Review

Stefanie Tietze, Susanne Michen, Gabriele Schackert and Achim Temme*

Prospects of immune checkpoint blockade and vaccine-based immunotherapy for glioblastoma

<https://doi.org/10.1515/iss-2020-0034>Received November 19, 2020; accepted January 10, 2021;
published online April 16, 2021

Abstract: Glioblastoma multiforme (GBM) is the most prevalent primary brain tumor endowed with a dismal prognosis. Nowadays, immunotherapy in a particular immune checkpoint blockade and therapeutic vaccines are being extensively pursued. Yet, several characteristics of GBM may impact such immunotherapeutic approaches. This includes tumor heterogeneity, the relatively low mutational load of primary GBM, insufficient delivery of antibodies to tumor parenchyma and the unique immunosuppressive microenvironment of GBM. Moreover, standard treatment of GBM, comprising temozolomide chemotherapy, radiotherapy and in most instances the application of glucocorticoids for management of brain edema, results in a further increased immunosuppression. This review will provide a brief introduction to the

principles of vaccine-based immunotherapy and give an overview of the current clinical studies, which employed immune checkpoint inhibitors, oncolytic viruses-based vaccination, cell-based and peptide-based vaccines. Recent experiences as well as the latest developments are reviewed. Overcoming obstacles, which limit the induction and long-term immune response against GBM when using vaccination approaches, are necessary for the implementation of effective immunotherapy of GBM.

Keywords: glioblastoma; immune checkpoint blockade; immunosuppressive microenvironment; immunotherapy; vaccine.

Introduction

In the industrialized countries, malignant glioma represents the most common primary adult brain tumor [1]. It is generally concluded that gliomas and its highest-grade form, designated glioblastoma multiforme (GBM) originate from neuroglial progenitor cells [2]. Besides the histopathological appearance, the newest World Health Organization (WHO) classification of glioblastomas employs molecular markers to classify glioma subsets [1]. In particular, isocitrate dehydrogenase gene 1/2 (IDH1/2) mutational status defines two major subtypes of glioblastoma. IDH1-wildtype-GBM corresponds to the clinically well-defined primary GBM, which represents about 90% of all GBMs and develops *de novo* without signs of prior precursor lesions. A significant fraction of primary GBMs, ranging from 35 to 45%, show amplifications of the gene for the epidermal growth factor receptor (*EGFR*) and high frequencies in loss of *PTEN* as well as *TERT* promoter mutations. A MGMT promoter methylation is seen in ~30–50% of primary GBM. Decreased expression of MGMT, an O⁶-alkylguanine DNA alkyltransferase may render a favorable prognosis and response to standard alkylating chemotherapy with temozolomide (TMZ) [1, 3]. Of note, *EGFR* gene amplification is frequently accompanied by the heterogeneous appearance of a truncated oncogenic *EGFR* variant containing a neoepitope, designated EGFRvIII, in

***Corresponding author: Prof. Dr. Achim Temme**, Department of Neurosurgery, Section Experimental Neurosurgery/Tumor Immunology, University Hospital Carl Gustav Carus, Technical University Dresden, Fetscherstrasse 74, 01307 Dresden, Germany; German Cancer Consortium (DKTK), Dresden, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany; and National Center for Tumor Diseases, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany, Phone: +49 351 4587011, E-mail: achim.temme@ukdd.de. <https://orcid.org/0000-0001-5084-1180>

Stefanie Tietze and Susanne Michen, Department of Neurosurgery, Section Experimental Neurosurgery/Tumor Immunology, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany, E-mail: stefanie.tietze@ukdd.de (S. Tietze), susanne.michen@ukdd.de (S. Michen). <https://orcid.org/0000-0003-0313-7646> (S. Tietze). <https://orcid.org/0000-0002-5726-386X> (S. Michen)

Gabriele Schackert, Department of Neurosurgery, Section Experimental Neurosurgery/Tumor Immunology, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany; German Cancer Consortium (DKTK), Dresden, Germany; German Cancer Research Center (DKFZ), Heidelberg, Germany; and National Center for Tumor Diseases, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany, E-mail: gabriele.schackert@ukdd.de

tumor tissue [4]. On the contrary, IDH-mutant glioblastoma (historically classified as secondary GBM) is thought to arise from progenitor precursor cells with IDH1/2 mutations and to stepwise develop into secondary GBM through lower grade precursor lesions characterized as diffuse and anaplastic astrocytoma (WHO grade II/III) [1]. On the molecular level, secondary GBM can be distinguished from primary GBM by higher frequencies of mutations in the tumor suppressor proteins *ATRX* and *TP53*, which eventually may facilitate stepwise tumor progression [1].

From the neuropathologist's view, both major subtypes of glioblastoma are characterized as high-grade astrocytic tumors, often with necrotic proportions and areas of neovascularization.

Despite aggressive treatment of each subtypes of GBM by surgical resection followed by multimodal therapies, these tumors ultimately recur [5]. Currently, no standard of care is established for recurrent or progressive GBM. The median overall survival (OS) of GBM patients has not improved significantly over the past decades and remains at about 14.6–16.7 months [5, 6]. The addition of tumor-treating fields (TTFields) to standard therapy was demonstrated to increase the time without progression by 2.7–6.7 months and overall survival from 15.6 to 20.5 months in newly diagnosed glioblastoma without reducing quality of life [6]. However, there was no significant prolongation of survival in recurrent GBM [7].

To date, immunotherapeutic approaches hold promise for improved outcome when treating solid tumors. This review focuses on state-of-the art vaccination approaches for GBM and immune checkpoint inhibition. In parallel, the review gives the reader a brief introduction of immunological principles. Although, vaccination against GBM represents not a novel approach *per se*, understanding of immunosuppressing circuits and the factors affecting immunotherapy of GBM as well as the identification of novel immunological targets have contributed significantly to innovative approaches for vaccine-based immunotherapy of GBM.

Fundamentals of T cell-mediated immunosurveillance

The immune system can be divided into two arms: the innate and the adaptive immunity. The cells of the innate immune system are decisive for initial induction of a proinflammatory response, which simultaneously controls the adaptive immunity. Crucial to almost all functions of the adaptive immune response is the activation and programming of T cells from their naïve/dormant state. The T cell

mediated immune response can be broadly divided into two main categories: the generation of “helper” T cells ($CD4^+$ T cells) and the generation of “cytotoxic” T cells ($CD8^+$ T cells). $CD4^+$ T cells support the immune response through the production of cytokines, especially interleukin-2 (IL-2) and chemokines. $CD8^+$ T cells focuses on the elimination of pathogen-infected cells or tumor cells by release of cytotoxic substances, such as perforin and granzymes, from granules or expression of FasL or TRAIL on the cell surface. Yet, they also contribute to inflammation by release of interferon-gamma (IFN- γ) and TNF- α . To avert uncontrolled, persistent or exuberant immune responses, the immune system produces regulatory T cells (Tregs) that slows down a variety of inflammatory processes. After the elimination of the pathogen, the majority of T cells die, leaving behind memory T cells that can mediate a rapid and protective immune response after reinfection [8].

T cell surveillance of neoplastic development and growth depends primarily on recognition of intracellularly processed tumor-antigens, presented by major histocompatibility complex (MHC) class I molecules on the surface of tumor cells. Principally, there are two types of tumor-antigens. The first group comprise “neoantigens”, originating from transforming viruses and hypermutation in tumor cells. Toward these tumor-antigens, the host is not tolerant. The second group comprises “self-antigens”, commonly termed tumor-associated antigens (TAAs). TAAs are mainly proliferation and differentiation markers overexpressed in tumors as well as testis-related and normal embryonic antigens aberrantly expressed in the course of epigenetic changes and cellular dedifferentiation during tumor progression [9]. However, T cells with high affinity receptors for MHC/self-peptides are eliminated during development in the thymus to establish central tolerance [9]. Therefore, the repertoire of T cells specific for TAA/self-peptides in the periphery is restricted to those T cells displaying a low affinity T cell receptor. Under physiological circumstances, T cells reactive against neoepitopes and self-peptides are in an ignorant state called peripheral tolerance and need to be activated by professional antigen presenting cells (APCs), in particular dendritic cells (DCs), in a process termed cross-priming, before they can exert their effector functions. Briefly, immunogenic responses of T lymphocytes are achieved when DCs simultaneously engulf antigen and encounter evolutionary conserved “pathogenic associated molecular patterns” (PAMPs), i.e. lipopolysaccharides (LPS), CpG rich DNA, viral RNA from pathogenic microorganisms or when they have been activated with vaccine components, such as complete Freund's adjuvant [9]. After sensing PAMPs via pattern recognition receptors such as toll like receptors

(TLRs), the DCs convert to mature DCs with stabilized MHC I/peptide complexes and capable of homing into the lymph nodes. In parallel, DCs upregulate co-stimulatory molecules CD80 and CD86, secrete T cell-supporting cytokines and from now on can fully activate naïve T cells displaying a T cell receptor which fits to the MHC I/peptide complex. Besides their role in DC activation, PAMPs are also essential for B-cell responses by inducing B-cell proliferation and immunoglobulin isotype class switching [10].

