Concepts for Immunotherapies in Gliomas

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Abstract

Strategies to empower the immune system to successfully attack cancers, including vaccination approaches, adaptive T cell therapies, and immune checkpoint modulators, have recently achieved remarkable success across a spectrum of cancer indications. Nonetheless, with rare exception, only a minority of patients with a given type of cancer respond to an immunotherapeutic when administered as single-agent therapy. Although under extensive laboratory and clinical investigation, the role of these approaches for glioma patients remains to be determined. While the central nervous system (CNS) is no longer regarded as an immunoprivileged sanctuary, nuances regarding immune responses in the CNS may impact on the activity of immunotherapy treatments of brain tumor patients. Furthermore, many common CNS tumors such as World Health Organization grade III and IV (high grade) gliomas utilize myriad, nonoverlapping strategies to dampen or extinguish antitumor immune responses. For these reasons, critical research efforts are focused on identifying biomarkers that predict patients with a heightened likelihood of therapeutic benefit as well as evaluating rationally designed combinatorial immunotherapy approaches with potentially complementary mechanisms of immune-activation for brain cancer patients.

Keywords

► glioma
► Immunotherapy
► Immunosuppression
► immune checkpoint
► vaccine

Malignant gliomas remain a major therapeutic challenge despite considerable progress in the understanding of their underlying genetic and biologic evolution. Localization, the remarkable infiltrative growth, and the autochthonous and microenvironment-driven resistance to genotoxic and targeted therapies limit the efficacy of current therapeutic approaches. Analogous resistance mechanisms impact immunotherapeutic approach to gliomas, which differ from other types of tumors in a variety of aspects. Gliomas are generally regarded as “cold” tumors, meaning that intratumoral immune-activation is suppressed. The underlying mechanisms are diverse but involve the immunoinhibitory function of the blood–brain barrier (BBB), paucity of specific antigens, and the immunosuppressive glioma microenvironment. A detailed understanding of the specific aspects of immune tolerance within the central nervous system (CNS) as well as knowledge regarding the cellular and molecular mechanisms of glioma-associated immune suppression is required to overcome these limitations. Reversal of glioma-associated immune suppression is a prerequisite for enabling an effective tumor-specific immune response, the specificity, and magnitude of which is determined by the presence of appropriate antigens. In addition to the regulatory and practical challenges associated with vaccines targeting patient-specific antigens, there are glioma-specific aspects of target antigen selection with respect to specific self and neoantigens as well as clonal representation of the target antigen.

Nuances of CNS Immunity

The CNS is generally considered an immunoprivileged site, which is protected from—among others—insults by the
immune system, which may cause transient or permanent neuronal damage. This does not, however, imply that immune responses against CNS intrinsic antigens do not occur. Also, a disrupted BBB is not a prerequisite for an effective immune response in the CNS. Multiple sclerosis is an exemplary disease, where focal inflammation sustained by CNS resident cells is caused by an antigen-specific immune response triggered in the peripheral immune system and transmitted through an initially intact BBB. Another example is constituted by classic paraneoplastic syndromes, such as cerebellar degeneration, where tumor-specific antibodies or T cells cross-reactive with neuronal structures may cause severe CNS damage in an otherwise unperturbed CNS. In fact, the CNS is constantly surveyed by T cells to prevent activation of opportunistic infections. When this surveillance fails, for instance, in the case of prolonged and severe immunosuppression, latent infections such as *John Cunningham* virus infection may become active and cause life-threatening CNS destructive progressive multifocal leukoencephalopathy. Animal models of CNS autoimmunity suggest that the immune privilege is not primarily sustained by preventing entry of immune cells in the CNS through a tight BBB but is rather an active process requiring various cellular and soluble factors actively suppressing unwanted immune responses. CNS immune homeostasis is geared toward protecting the CNS from inflammatory insults to ensure proper neuronal function. This protection is—in large part—conferred by CNS resident immune cells, namely microglial cells and perivascular myeloid cells. These myeloid cells in the CNS parenchyma and at the BBB interface are highly specialized yet display extraordinary morphologic and functional plasticity in response to changes in the CNS microenvironment. This plasticity in the CNS myeloid compartment is exploited by gliomas to suppress antitumor immune responses. Understanding these mechanisms is important for developing strategies to alleviate glioma-associated immunosuppression.

