting the same question observed in earlier vaccine trials: does

Table 3 Adoptive lymphocyte transfer clinical trials

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
CAR T cells											
NCT02209376	EGFRvIII CAR T cells	EGFRvIII	Phase I, single arm	Adults, recurrent GBM, EGFRvIII(+)	CAR T cells infused ×1	None	10	OS: 8.2	AEs: 3 (neurologic, possibly related)	Published	(136)
NCT01454596	EGFRvIII CAR T cells with Chemo and IL-2	EGFRvIII	Phase I, dose escalation	Adults, recurrent GBM, EGFRvIII(+)	Conditioning chemotherapy (flu/cy), then CAR T cells with IL-2 ×1	None	18	PFS: 1.3 (IQR, 1.1–1.9); OS: 6.9 (IQR, 2.8–10.0)	2 treatment related adverse events (hypoxia), including 1 death (highest dose level)	Published	(137)
NCT03170141	EGFRvIII CAR T cells with Chemo	EGFRvIII	Phase I, single arm	Adults, recurrent GBM, EGFRvIII(+)	Conditioning chemotherapy (flu/cy), then CAR T cells ×1	None	20 expected	N/A	Primary: toxicity; secondary: OS, PFS, ORR, immunogenicity	Enrolling by invitation	N/A
NCT02664363	ExCeL	EGFRvIII	Phase I, single arm	Adults, new GBM, EGFRvIII(+)	Up to 3 cycles of standard adjuvant TMZ, followed by CAR T cells ×1, followed by additional adjuvant TMZ	None	3	N/A	Primary: MTD; secondary: toxicity	Terminated (study funding ended)	N/A
NCT01109095	HERT-GBM	HER2	Phase I, single arm	Adults (n=10) and children (n=7), recurrent GBM, HER2(+)	CAR T cells ×1, up to 6 times if objective response	None	17	PFS: 3.5; OS: 11.1 (4.1–27.2); PR: 1; SD: 7	Serious AEs: 0	Published	(138)
NCT02442297	iCAR	HER2	Phase I, 2 arms	Adults and children, recurrent GBM, HER2(+)	CAR T cells ×3 at various dose levels; Arm I: high risk (51–100% HER2 positive); Arm II: standard risk (1–50%)	None	28 expected	N/A	Primary: toxicity; secondary: ORR	Recruiting	N/A
NCT00730613	IL13Rα2 CAR T cells (single arm)	IL13Rα2	Phase I, single arm	Adults, recurrent GBM	Resection and catheter placement, followed by intracavitary or intratumoral CAR T cell infusion, up to 12 times	None	3	OS: 10.3 (range, 8.6–13.9)	G3 AE: 2 (headache, transient neurologic symptoms)	Published	(139)
NCT02208362	IL13Rα2 CAR T cells (5 arms)	IL13Rα2	Phase I, 5 arms	Adults and children 12+ years old, recurrent HGG, IL13Rα2(+)	CAR T cell infusion weekly ×3, then repeated at least 1 week later until progression or depletion of product: Arm I: intratumoral; Arm II: intracavitary; Arm III: intraventricular; Arm IV: intratumoral and intraventricular; Arm V: intratumoral and intraventricular vaccine therapy	None	92 expected	Case report: pt in arm IV had CR of all intracranial and spinal tumors, maintained for 7.5 mo, no G3+ toxicities	Primary: toxicity; secondary: OS, PFS, ORR, immunogenicity, QoL	Recruiting, case report published	(140)
NCT02575261	SOC treatment +/– EphA2 CAR T cells	EphA2	Phase I/II, randomized	Adults, new or recurrent GBM, EphA2(+)	CAR T cell infusion	SOC treatment	60 enrolled	N/A	Primary: immunogenicity; secondary: ORR, PFS, toxicity	Completed, not published	N/A
CTLs											
ACTRN12609000338268	CMV-specific T cells	CMV peptides	Phase I, single arm	Adults, recurrent GBM, CMV(+) serology	Activated T cell infusions every 4 weeks ×3 or until depletion, concomitant chemotherapy or bevacizumab allowed (n=9)	None	11	PFS: 8.1 (range, 3.6–58.7); OS: 13.3 (range, 4.4–79.9+)	Serious AEs: 0	Published	(141)
NCT02060955	ALECSAT vs. bevacizumab and irinotecan	Tumor antigens (to activate CD8+, CD4+ T cells, NK cells)	Phase II, randomized	Adults, recurrent GBM	ALECSAT infusion weeks 4, 9, 14, 26, 46, crossover permitted	Bevacizumab and irinotecan every 2 weeks	Experimental: 15; Control: 10 (175 planned)	PFS: 1.0 vs. 5.4, HR 0.16, P<0.001; OS: 5.0 vs. 6.8, HR 0.45, P=0.19	–	Terminated, abstract only	(142)
NCT02799238	ChemoRT +/– ALECSAT	Tumor antigens (to activate CD8+, CD4+ T cells, NK cells)	Phase II, randomized	Adults, new GBM	ALECSAT infusion starting concomitantly with chemoRT, every 4 weeks ×3, then every 3 months	SOC treatment	62 expected	N/A	Primary: PFS; secondary: OS, ORR, toxicity, QoL	Active, not recruiting	N/A
TILs											
NCT03347097	Autologous TILs vs. PD1-TILs	Tumor antigens	Phase I, 2 arms	Adults, new GBM	Starting 10 days after standard chemoRT, infused twice every 30 days without adjuvant TMZ: Arm I: TIL infusion; Arm II: PD-1-TIL infusion (transgenic modified TILs expressing PD-1)	None	40 expected	N/A	Primary: toxicity; secondary: OS, PFS, ORR	Recruiting	N/A