Several lines of evidence indicate that tumor cell death can also deliver danger signals required for DC priming. For instance, it has been shown that release of calreticulin and high-mobility group box1 protein (HMGB1) from dying cancer cells, can co-deliver so called “damage-associated molecular pattern” (DAMPs) signals to DCs, which in turn can break peripheral tolerance of T lymphocytes [11, 12]. In particular, for GBM it appears reasonable that DAMPs released from necrotic areas may facilitate adaptive immune responses (Figure 1). In addition, calreticulin serves as “eat-me” signal [13]. Thus, immunogenic cell death is characterized by the translocation of calreticulin from the cell interior to the tumor cell surface, which significantly increases the phagocytosis of tumor cells by DCs.

In summary, immunogenic cell death of tumor cells can deliver tumor antigens and essential danger signals to DCs, which are now able to activate tumor-specific T cells. So far, tumor-infiltrating lymphocytes (TILs) which were proven to specifically react against tumor cells or which were associated with a better clinical outcome, were reported for several cancer types [9]. However, the appearance of TILs in patients *per se* did not predict an efficient immune response or improved clinical outcome, as exemplified in GBM, since various mechanisms can impair T cell-mediated tumor immunosurveillance.

GBM, a tumor suited for immunotherapy?

The central nervous system (CNS), in particularly the brain, has been traditionally viewed as an immune-privileged organ which is not targetable by systemic immunotherapy approaches since it lacks lymphatic drainage and since tight endothelial barriers (i.e. blood brain barrier, BBB) prevent entry of immune cells and macromolecules. Yet, recent studies established that lymphatic structures exist in the dura and arachnoid meninges and that lymphocytes and probably APCs, in particular DCs, exit the brain via this system and reach cervical lymph nodes [14, 15]. In healthy brain, lymphocytes cannot cross the BBB but can traffic

from meningeal blood vessels into the cerebrospinal fluid, where they can gain access to the brain parenchyma via the pia mater or choroid plexus [16].

Noteworthy, during tumor disease, injury or by pharmacological modification of brain endothelial cells, the BBB can be disrupted [16–19]. Subsequently, this facilitates the presentation of CNS antigens to cervical lymph nodes, allowing the influx of antibodies and infiltration of immune cells, such as T cells, into the tumor tissue (Figure 1). Furthermore, in most GBMs increased expression levels of vascular endothelial growth factor (VEGF) as well as immature, pathologically structured vessels can lead to a local loss of the endothelial barrier [20, 21].

GBM cells express classical MHC-class I molecules and therefore are able to present peptides derived from mutated genes to become a legitimate target for antigen-experienced cytotoxic T cells (CTLs) [22, 23]. In addition, necrotic cell death in GBM and increased release of exosomes by tumor cells can provide tumor antigens and DAMPs for provoking cellular and humoral immune responses. That GBM-derived antigens indeed can elicit immune responses was demonstrated in a study from Schuster et al. (2014) showing an existing humoral response of patients against the GBM neoantigen IDH1^{R132H} [24] (described later) and from Pallasch et al. (2005) which identified autoantibodies to GBM-specific glioma-expressed antigen 1 and 2 (GLEA1, GLEA2) and PHD finger protein 3 (PHF3) [25]. In addition, an earlier study using patient’s tumor specimens identified cellular immune response by CD8⁺ TILs, which were detected in close proximity to GBM tumor cells and characterized by T cell receptor (TCR)-clustering, phosphoZAP70 and increased granzyme B immunosignals at the immunological synapse [26].

Another important issue when considering adaptive immune responses against glioblastoma is related to T cell homing. However, the homing of antigen-experienced T cells to brain tumors has been demonstrated in an elegant preclinical study performed by Calzascia et al. [27], which demonstrated that antigens from intracerebral MC57-GP fibrosarcoma can be presented by DCs in cervical lymph nodes of P14 transgenic C57BL/6 mice and promote intracerebral homing of T cells. P14 transgenic mice express a Vα2Vβ8.1 TCR specific for the MC57-GP-derived neopeptide GP_{33–41}. Remarkably, activation of naïve Vα2Vβ8.1-CD8⁺ cells in cervical lymph nodes by brain-derived DCs led to tumor-infiltrating CTLs, which displayed a higher expression of integrin α₄β₁ (VLA-4) that favors CNS tropism [27].

Subsequent studies of the same group showed that the brain microenvironment of these mice and also of glioma patients led to acquisition of α_E(CD103)β₇

Figure 1: T cell-mediated immunosurveillance and vaccination approaches for GBM.

Induction of immunogenic cell death (i.e. by necrotic cell death of tumor cells or mediated by intratumoral oncolytic virotherapy) causes release of “damage associated molecular patterns” (DAMPs/PAMPs) and tumor antigens. Resident APCs, in particular DCs endocytose cellular debris containing tumor antigens and after recognition of PAMPs/DAMPs via pattern recognition receptors, DCs convert into mature DCs with stabilized MCH I/tumor peptide complexes. Mature DCs express costimulatory B7.1/2 ligands for CD28 costimulatory receptors on T cells, secrete T cell-activating cytokines (i.e. IL-12 for Th1 responses) and home to lymph nodes. In lymph nodes, DCs activate naive $CD4^+$ T and $CD8^+$ CTLs with appropriate TCR via MHC class II/peptide complex and MHC class I/peptide complex, respectively. Such activated T cells clonally expand and home to GBM tissue. Tumor infiltrating T cells (TILs) can selectively kill tumor cells. Current state-of-the-art vaccination against GBM employs (i) injections of *ex vivo*-generated matured DCs loaded with either tumor cell lysate or peptides, (ii) injection of synthetic peptides plus immune adjuvant (peptide vaccines) and includes intratumoral oncolytic virotherapy (*in situ* vaccination).

phenotype of infiltrated CTLs which was associated with retention of cells at the tumor site and increased levels of granzyme B [28]. This data and similar data from other groups, which analyzed T cell homing to further tissues, suggest that the homing phenotype of T cells is in part dictated at the site of antigen capture by cross-presenting APCs/DCs [29].

More recently, a phase Ib study employing a subcutaneously applied neoantigen vaccine for treatment of glioblastoma demonstrated infiltration of neoantigen-experienced TILs in GBM, which were identified by their individual TCR using single cell RNAseq [30].

A completely novel approach are genetically engineered T cells with chimeric antigen receptors (CARs) that can recognize TAAs with high specificity in an MHC-independent manner. Despite promising preclinical results, only limited anti-tumor response has been observed in early clinical trials. The limited T-cell persistence and the loss of TAAs in the relapses suggest that the current settings used in CAR T-cell therapies do not establish long-lasting anti-tumor immune responses in patients with GBM [31].

In conclusion, there is growing evidence that the CNS is not a complete sanctuary site for the immune system, since humoral and cellular adaptive immune responses can be observed. However, immune responses to GBM are eventually blocked by immune evasion mechanisms described below.