**Mechanisms of Glioma Immunosuppression**

Patients with malignant gliomas are generally considered in an immunocompromised state independent of treatment with steroids. Glioblastoma patients display reduced CD4 T cell counts, suppressed CD4 T cell memory function and accumulate myeloid-derived suppressor cells (MDSC) in the peripheral circulation. However, there is insufficient evidence that these signs of immunosuppression in the peripheral circulation predict response to immunotherapies or even reflect the tumor immune microenvironment. Here, the BBB provides a significant barrier prohibiting the free transfer of immune cells from the periphery to the intratumoral compartment and vice versa. On the other hand, leakage of the BBB, hypoxia, the accumulation of soluble mediators, and the attraction of myeloid cells from the peripheral circulation profoundly alter the immunobiology of high-grade gliomas. The glioma microenvironment is particularly hostile to an effective antiglioma immune response and will therefore hamper attempts to transfer, induce, or amplify glioma-specific T cells. Therefore, glioma-associated immune suppression requires therapeutic targeting to allow glioma-specific T cells to become effective. Such therapeutic targeting requires a detailed understanding of the immunologic alterations in the glioma microenvironment and its cellular and molecular mechanisms. While it has long been known that glioma cells themselves suppress T cell responses, this cannot be solely explained by cell-mediated mechanisms as glioma patients also display deficits in systemic immune responses. Pivotal soluble mediators of glioma-associated immune suppression include transforming growth factor β (TGF-β) and kynurenines. TGF-β has been discovered as the “glioma-derived T cell suppressor factor” capable of suppressing immune responses by suppressing T cell proliferation, inducing T cell apoptosis, as well as suppressing natural killer (NK) cell and myeloid cell activity. Preclinical models have established TGF-β as a viable therapeutic target. While clinical trials using TGF-β kinase inhibitor galunisertib or the antisense oligonucleotide trabedersen did not show efficacy as single agents in recurrent glioblastoma, they may enhance antitumor immunity when combined with active immunotherapy.

The catabolism of the essential amino acid tryptophan is an additional important microenvironmental factor suppressing antitumor immune responses. Tryptophan is catabolized by the dioxygenases indoleamine-2,3-dioxygenase (IDO) and tryptophan dioxygenase (TDO), creating an immunosuppressive milieu by accumulating immunosuppressive tryptophan catabolites such as kynurenine. Of note, there is also evidence of nonimmune functions of tryptophan catabolism in gliomas regulating tumor supportive activities such as invasiveness, resistance to therapy, and excitotoxicity. Blocking IDO or TDO alleviates tumor-associated immune suppression in preclinical cancer models. The competitive IDO inhibitor 1-methyl-D-tryptophan (indoximod), which may have considerable off-target effects as a result of mitogen-activate protein kinase inhibition, and the noncompetitive IDO inhibitor INCBO24360 (epacadostat) are currently investigated in clinical trials in patients with cancer including gliomas in combination with chemotherapy or checkpoint inhibition. Currently, fundamental questions such as CNS availability or the exact cellular and molecular mechanism of action of indoximod or epacadostat are still elusive. Other agents targeting regulators of tryptophan metabolism are still in preclinical development including the TDO inhibitor LM10, the prodrug of 680C91, and inhibitors of the aroyl hydrocarbon receptor, such as reservatrol, which is an orally available flavonoid with AHR-antagonistic properties capable of blocking T cell differentiation into regulatory T cells and may thus be an interesting immunotherapeutic approach to gliomas.

In addition to releasing soluble mediators, glioma cells instruct CNS resident or tumor-infiltrating cells to become immunosuppressive. Glioma-infiltrating monocytes and resident microglial cells have been described as mediators of immunosuppression in gliomas since the 1980s. Analyses of animal models and glioma tissue have shown that monocyteic cells in gliomas, which may constitute up to 50% of the...
total cellular content, are shaped in their function and enumeration by glioma-derived soluble factors such as the chemokine ligand 2 (CCL2)\textsuperscript{23} or colony-stimulating factor (CSF-1).\textsuperscript{24} Such “educated” myeloid cells amplify tumor cellular proliferation, angiogenesis, and immunosuppression.\textsuperscript{25} Immunosuppressive mononuclear cells in the glioma microenvironment are referred to as MDSC. This incompletely characterized cell population signified by co-expression of granulocytic and monocytic markers suppresses antigen-specific T cell responses through various soluble and membrane-bound mechanisms.\textsuperscript{26,27} MDSC are found in gliomas where they suppress T cell activation by various mechanisms.\textsuperscript{7}

