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Quo Vadis—Do Immunotherapies Have a Role in Glioblastoma?

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Abstract

Purpose of review More effective therapies for glioblastoma are urgently needed. Immunotherapeutic strategies appear particularly promising and are therefore intensively studied. This article reviews the current understanding of the immunosuppressive glioblastoma microenvironment, discusses the rationale behind various immunotherapies, and outlines the findings of several recently published clinical studies.
Recent findings The results of CheckMate-143 indicated that nivolumab is not superior to bevacizumab in patients with recurrent glioblastoma. A first-in man exploratory study evaluating EGFRvIII-specific CAR T cells for patients with newly diagnosed glioblastoma demonstrated overall safety of CAR T cell therapy and effective target recognition. A pilot study evaluating treatment with adoptively transferred CMV-specific T cells combined with a CMV-specific DC vaccine was found to be safe and resulted in increased polyclonality of CMV-specific T cells in vivo.
Summary Despite the success of immunotherapies in many cancers, clinical evidence supporting their efficacy for patients with glioblastoma is still lacking. Nevertheless, the recently published studies provide important proof-of-concept in several areas of immunotherapy research. The careful and critical interpretation of these results will enhance our understanding of the opportunities and challenges of immunotherapies for high-grade gliomas and improve the immunotherapeutic strategies investigated in future clinical trials.

Introduction

Glioblastoma is the most common malignant primary brain tumor and ~ 12,000 patients are diagnosed with this tumor in the USA each year [1]. Despite enormous research efforts to better understand the pathobiology of this incurable disease and develop more effective therapies, the established standard of care therapy has remained unchanged for more than a decade [2]. Prognosis for patients with glioblastoma remains poor with a median survival time of 14.9 months and 5-year overall survival rates of only 5.5% [1]. Fueled by the success of immunotherapies for many non-CNS cancers, the idea of harnessing the immune system to treat glioblastoma has gained great interest over the past years. Various immunotherapeutic strategies are currently evaluated including immune checkpoint blockade, anti-tumor vaccination, and cellular therapies. Here, we review the current understanding of the immunosuppressive processes in the glioblastoma tumor microenvironment and the immunotherapeutic strategies that are currently being investigated.

The immunosuppressive tumor microenvironment in glioblastoma

Glioblastomas create an immunosuppressive tumor microenvironment which allows tumor cells to escape from immune surveillance and effectively disrupts and modulates effector immune cell function via multiple mechanisms.

Change in the expression of surface molecules on glioma and glioma stem cells

Glioma cells downregulate the presentation of cellular antigens via the MHC class I and MHC II pathways [3, 4]. Instead of classical HLA class I molecules (HLA-A, HLA-A, and HLA-C), GBM cells express non-classical HLA class I molecules such as HLA-G and HLA-E. HLA-G affects the function of tumor-infiltrating lymphocytes [5]. The upregulation of HLA-E and lectin-like transcript-1 (LLT-1) on glioma cells impairs the function of natural killer (NK) cells [6–8]. In addition, glioma cells express Fas ligand (FasL, CD95) which, upon binding to the receptor Fas, induces apoptosis in tumor infiltrating lymphocytes [9].

Upregulation of STAT3 and key immunosuppressive cytokines in glioblastoma

Hypoxia-mediated upregulation of signal transducer and activator of transcription 3 (STAT3) is one of the hallmark pro-oncogenic alterations in glioblastoma. STAT3 activation in glioblastoma cells and glioma stem cells (GSCs) induces the production of key immunosuppressive cytokines including TGF-β, IL-6, IL-10, and IL-23 with important downstream effects on immune cell composition and immune cell activity in the glioma microenvironment [10–12]. In dendritic cells, STAT3 activation leads to reduced antigen presentation [13] and downregulation of CD40, CD80, CD86, and MHCII [14•]. Antigen presentation by these dendritic cells in the presence of IL-10 and TGF-β induces FoxP3 expression in $CD4^+$ cells and leads to recruitment of regulatory T cells (Tregs) to the tumor [15]. STAT3 therefore represents an attractive treatment target and preclinical studies have demonstrated that STAT3 blockade results in upregulation of the co-stimulatory molecules CD80 and CD86 and increases the secretion of the pro-inflammatory cytokines [16, 17]. Unfortunately, this

has not yet translated into successful clinical therapies for patients with glioblastoma although the development of small molecular inhibitors is ongoing [18–20].

Transforming growth factor (TGF)-β secretion is one of the key immunosuppressive cytokines secreted by glioma cells and leads to upregulation of FoxP3 in $CD4^+$ cells thereby promoting their differentiation into Tregs [21, 22]. TGF-β-mediated activation of the SMAD/ATF1 pathways also dampens effector $CD8^+$ T cell function by downregulating IFN-γ and granzyme B production. TGF-β has further been described to impair NK cell function via downregulation of NKG2D, a key molecule for NK cell activation. Despite encouraging preclinical work suggesting that TGF-β inhibition increases immunogenicity in murine glioma models [23, 24], the TGF-β inhibitor galunisertib was not more effective than lomustine for patients with recurrent glioblastoma in a randomized phase II study [25].