The immunosuppressive microenvironment in glioblastoma affects immune responses

For glioblastoma it is generally acknowledged, that the tumor microenvironment comprises manifold and inter-relating immunosuppressive factors. This eventually results in high frequencies of $CD4^+$ and $CD8^+$ TILs positive for immune checkpoint receptors programmed cell death protein-1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), and lymphocyte-activation gene 3 (LAG-3) when compared to phenotypes of PBMC derived T cells

from the same patient showing less exhausted T cells [32]. Another study on TILs prepared from GBM tissue revealed that besides strong expression of immune checkpoint markers such T cells show significantly reduced cytokine production indicative of nonfunctional T cells [33]. Of particular interest remains B7-H1/“programmed cell death ligand 1” (PD-L1) which in an earlier study was reported to be strongly expressed in commonly used GBM cell lines and also robustly expressed in GBM specimens [23]. In contrast, a recent study from Nduom et al. (2016) found that expression of PD-L1 was mostly confined to a minor GBM cell subpopulation [34]. Moreover, the authors reported that PD-1-positive TILs account for the vast majority of PD-L1 expressing cells in GBM. Additionally, mRNA expression analyses linked higher expression of PD-1/PD-L1 to TILs, which was correlated with worse outcome. In conclusion, the data on PD-1/PD-L1 expression are intriguing, deserve further investigation and suggests that exhaustion and anergy of TILs might be induced by so far not identified factors that may be effective in GBM.

Concerning CTLA-4, in a mouse glioma model, blockade of CTLA-4 improved the number of CD4⁺/CD25⁺ T cells, CD8⁺ infiltrating CTLs and survival of mice [35]. However, to date, there are no reports decisively demonstrating expression of CD80 (B7.1) and of CD86 (B7.2), the ligands for CTLA-4, in tumor specimens of glioblastoma or GBM-derived stromal cells. Since, commonly used GBM cells lines as well as murine GBM models cannot exactly recapitulate the situation in human GBM, a comprehensive analysis of CD80 and CD86 in respect to expression levels, correlation to survival and immunomodulatory effects, remains mandatory.

Noteworthy, GBM cells also release several factors, which enhance proliferation in autocrine manner but also further shape the tumor environment. In particular, transforming growth factor beta (TGF- β) secreted by tumor cells and infiltrating regulatory T cells (Tregs) [36, 37] suppresses CTLs, CD4⁺ Th1 cells and natural killer (NK) cells [38]. Additionally, the CXCL12/CXCR4 axis increases tumor aggressiveness [39] and together with secreted CCL7/MCP-3 [40], granulocyte-macrophage colony stimulating factor (GM-CSF) [41] and CX3CL1 (fractaline) [42] as well as increased levels of the extracellular matrix component periostin [43] is thought to result in GBM-infiltration of resident microglia, of extra-parenchymal macrophages and of attraction/proliferation of myeloid progenitor cells. Recruitment of myeloid-derived stroma cells might also be related to increased levels of hepatocyte growth factors/scatter factor (HGF/SF) which was observed in human glioblastoma specimens [44] and cerebrospinal fluid of GBM patients [45]. Increased levels of HGF/SF and its cognate

receptor c-Met (hepatocyte growth factor receptor) has recently be described to support proliferation of myeloid progenitor cells [46], which in GBM might further cooperate with GM-CSF to contribute to the development of tumor stroma. In GBM, the tumor stroma containing the above-described cell types can account for up to 30% of tumor mass. By the influence of GBM cells, microglia/macrophages acquire an M2-like immunosuppressive phenotype leading to so called glioma-associated microglia/macrophages (GAMs). Furthermore, infiltrating myeloid precursors, designated myeloid-derived suppressor cells (MDSCs) have the ability to suppress adaptive immune response mediated by CD4⁺ T cells and CD8⁺ cytotoxic T cells in antigen dependent or non-dependent manner [47]. GAMs and MDSCs are characterized by enhanced secretion of immunosuppressive IL-10 and prostaglandin E2 (PGE2) [47, 48] which together with glioma cell-secreted TGF- β likely induce tumor-reactive CD4⁺ T cells to become “induced Tregs” or potentially contribute to induction of anergy by inducing PD1/PD-L1 expression of TILs (see above) [49]. In addition to the aforementioned immunosuppressive factors, metabolic alterations in the GBM microenvironment can contribute to T cell exhaustion. The inducible tryptophan catabolic enzyme, indoleamine 2,3 dioxygenase 1 (IDO1) can negatively affect T cells. The primary function of IDO1 is linked with conversion of the essential amino acid, tryptophan, into downstream catabolites known as kynurenines. The depletion of tryptophan and subsequent accumulation of kynurenine anergize T cells and increase Treg frequencies in preclinical murine GBM models [50]. While IDO1 is expressed at moderate levels under physiological circumstances, in the adult central nervous system, a recent study using GBM specimens and patient-derived xenograft (PDX)-models demonstrated that IDO1 is rapidly upregulated upon inflammatory stimulus such as IFN- γ release by activated T cells [51].

Other metabolic alterations, which may inhibit or modulate T cell function, include the production of amino acid metabolizing enzymes such as arginase, nitric oxide synthetase (NOS) and the production of reactive oxygen species (ROS) by MDSCs or GAMs [52–54]. In addition, T cells depend on similar glycolytic pathways as tumor cells for survival [55]. Competition for glucose, lactate production and subsequently the generation of an acidic tumor microenvironment, may modulate metabolism of TILs, affecting their immune effector functions, their exhaustion and/or their survival [56]. A brief overview of relevant immunosuppressive factors is depicted in Figure 2.

In addition, to the endogenous immunosuppression in GBM, common treatment of patients before and after

Figure 2: Immunosuppressive glioblastoma tumor microenvironment.

Overview of immunosuppressive evasion mechanisms of GBM. Tumor cells and tumor-infiltrating Tregs and tumor stroma containing GAMs and MDSC, can employ a plethora of immunosuppressive factors and molecules that impair T-cell function or lead to T-cell anergy and/or apoptosis. Depicted are immunosuppressive self-inhibitory circuits of activated T cells and immunosuppressive mechanisms of GBM.

surgery include relatively high doses of immunosuppressive glucocorticoids. This treatment counteracts vasogenic edema but on the other hand may diminish T cell activity and may interfere with immune checkpoint blockade discussed below.

Immune checkpoint blockade and GBM

To date, it is well known that self-restricting circuits control activated T cells to prevent damage to normal somatic tissue. Such self-restricting circuits include activation-induced cell death (AICD) of T cells, governed by Fas-receptor-Fas ligand (FasL)-induced suicide and fratricide, but also induction of adaptive tolerance in the periphery by upregulation of ligands of the B7-family (CD80, CD86, PD-L1) to which activated T cells express cognate inhibitory CTLA-4 and PD-1 receptors [9]. In particular, activation of PD-1 receptors on T cells lead to hyporesponsive/exhausted effector T cells [9]. Over the last decade, immunotherapy has gained much impetus by the development of antibodies inhibiting the activation of self-restricting immune checkpoint receptors of T cells. In particular, treatment of exhausted tumor-reactive T cells with ipilimumab (anti-CTLA-4, Bristol-Meyers-Squibb)

and nivolumab (anti-PD-1, Bristol-Meyers-Squibb), pembrolizumab (anti-PD-1, Merck) reactivated anti-tumor responses of T cells and showed clinical efficiency when treating advanced melanoma [57]. In this regard, a recent study demonstrated that melanoma samples from patients who showed a clinical response to anti-PD-1 therapy prior to treatment had higher CD8⁺ T cell frequencies than samples from patients with progressive disease during therapy, suggesting that pre-existing tumor-infiltrating CD8⁺ T cells are predictors of clinical response to PD-1 blockade [58]. Also, in patients with other tumor entities, including non-small lung cancer and renal cell carcinoma, treatment with immune checkpoint inhibitors promotes efficient antitumor effects with objective clinical responses [59, 60]. However, GBM initially possesses only moderate mutational burden when compared to immunogenic tumors such as melanoma and lung cancers [61]. In addition, as stated above the immunosuppressive microenvironment of GBM affects TILs. Therefore, it is plausible that immune checkpoint blockade, which works well in some tumors by releasing the immunosuppressive brake of a surplus of TAA/neoantigen-experienced T cells, might not work well in GBM. Immune checkpoint inhibition using nivolumab has been currently evaluated in clinical phase III trials for newly diagnosed MGMT-methylated GBM (CheckMate 548, NCT02667587) and MGMT-unmethylated GBM (CheckMate 498, NCT02617589) in combination with standard of care TMZ and radiation therapy (Table 1). Thus far, data is disappointing and most recently the sponsor announced cancelation of the trials. Likewise, data from an exploratory trial and clinical phase III trials (CheckMate 143, NCT02017717), which investigated immune checkpoint inhibition monotherapy with nivolumab in patients after glioblastoma at first recurrence following standard chemo- and radiotherapy, was similarly disappointing [62, 63]. However, a documented successful immune checkpoint inhibition (i.e. showing immune response and tumor regression) when using atezolizumab (anti-PD-1L, Roche), was reported for rare GBM patients bearing germline defects in mismatch repair genes [64]. Another case report showed clinical response in a patient with germline *POLE* mutation and treated with nivolumab. This mutation in the catalytic subunit of DNA-dependent DNA polymerase ϵ negatively affects base excision repair of DNA. After receiving standard radiochemotherapy, this patient suffered from recurrent GBM and metastatic disease to the spine, which was associated with the appearance of 2,040–3,245 novel neoantigenic mutations. Noteworthy, the patient then responded well to checkpoint inhibition with pembrolizumab and showed improved CTL infiltration in tumor tissues as well as objective radiographic