Table 3 (continued)

Table 3 *(continued)*

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
TCR T cells											
NCT01082926	Intratumoral GRm13Z40-2 T cells	IL-13-zetakine, HyTK	Phase I, single arm	Adults, recurrent HGG	Intratumoral GRm13Z40-2 CTL infusion on days 1 and 3 and IL-2 on days 2–5, weekly ×2	None	6 enrolled	N/A	Primary: toxicity	Completed, not published	N/A
Gamma-delta T cells											
NCT04165941	Intratumoral γδ T cells	Tumor antigens	Phase I, single arm	Adults, new GBM	Intratumoral γδ T cells 1–3 times with standard adjuvant TMZ	None	12 expected	N/A	Primary: MTD; secondary: OS, PFS, immunogenicity	Recruiting	N/A

CAR, chimeric antigen receptor; EGFRvIII, epidermal growth factor variant III; GBM, glioblastoma; OS, overall survival; AE, adverse event; IL-2, interleukin-2; Flu/cy, fludarabine/cyclophosphamide; PFS, progression free survival; IQR, interquartile range; ORR, objective response rate; TMZ, temozolomide; MTD, maximum tolerated dose; HER2, human epidermal growth factor receptor 2; PR, partial response; SD, stable disease; IL13Rα2, interleukin 13 receptor subunit alpha 1; G3, grade 3; HGG, high grade glioma (i.e., WHO Grade III or IV glioma); Pt, patient; QoL, quality of life; SOC, standard of care; CTL, cytotoxic T lymphocyte; EphA2, EPH receptor A2; CMV, cytomegalovirus; n, number of patients; NK, natural killer; HR, hazard ratio; ChemoRT, chemoradiation; TIL, tumor infiltrating lymphocyte; PD-1, programmed death-1; TCR, T cell receptor; IL-13, interleukin 13.

this represent selective eradication of antigen positive tumor cells, or downregulation of antigen presentation as a mechanism of adaptive immune escape? Surprisingly, the patient with the lowest pre-treatment expression of IL13Rα2 was one of 2 who had an objective response and a favorable OS of 10.3 months, compared to 8.6 and 13.9 months in the other two patients. A larger follow-up study is now recruiting patients 12 years or older with recurrent HGG and allocating to 5 arms with various modes of delivery, including intratumoral, intracavitary, intraventricular, or a combination (NCT02575261). The investigators published a case report on one study participant with recurrent multifocal GBM who received 6 intracavitary and 10 intraventricular infusions with a complete response of all intracranial and spinal tumors, maintained 7.5 months later with no grade 3 or higher adverse events (140).

Similar to the use of ICIs, the existing clinical trials utilizing ALT have shown impressive durable responses in subsets of patients, though the majority of study patients experience a disease course similar to those receiving standard care. One limitation of ALT is that the patient must have a tumor that expresses the target of interest. In the initial trial of EGFRvIII CAR T cells, the authors reported that the tumors of 369 patients were evaluated for possible study entry, but only 79 (21%) were EGFRvIII positive (136). The heterogeneity of glioma tumors also poses a challenge. As seen in the trial of IL13Rα2 CAR T cells, there may only be selective killing of tumor cells that express the target antigen, leaving the remaining tumor cells free to divide and repopulate. Researchers are developing T cells loaded with multiple CARs (147) or bispecific CARs (148) in attempts to overcome this issue. Alternatively, the lack of antigen positive tumor cells after ALT may represent adaptive immune escape, in which case a combined modality approach may be more efficacious, a topic that is discussed further below.

In terms of safety, the two most concerning ALT-related toxicities that have been seen in the treatment of other malignancies are cytokine release syndrome and on-target/off-tumor effects (149). Cytokine release syndrome occurs as a result of diffuse triggering of T cells leading to overproduction of proinflammatory cytokines, manifesting as a combination of fever, hypotension, hypoxia, and tachycardia. On-target/off-tumor effects refer to cross reactivity between tumor cells and normal tissue cells that also express the target antigen. This was observed in the early trials of ALT in melanoma, in which there was damage to melanocytes of the skin, eye, and cochlea (150). These effects have not been observed in the published clinical trials of ALT in HGG. With the exception of a treatment-related death in the dose escalation study of EGFRvIII CAR T cells, the observed toxicities have been infrequent and transient.