Lastly, increased interleukin-10 (IL-10) secretion in glioblastoma impairs antigen presenting cell (APC) and effector T cell function and promotes Treg activity and proliferation [26]. Other immunosuppressive cytokines include prostaglandin E_2 (PGE_2) which suppresses lymphocyte proliferation [27] and monocyte chemoattractant protein-1 (MCP-1, CCL2) which promotes Treg recruitment [28, 29].

Recruitment of immunosuppressive cells to the glioblastoma microenvironment

Tregs are characterized by FoxP3 and CD25 (IL-2α) expression and suppress the activity and proliferation of effector T cells via the production of the immunosuppressive cytokines TGF-β and IL-10. They comprise up to 15% of cells within the tumor [30] and the degree of tumor infiltration by Tregs has been correlated with tumor grade and prognosis [31–33].

Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells that originate from the bone marrow and are recruited to the glioma microenvironment, mainly driven by upregulation of hypoxia-factor-1α (HIF-1α) in the tumor [34, 35]. Through increased secretion of arginase I, nitric oxide (NO), and other reactive oxygen species (ROS), MDSCs induce apoptosis of activated T cells and further stimulate the proliferation and recruitment of Tregs [36, 37].

Glioblastomas are also heavily infiltrated by $CD45^{high}$ monocytes/macrophages and $CD45^{low}$ microglia. These cells have been reported to comprise up to 30% of the tumor mass and the extent of tumor infiltration by these cells may correlate with prognosis [38, 39]. Monocytes/macrophages and brain-resident microglia are extraordinarily difficult to distinguish phenotypically and therefore have been categorized based on their response to key cytokines: While IFN-γ and GM-CSF increase inducible nitric oxide synthase (iNOS) expression in the pro-inflammatory M1 phenotype, IL-4 and M-CSF induce secretion of arginase I, TGF-β, matrix metalloproteinase (MMP)-2, and MMP-9, IL-10, thereby characterizing the immunosuppressive M2 phenotype [40–42•]. In the glioblastoma environment, macrophages have been described to be predominantly shifted towards the pro-tumor M2 phenotype [43] and the binding of colony-stimulating-factor (CSF)-1 to CSF-1 receptor (CSF-1R) on macrophages appears to induce this shift from M1 to M2 [44]. It is important to note, however, that the various activity states of macrophages and microglia remain incompletely understood and in fact, the M1/M2 classification concept has

recently been challenged [42•, 45]. Our understanding of the role of macrophages and microglia in glioblastoma will therefore continue to evolve.

Immune checkpoints in glioblastoma

Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) is predominantly expressed by T cells in secondary lymphoid organs and is upregulated upon immune activation. Binding of CTLA-4 to its ligands CD 80 (B7.1) or CD86 (B7.2) leads to reduced activation and proliferation of effector T cells and increases the activation and recruitment of Tregs and MDSCs to the glioblastoma microenvironment [46].

Programmed cell death (PD)-1 is expressed and upregulated in T cells, other immune cells, and tumor cells. The PD-1 receptor has two ligands, PD-L1 (B7-homologue 1, B7-H1) and PD-L2 (B7-homologue 2, B7-H2). While PD-L1 is found on immunosuppressive Tregs, tumor-associated macrophages, and other cells within the tumor microenvironment including tumor cells [47], PD-L2 expression is restricted to reactive immune cells such as dendritic cells and macrophages [48]. Binding to of either ligand to PD-1 results in reduced function and proliferation of effector T cells [49, 50] and augments the activity, proliferation, and recruitment of Tregs to the tumor [51]. PD-L1 expression levels in glioblastoma range from 7.8 to 37.5% of tumor-resident cells and appear overall lower as compared to other cancers [52–57].

Other inhibitory checkpoint molecules in the tumor microenvironment include T cell immunoglobulin and mucin-domain containing-3 (TIM-3) and lymphocyte-activation gene 3 (LAG-3). TIM-3 is a surface marker expressed on TH1 cells and is considered a marker of T cell exhaustion which induces T cell apoptosis upon binding to its ligand galectin-9 (Gal-9) [58]. In glioblastoma, TIM-3 expression levels have been correlated with tumor grade and functional status [59, 60]. Anti-TIM-3 therapy was recently demonstrated to increase survival when combined with anti-PD-1 blockade and radiation in a murine glioma model [61]. LAG-3 is a membrane molecule upregulated on tumor infiltrating lymphocytes and LAG-3 binding to MHC class II on APCs and tumor cells results in reduced T cell and NK cell activation [62]. While LAG-3 upregulation has been reported to have important pro-oncogenic implications in other cancers including melanoma [63], there are no preclinical studies to date characterizing the role of LAG-3 in glioblastoma.

In addition to these immunosuppressive checkpoint molecules, a number of stimulatory checkpoint markers have been identified. CD137 (4-1BB) acts as a co-stimulatory protein of the T cell receptor (TCR) and promotes T cell activation and proliferation. CD137 stimulation has been demonstrated to increase survival in murine glioma models when used in combination with anti-CTLA4 antibodies and radiation [64]. Similarly, OX40L is a co-stimulatory molecule of the TCR and its expression in gliomas has been correlated with improved survival. The induction of OX40L expression improved survival in a murine glioma model [65].