response of intracranial recurrent GBM [65]. In conclusion, these reports show promising effects of immune checkpoint blockade in hypermutated gliomas. In addition, the above-mentioned reports suggest, that TMZ-treatment of patients without germline mutations affecting DNA repair, might not provide the quantity of hypermutations required to induce immune responses and objective clinical responses. They furthermore indicate that most GBM patients treated in the current setting for disease management (in particular when glucocorticoids are systemically applied) might not be eligible for immune checkpoint blockade. Conceptually, checkpoint blockade might be more effective when treating GBM patients who have not been treated with glucocorticoids and/or bearing a hypermutation-phenotype, the latter which can be identified by next generation sequencing-based profiling. Also, in the setting of vaccination approaches, immune checkpoint blockade could be of value since it might restrict adaptive tolerance mechanisms of tumor cells and *vice versa* may safeguard effector functions of antigen-experienced T cells. In this regard, one might hypothesize that engineered antibodies, such as low molecular weight (30–35 kD) single chain antibody variable fragments (scFv) against PD-1 and PD-1L, respectively, might achieve better intratumoral concentration and checkpoint blockade than the large (145–149 kD) anti-PD-1 and anti-PD-1L full-length (Fc-containing) antibodies.

Vaccine-based immunotherapy

As described above, neoantigen-peptides are excluded from central tolerance. Likewise, processed neoantigens, designated neopeptides, when presented in complex with MHC can generate strong immune responses of T cell clones expressing a high affinity TCR. Only a minority of neoantigens are processed into peptides that fit into the peptide-binding groove of MHC-class I molecules and can be presented by APCs to elicit a T cell response. Importantly, many potential tumor antigens are TAAs originating from mis- or overexpressed normal proteins [9]. For TAAs, which principally represents “self-antigens” it is generally considered that a proportion of T cells with low affinity TCRs, which escaped deletion in the thymus, may elicit immune responses when properly activated. However, activation of such T cells using self-antigens (either provided by tumor cell lysates or chemically produced) bears the risk of collateral autoimmunity. Likewise, most of the state-of-the-art vaccination approaches for GBM focus on neoantigens and their derived MHC-neopeptides. Current vaccine approaches for GBM treatment can be divided into three main categories: tumor

cell lysate- or peptide-pulsed-DC vaccines, peptide vaccines and *in situ*-vaccination by genetically engineered oncolytic viruses (Figure 3). A selection of recent clinical trials on vaccine-based approaches for the treatment of GBM is summarized in Table 1.

Tumor cell-based and DC-based vaccination of GBM

Conceptually tumor cell vaccination approaches, relying on application of irradiated autologous GBM cells/tumor or their cell lysates, deliver all tumor antigens present in the tumor and may favor the generation of polyclonal effector T cells. The priming of peripheral DCs can be accomplished by adjuvants/immunomodulatory factors like type-1 and type-2 interferons, TLR ligands or GM-CSF, which all enhance the antigen presenting and T cell activating capacities of DCs [66]. A recent vaccination approach for GBM includes the delivery of irradiated tumor cells together with irradiated allogenic K562 cells genetically modified to express GM-CSF (GM-K562). The results of a recent phase I study in patients with recurrent malignant glioma demonstrated safety and showed induction of a humoral response toward angiogenic factors in some patients, which was not seen before treatment [67]. Yet, in patients, a general increase in activated T cells in peripheral blood was accompanied by an increase in the frequency of regulatory CD4⁺ T-lymphocytes that might limit this vaccination approach [67]. From the results of this phase I study, it moreover might be concluded that adverse autoimmune effects cannot be excluded when using the combined full antigenic repertoire of autologous tumor cells and of GM-K562 cells for vaccination.

For DC-based vaccines, DC precursors of patients are obtained by leukapheresis. In addition, monocytes can be isolated from peripheral blood and mature *ex vivo* into professional DCs after addition of GM-CSF and IL-4 [66]. DCs are subsequently loaded with tumor-specific peptides and autologous tumor lysate. Furthermore, a more tedious and regulatory complicated approach encompasses DCs that genetically express TAAs from tumor cells [68].

Recent phase III studies (NCT00045968 and NCT02146066) performed with patients suffering from newly diagnosed glioblastoma and treated with standard therapy employed matured DCs *ex vivo* loaded with full tumor lysate (DCVax[®]-L vaccine, Northwest Biotherapeutics) revealed encouraging results [69]. Briefly, patients with methylated MGMT showed an improved median overall survival (mOS) of 34.7 months and an impressive three-year survival of 46.4%

Table 1: Summary of clinical trials on immune checkpoint inhibitors and vaccine-based approaches for the treatment of GBM.

Trial name	Phase	Enrollment	Status	Experimental design	Immunological response	PFS, months	mOS, months	References
Immune checkpoint blockade								
CheckMate 548	III	693	Active	Anti-PD-1 antibody	–	–	–	NCT02667587
CheckMate 498	III	550	Active	Anti-PD-1 antibody	–	–	–	NCT02617589
CheckMate 143	III	626	Active	Anti-PD-1 antibody	–	–	9.5	NCT02017717 [62, 63]
Tumor cell-based and DC-based vaccines								
GVAX	I	11	Completed	Irradiated autologous glioma cells mixed with irradiated GM-K562 cells	– CD8 ⁺ T lymphocyte activation – Induction of regulatory CD4 ⁺ Tregs	–	8.8	NCT00694330 [67]
DCVax-L	III	331	Completed	Autologous tumor lysate-pulsed DCs	Increased frequencies of CD4 ⁺ T cells	–	34.7/19.8	NCT00045968, NCT02146066 [69]
DEN-STEM	II/III	60	Recruiting	DCs with mRNA from autologous tumor stem cells, survivin and hTERT	–	–	–	NCT03548571
CMV-DC	I/II	42	Active	CMV pp65-LAMP mRNA electroporated autologous DCs	Patient CMV pp65 immune response via increased IFN- γ secretion	25.3	41.1	NCT00639639 [71]
Trial name	Phase	Enrollment	Status	Experimental design	Immunological response	PFS, months	mOS, months	References
Peptide vaccines								
Rindopepimut (CDX-110)	II	82	Completed	Rindopepimut admixed with GM-CSF	– Increased anti-EGFRvIII antibody titers – EGFRvIII was eliminated in tumor samples	5.5	21.8	NCT00458601 [73]
Rindopepimut (CDX-110)	III	745	Completed	Rindopepimut admixed with GM-CSF	Increased anti-EGFRvIII antibody titers	–	20.1	NCT01480479 [74]
NOA-16	I	39	Completed	IDH1 peptide	Activation of mutation-specific T cell or humoral immune response	–	–	NCT02454634 [75]
Neoantigen vaccine (NeoVax)	I/Ib	16	Active	Personalized neoantigens	– Increased frequencies of neoantigen-specific CD4 ⁺ and CD8 ⁺ T cells – Increased number of TILs	7.6	16.8	NCT02287428 [30]
Viral-based vaccines								
HSV-1-G47delta	II	13	Completed	Third generation oncolytic HSV-1	–	–	–	[76]
DNX-2401	I	37	Completed	Tumor-selective, replication-competent oncolytic adenovirus	Immune cell infiltration of CD4 ⁺ and CD8 ⁺ T cells	–	9.5	NCT00805376 [79]
DNX-2401	I	36	Recruiting	Allogeneic BM-hMSCs loaded with the oncolytic adenovirus DNX-2401	–	–	–	NCT03896568
PVS-RIPO	I/II	61	Active	Poliovirus-rhinovirus chimera	–	–	12.5	NCT01491893 [81]

Figure 3: Current vaccine approaches to treat glioblastoma.