In contrast to resident or infiltrating monocytes, there is little NK and T cell infiltration in gliomas. As in systemic tumor entities, however, the extent of T cell infiltration appears to be associated with favorable outcome,\textsuperscript{27,28} which is also supported by gene expression studies.\textsuperscript{29} Hence, glioma-infiltrating T cells are not inherently defective in executing tumor-cell lysis as also evidenced by in vitro studies using ex vivo cultivated glioma-infiltrating T cells.\textsuperscript{30} This in turn suggests that glioma-infiltrating T cells may be reinvigorated by reversing the immunosuppressive glioma microenvironment. Cytotoxic T-effector cells are inhibited by regulatory T cells (Treg), which are commonly identified as natural CD25\textsuperscript{+} FoxP3\textsuperscript{+} CD4\textsuperscript{+} T cells. While more detailed analyses of the Treg phenotype(s) infiltrating human gliomas are sparse, it remains unclear if the degree of natural Treg infiltration or their functional properties in gliomas impacts outcome.\textsuperscript{31–33} In experimental glioma models, the systemic depletion of Tregs aids in inducing an antitumor immune response,\textsuperscript{34,35} which builds a rationale for future intervention trials in glioma patients.\textsuperscript{36}

## Vaccination Strategies

Vaccines aim to induce or enhance a tumor-specific immune response by immunizing with tumor lysate or nucleic acids, with tumor-associated antigens, viral antigens, or tumor antigens. Here, we will focus on vaccines with tumor-associated antigens, which are primarily embryonal self-antigens. These antigens are not unique for gliomas. In fact, there are only a few glioma-specific self-antigens reported to date. While these self-antigens are expressed in the majority of gliomas albeit at highly variable levels,\textsuperscript{37} the degree of presentation on MHC class I in the tumor tissue often remains unclear. Mass-spectrometry-based human leukocyte antigen (HLA) ligandome analyses from tumor tissue provide useful information on antigen presentation and thus help enrich the pool of relevant self-antigens.\textsuperscript{38} Vaccines targeting self-antigens are generally viewed as safe, although autoimmunity to the CNS caused by cross-reactivity may occur.\textsuperscript{39} The more pertinent problem is that the induction of an efficacious antitumor immunity may be hampered by the expression of self-antigens in the thymus, resulting in central T cell tolerance and the development of antigen-specific suppressive T-regulatory cells.\textsuperscript{40,41}

Hence, many current clinical trials targeting self-antigens combine multiple epitopes. Some approaches select one or multiple vaccines from a warehouse of vaccines based on individual antigen expression, antigen presentation, and/or preexisting immune responses. These vaccines are typically restricted to common class I allelotypes such as HLA-A2 (IMA950, ICT-107) or HLA-A24 (ITK-1) and involve intradermal or subcutaneous injection with or without prior loading on autologous dendritic cells. IMA950, a warehouse vaccine selected based on all former criteria, has demonstrated safety and immunogenicity in a phase I clinical trial (NCT01920191) in HLA-A2\textsuperscript{+} patients with newly diagnosed glioblastoma.\textsuperscript{42} ICT-107 (NCT01280552), a multipeptide vaccine targeting stem cell antigens, is being tested in a phase III clinical trial in patients with newly diagnosed glioblastoma based on encouraging results from a randomized phase II clinical trial (EORTC1587, NCT02546102), although accrual to this study was recently suspended due to sponsor financial issues.

True tumor antigens are antigens specifically present only in the tumor tissue and usually arise de novo from mutated or variant proteins. The random nature of nonsynonymous mutation renders these neoantigens patient-specific or private antigens. There are only a few, but relevant examples of shared neoantigens, which are targeted in gliomas using specific vaccines. The variant III of the epidermal growth factor receptor (EGFRvIII) is a frequently expressed glioma antigen that has been targeted as a vaccine antigen.\textsuperscript{37,38}