Radiation and immunotherapy

The term abscopal effect was first used in 1953 to describe the phenomenon of radiation's effect on decreasing the size of tumors "at a distance from the irradiated volume" (151). This distant effect on non-radiated tumors has since been shown to be immune-mediated in preclinical studies and case reports (152-154). It is hypothesized that radiation's direct effects of tumor cell killing initiate a cascade of proinflammatory cytokine release and ingestion of dead cells by APCs, which in turn release immunostimulatory cytokines and present TAAs to CD8$^+$ T cells, which differentiate into CTLs to produce a tumor-specific immune response, a process termed immunogenic cell death (155,156). Apart from direct cytotoxic effects of DNA damage, radiation may increase the immunogenicity of tumor cells by various mechanisms. Non-lethal DNA mutations caused by radiation may lead to the transcription of neoantigens, increasing mutational burden and likelihood of immune recognition (157). Double-stranded DNA fragments that enter the cytoplasm indirectly activate stimulator of interferon genes (STING), which leads to the production of IFN-I, which is involved in the maturation of glioma-directed DCs (158,159). In radiation-induced cell death, damage-associated molecular patterns (DAMPs), such as high-mobility-group box 1 (HMGB1) (160), are released and cause increased expression of costimulatory molecules on DCs, which in turn activate CTLs (161). Microglia also express DAMP receptors which, when bound, activate these cells to enhance antigen presentation (162). Radiation also increases MHC-I expression, thereby increasing the recognition of TAAs and neoantigens by CD8$^+$ T cells (163). Given the role of radiation in SOC treatment for gliomas, and its apparent role in increasing tumor immunogenicity, its combination with immune therapies may have a synergistic effect. This has in fact been seen in a number of preclinical trials in which radiation delivered in combination with blockade of PD-1, CTLA-4, and other checkpoint proteins leads to improved tumor response and survival (164-166).

When considering the addition of immunotherapy to

SOC treatment, the appropriate sequencing and timing of therapies is unknown. Most of the patients with newly diagnosed HGG included in the studies discussed in the previous sections (see *Tables 1-3*) underwent standard chemoradiation with neoadjuvant, concurrent, and/or adjuvant experimental immunotherapy. These studies are categorized by timing of immunotherapy relative to radiation in *Table 4*. One of these studies compared neoadjuvant and concurrent immunotherapy to adjuvant immunotherapy (45). Namely, newly diagnosed GBM patients in the Cancer Research UK IMA950-101 trial received a multi-peptide vaccine starting 7–14 days prior to chemoradiation that was continued concurrently (arm I,

n=22) or started 7 days after completion of chemoradiation (arm II, n=23). Overall, there was a trend toward more TAA responses per patient in arm II (mean, 1.6 in arm I *vs.* 2.2 in arm II, P=0.295). Furthermore, when patients were assessed for any vaccine response at three different timepoints relative to the first vaccination, patients in arm I had a higher rate of response at timepoint 1 (83% *vs.* 57%), but a lower rate at timepoints 2 (7% *vs.* 21%) and 3 (10% *vs.* 21%). In other words, patients in arm I had a more robust response prior to chemoradiation that was not maintained during the course of treatment, whereas patients in arm II had a response that was less robust upfront but was more durable. These findings suggest chemoradiation

Table 4 Clinical Trials of Immunotherapy with Conventionally Fractionated Radiation, Categorized by Timing of Immunotherapy

Trial number	Abbreviated trial name	Target	Additional details	Reference
Neoadjuvant and adjuvant				
NCT02550249	Neoadjuvant and adjuvant nivolumab	PD-1	Table 2	(100)
NCT01006044	Autologous tumor lysate DC vaccine (phase II)	Tumor lysate (autologous)	Table 1	(63)
Adjuvant				
NCT03047473	The SEJ Study	PD-L1	Table 2	(109)
NCT02311920	Ipilimumab and/or nivolumab	PD-1, CTLA-4	Table 2	N/A
NCT03347097	Autologous TILs *vs.* PD1-TILs	Tumor antigens	Table 3	N/A
NCT04165941	Intratumoral $\gamma\delta$ T Cells	Tumor antigens	Table 3	N/A
NCT00643097	ACTIVATE	EGFRvIII	Table 1	(38)
NCT00643097	ACT II	EGFRvIII	Table 1	(39)
NCT00458601	ACT III	EGFRvIII	Table 1	(40)
NCT01480479	ACT IV	EGFRvIII	Table 1	(41)
NCT02454634	NOA-16	IDH1R132H	Table 1	(43)
NCT01920191	IMA950 multi-peptide vaccine with poly-ICLC	11 GBM TAAs	Table 1	(46)
NCT02149225	GAPVAC-101	5–10 unmutated TAAs and 1–2 mutated TAAs based on tumor mutation/transcriptome analysis	Table 1	(49)
UMIN000001426	An autologous tumor vaccine with adjuvant TMZ	Autologous tumor peptides	Table 1	(51)
NCT00905060	HeatShock	HSPPC-96	Table 1	(56)
NCT03650257	Adjuvant TMZ +/– HSPPC-96 vaccine	HSPPC-96	Table 1	N/A
N/A	EGFRvIII-targeted DC vaccine	EGFRvIII	Table 1	(57)

Table 4 *(continued)*

Table 4 (*continued*)