IDO and tryptophan metabolism

There has been a growing interest in studying the effects of altered tumor metabolism on tumor growth and the immunosuppressive tumor environment. The indoleamine-2, 3-dioxygenase (IDO) is a key enzyme of tryptophan

metabolism and metabolizes tryptophan into kynurenine. Increased kynurenine levels appear to induce T cell anergy and immune cell apoptosis [66]. IDO has been found to be upregulated in up to 90% of gliomas and is associated with higher grade, shorter survival, and increased recruitment of Tregs and MDSCs to the tumor microenvironment [67–69]. Simultaneous targeting of IDO, CTLA4, and PD-L1 resulted in durable treatment responses in a murine glioma model [70]. Therefore, IDO inhibitors are currently explored for patients with high-grade gliomas, either as single agent (NCT02052648) or in combination with other immune checkpoint inhibitors (NCT03192943, NCT02658890).

Immunotherapies for glioblastoma

Immune checkpoint blockade

Given the prominent expression of CTLA-4, PD-1 and their ligands in glioblastoma and fueled by the enthusiasm about the success of immune checkpoint blockade in melanoma, non-small cell lung cancer (NSCLC), and other malignancies, a multitude of clinical trials is currently evaluating the role of checkpoint inhibitors either alone or in combination for newly diagnosed and recurrent high-grade glioma. These include the anti-PD-1 antibodies nivolumab (Opdivo) and pembrolizumab (Keytruda), the anti-PD-L1 antibodies avelumab (Bavencio) and durvalumab (Imfinzi), and the anti-CTLA-4 antibodies ipilimumab (Yervoy) and tremelimumab (formerly ticilimumab). In addition, alternative checkpoint-targeting antibodies such as the anti-LAG-3 antibody urelumab and an anti-CD137 antibody are investigated, either alone or in combination with nivolumab (Table 1).

While the majority of these studies are still ongoing, the results of the first clinical study evaluating PD-1 and CTLA-4 blockade in recurrent high-grade gliomas recently became available. In the exploratory phase I part of CheckMate-143, the safety of nivolumab versus nivolumab combined with ipilimumab was investigated. Because of dose-limiting toxicities predominantly in the combination arm, the subsequent phase III part of the study investigated nivolumab alone compared to bevacizumab. Unfortunately, the primary endpoint of superior survival with nivolumab was not met in this study given that median overall survival for patients treated with nivolumab was 9.8 months compared to 10 months for those receiving bevacizumab. Response rates were 8% with nivolumab and 23% with bevacizumab [71••, 72••].

In order to understand the reasons for failure of this study, it is important to recognize some of the emerging key concepts that may influence the efficacy of checkpoint blockade in cancer. One of these is the significance of PD-1 and PD-L1 expression as a predictive biomarker of treatment response to PD-1/PD-L1 blocking therapy. In a phase I study exploring nivolumab in multiple cancer types, 36% of patients with PD-L1 positive tumors (defined as ≥ 5% of cells PD-L1^{+}) demonstrated a treatment response whereas none of the patients with PD-L1 negative tumors had a treatment response [73]. Similarly, progression-free and overall survival were significantly prolonged in patients with NSCLC whose tumors had a proportional PD-L1 expression ≥ 50% compared to patients whose tumors had 50% proportional PD-L1 expression [74].

PD-L1 expression levels were also evaluated in CheckMate-143. Thirty-two percent of the tumors expressed PD-L1 in < 1% of tumor cells and only 27% of

Table 1. Currently ongoing clinical trials investigating immune checkpoint targeting agents and anti-tumor vaccination for high-grade gliomas

Name/ClinicalTrials.gov ID	Target(s)	Active agent(s)	Phase	Disease stage	Description and comments
Immune checkpoints					
NCT02648633	PD-1	Nivolumab	I	Recurrent	SRS with nivolumab and VPA
NCT02667587	PD-1	Nivolumab	II	Newly diagnosed	RT + TMZ ± nivolumab (CheckMate 548)
NCT02617589	PD-1	Nivolumab	III	Newly diagnosed	RT + TMZ vs. RT + nivolumab (CheckMate 498)
HSPPC-96/NCT03018288	PD-1, HSPPC-96	Pembrolizumab, HSPPC-96	II	Newly diagnosed	RT + TMZ + pembrolizumab ± HSPPC-96
NCT02530502	PD-1	Pembrolizumab	I/II	Newly diagnosed	RT + TMZ + pembrolizumab
NCT02337686	PD-1	Pembrolizumab	I/II	1st or 2nd recurrence	Pharmacodynamic study
NCT02337491	PD-1, VEGF	Pembrolizumab, bevacizumab	II	Recurrent	Pembrolizumab ± bevacizumab
NCT02311582	PD-1	Pembrolizumab	I	Recurrent	Pembrolizumab + laser interstitial thermal therapy
NCT02658279	PD-1	Pembrolizumab	I	Recurrent	Pembrolizumab for recurrent high-grade glioma with hypermutated phenotype
NCT02968940	PD-L1	Avelumab	II	Recurrent	Avelumab + hypofractionated RT in patients with progressive IDH-1 mutant glioblastoma
NCT03047473	PD-L1	Avelumab	II	Newly diagnosed	RT + TMZ + avelumab
STERIMGLI/NCT02866747	PD-L1	Durvulumab	I/II	Recurrent	RT + durvulumab for patients with recurrent glioblastoma
NCT02336165	PD-L1, VEGF	Durvulumab, bevacizumab	II	Cohort A: newly diagnosed; cohort B: recurrent, bevacizumab-naïve subjects; cohort C: recurrent, bevacizumab-refractory subjects	Cohort A: RT + TMZ + durvulumab; Cohort B: durvulumab ± bevacizumab; cohort C: durvulumab + continued bevacizumab
NCT02311920	CTLA-4, PD-1	Ipilimumab	I	Newly diagnosed	Arm I: ipilimumab; arm II: nivolumab; arm III: ipilimumab + nivolumab
NCT03367715	CTLA-4, PD-1	Ipilimumab, nivolumab	II	Newly diagnosed	Hypofractionated RT + ipilimumab + nivolumab in MGMT unmethylated glioblastoma