<table>
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<tr>
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<td>20</td>
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CAR, chimeric antigen receptor; CMV, cytomegalovirus; EGFRvIII, variant III of epidermal growth factor receptor; PD-L1, programmed death ligand 1.
factor receptor (EGFRvIII) is a tumor-specific antigen generated by alternative splicing of exons 2 to 7. The neoepitope is generated by the peptide sequence of the fused exons 1 and 8. EGFRvIII is detected in approximately 20% to 30% of glioblastoma samples, and in general is coexpressed with the wild-type variant on a single-cell level.\(^{52}\) A peptide vaccine conjugated to the adjuvant keyhole limpet hemocyanin (KLH) induces robust anti-EGFRvIII antibody responses in patients with EGFRvIII-positive tumors.\(^{43}\) After encouraging results from noncontrolled phase I/II studies, the randomized ACT-IV registration trial, tested the efficacy of EGFRvIII Pep-KLH (rindopepimut) compared with placebo when combined with temozolomide radiochemotherapy in patients with newly diagnosed EGFRvIII-positive glioblastoma (NCT01480479). The primary outcome of this trial, overall survival, did not differ between vaccine and placebo groups. In contrast, a placebo-controlled, randomized phase II study in patients with recurrent glioblastoma, where rindopepimut was given in combination with the antiangiogenic agent bevacizumab (ReACT, NCT01498328), demonstrated an increase in overall survival. While the majority of patients in the phase I/II program with recurrent disease after the vaccine had lost expression of EGFRvIII in the tumor tissue,\(^{44}\) this cannot be viewed as a firm sign of immune escape as loss of EGFRvIII in glioblastoma also occurs naturally in the absence of EGFRvIII-directed therapies in 50% of the patients.\(^{45}\) In light of the negative registration trial, further development of this vaccine approach will depend on the rationalization of biological efficacy in the ReACT trial.

The experience with the vaccine targeting EGFRvIII, a subclonal antigen not expressed in all cells of a given tumor, illustrates the challenges associated with tumor heterogeneity, which often results in antigen heterogeneity and therefore antigen loss after vaccination. In contrast, targeting a true driver mutation should circumvent heterogeneity-driven immune escape. A mutation in the gene for isocitrate dehydrogenase type 1 (IDH1) occurs in 70% to 80% of diffuse and anaplastic gliomas and results in an amino acid exchange (Arg to His) at position 132 of the protein (IDH1R132H) in > 90% of cases. It is the earliest mutation in these tumors, rendering all tumor cells positive for IDH1R132H even during malignant progression.\(^{46,47}\) A subset of patients with IDH1R132H-mutated gliomas harbors spontaneous mutation-specific CD4+ T helper cells and antibodies, indicating that IDH1R132H is specifically presented to and recognized by the immune system in a mutation-specific manner.\(^{58}\) An IDH1R132H vaccine differs from other vaccines as it represents as a CD4 epitope, which requires endosomal/lysosomal processing and—most likely—cross-presentation by professional antigen-presenting cells. After preclinical testing, a multicenter phase I trial (NCT02454634) has completed accrual with 33 evaluable patients with newly diagnosed high-grade astrocytoma at eight German sites. Patients have received a total of eight vaccines comprised of a 20-mer peptide emulsified in Montanide-ISA51 in addition to combined radiochemotherapy. Importantly, the vaccine is integrated into the primary therapy. Mature outcome data are expected in Q4 2017.

The private nature of most neoepitopes constitutes the need for personalized vaccine concepts. Based on whole exome sequencing (WES) and a computational pipeline predicting HLA-binding of mutated epitopes,\(^{49}\) a phase I study in patients with newly diagnosed O6-methylguanine DNA methyltransferase (MGMT)-promoter unmethylated glioblastoma is underway to test the safety and immunogenicity of a personalized peptide vaccine (NeoVax) encompassing neoepitopes relevant for the individual patient (NCT02287428). The vaccine is given after completion of radiotherapy. In addition, the European Glioma Actively Personalized Vaccine Consortium (GAPVAC) has conducted a multicenter phase I clinical trial in patients with newly diagnosed glioblastoma (GAPVAC-101, NCT02149225). The selection and production of the personalized peptide vaccine in this trial is based not only on WES but also on HLA-ligandome analyses providing additional information of the actual presentation of relevant epitopes on HLA molecules in tumor tissue. While efficacy and immunogenicity data in this trial are of keen interest, this trial also illustrates the feasibility of performing complex epitope discovery for generating a personalized vaccine product which allowed for applying the personalized vaccine within three adjuvant temozolomide cycles.