Vaccine approaches for GBM treatment can be divided into three main categories: tumor cell lysate- or peptide-pulsed dendritic cell (DC) vaccines, peptide vaccines and *in situ* vaccination by genetically engineered oncolytic viruses. DCs either can be stimulated *in vivo* with synthetic peptides (peptide vaccines) or by *ex vivo* generated autologous DCs loaded with tumor cell lysates or peptides (dendritic cell vaccines). Contrary, oncolytic virotherapy is based on genetically engineered replicating viruses that can selectively kill cancer cells. GBM cells succumbing to virus infection release tumor antigens and DAMPs. Additional surplus of PAMPs provided by the oncolytic virus can amplify inflammation (i.e. activation of DCs) and improve adaptive anti-tumor immunity.

[69]. Yet, the cross-over trial design, where approximately 90% of the patients received DCVax[®]-L, and then reported highly selected patient cohort remains a matter of debate [70]. Most recently, a phase II study of DCVax-L and nivolumab, an anti-PD-1 antibody, for the treatment of patients with recurrent glioblastoma was withdrawn before the start of the study in end of 2019 (NCT03014801). Currently, there is one active phase III study for patients with glioblastoma IDH wild-type using DCs transfected with mRNA encoding the TAAs survivin and hTERT (NCT03548571) with so far no published results.

Another vaccine approach is based on human cytomegalovirus (HCMV) components detected in GBM tumor tissue. Remarkably, a first clinical trial with patients treated with a DC vaccine carrying mRNA from HCMV showed that almost one third of the study participants were without tumor recurrence five years after diagnosis (NCT00639639, [71]).

Peptide vaccines for immunotherapy of GBM

Up to now, the relative lack of highly expressed neoantigens and therefore the lack of MHC- neoepitopes is limiting the development of peptide-vaccines for immunotherapy of glioblastoma. So far, the oncogenic epidermal growth factor receptor variant III (EGFRvIII), which was already identified nearly three decades ago, remains a

promising tumor-antigen for vaccination. In primary GBM, EGFRvIII is found in 20–30% of tumors, yet with heterogeneous expression levels in tumor areas [1, 72]. EGFRvIII shows an in-frame deletion of exons 1–7 with a new glycine at the fusion point. The junctional sequence represents a *bona fide* neoantigen to which the host is not tolerant. A vaccine, termed rindopepimut, containing the 13-mer peptide “LEEKKgNYVVTDH” plus an additional C-terminal cytosine for chemical coupling to lysine residues of the nonspecific immunomodulatory macromolecule keyhole limpet hemocyanine (KLH), was recently investigated in phase II (ACT III) and phase III (ACT IV) clinical trials [73, 74]. Although rindopepimut treatment resulted in a significant improvement in overall survival for EGFRvIII + GBM patients when matched to historic controls in the ACT III trial, the results from the successive ACT IV study were disappointing. In particular, overall survival of the treatment group was not improved when compared to the trial control group of patients, which in contrast to the aforementioned ACT III trial were treated with KLH alone. Several key issues can be drawn from the ACT III and ACT IV experience. It might be not advisable to target a single subclonal neoantigen due to selective pressure on escape variants. Instead, it may be mandatory to target multiple neoantigens if a high priority clonal neoantigen is not present. A further important issue is to include patients in the respective trial that contain MHC class I alleles providing high affinity binding of the processed neoepitope. In addition, MHC epitopes from the EGFRvIII 13-mer peptide of rindopepimut may represent weak MHC I

binder. Finally, in the ACT IV control group, the applied strong immunomodulator KLH might have some stimulatory impact on the immune system of patients leading to better outcome when compared to the historic control group of the ACT III trial. Of note, the ACT III and ACT IV studies mainly demonstrated a humoral immune response rather than a cell-mediated response. Conceptually, a cell-mediated immune response, executed by CTLs and Th1 CD4⁺ cells could be more efficacious since this might better facilitate epitope spreading resulting in improved successive polyclonal T cell responses. In summary, ACT IV's unsatisfactory outcome has stalled vaccine approaches using EGFRvIII as neoantigen. Yet, EGFRvIII is still an attractive target for combinatorial use with immune checkpoint blockade inhibitors or for including in multi-antigen vaccines. Likewise, EGFRvIII remains a suitable target for selective delivery of immunotoxins or immunoconjugates capable of modulating the tumor microenvironment of GBM.

A further current vaccination approach focuses on secondary GBM. Here, recurrent mutations of the *IDH1* gene, of which 90% displays a R132H substitution (IDH1^{R132H}), represents a promising neoantigen for vaccination. Noteworthy, IDH1 mutations are linked to initiation of gliomagenesis and persist during progression to IDH1-mutant glioblastoma [1]. IDH1^{R132H} is therefore a genuine clonal marker expressed by all tumor cells. Recently, the effects of vaccination using an IDH1^{R132H}-peptide spanning positions 123–142, which include the neoepitope was explored in a humanized mouse model and revealed CD4⁺ T cells response, which were accompanied by the appearance of murine anti-IDH1^{R132H}-antibodies. Noteworthy, Schumacher et al., also demonstrated cellular as well as humoral immune responses against the IDH1^{R132H}-peptide in patients with glioma prior vaccination [24] highlighting the clinical relevance of the IDH1^{R132H} neoantigen. *In silico* analysis predicted that processed IDH1^{R132H}-peptide bind to MHC class II molecule HLA-DRB, indicating an immunodominant neoantigenic target for CD4⁺ T cells. An early report of the NOA-16 clinical phase I trial (NCT02454634) demonstrated safety of the IDH1^{R132H}-peptide vaccine in patients with in WHO Grade III-IV IDH1^{R132H}-mutated gliomas. In addition, an encouraging appearance of mutation-specific T cells in 80% and humoral responses in 87% of patients not detectable before vaccination was reported [75]. This preliminary report provides strong support for further pursuing the IDH1^{R132H}-vaccine and indicate so far, an underestimated role of CD4⁺ T lymphocytes in tumor immunosurveillance in secondary GBM.

To date, exciting advances in next-generation sequencing technology, bioinformatics as well as sophisticated prediction tools for determining affinities of

neoepitopes for MHC molecules pave the way to personalized vaccination of GBM. By comprehensive genomic and transcriptome analysis of tumors and normal tissue, it becomes possible to address tumor heterogeneity, driver mutations and to identify optimal neoantigens for immunotherapy. This approach was elegantly performed in a study by Keskin et al. (2019), which conducted phase Ib clinical vaccination studies using personalized neoantigen vaccines for patients with newly diagnosed MGMT-unmethylated GBM [30]. Patients were treated with multiple subcutaneous injections of vaccines containing up to 20 amino acids long peptides, which all represented high-affinity MHC class I binders. It is noteworthy that patients did not receive TMZ due to MGMT status. Strikingly, in some patient's immunosuppressive glucocorticoid-treatment could be avoided and was accompanied by promising immune responses, which were not observed in patients receiving glucocorticoids. In particular, such patients generated circulating polyfunctional neoantigen-experienced CD4⁺ and CD8⁺ T cells that were enriched in memory phenotype. Furthermore, neoantigen-targeting vaccine leads to an increase in antigen-experienced TILs in GBM, demonstrating that T cells from peripheral blood can migrate into intracranial tumors.