Table 1. (continued)

Name/ClinicalTrials.gov ID	Target(s)	Active agent(s)	Phase	Disease stage	Description and comments
NCT02794883	CTLA-4, PD-L1	Tremelimumab, durvulumab	II	Recurrent	Arm I: tremelimumab; arm II: durvulumab; arm III: tremelimumab + durvulumab
NCT02658981	LAG-3, PD-1	Urelumab	I	Recurrent	Urelumab ± nivolumab
Vaccine therapies					
NOA-16/NCT02454634	IDH-1^{R132H}	IDH1 peptide vaccine	I	Recurrent	First-in man trial of the IDH1 peptide vaccine
DCVax-L newly diagnosed GBM/NCT00045968	Tumor lysate	DCVax-L	III	Newly diagnosed	Autologous DCs are pulsed with tumor-lysate
DCVax-L recurrent GBM/ NCT00045968	Tumor lysate	DCVax-L	II	Recurrent	Autologous DCs are pulsed with tumor-lysate
STING/NCT02546102	AIM-2, MAGE-1, TRP-2, gp100, HER-2, IL-13Rα2	ICT-107	III	Newly diagnosed	DCs are pulsed with cocktail containing six TAA-peptides
ATTAC-II/NCT02465268	CMV pp65	CMV-pulsed DCs, GM-CSF, Td-boost	II	Newly diagnosed	DCs are electroporated with CMV pp65 shLAMP, GM-CSF/Td-boost
ELEVATE/NCT02366728	CMV pp65, IL-2	CMV-pulsed DCs, Td-boost, basilixumab	I	Newly diagnosed	Arm I: CMV-specific DC vaccine without Td boost; arm II: CMV-specific DC vaccine with Td boost; arm III: CMV-specific DC vaccine + basilixumab + Td boost
AVeRT/NCT02529072	CMV pp65	hCMV pp65-LAMP mRNA-pulsed autologous DCs		Recurrent	Arm I: nivolumab monotherapy → tumor resection → CMV-specific DC vaccine + nivolumab; arm II: nivolumab + CMV-specific DC vaccine → tumor resection → CMV-specific DC vaccine + nivolumab
ALLIANCE/NCT01814813	HSPPC-96, VEGF	HSPPC-96	II	Recurrent	HSPPC-96 vaccine ± bevacizumab
HSPPC-96/NCT03018288	HSPPC-96, PD-1	HSPPC-96	II	Newly diagnosed	RT + TMZ + pembrolizumab ± HSPPC-96
SurVaxM/NCT02455557	IL-13Rα2, survivin and EphA2	SurVaxM	II	Newly diagnosed	Multi-peptide vaccine

Table 1. (continued)

Name/ClinicalTrials.gov ID	Target(s)	Active agent(s)	Phase	Disease stage	Description and comments
GAPVAC/NCT02149225	Tumor-specific antigens	APVAC1 and APVAC2 vaccine plus poly-ICLC and GM-CSF	I	Newly diagnosed	Personalized anti-tumor vaccine
NeoVax/NCT02287428	Tumor-specific neoantigens	Tumor-specific neoantigen peptides plus poly-ICLC	I	Newly diagnosed	Personalized anti-tumor vaccine
NeoVax+PD-1 inhibition/NCT03422094	Tumor-specific neoantigens, PD-1	Tumor-specific neoantigen peptides plus poly-ICLC, nivolumab	I	Newly diagnosed	Personalized anti-tumor vaccine + nivolumab