Trial number	Abbreviated trial name	Target	Additional details	Reference
N/A	ICT-107	6 GBM TAAs	Table 1	(58)
NCT01280552	Adjuvant TMZ +/− ICT-107	6 GBM TAAs	Table 1	(59)
NCT00068510	Autologous tumor lysate DC vaccine (phase I)	Tumor lysate (autologous)	Table 1	(60)
EY-DOH-MD #0910072504	Autologous tumor lysate DC vaccine (phase I/II)	Tumor lysate (autologous)	Table 1	(61)
N/A	ChemoRT +/− autologous tumor lysate DC vaccine	Tumor lysate (autologous)	Table 1	(62)
NCT01213407	Adjuvant TMZ +/− Audencel	Tumor lysate (autologous)	Table 1	(65)
NCT00045968	Adjuvant TMZ +/− DCVax®-L	Tumor lysate (autologous)	Table 1	(66)
NCT01957956	Allogenic tumor lysate DC vaccine (new GBM)	Tumor lysate (allogeneic)	Table 1	N/A
NCT02010606	Allogenic tumor lysate DC vaccine from a GBM stem-like cell line	Tumor lysate (allogeneic)	Table 1	N/A
NCT00846456	GSC antigen mRNA DC vaccine	Autologous GSC antigens	Table 1	(67)
NCT02649582	ADDIT-GLIO	WT1	Table 1	N/A
NCT02709616	PERCELLVAC	Autologous TAAs	Table 1	N/A
NCT00639639	CMV pp65 DC vaccine	pp65	Table 1	(69)
NCT03927222	I-ATTAC	pp65	Table 1	N/A
NCT02465268	ATTAC-II	pp65	Table 1	N/A
Concurrent				
NCT02617589	CheckMate-498	PD-1	Table 2	(103)
NCT02667587	CheckMate-548	PD-1	Table 2	(104)
NCT02530502	Pembrolizumab with chemoRT	PD-1	Table 2	N/A
NCT02336165	Durvalumab with RT (new) or with/without bevacizumab (recurrent)	PD-L1	Table 2	(106)
NCT04047706	Nivolumab and IDO1 inhibition (BMS-986205) with RT or ChemoRT	PD-1, IDO1	Table 2	N/A
NCT02799238	ChemoRT +/− ALECSAT	Tumor antigens (to activate CD8+, CD4+ T cells, NK cells)	Table 3	N/A
UMIN000000002	An autologous tumor vaccine with RT	Autologous tumor peptides	Table 1	(50)
NCT01567202	ChemoRT +/− autologous GSC DC vaccine	Autologous GSC antigens	Table 1	(68)
Other				
NCT01222221	Cancer Research UK IMA950-101	11 GBM TAAs	Table 1	(45)

PD-1, programmed death-1; DC, dendritic cell; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; TIL, tumor infiltrating lymphocyte; EGFRvIII, epidermal growth factor variant III; Poly-ICLC, polyinosinic-polycytidylic acid-poly-l-lysine carboxymethylcellulose; GBM, glioblastoma; TAA, tumor associated antigen; TMZ, temozolomide; HSPPC-96, heat shock protein peptide complex 96; ChemoRT, chemoradiation; GSC, glioma stem cell; WT1, Wilms tumor 1; CMV, cytomegalovirus; pp65, phosphoprotein 65; RT, radiation; IDO1, indoleamine-2,3-dioxygenase 1; NK, natural killer.

may limit induction and maintenance of a tumor-specific immune response, possibly due to chemotherapy- and radiation-induced lymphodepletion; on the other hand, chemotherapy-induced lymphodepletion in the adjuvant setting may cause a subdued induction response followed by lymphoid recovery allowing for expansion and maintenance of the response. While clinical outcomes were the same between groups, the study was not randomized in design or powered to detect survival differences. The vast majority of vaccine therapy trials in newly diagnosed gliomas listed in *Table 4* deliver the experimental therapy adjuvantly, whereas most of the ICI trials utilize concurrent delivery. These studies may provide clues as to how sequencing and timing can be optimized for various types of therapeutics, but larger randomized trials are needed to improve our understanding of these details. Particularly, the potential advantages of initiating immunotherapy prior to radiation are unknown due to a paucity of studies designed in this fashion (see *Table 4*).

While the prior discussion focused on the use of conventionally-fractionated radiation therapy [CFRT, using smaller doses of 1.8–2.0 Gray (Gy) per fraction daily over 6 or more weeks], some have hypothesized that fractionated stereotactic radiosurgery (fSRS, using doses of 6–8 Gy per fraction over 3–5 treatments) may be more effective in generating an immune response. Such "ablative" doses may lead to increased tumor antigen release and, in preclinical studies, have shown favorable changes to the TME (167,168). Though the use of fSRS has not proven beneficial in the treatment of GBM (169), its use in combination with immunotherapy has not been well studied. A number of clinical trials are underway to investigate the benefit of fSRS and ICIs (and one study using EGFRvIII CAR T cells) in recurrent glioma (*Table 5*). One phase I, single arm study published interim results on 23 patients treated with fSRS to a dose of 30 Gy in 5 fractions with bevacizumab and concurrent dose-escalated pembrolizumab with 53% of patients experiencing a complete or PR lasting at least 6 months, and 6- and 12-month OS rates of 94% and 64%, respectively (170). There was one grade 3 treatment-related toxicity of elevated liver transaminases. As an unstudied domain, these upcoming trials are an exciting source of data to potentially support new and meaningful treatment approaches.