### Adoptive T Cell Therapies

For over 30 years, strategies to genetically engineer T cells to successfully attack tumor cells have been pursued.\(^{50}\) Such approaches have evolved considerably with modern era gene transfer technology allowing very high gene transfer efficiencies and greater understanding of T cell biology. Initially autologous tumor-infiltrating lymphocytes (TILs) harvested from surgically resected tumors were infused back to the patient. Modest but short-lived tumor regression was achieved in some patients with melanoma\(^{51}\) and the subsequent incorporation of lymphocyte depleting chemotherapy and/or whole body irradiation improved the overall benefit rate and durability.\(^{52}\) Subsequent efforts focused on genetic transfer of T cell receptor (TCR) α and β chains specific to a defined tumor target antigen to generate antigen-specific T cells for infusion.\(^{53}\) Key limitations to these TCR-based approaches include restriction to a single HLA type and inability to target nonprotein tumor antigens.

To overcome these deficiencies, subsequent efforts focused on the development of chimeric antigen receptor (CAR) T cells which are autologous T cells harvested from patients and engineered to express a tumor-antigen binding domain of a single chain antibody (scFv) fused with intracellular signaling proteins.\(^{54}\) CAR T cells are typically expanded ex vivo and then systemically administered. Optimal tumor antigens for CAR T cell targeting are tumor-specific and therefore not expressed by normal tissues, are widely expressed within the tumor and localize to the tumor cell surface. Upon binding to the tumor antigen target, CAR transduced T cells directly activate to mediate tumor cell killing. More recent generation CAR T cells are engineered to also express costimulatory signaling molecules such as CD28 and/or 41BB that enhance cell proliferation.
upon recognition of target antigen as well as cytokine release and cell lysis.\textsuperscript{55,56} In addition, conditioning chemotherapy prior to CAR T cell administration may enhance systemic persistence.\textsuperscript{57} Because they rely on antibody rather than TCR recognition of tumor antigen targets, CAR T cells are not HLA-restricted. This feature provides a substantial advantage over immunotherapeutic strategies dependent on TCR interaction with tumor antigen expressed in the context of HLA because many tumors are known to downregulate HLA as a strategy to avoid immune system attack.\textsuperscript{58} Another advantage of CAR T cells is that they can be engineered to recognize nonprotein surface molecules including tumor-specific carbohydrate and glycolipid targets. Nonetheless, once successfully engineered and infused, CAR T cells face several challenges including (1) adequately expanding and persisting, (2) maintenance of durable antitumor reactivity, (3) the ability to overcome immunosuppressive factors in the tumor microenvironment, and (4) the development of tumor resistance by antigen escape.

Proof of concept for CAR T cells has been demonstrated by the achievement of remarkable rates of durable remission among heavily pretreated patients with refractory B-lineage acute lymphoblastic leukemia following administration of CD19 or CD20 targeting CAR T cells.\textsuperscript{59,60} In these studies, unanticipated adverse events were noted including the development of cytokine release syndrome (CRS) characterized by fever, hypotension, and malaise with possible progression to life-threatening vasodilatory shock.\textsuperscript{60} CRS occurs in the context of high level expression of inflammatory markers and cytokines, while the use of tocilizumab, an interleukin-6 receptor antibody, has been shown to improve CRS outcome.\textsuperscript{61} In addition, CD19 targeting CAR T cell therapy has also been associated with a wide array of neurologic complications including encephalopathy, delirium, speech difficulties, and seizures.\textsuperscript{62} The mechanism leading to such neurologic complications is unclear and they are typically self-limited.