AIM-2 interferon-inducible protein AIM2; *CAR* chimeric antigen receptor; *CD* cytosine deaminase; *CMV* cytomegalovirus; *CMV pp65* CMV phosphoprotein 65; *CTLA-4* cytotoxic T lymphocyte associated antigen-4; *CRAd* conditionally replicative adenovirus; *DC* dendritic cell; *EGFRvIII* endothelial growth factor receptor, variant III; *FC* flucytosine; *Flt3L* Fms-like tyrosine kinase 3 ligand; *gp100* glycoprotein 100; *M-CSF* granulocyte/monocyte stimulating factor; *HER2* human epidermal growth factor receptor 2; *hIL-12* human interleukin-12; *HSPPC-96* heat-shock protein peptide complex-96; *HSV* herpes simplex virus; *IL-2* interleukin-2; *IL-13Rα2* interleukin-13 receptor subunit α2; *LAG-3* lymphocyte activation gene-3; *MAGE-1* melanoma-associated antigen 1; *NSC* neural stem cell; *oADV* oncolytic adenovirus; *PD-1* programmed cell death-1; *PD-L1* programmed cell death ligand-1; *pK7* polylysine fiber pK7-modified viral vector enhances infectivity; *RT* radiotherapy; *SRS* stereotactic radiosurgery; *Td* tetanus toxoid; *TK* thymidine kinase; *TMZ* temozolomide; *TRP-2* tyrosinase-related protein 2; *VEGF* vascular endothelial growth factor; *4-1BB* CD137

patients had expression levels ≥ 10% [71••]. Under the assumption that the level of PD-L1 expression in glioblastoma has similar implications as in other cancers, the PD-L1 expression found in the patient population of CheckMate-143 may therefore have been too low to result in a treatment response. However, if PD-1 and PD-L1 expression actually have a role as predictive biomarkers that can identify glioblastoma patients who are likely to respond to checkpoint blockade remains insufficiently understood at this time. This continues to be investigated in the correlative studies accompanying many of currently ongoing clinical trials.

A similarly important predictor of response to PD-1/PD-L1 targeting therapies is the mutational load of the individual tumor. The abundance of genetic alterations in a given tumor is thought to increase the amount of available tumor-specific neoantigens to which a robust anti-tumor immune response can be directed. This is supported by clinical evidence showing that patients with colorectal cancer respond better to pembrolizumab if their tumors demonstrate deficiency in DNA mismatch repair (MMR) enzymes [75]. Similarly, the mutational burden in melanoma or NSCLC appears to correlate with response to immune checkpoint blockade [76, 77]. Several case reports have demonstrated that dramatic responses can also be seen in patients with glioblastoma who have germline mutations in MMR genes [78] or *POLE* [79, 80]. Interestingly, treatment with temozolomide has been associated with mutational loss of MMR genes (*MSH6*, *MLH1*, and *MSH2*) at time of high-grade glioma recurrence [81–83]. Based on these results, several clinical trials are exploring the efficacy of immune checkpoint inhibition in patients with hypermutated glioblastoma (Table 1).

Other clinical studies are investigating checkpoint inhibition combined with radiation (Table 1) because radiation has been demonstrated to increase the pool of potential neoantigens and to improve MHC-I-mediated presentation of these antigens by tumor cells and APCs [84, 85].

Lastly, the average number of effector immune cells in the glioblastoma microenvironment, in particular CD4+ and CD8+ T cells, may be too low to result in an effective anti-tumor immune response even if the activity of these cells can be augmented via checkpoint inhibition. Therefore, several clinical studies investigate the combination of checkpoint inhibitors with vaccine or viral vector-based strategies with the goal to increase T cell recruitment to the tumor (Table 1). In addition, there may be a potential synergistic effect of immunotherapies combined with targeted therapies. For example, CDK4 inhibition appears to increase antigen presentation and decreases regulatory T cells in the tumor microenvironment and therefore may lead to an improved anti-tumor immune response when combined with PD1 inhibitors [86].

Vaccine strategies for glioblastoma

Anti-tumor vaccination strategies follow the idea that improved immunization with tumor antigens augments recruitment of antigen-specific effector T cells to the tumor. Vaccination strategies are inherently complex because they require the optimal interplay of multiple factors including appropriate antigen selection, efficient presentation of these antigens by APCs, efficient expansion and migration of immunized T cells to the tumor, and effective cytotoxic function of these T cells in the tumor microenvironment.

Tumor antigens used for vaccine development can be broadly categorized into tumor-specific antigens (TSA) and tumor-associated antigens (TAAs). TSAs are exclusively expressed on glioblastoma cells and typically elicit a quite robust tumor-specific immune response with a low risk of cross-reactivity. In high-grade gliomas, EGFRvIII represents the prototype of such a tumor-specific antigen and ~ 30% of glioblastomas express this mutation [87]. Based on the robust anti-tumor response seen in mice treated with anti-EGFRvIII vaccination [88], the EGFRvIII-peptide vaccine rindopepimut (Rintega) was developed and extensively studied in phase I through phase III clinical trials for patients with newly diagnosed and recurrent glioblastoma [89–93]. While the early-phase clinical studies demonstrated general safety of the vaccine and impressive EGFRvIII-specific humoral and cellular immune responses [91, 90, 93], an international multicenter phase III clinical trial failed to demonstrate a survival benefit when rindopepimut was added to standard radiochemotherapy [94••]. It has been speculated that the vaccine was ineffective because the majority of tumors lost EGFRvIII expression over time. Antigen loss and the intratumoral as well as interindividual heterogeneity are hallmark characteristics of glioblastomas and illustrate additional immune escape mechanisms.