Of note, while it appears radiation may augment tumor-specific immune responses, concerns have been raised regarding the potential immunosuppressive effects of standard treatment for HGG. Patients treated with radiation and TMZ (and, in many cases, glucocorticoids) often have significant reductions in CD4+ T cell counts that are long-lasting and have been associated with early death due to tumor progression (173). While chemotherapy-induced lymphodepletion is a well-understood phenomenon, radiation-induced lymphodepletion may impede immune-mediate tumor control due to direct damage to lymphocytes, though these mechanisms are not well understood (37). One published phase I/IIa study of 22 patients with newly diagnosed GBM received an autologous tumor peptide vaccine concomitant with and after standard radiation without TMZ with favorable PFS and OS outcomes of 7.6 months (95% CI, 4.3–13.6) and 19.8 months (95% CI, 13.8–31.3), respectively (50). However, the unpublished results of the large CheckMate-498 trial, which compared SOC treatment to concurrent and adjuvant nivolumab without TMZ, showed no survival benefit [Bristol-Myers Squibb, 2019, unpublished data (103)]. Additional studies of various immunotherapy modalities with or without TMZ are needed to further characterize the effects of chemotherapy on tumor-directed immune responses and clinical outcomes. Several ongoing studies are omitting concurrent or adjuvant TMZ, including one phase I trial of nivolumab and IDO1 inhibition in newly diagnosed GBM that includes separate treatment arms with or without TMZ (NCT04047706). Details regarding chemotherapy use in these studies are summarized in *Table 4*.

Combination immunotherapies

One of the predominant approaches in recently opened clinical trials for newly diagnosed and recurrent HGG is combination immunotherapy, i.e., utilizing a combination of therapeutic vaccination, ICI, and ALT. Each individual therapy stimulates or augments the immune system by a different mechanism: ICIs overcome local immunosuppression of the TME and T cell anergy, while vaccines and ALT overcome nonimmunogenic properties of the tumor and augment the limited specificity and size of the pre-existing T cell population, respectively. Preclinical studies have, indeed, shown improved immune and tumor responses with the use of an ICI in conjunction with a vaccine or ALT (174-177). Moreover, hypotheses of improved responses to combination therapy are also derived from observations of some of the early single-modality clinical trials discussed above. For example, O'Rourke *et al.* observed that after administration of EGFRvIII CAR T

Table 5 Clinical trials of immunotherapy with fractionated stereotactic radiosurgery

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
NCT02313272	Pembrolizumab with fSRS and bevacizumab	PD-1	Phase I, single arm, 3+3	Adults, recurrent HGG	fSRS (30 Gy/5 fx) with concomitant dose-escalated pembrolizumab every 3 weeks and bevacizumab every 2 weeks	None	32 enrolled; 23 analyzed	6-mo OS: 94%; 12-mo OS: 64%; CR or PR ≥6 mo: 53%	G3+ AE: 1 (elevated transaminases)	Active, not recruiting, abstract only	(170)
NCT02866747	STERIMGLI	PD-L1	Phase I/II, randomized	Adults, recurrent GBM	fSRS (24 Gy/3 fx) with durvalumab starting day of 3rd fx, then every 4 weeks	fSRS (24 Gy/3 fx) alone	62 expected	Phase I results: G3+ AE: 1 (vestibular neuritis)	Primary: DLT, ORR; secondary: OS, PFS, QoL, toxicity	Suspended (Interim analysis), abstract only (phase I)	(171)
NCT02968940	Avelumab with fSRS	PD-L1	Phase II, single arm	Adults, WHO II or III IDH-mutant glioma transformed to GBM	fSRS (30 Gy/5 fx) with avelumab every 2 weeks	None	43 expected	N/A	Primary: toxicity, PFS; secondary: OS, ORR	Active, not recruiting	N/A
NCT03961971	Anti-Tim-3 (MBG453) and spartalizumab (anti-PD-1) with fSRS	Tim-3, PD-1	Phase I, single arm	Adults, recurrent GBM	Anti-Tim-3 and spartalizumab ×1, then fSRS, then anti-Tim-3 and spartalizumab every 4 weeks until progression or intolerance	None	15 expected	N/A	Primary: toxicity; secondary: OS, PFS, ORR	Active, not recruiting	N/A
NCT02829931	Nivolumab with fSRS	PD-1	Phase I, single arm	Adults, recurrent HGG	fSRS (30 Gy/5 fx) then nivolumab every 2 weeks ×8, then nivolumab every 4 weeks ×4	None	26 expected	N/A	Primary: toxicity; secondary: OS, ORR, biomarkers	Recruiting	(172)
NCT03743662	Nivolumab with fSRS and bevacizumab	PD-1	Phase II, 2 arms	Adults, recurrent GBM, MGMT-methylated	Arm I: nivolumab every 2 weeks ×2, then fSRS (30 Gy/5 fx) with bevacizumab every 2 weeks ×2, then nivolumab every 2 weeks until progression or intolerance; Arm II: Addition of surgery after first 2 cycles of nivolumab, prior to fSRS	None	94 expected	N/A	Primary: OS; secondary: PFS, ORR	Recruiting	N/A
NCT04225039	Anti-GITR (INCAGN01876) and anti-PD-1 (INCMGA00012) with fSRS	GITR, PD-1	Phase II, 3 arms	Adults, recurrent GBM	Arm I: anti-GITR and anti-PD-1 ×1, then fSRS (24 Gy/3 fx), then anti-PD-1 every 4 weeks and anti-GITR every 2 weeks until progression or intolerance; Arm II: Arm I with surgery following fSRS; Arm III: Arm I with surgery and no RT	None	32 expected	N/A	Primary: ORR; secondary: OS, PFS, toxicity	Not yet recruiting	N/A
NCT03283631	INTERCEPT	EGFRvIII	Phase I, single arm	Adults, recurrent GBM, EGFRvIII(+)	fSRS followed by same-day intratumoral infusion of CAR T cells	None	24 expected	N/A	Primary: MTD; secondary: OS, PFS, immunogenicity	Recruiting	N/A