Viral-based vaccination approaches for treatment of glioblastoma

Viral-based therapies for treatment of gliomas represent an alternative vaccination approach as they co-deliver PAMPs and facilitate release of tumor-antigens by dying virus-infected cells. Literally, viral-based therapy can be characterized as an *in situ*-vaccination of the tumor, which encompasses the complete repertoire of neoantigens. Furthermore, this strategy holds promise in modulating the immunosuppressive tumor into a “hot” tumor supporting Th1-primed T cell responses. Among the current clinical trials treating for the treatment of patients with high-grade gliomas, promising gene-engineered oncolytic viruses are herpes virus-1 (HSV-1)-G47delta, adenovirus DNX-2401 and poliovirus PVS-RIPO.

HSV-1-G47delta represents a third generation oncolytic HSV-1 in which factors for neurovirulence are deleted and a tumor-selective replication is accomplished by deletion of the *ICP6(UL39)*, a gene encoding a subunit of viral ribonucleotide reductase, which is the key enzyme for DNA synthesis in non-dividing cells [76]. Recent data from a phase II trial employing HSV-1-G47delta treatment together with

TMZ maintenance therapy of recurrent or residual GBM demonstrated a one-year OS of 92.3%, which was significantly higher when compared to one-year OS of 78.5% in a historic control [76]. So far, no data on immune monitoring, including anti-virus immune response and induction of anti-tumor immune responses in GBM, are available.

DNX-2401 is an oncolytic adenovirus with restricted replication to tumor cells. This genetically engineered virus is characterized by a 24 bp deletion in the E1A gene, a protein that binds to the retinoblastoma tumor suppressor protein. Thus, the recombinant virus can replicate within tumor cells but not in normal somatic cells. Furthermore, the virus receptor was modified with an Arg-Gly-Asp (RGD) tripeptide, which specifically recognizes glioma-associated integrins, facilitating selective viral entry into tumor cells [77]. Preclinical studies demonstrated immunostimulatory effect using DNX-2401 in the syngeneic and immunocompetent murine glioma model GL261. Intratumoral injection in glioma-bearing GL261 mice induced anti-glioma immunity with immune cell infiltration, reduced tumor growth and prolonged survival [78]. It is noteworthy, that in a phase I clinical trial of DNX-2401 involving 37 patients with recurrent malignant glioma, 20% of patients survived more than three years after a single administration of DNX-2401. Histopathological examinations of tumor samples revealed immune cell infiltration of CD8⁺ and CD4⁺ T cells expressing the Th1 specific transcription marker T-bet which was associated with reduced expression of the exhaustion marker immunoglobulin mucin-3 (TIM-3) [79]. Currently, DNX-2401 is investigated in a further phase I clinical trial (NCT03896568). Instead of intratumoral injection, DNX-2401 is delivered to recurrent GBM by DNX-2401-loaded mesenchymal stem cells administered via intraarterial injection.

An efficient oncolytic capacity of poliovirus PVS-RIPO correlates with its characteristic own viral receptor which binds to CD155/poliovirus receptor (PVR) expressed on human glioma cells. To avoid neurotoxicity, the internal ribosomal entry site (IRES) was replaced with the non-virulent human rhinovirus type 2 (HRV2) [80]. In a phase I clinical trial involving 61 patients with recurrent GBM, intratumoral injection of PVS-RIPO induced inflammation of GBMs, characterized by pseudoprogression when analyzed by magnetic resonance imaging. Yet, no further major adverse effects were reported, and overall survival rate of patients were 21% at 36 months when compared to historic control groups exhibiting OS of 4% at 36 months [81]. The PVS-RIPO viral therapy is currently further pursued in a clinical phase II study (NCT01491893).

Despite the potential immunotherapeutic effects observed in oncolytic virotherapy, some limitations are

apparent. These include a counteracting innate antiviral response and limited virus spreading in tumor tissue. As a first line of defense, the innate antiviral response is activated after a viral infection, which is mainly mediated by the release of type I interferons (IFN-I). The virus-induced release of IFN-I activates innate immune cells. Although viral PAMPs should favour the reprogramming into pro-inflammatory M1-phenotype, the increased production of the tumour necrosis factor α (TNF- α) by macrophages most likely induces apoptosis of infected tumor cells and inhibits virus spread [82]. A potential strategy to amplify beneficial immune effects of oncolytic virotherapy might include initial treatment with inhibitors for the type I-interferon signalling pathway which may improve viral titers and subsequent virus spreading along GBM cells.

Conclusions

In the last years intense research efforts focused on redirection and reactivation of adaptive immune responses to treat glioblastoma. When using vaccination and immune checkpoint blockade, almost all clinical studies revealed promising signs of immune responses and in part increased overall survival. Yet, hurdles affecting the efficacy of vaccines, especially for state-of-the-art peptide-based vaccinations, and immune checkpoint blockade became apparent. These include intrinsic restricting factors linked to tumor biology such as the immunosuppressive microenvironment of GBM, the potential lack of efficient T cell homing and the tumor heterogeneity in GBM combined with a lack of sufficient numbers of clonal neoantigens. In addition, treatment related factors limiting immunotherapy include the frequent use of glucocorticoids, insufficient intratumoral bio-distribution of therapeutic antibodies (i.e. anti-PD-1, anti-PD-L1), induction of peripheral resistance mechanisms of tumors, the lack of knowledge about the best vaccine formulation (what adjuvant), application schedule (one shot vs. repetitive vaccination) and the best localization to inject/inoculate the vaccine (subcutaneous vs. nasal vaccine application). It is foreseeable that GBM-vaccines alone will not be able to establish long-lasting immune effects and it might be advisable to set up rational designs, which implement combinatorial treatments to dampen the immunosuppressive microenvironment of GBM. This might include for example levo-1-methyl-tryptophan to block IDO, s-DAB-IL-2(V6A), a second-generation version of denileukin diftitox fusion protein to target CD25⁺ Tregs in GBM [83], but also new approaches to block TGF- β signaling [9]. Furthermore, one might speculate on transiently modifying the BBB barrier to improve influx of macromolecular-sized

therapeutics or, in the case of anti-PD-1 and anti-PD-L1, generating low molecular weight antibodies that achieve enhanced intratumoral concentration and efficiency. Moreover, it is important to study such combinatorial treatments with standard of care TMZ and radiotherapy. Finally, for the benefit of patients, future testing of such novel combinatorial treatments must be accompanied by detailed monitoring of adverse effects, immune responses and immune architecture of GBMs.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