Similarly, the *IDH-1*^{R132H} mutation found in 70% of diffuse gliomas and ~ 5% of glioblastomas [95] harbors a tumor-specific neoantigen that was demonstrated to be effectively presented by professional APCs and to generate a robust mutation-specific CD4+ T cell response in a syngeneic mouse glioma model [96]. NOA-16, a phase I clinical study is therefore exploring an IDH-1^{R132H} targeting vaccine for patients with recurrent *IDH-1*^{R132H} mutant high-grade gliomas (Table 1).

In contrast, TAAs are native proteins that are overexpressed in glioblastoma cells but also have limited expression in other tissues, e.g., survivin [97], telomerase [98], HER2/neu [99], EphA2 [100, 101], and IL-13Rα [102]. The low-level expression in other body tissues typically causes a certain level of central immune tolerance and vaccination with these peptides therefore generates an overall weaker immune response but also lowers the risk of cross-reactivity and auto-immune complications. In order to augment the anti-tumor immune response and to reduce the chance of immune escape, various vaccines combine different TAAs.

SL-701 for example is a multi-peptide vaccine targeting IL-13Rα2, survivin, and EphA2. A multicenter phase I/II clinical study evaluating the safety and efficacy of this vaccine is currently under way (Table 1) [103]. ICT-107 represents a dendritic cell (DC) vaccine that is generated by pulsing DCs with a synthetic TAA cocktail containing the peptides AIM-2, MAGE-1, TRP-2, gp100, HER-2, and IL-13α2. In a single-arm phase 1 pilot study, a robust immune response to gp100 and HER2 was observed and median progression-free and overall survival appeared prolonged compared to historical controls [104]. This prompted further evaluation of ICT-107 in a multicenter double-blinded phase IIb study randomizing patients to receive DCs pulsed with the TAA cocktail or unpulsed DCs. While progression-free survival appeared prolonged in the treatment arm, the trend towards improved overall survival in the treatment group was not statistically significant. However, a possible survival benefit was seen in the subgroup of HLA-A2 positive individuals [105]. Therefore, a phase

III clinical trial was launched in 2016 accruing HLA-A2 positive patients only. Unfortunately, further patient enrollment was halted in 2017 due to funding issues.

Other vaccines target viral antigens expressed in malignant gliomas such as the cytomegalovirus (CMV) antigens immediate early-1 (IE1), phosphoprotein 65 (pp65), and glycoprotein B (gB) [106–108]. In addition to CMV peptides, CMV mRNA can be used to load and activate DCs. In a randomized and blinded pilot study, DCs were pulsed with CMV pp65-lysosomal-associated membrane protein (*LAMP*) mRNA either with or without preconditioning of the vaccination site by tetanus toxoid (Td) injection. This study demonstrated prolonged survival in patients receiving the DCs in conjunction with the Td booster [109]. A multicenter, randomized, blinded, and placebo-controlled phase II study is currently underway to further investigate the efficacy of this vaccine (Table 1).

In addition, heat-shock protein (HSP)-96 has been demonstrated to bind TAAs in the tumor microenvironment. A vaccine targeting the HSP-96 peptide complex (HSPPC-9) has therefore been studied in early-phase clinical trials and was demonstrated to generate robust humoral and cellular antitumor responses [110, 111]. A randomized phase II clinical trial investigating this approach was recently reported as being negative (Table 1).

Lastly, personalized vaccination strategies are currently explored. The general safety and feasibility of this approach has been demonstrated in several pilot clinical studies using DC vaccines generated by DC exposure to antigens isolated from autologous tumor lysates [112, 113]. In a phase I clinical study for newly diagnosed glioblastoma patient, a personalized neoantigen cancer vaccine is manufactured by combining autologous tumor-specific peptides with the immunoadjuvant agent poly-ICLC (Table 1). In melanoma patients, this approach was recently demonstrated to result in an effective anti-tumor T cell response [114••]. While the advantages of individualized, tumor-specific anti-tumor vaccination appear obvious, the production of these vaccines is labor-, time-, and cost-intense. In addition, there is at least a theoretical risk of auto-immunity given that the tumor-lysate may contain self-antigens expressed in other body tissues.

Cellular therapies

Adoptive T cell transfer involve the isolation of tumor-specific T cells from tumor or tumor-draining lymph nodes, the expansion of these cells ex vivo followed by their intravenous infusion back to the patient [115, 116]. This concept has recently gained new momentum when it was demonstrated that therapy with ex vivo expanded CMV-specific T cells resulted in encouraging progression-free and overall survival in patients with recurrent glioblastoma [117]. Most recently, a pilot study evaluated the combination of CMV pp65-specific T cells with a CMV pp65 RNA-loaded DC vaccine compared to treatment with CMV-specific T cells alone and demonstrated encouraging survival times in the treated individuals [118••]. Further studies evaluating CMV-specific T cell therapies are under way (Table 2).