fSRS, fractionated stereotactic radiosurgery; PD-1, programmed death-1; HGG, high grade glioma (i.e., WHO Grade III or IV glioma); Gy, Gray; Fx, fractions; OS, overall survival; CR, complete response; PR, partial response; G3, grade 3; AE, adverse event; PD-L1, programmed death ligand 1; GBM, glioblastoma; DLT, dose limiting toxicity; ORR, objective response rate; PFS, progression free survival; QoL, quality of life; MGMT, O⁶-methylguanine DNA methyltransferase; GITR, glucocorticoid-induced TNFR family related gene; EGFRvIII, epidermal growth factor variant III; CAR, chimeric antigen receptor; MTD, maximum tolerated dose.

cells to patients with recurrent GBM, resection specimens expressed higher levels of inhibitor cell surface proteins, including PD-L1, with similar levels of PD-1 expression on TILs (136). Thus, the addition of an ICI that blocks interactions between PD-1 and PD-L1 would presumably limit this mechanism of adaptive immune escape. This hypothesis is the basis of an ongoing phase I, single arm trial of patients with newly diagnosed MGMT-unmethylated GBM, utilizing short-course radiation (40 Gy in 15 fractions) without TMZ followed by combined EGFRvIII CAR T cell and pembrolizumab infusions every 3 weeks for 3 and 4 cycles, respectively [NCT03726515, (178)].

In another single-modality trial, Schuessler *et al.* reported the results of a phase I, single arm trial of 11 adults with recurrent GBM and CMV-positive serology, who received autologous CMV-specific T cell infusions (141). Notably, one patient who enrolled after a second recurrence was still alive more than 6 years later at the time of publication. The investigators observed that patients with less durable control (i.e., PFS <100 days) had significant differences in gene expression compared to durable responders, including higher expression of CTLA-4, again implying the potential benefit of combined treatment with an ICI. Moreover, one subject underwent subsequent resection, and analysis of TILs showed that only approximately one third of antigen-specific T cells were polyfunctional. Polyfunctional T cells (i.e., those that express multiple pro-inflammatory cytokines and cytotoxic markers), have been associated with prolonged survival in melanoma patients treated with DC vaccination (179), and Reap *et al.* hypothesized that the addition of pp65 mRNA-loaded DCs to CMV-specific T cell therapy would generate more polyfunctional T cells (180). In their phase I study, 17 patients with newly diagnosed GBM were randomized to receive activated T cells once and either the DC vaccine or placebo every 2 weeks for three cycles, concomitant with standard adjuvant TMZ. They did in fact find that the presence of polyfunctional T cells (positive for IFN-γ, TNF-α, and CCL3) was correlated with OS (R =0.7371, P=0.0369) in patients that received combination therapy.

Limited data exist on clinical outcomes of combination immunotherapy in HGG at this time, but current reports include a phase I trial published in 2000 (181) and unpublished data from the AVeRT study [Archer G, 2020, unpublished data (182)]. In the former, a phase I, single arm trial reported by Sloan *et al.*, 19 adults with recurrent HGG (2 with anaplastic astrocytoma, 1 with gliosarcoma, and 16

with GBM) underwent resection followed by administration of an autologous whole tumor cell vaccine (with GM-CSF) and anti-CD3 T cell therapy with a favorable median OS of 12 months (range, 6–28) (181). In the phase I AVeRT study, 6 adults with recurrent HGG were randomized to receive 4 cycles of biweekly nivolumab with (n=3) or without (n=3) three additional biweekly cycles of nivolumab in combination with a pp65 mRNA-loaded DC vaccine. Patients in both arms then underwent re-resection followed by biweekly nivolumab until progression and 5 cycles of monthly DC vaccine infusions. The 3 patients who received both nivolumab and the DC vaccine prior to resection had PFS and OS of 6.3 months (4.7–10.7) and 15.3 months (4.73–not reached), respectively, compared to 4.3 months (2.1–5.3) and 8.0 months (5.7–8.3) in the patients who received nivolumab only prior to resection [Archer G, 2020, unpublished data (182)]. To date, the number of HGG patients treated with combination immunotherapy is small, but these early data and the scientific basis of their possible synergy are encouraging. Additional ongoing studies are summarized in *Table 6*.