References

- Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. *Acta Neuropathol* 2016;131:803–20.
- Canoll P, Goldman JE. The interface between glial progenitors and gliomas. *Acta Neuropathol* 2008;116:465–77.
- Hegi ME, Liu L, Herman JG, Stupp R, Wick W, Weller M, et al. Correlation of O⁶-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. *J Clin Oncol* 2008;26:4189–99.
- Fuller GN, Bigner SH. Amplified cellular oncogenes in neoplasms of the human central nervous system. *Mutat Res* 1992;276:299–306.
- Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005;352:987–96.
- Stupp R, Taillibert S, Kanner A, Read W, Steinberg D, Lhermitte B, et al. Effect of tumor-treating fields plus maintenance temozolomide vs. maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. *J Am Med Assoc* 2017;318:2306–16.
- Stupp R, Wong ET, Kanner AA, Steinberg D, Engelhard H, Heidecke V, et al. NovoTTF-100A versus physician's choice chemotherapy in recurrent glioblastoma: a randomised phase III trial of a novel treatment modality. *Eur J Canc* 2012;48:2192–202.
- Pennock ND, White JT, Cross EW, Cheney EE, Tamburini BA, Kedl RM. T cell responses: naive to memory and everything in between. *Adv Physiol Educ* 2013;37:273–83.
- Topfer K, Kempe S, Muller N, Schmitz M, Bachmann M, Cartellieri M, et al. Tumor evasion from T cell surveillance. *J Biomed Biotechnol* 2011;2011:918471.
- Buchta CM, Bishop GA. Toll-like receptors and B cells: functions and mechanisms. *Immunol Res* 2014;59:12–22.
- Tesniere A, Schlemmer F, Boige V, Kepp O, Martins I, Ghiringhelli F, et al. Immunogenic death of colon cancer cells treated with oxaliplatin. *Oncogene* 2010;29:482–91.
- Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002;418:191–5.
- Chao MP, Jaiswal S, Weissman-Tsukamoto R, Alizadeh AA, Gentles AJ, Volkmer J, et al. Calreticulin is the dominant pro-phagocytic signal on multiple human cancers and is counterbalanced by CD47. *Sci Transl Med* 2010;2:63ra94.
- Aspelund A, Antila S, Proulx ST, Karlson TV, Karaman S, Detmar M, et al. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J Exp Med* 2015;212:991–9.
- Kipnis J. Multifaceted interactions between adaptive immunity and the central nervous system. *Science* 2016;353:766–71.
- Elliott PJ, Hayward NJ, Dean RL, Bartus RT. Dissociation of blood-brain barrier permeability and the hypotensive effects of the bradykinin B2 agonist, RMP-7. *Immunopharmacology* 1996;33:205–8.
- Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, Peske JD, et al. Structural and functional features of central nervous system lymphatic vessels. *Nature* 2015;523:337–41.
- Stamatovic SM, Keep RF, Andjelkovic AV. Brain endothelial cell-cell junctions: how to “open” the blood brain barrier. *Curr Neuropharmacol* 2008;6:179–92.
- Watkins S, Robel S, Kimbrough IF, Robert SM, Ellis-Davies G, Sontheimer H. Disruption of astrocyte-vascular coupling and the blood-brain barrier by invading glioma cells. *Nat Commun* 2014;5:4196.
- Argaw AT, Gurfein BT, Zhang Y, Zameer A, John GR. VEGF-mediated disruption of endothelial CLN-5 promotes blood-brain barrier breakdown. *Proc Natl Acad Sci U S A* 2009;106:1977–82.
- Hardee ME, Zagzag D. Mechanisms of glioma-associated neovascularization. *Am J Pathol* 2012;181:1126–41.
- Zhang JG, Eguchi J, Kruse CA, Gomez GG, Fakhrai H, Schroter S, et al. Antigenic profiling of glioma cells to generate allogeneic vaccines or dendritic cell-based therapeutics. *Clin Canc Res* 2007;13:566–75.
- Wintterle S, Schreiner B, Mitsdoerffer M, Schneider D, Chen L, Meyermann R, et al. Expression of the B7-related molecule B7-H1 by glioma cells: a potential mechanism of immune paralysis. *Canc Res* 2003;63:7462–7.
- Schumacher T, Bunse L, Pusch S, Sahm F, Wiestler B, Quandt J, et al. A vaccine targeting mutant IDH1 induces antitumor immunity. *Nature* 2014;512:324–7.
- Pallasch CP, Struss AK, Munnia A, Konig J, Steudel WI, Fischer U, et al. Autoantibodies against GLEA2 and PHF3 in glioblastoma: tumor-associated autoantibodies correlated with prolonged survival. *Int J Canc* 2005;117:456–9.
- Barcia C Jr., Gomez A, Gallego-Sanchez JM, Perez-Valles A, Castro MG, Lowenstein PR, et al. Infiltrating CTLs in human glioblastoma establish immunological synapses with tumorigenic cells. *Am J Pathol* 2009;175:786–98.
- Calzascia T, Masson F, Di Bernardino-Besson W, Contassot E, Wilmotte R, Aurrand-Lions M, et al. Homing phenotypes of tumor-specific CD8 T cells are predetermined at the tumor site by crosspresenting APCs. *Immunity* 2005;22:175–84.
- Masson F, Calzascia T, Di Bernardino-Besson W, de Tribolet N, Dietrich PY, Walker PR. Brain microenvironment promotes the

- final functional maturation of tumor-specific effector CD8⁺ T cells. *J Immunol* 2007;179:845–53.
29. Mora JR, Cheng G, Picarella D, Briskin M, Buchanan N, von Andrian UH. Reciprocal and dynamic control of CD8 T cell homing by dendritic cells from skin- and gut-associated lymphoid tissues. *J Exp Med* 2005;201:303–16.
 30. Keskin DB, Anandappa AJ, Sun J, Tirosh I, Mathewson ND, Li S, et al. Neoantigen vaccine generates intratumoral T cell responses in phase Ib glioblastoma trial. *Nature* 2019;565:234–9.
 31. Land CA, Musich PR, Haydar D, Krenciute G, Xie Q. Chimeric antigen receptor T-cell therapy in glioblastoma: charging the T cells to fight. *J Transl Med* 2020;18:428.
 32. Park J, Kwon M, Kim KH, Chang JH, Shin EC. Expression of immune checkpoint molecules on tumor infiltrating lymphocytes in glioblastoma multiforme. *J Immunol* 2017;198:196.3.
 33. Woroniecka K, Chongsathidkiet P, Rhodin K, Kemeny H, Dechant C, Farber SH, et al. T-cell exhaustion signatures vary with tumor type and are severe in glioblastoma. *Clin Canc Res* 2018;24:4175–86.
 34. Nduom EK, Wei J, Yaghi NK, Huang N, Kong LY, Gabrusiewicz K, et al. PD-L1 expression and prognostic impact in glioblastoma. *Neuro Oncol* 2016;18:195–205.
 35. Fecci PE, Ochiai H, Mitchell DA, Grossi PM, Sweeney AE, Archer GE, et al. Systemic CTLA-4 blockade ameliorates glioma-induced changes to the CD4⁺ T cell compartment without affecting regulatory T-cell function. *Clin Canc Res* 2007;13:2158–67.
 36. El AA, Lesniak MS. An increase in CD4⁺CD25⁺FOXP3⁺ regulatory T cells in tumor-infiltrating lymphocytes of human glioblastoma multiforme. *Neuro Oncol* 2006;8:234–43.
 37. Bodmer S, Strommer K, Frei K, Siepl C, de Tribolet N, Heid I, et al. Immunosuppression and transforming growth factor-beta in glioblastoma. Preferential production of transforming growth factor-beta 2. *J Immunol* 1989;143:3222–9.
 38. Malygin AM, Meri S, Timonen T. Regulation of natural killer cell activity by transforming growth factor-beta and prostaglandin E2. *Scand J Immunol* 1993;37:71–6.
 39. Gagliardi F, Narayanan A, Reni M, Franzin A, Mazza E, Boari N, et al. The role of CXCR4 in highly malignant human gliomas biology: current knowledge and future directions. *Glia* 2014;62:1015–23.
 40. Okada M, Saio M, Kito Y, Ohe N, Yano H, Yoshimura S, et al. Tumor-associated macrophage/microglia infiltration in human gliomas is correlated with MCP-3, but not MCP-1. *Int J Oncol* 2009;34:1621–7.
 41. Kohanbash G, McKaveney K, Sakaki M, Ueda R, Mintz AH, Amankulor N, et al. GM-CSF promotes the immunosuppressive activity of glioma-infiltrating myeloid cells through interleukin-4 receptor-alpha. *Canc Res* 2013;73:6413–23.
 42. Held-Feindt J, Hattermann K, Muerkoster SS, Wedderkopp H, Knerlich-Lukoschus F, Ungefroren H, et al. CX3CR1 promotes recruitment of human glioma-infiltrating microglia/macrophages (GIMs). *Exp Cell Res* 2010;316:1553–66.
 43. Zhou W, Ke SQ, Huang Z, Flavahan W, Fang X, Paul J, et al. Periostin secreted by glioblastoma stem cells recruits M2 tumour-associated macrophages and promotes malignant growth. *Nat Cell Biol* 2015;17:170–82.
 44. Arrieta O, Garcia E, Guevara P, Garcia-Navarrete R, Ondarza R, Rembao D, et al. Hepatocyte growth factor is associated with poor prognosis of malignant gliomas and is a predictor for recurrence of meningioma. *Cancer* 2002;94:3210–8.
 45. Garcia-Navarrete R, Garcia E, Arrieta O, Sotelo J. Hepatocyte growth factor in cerebrospinal fluid is associated with mortality and recurrence of glioblastoma, and could be of prognostic value. *J Neuro Oncol* 2010;97:347–51.
 46. Yen BL, Yen ML, Hsu PJ, Liu KJ, Wang CJ, Bai CH, et al. Multipotent human mesenchymal stromal cells mediate expansion of myeloid-derived suppressor cells via hepatocyte growth factor/c-met and STAT3. *Stem Cell Rep* 2013;1:139–51.
 47. Gieryng A, Pszczolkowska D, Walentynowicz KA, Rajan WD, Kaminska B. Immune microenvironment of gliomas. *Lab Invest* 2017;97:498–518.
 48. Nakano Y, Kuroda E, Kito T, Yokota A, Yamashita U. Induction of macrophagic prostaglandin E2 synthesis by glioma cells. *J Neurosurg* 2006;104:574–82.
 49. Walker MR, Kasprowicz DJ, Gersuk VH, Benard A, Van Landeghen M, Buckner JH, et al. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4⁺. *J Clin Invest* 2003;112:1437–43.
 50. Wainwright DA, Balyasnikova IV, Chang AL, Ahmed AU, Moon KS, Auffinger B, et al. IDO expression in brain tumors increases the recruitment of regulatory T cells and negatively impacts survival. *Clin Canc Res* 2012;18:6110–21.
 51. Zhai L, Ladomersky E, Lauing KL, Wu M, Genet M, Gritsina G, et al. Infiltrating T cells increase IDO1 expression in glioblastoma and contribute to decreased patient survival. *Clin Canc Res* 2017;23:6650–60.
 52. Rodriguez PC, Hernandez CP, Morrow K, Sierra R, Zabaleta J, Wyczechowska DD, et al. L-arginine deprivation regulates cyclin D3 mRNA stability in human T cells by controlling HuR expression. *J Immunol* 2010;185:5198–204.
 53. Schmielau J, Finn OJ. Activated granulocytes and granulocyte-derived hydrogen peroxide are the underlying mechanism of suppression of T-cell function in advanced cancer patients. *Canc Res* 2001;61:4756–60.
 54. Nagaraj S, Gupta K, Pisarev V, Kinarsky L, Sherman S, Kang L, et al. Altered recognition of antigen is a mechanism of CD8⁺ T cell tolerance in cancer. *Nat Med* 2007;13:828–35.
 55. Wang RN, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* 2011;35:871–82.
 56. Sugiura A, Rathmell JC. Metabolic barriers to T cell function in tumors. *J Immunol* 2018;200:400–7.
 57. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. *Nat Commun* 2020;11:3801.
 58. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* 2014;515:568–71.
 59. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015;373:1627–39.
 60. Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. *N Engl J Med* 2018;378:1277–90.
 61. Zhang Y, Nagata Y, Yu G, Nguyen HG, Jones MR, Toselli P, et al. Aberrant quantity and localization of Aurora-B/AIM-1 and survivin during megakaryocyte polyploidization and the consequences of Aurora-B/AIM-1-deregulated expression. *Blood* 2004;103:3717–26.
 62. Omuro A, Vlahovic G, Lim M, Sahebjam S, Baehring J, Cloughesy T, et al. Nivolumab with or without ipilimumab in patients with