An alternative strategy to utilize tumor-specific T cells is the generation of chimeric antigen receptor (CAR) T cells. CAR T cells are genetically modified T cells that express a CAR construct consisting of an extracellular ligand

Table 2. Currently ongoing clinical trials investigating oncolytic viruses, viral vector-based, and cellular therapies for high-grade gliomas

Name/ClinicalTrials.gov ID	Target(s)	Active agent(s)	Phase	Disease stage	Description and comments
Oncolytic viruses and viral vector-based therapies					
NCT01811992	Tumor cells	Ad-hCMV-TK, Ad-hCMV-Flt3L	I	Newly diagnosed	Dose escalation study of adenovirus-mediated delivery of TK leading to valacyclovir induced glioma cell death and Flt3L inducing dendritic cell recruitment to the tumor
NCT03072134	Glioma stem cells	Neural stem cells loaded with NSC-CRAd-Survivin-pK7	I	Newly diagnosed	Neural stem cell-mediated transfer of an oncolytic adenovirus to the resection cavity/biopsy site in newly diagnosed glioblastoma
NCT03152318	Tumor cells	rQNestin34.5v.2	I	Recurrent	Dose escalation study of rQNestin34.5v.2, genetically modified, neuroattenuated HSV-1, preferentially infects glioma cells and subsequently replicates and causes tumor cell lysis.
NCT02062827	Tumor cells	M032-HSV-1	I	Recurrent	Transfer of directly oncolytic herpes-simplex-virus (oHSV) which also acts as a vector for transfer of hIL-12 to locally promote anti-tumor immunity
Toca 511 and Toca FC/NCT02414165	Tumor cells, retrovirally mediated transfer of CD	Toca 511 and Toca FC	II/III	Recurrent	Toca 511 and Toca FC versus standard of care therapy. Toca 511 = retroviral vector which transfers the CD gene to the tumor cells. CD converts 5-flurocytosine to cytotoxic 5-fluorouracil. Toca FC = extended release formulation of 5-flucytosine administered orally following Toca 511 injection into the resection cavity

Table 2. (continued)

Name/ClinicalTrials.gov ID	Target(s)	Active agent(s)	Phase	Disease stage	Description and comments
NCT02026271	Tumor cells, adenovirally mediated transfer of hIL-12	Ad-RTS-hIL-12, veledimex	I	Recurrent	Adenovirally mediated transfer of hIL-12 which is conditionally expressed in presence of the activator ligand veledimex
CAPTIVE/NCT02798406	Tumor cells, PD-1	oAdV, pembrolizumab	II	Recurrent	Investigation for the efficacy of DNX-2401, an oncolytic adenovirus
Cellular therapies					
NCT02442297	HER2	HER2-CAR T cells	I	Recurrent	HER2-CAR T cells are injected into the tumor resection cavity at time of tumor re-resection
HERT-GBM/ NCT01109095	HER2	CMV-specific autologous T cells modified to express a HER2-CAR	I	Recurrent	Single injection with autologous CMV-specific T cells expressing a HER2-CAR
NCT03389230	HER2	HER2(EQ)BBζ/ CD19t+ -expressing T cells enriched for memory T cells	I	Recurrent	3 weekly intracavitary catheter injection of HER2-CAR T cells enriched for memory T cells
NCT02209376	EGFRvIII	EGFRvIII-CAR T cells	Pilot	Recurrent or residual	No preconditioning, single CAR T cell dose
NCT03283631	EGFRvIII	EGFRvIII-CAR T cells	I	Recurrent	Convection-enhanced delivery of autologous T cells modified to express a EGFRvIII-CAR
ExCeL/NCT02664363	EGFRvIII	EGFRvIII-CAR T cells	I	Newly diagnosed	No preconditioning, single CAR T cell dose

Table 2. (continued)

Name/ClinicalTrials.gov ID	Target(s)	Active agent(s)	Phase	Disease stage	Description and comments
NCT01454596	EGFRvIII	EGFRvIII-CAR T cells	I/II	Recurrent	EGFRvIII-CAR T cells following non-myeloablative but lymphoid depleting chemotherapy
NCT02208362	IL-13Rα2	IL-13Rα2-CAR T cells	I	Recurrent	IL13Rα2-specific, hinge-optimized, 41BB-co-stimulatory CAR

AIM-2 interferon-inducible protein AIM2; *CAR* chimeric antigen receptor; *CD* cytosine deaminase; *CMV* cytomegalovirus; *CMV pp65* CMV phosphoprotein 65; *CTLA-4* cytotoxic T lymphocyte associated antigen-4; *CRAd* conditionally replicative adenovirus; *DC* dendritic cell; *EGFRvIII* endothelial growth factor receptor, variant III; *FC* flucytosine; *Flt3L* Fms-like tyrosine kinase 3 ligand; *gp100* glycoprotein 100; *M-CSF* granulocyte/monocyte stimulating factor; *HER2* human epidermal growth factor receptor 2; *hIL-12* human interleukin-12; *HSPPC-96* heat-shock protein peptide complex-96; *HSV* herpes simplex virus; *IL-2* interleukin-2; *IL-13Rα2* interleukin-13 receptor subunit α2; *LAG-3* lymphocyte activation gene-3; *MAGE-1* melanoma-associated antigen 1; *NSC* neural stem cell; *oADV* oncolytic adenovirus; *PD-1* programmed cell death-1; *PD-L1* programmed cell death ligand-1; *pK7* polylysine fiber pK7-modified viral vector enhances infectivity; *RT* radiotherapy; *SRS* stereotactic radiosurgery; *Td* tetanus toxoid; *TK* thymidine kinase; *TMZ* temozolomide; *TRP-2* tyrosinase-related protein 2; *VEGF* vascular endothelial growth factor; *4-1BB* CD137