Conclusions

This discussion has attempted to comprehensively review the existing data and ongoing studies of immunotherapy and radiation in HGG, including therapeutic vaccination, immune checkpoint inhibition, and ALT. Though larger randomized controlled trials have not consistently shown improvements in clinical outcomes, this area of research is still in its early stages and a number of important lessons can be taken away from the studies that have been completed to date. Many studies found a subset of patients who experienced durable responses, and analysis of their immune cells and tumor cells can be used to identify biomarkers that predict therapeutic response, as well as additional glioma-specific targets that can enhance therapeutic efficacy. Given the rationale for potential synergy with radiation and combination immunotherapies, future research should focus on these approaches. Identification of biomarkers to predict the efficacy of radiation combined with immunotherapy is of great importance in appropriately selecting patients for more aggressive treatment regimens. This specific area of combinatorial therapy is only in its infancy, and the results of ongoing trials are anticipated to push us further along the path to achieving long-term survival for patients with GBM and other HGGs.

Table 6 Combination immunotherapy clinical trials

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
Vaccine + ICI											
NCT04013672	SurVaxM peptide vaccine and pembrolizumab	Survivin, PD-1	Phase II, 2 arms	Adults, recurrent GBM	SurVaxM/GM-CSF/Montanide ISA 51 every 2 weeks times 4 then every 3 months, with pembrolizumab every 3 weeks, until progression or intolerance; Arm I: anti-PD-1 therapy naïve; Arm II: failed previous anti-PD-1 therapy	None	51 expected	N/A	Primary: PFS; secondary: toxicity	Recruiting	N/A
NCT03893903	AMPLIFY-NEOVAC	IDH1R132H, PD-L1	Phase I, 3 arms, randomized	Adults, recurrent HGG excluding 1p/19q co-deletion and nuclear ATRX loss	Experimental drug every 2 weeks ×3, followed by re-resection, followed by maintenance experimental drug: Arm I: IDH1R132H peptide vaccine; Arm II: IDH1R132H peptide vaccine with avelumab; Arm III: avelumab	None	48 expected	N/A	Primary: RLT; secondary: immunogenicity, AEs, ORR, OS, PFS	Recruiting	N/A
NCT03665545	IMA950-106	11 GBM TAAs, PD-1	Phase I/II, 2 arms, randomized	Adults, recurrent GBM, HLA-A*02(+)	Arm I: IMA950/poly-ICLC vaccine alone; Arm II: IMA950/poly-ICLC vaccine with pembrolizumab	None	24 expected	N/A	Primary: toxicity; secondary: PFS, OS, QoL, immunogenicity	Recruiting	N/A
NCT03018288	Pembrolizumab +/− HSPPC-96 vaccine	PD-1, HSPPC-96	Phase II, randomized, double-blind	Adults, new GBM	Arm I: pembrolizumab every 3 weeks ×3 concomitant with standard chemoRT with TMZ; Arm II: Arm I with addition of HSPPC-96 vaccine post-chemoradiation weekly ×4, then every 3 weeks ×6	Arm III: Arm II with placebo vaccine	14 enrolled	N/A	Primary: OS	Suspended (root cause analysis being conducted)	N/A
NCT04201873	Autologous tumor lysate DC vaccine +/− pembrolizumab	Tumor lysate (autologous), PD-1	Phase I, randomized	Adults, recurrent GBM	Pembrolizumab ×1 2 weeks prior to re-resection, then every 3 weeks after resection until progression or intolerance. DC vaccine/poly-ICLC after resection every 2 weeks ×3	Placebo pembrolizumab with experimental DC vaccine/poly-ICLC on same schedule	40 expected	N/A	Primary: toxicity, immunogenicity; secondary: OS, PFS, biomarkers	Recruiting	N/A
NCT02529072	AVeRT	pp65, PD-1	Phase I, randomized	Adults, recurrent HGG	Arm I: nivolumab every 2 weeks ×4, then re-resection, then biweekly nivolumab until progression and monthly CMV pp65 DC vaccine ×5; Arm II: Nivolumab every 2 weeks ×4, then nivolumab and CMV pp65 DC vaccine every 2 weeks ×3, then re-resection, then biweekly nivolumab until progression and monthly CMV pp65 DC vaccine ×5	None	Arm I: 3; Arm II: 3	PFS, OS: Arm I: 4.3 (2.1–5.3), 8.0 – (5.7–8.3); Arm II: 6.3 (4.7–10.7), 15.3 (4.73–not reached)		Completed, unpublished data	(182)
NCT02798406	DNX-2401 oncolytic adenovirus and pembrolizumab	E1A mutant, PD-1	Phase II, single arm	Adults, recurrent GBM	DNX-2401 vaccine intratumoral injection ×1, then pembrolizumab every 3 weeks for 2 years or until progression or intolerance	None	49 enrolled	N/A	Primary: ORR; secondary: OS, PFS	Active, not recruiting	N/A
Vaccine + other											
NCT02366728	ELEVATE	pp65, IL-2R	Phase II, randomized, double-blind, placebo-controlled	Adults, new GBM s/p resection with <1 cm residual in maximal dimension	Arm I: placebo DC vaccine; Arm II: DC vaccine; Arm III: DC vaccine with basiliximab (anti-IL-2R)	Arm I: placebo vaccine	100 expected	N/A	Primary: OS, immunogenicity; secondary: PFS	Active, not recruiting	N/A
Vaccine + ALT											
N/A	Autologous whole tumor vaccine and anti-CD3 T cells	Tumor antigens, CD3	Phase I, single arm	Adults, recurrent HGG	Re-resection followed by vaccine/GM-CSF ×2, followed by activated T cells ×1	None	19	OS: 12 mo (range, 6–28)	–	Published	(181)
NCT00693095	CMV-specific T cells +/− CMV pp65 DC vaccine	pp65	Phase I, randomized	Adults, new GBM	Arm I: activated T cells ×1 and DC vaccine every 2 weeks ×3 concomitant with standard adjuvant TMZ; Arm II: Arm I with saline placebo instead of DC vaccine	None	Arm I: 9; Arm II: 8	PFS and OS N/R; polyfunctional T cells correlated with OS (R =0.7371, P=0.0369) in Arm I	G3+ AEs: 0	Published	(180)