- recurrent glioblastoma: results from exploratory phase I cohorts of CheckMate 143. *Neuro Oncol* 2018;20:674–86.
63. Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, et al. Effect of nivolumab vs. bevacizumab in patients with recurrent glioblastoma the CheckMate 143 phase 3 randomized clinical trial. *JAMA Oncol* 2020;6:1003–10.
 64. Bouffet E, Larouche V, Campbell BB, Merico D, de Borja R, Aronson M, et al. Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. *J Clin Oncol* 2016;34:2206–11.
 65. Johanns TM, Miller CA, Dorward IG, Tsien C, Chang E, Perry A, et al. Immunogenomics of hypermutated glioblastoma: a patient with germline POLE deficiency treated with checkpoint blockade immunotherapy. *Canc Discov* 2016;6:1230–6.
 66. Tuyaerts S, Aerts JL, Corthals J, Neyns B, Heirman C, Breckpot K, et al. Current approaches in dendritic cell generation and future implications for cancer immunotherapy. *Cancer Immunol Immunother* 2007;56:1513–37.
 67. Curry WT Jr., Gorrepati R, Piesche M, Sasada T, Agarwalla P, Jones PS, et al. Vaccination with irradiated autologous tumor cells mixed with irradiated GM-K562 cells stimulates antitumor immunity and T lymphocyte activation in patients with recurrent malignant glioma. *Clin Canc Res* 2016;22:2885–96.
 68. Temme A, Morgenroth A, Schmitz M, Weigle B, Rohayem J, Lindemann D, et al. Efficient transduction and long-term retroviral expression of the melanoma-associated tumor antigen tyrosinase in CD34(+) cord blood-derived dendritic cells. *Gene Ther* 2002;9:1551–60.
 69. Liao LM, Ashkan K, Tran DD, Campian JL, Trusheim JE, Cobbs CS, et al. First results on survival from a large phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. *J Transl Med* 2018;16:142.
 70. Wick W, van den Bent MJ. First results on the DCVax phase III trial: raising more questions than providing answers. *Neuro Oncol* 2018;20:1283–4.
 71. Batich KA, Reap EA, Archer GE, Sanchez-Perez L, Nair SK, Schmittling RJ, et al. Long-term survival in glioblastoma with cytomegalovirus pp65-targeted vaccination. *Clin Canc Res* 2017;23:1898–909.
 72. Nishikawa R, Sugiyama T, Narita Y, Furnari F, Cavenee WK, Matsutani M. Immunohistochemical analysis of the mutant epidermal growth factor, deltaEGFR, in glioblastoma. *Brain Tumor Pathol* 2004;21:53–6.
 73. Schuster J, Lai RK, Recht LD, Reardon DA, Paleologos NA, Groves MD, et al. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. *Neuro Oncol* 2015;17:854–61.
 74. Weller M, Butowski N, Tran DD, Recht LD, Lim M, Hirte H, et al. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol* 2017;18:1373–85.
 75. Platten M, Schilling D, Bunse L, Wick A, Bunse T, Riehl D, et al. ATIM-33. NOA-16: a first-in-man multicenter phase I clinical trial OF the German neurooncology working group evaluating a mutation-specific peptide vaccine targeting IDH1R132H IN patients with newly diagnosed malignant astrocytomas. *Neuro Oncol* 2018;20(6 Suppl):vi8–9.
 76. Todo T. ATIM-14. Results of phase II clinical trial of oncolytic herpes virus G47Δ IN patients with glioblastoma. *Neuro Oncol* 2019;21(6 Suppl):vi4.
 77. Suzuki K, Fueyo J, Krasnykh V, Reynolds PN, Curiel DT, Alemany R. A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. *Clin Canc Res* 2001;7:120–6.
 78. Jiang H, Clise-Dwyer K, Ruisaard KE, Fan X, Tian W, Gumin J, et al. Delta-24-RGD oncolytic adenovirus elicits anti-glioma immunity in an immunocompetent mouse model. *PLoS One* 2014;9:e97407.
 79. Lang FF, Conrad C, Gomez-Manzano C, Yung WKA, Sawaya R, Weinberg JS, et al. Phase I study of DNX-2401 (Delta-24-RGD) oncolytic adenovirus: replication and immunotherapeutic effects in recurrent malignant glioma. *J Clin Oncol* 2018;36:1419–27.
 80. Gromeier M, Alexander L, Wimmer E. Internal ribosomal entry site substitution eliminates neurovirulence in intergeneric poliovirus recombinants. *Proc Natl Acad Sci U S A* 1996;93:2370–5.
 81. Desjardins A, Gromeier M, Herndon JE 2nd, Beaubier N, Bolognesi DP, Friedman AH, et al. Recurrent glioblastoma treated with recombinant poliovirus. *N Engl J Med* 2018;379:150–61.
 82. Meisen WH, Wohleb ES, Jaime-Ramirez AC, Bolyard C, Yoo JY, Russell L, et al. The impact of macrophage- and microglia-secreted TNF alpha on oncolytic HSV-1 therapy in the glioblastoma tumor microenvironment. *Clin Canc Res* 2015;21:3274–85.
 83. Cheung LS, Fu J, Kumar P, Kumar A, Urbanowski ME, Ihms EA, et al. Second-generation IL-2 receptor-targeted diphtheria fusion toxin exhibits antitumor activity and synergy with anti-PD-1 in melanoma. *Proc Natl Acad Sci U S A* 2019;116:3100–5.

Supplementary Material: The online version of this article offers reviewer assessments as supplementary material (<https://doi.org/10.1515/iss-2020-0034>).