recognition domain often derived from a monoclonal antibody which is linked to an intracellular signaling domain typically derived from a signaling subunit of the T cell receptor (CD3ζ,). Additional co-stimulatory endodomains enhance T cell cytokine production, expansion, and in vivo persistence. Upon recognition of the CAR-specific target antigen, CAR T cells are activated and proliferate and recruit other immune cells via cytokine and chemokine secretion. Importantly, the target recognition is independent of MHC presentation and the same CAR construct therefore can be used as an "off the shelf" product [119]. CAR T cells targeting CD19 were demonstrated to be extraordinarily successful in the treatment of B cell acute lymphoblastic leukemia (B-ALL) and chronic lymphoblastic leukemia (CLL) [120, 121].

CARs targeting glioblastoma-relevant antigens have been developed, including IL13Rα2 [122], Her2 [123], EGFRvIII [124, 125], and EphA2 [126]. In an exploratory cohort of a first-in human clinical study investigating an anti-EGFRvIII CAR, nine of ten patients had progressed at 1-year of follow-up. Evaluation of tumor tissue resected at time of recurrence demonstrated that EGFRvIII-specific CAR T cells could be detected in small amounts in the tumor environment and EGFRvIII expression was lost over time. While this provided indirect evidence for successful CAR T cell trafficking to the tumor and effective antigen targeting, it again demonstrated antigen loss to be an important mechanism of immune escape in glioblastoma. Moreover, these tumors demonstrated increased PD-L1 and IDO expression and increased tumor infiltration with Tregs [127••]. The overall safety and potential efficacy of CAR T cells targeting IL13Rα2 was demonstrated in a small case series and a single case report [128, 129]. More recently, a phase I clinical study demonstrated the overall feasibility and safety of a HER2-CAR in patients with recurrent glioblastoma [130••]. Based on these encouraging results, several ongoing clinical trials continue to investigate the safety and efficacy of CAR T cell therapy for patients with glioblastoma (Table 2).

Oncolytic viruses

This strategy exploits the ability to modify certain viral vectors such that they selectively infect tumor cells, intracellularly replicate and induce tumor cell lysis, thereby allowing the release new infectious particles to the tumor microenvironment. In addition, they frequently induce an inflammatory response and upregulate PDL1 expression [131]. Commonly used viral vectors include oncolytic herpes simplex virus (oHSV), oncolytic measles virus (oMV), reovirus, polio virus, and conditionally replicating adenoviruses (CRAdVs) [132–137]. Several ongoing clinical trials investigate the value of immunotherapy utilizing viral vectors alone or in combination with checkpoint inhibitors for patients with recurrent and newly diagnosed glioblastoma (Table 2). In addition, viral vectors can be used to introduce therapeutic genes into the tumor cells. These genes most often encode for metabolic enzymes such as the thymidine kinase (TK) which then serves to transform a subsequently administered prodrug such as ganciclovir or flucytosine into cytotoxic agents within the tumor microenvironment [138, 131]. In addition, genes encoding for pro-inflammatory cytokines such as IL-2, IL-4, IL-12, IFN-γ, and IFN-β can be transferred to the tumor using various viral vectors [139–143]. Viral vector therapies are explored in

several ongoing clinical trials either alone or in combination with checkpoint inhibitors (Table 2).

Summary and future directions

Glioblastoma represents the most aggressive primary brain tumor and prognosis remains dismal despite the best current standard of care treatment. High-grade gliomas are well known to create an immunosuppressive tumor microenvironment through the alteration of cellular surface markers, the secretion of immunosuppressive cytokines, the activation of immune checkpoint inhibitory pathways, and the recruitment of immunosuppressive Tregs and MDSCs to the tumor. The recently published results of early-phase clinical studies exploring various immunotherapeutic strategies provide important proof-of-concept and support the continued investment in immunotherapies for high-grade gliomas. Nevertheless, the critical evaluation of these study results will be necessary to uncover conceptual and methodological difficulties and will be prerequisite for the improvement of the immunotherapeutic strategies investigated in future clinical studies.

Compliance with Ethical Standards

Conflict of Interest

Sylvia C. Kurz declares that she has no conflict of interest. Patrick Y. Wen receives research support from Agios, Angiochem, Astra Zeneca, Exelixis, Genentech/Roche, GlaxoSmith Kline, Karyopharm, Novartis, Sanofi-Aventis, Regeneron Pharmaceutical Inc., and Vascular Biogenics; he is on the advisory board for Abbvie, Cavion, Celldex, Genentech/Roche, Midatech, Momenta, Novartis, Novocure, SigmaTau, and Vascular Biogenics and is part of the Speaker's Bureau for Merck.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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