Table 6 (continued)

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Table 6 *(continued)*

Trial number	Abbreviated trial name	Target	Study design	Population	Intervention/arms	Control	Number of analyzed patients	Survival, median months (95% CI)	Additional outcomes	Trial status	Reference
ALT + ICI											
NCT03726515	EGFRvIII CAR T cells and pembrolizumab	EGFRvIII, PD-1	Phase I, single arm	Adults, new GBM, MGMT-unmethylated, EGFRvIII(+)	2–3 weeks after short-course RT (40 Gy/15 fx) without TMZ, CAR T cells and pembrolizumab given every 3 weeks ×3 and ×4, respectively	None	7 expected	N/A	Primary: toxicity; secondary: OS, PFS, ORR	Active, not recruiting	(178)
NCT04003649	IL13Rα2 CAR T cells and nivolumab +/– ipilimumab	IL13Rα2, PD-1, CTLA-4	Phase I, randomized	Adults, recurrent GBM, IL13Rα2(+)	Re-resection with intraventricular catheter placement, then: Arm I: ipilimumab and nivolumab ×1, then two weeks later, nivolumab and intraventricular CAR T cells ×4 or more; Arm II: nivolumab and intraventricular CAR T cells ×4 or more	None	60 expected	N/A	Primary: toxicity, feasibility; secondary: OS, PFS, ORR, immunogenicity, biomarkers, QoL	Recruiting	N/A
ICI + other											
NCT02423343	Galunisertib (anti-TGFβRI) and nivolumab	PD-1, TGFβRI	Phase Ib, single arm	Adults, recurrent GBM	Galunisertib for 14 days every 4 weeks ×4 and nivolumab every 2 weeks ×8	None	75 enrolled (entire cohort)	N/A	Primary: MTD	Active, not recruiting	N/A
NCT04160494	D2C7 (EGFR-targeted immunotoxin) and atezolizumab	EGFRwt/EGFRvIII, PD-L1	Phase I, single arm	Adults, recurrent GBM	Intratumoral D2C7 ×1, then atezolizumab ×1 2 weeks later, then possible re-resection 2 weeks later, then atezolizumab every 3 weeks for 2 years or until progression or intolerance	None	18 expected	N/A	Primary: toxicity	Recruiting	N/A

ICI, immune checkpoint inhibitor; PD-1, programmed death-1; GBM, glioblastoma; GM-CSF, granulocyte-macrophage colony-stimulating factor; PFS, progression-free survival; PD-L1, programmed death ligand 1; HGG, high grade glioma (i.e., WHO Grade III or IV glioma); ATRX, alpha-thalassemia/mental retardation, X-linked; RLT, regime limiting toxicity; AE, adverse event; ORR, objective response rate; OS, overall survival; TAA, tumor associated antigen; Poly-ICLC, polyinosinic-polycytidylic acid-poly-l-lysine carboxymethylcellulose; QoL, quality of life; HSPPC-96, heat shock protein peptide complex 96; ChemoRT, chemoradiation; TMZ, temozolomide; DC, dendritic cell; pp65, phosphoprotein 65; CMV, cytomegalovirus; E1A, early region 1A; IL-2R, interleukin 2 receptor; N/R, not reported; G3, grade 3; MGMT, O⁶-methylguanine DNA methyltransferase; EGFRvIII, epidermal growth factor variant III; RT, radiation; Gy, Gray; fx, fractions; CAR, chimeric antigen receptor; IL13Rα2, interleukin 13 receptor subunit alpha 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; TGFβRI, TGFβ receptor I; MTD, maximum tolerated dose; EGFRwt, epidermal growth factor wild type.

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Footnote

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