Several CARS are in clinical development for glioblastoma (\textit{\textsuperscript{- Table 2\textsuperscript{}}} including those targeting tumor antigens such as IL-13Rα2,\textsuperscript{63} EGFRVIII,\textsuperscript{64–67} cytomegalovirus antigens, and HER2.\textsuperscript{68} Although limited therapeutic benefit was noted, a recent report of 10 recurrent glioblastoma patients who received a single intravenous infusion of EGFRVIII targeting CAR T cells revealed evidence that these cells were able to traffic into intracranial regions of active glioblastoma.\textsuperscript{69} Validation of the robust antitumor killing capability of CAR T cells for glioblastoma patients was recently reported in a heavily pretreated, recurrent glioblastoma patient with multifocal disease.\textsuperscript{70} Initially, administration of IL-13Rα2 targeting CAR T cells into the tumor resection cavity via Rickham catheter led to a mixed response including regression of some lesions but progression of others and the development of new lesions. Thereafter, the patient received 15 intraventricular CAR T cell infusions which led to regression of all sites of intracranial and spinal tumor which was maintained for 7.5 months prior to the development of resistant tumor. This case report suggests that local delivery into the CNS may be an important consideration for these therapies among high-grade glioma patients. In addition, although it is not clear why this patient ultimately relapsed, antigen escape via acquired mutations and alternative splicing has been reported at the time of relapse among B cell leukemia patients following CD19 CAR T cell therapy.\textsuperscript{71} One strategy currently under development to mitigate antigen escape as a mechanism of resistance is the development of tandem CAR T cells designed to target multiple tumor antigens.\textsuperscript{72}

### Immune Checkpoint Therapies

The immune system normally modulates its physiologic response to foreign antigens via expression of enhancing (stimulatory) and inhibitory immune checkpoint molecules with the latter physiologically functioning to attenuate immune responses and thereby mitigate damage to normal tissues. Thus, blockade of inhibitory immune checkpoint molecules may result in augmentation of immune responses. Two highly studied inhibitory immune checkpoints for oncology are cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) which function in a nonredundant manner to negatively regulate immune responses. CTLA-4 is rapidly upregulated following activation of the TCR and attenuates early activation of naïve and memory T cells primarily at the level of regional lymph nodes by outcompeting CD28 for

<table>
<thead>
<tr>
<th>Study</th>
<th>Agent</th>
<th>Phase</th>
<th>Key eligibility</th>
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<th>ORR</th>
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Abbreviations: NR, not reported; ORR, overall response rate; OS, overall survival; OS-12, overall survival rate at 12 months; PD-1, programmed death-1; PD-L1, programmed death ligand 1; PFS-6, progression-free survival rate at 6 months.
binding to B7 costimulatory molecules expressed by antigen presenting cells.\textsuperscript{73} In contrast, PD-1 attenuates T cell activity primarily in peripheral tissues via interaction with its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC).\textsuperscript{74} The PD-1/PD-L1 signaling pathway has been implicated as a mechanism of immune evasion based on increased PD-L1 expression noted by a wide array of malignancies\textsuperscript{75} including glioblastoma,\textsuperscript{76,77} and the upregulation of PD-1 expression by TILs.\textsuperscript{78} Blocking antibodies to inhibit CTLA-4, PD-1, and PD-L1 have demonstrated striking benefit across a wide spectrum of cancer indications including patients with large, disseminated tumor burden.\textsuperscript{79,80} In some cancers, responses have been remarkably durable such as metastatic melanoma, a previously incurable cancer, where 22\% of patients remain alive 10 years following CTLA-4 blockade therapy.\textsuperscript{81} Nonetheless with the exception of recurrent Hodgkin’s disease,\textsuperscript{82} response to single agent immune checkpoint inhibitor therapy is limited to a minority of patients, while some cancers fail to respond at all.\textsuperscript{83,84} Thus, currently a highly active area of translational research is the identification of immunocorrelative biomarkers to define patients most likely to respond or, conversely, to fail to respond to immune checkpoint blockade therapy. Biomarkers associated with a heightened likelihood of therapeutic benefit include tumor PD-L1 expression,\textsuperscript{85,86} increased tumor mutation or neoantigen load,\textsuperscript{87,88} and presence of a “hot” tumor microenvironment characterized by significant immune cell infiltrate.\textsuperscript{85}

Preclinical studies utilizing immunocompetent, orthotopic, syngeneic glioblastoma murine models have demonstrated significant benefit associated with single agent immune checkpoint blockade that is enhanced with combinatorial therapy.\textsuperscript{89–93} Of note, these studies have been criticized due to the fact that most of the immunocompetent glioblastoma cell lines utilized are highly immunogenic and thus may not recapitulate typical human glioblastoma tumors.

Results of clinical trials evaluating inhibitory immune checkpoint molecules among recurrent glioblastoma patients have reported preliminary results (\textsuperscript–Table 3).\textsuperscript{94–96} In these studies, evidence of efficacy was limited to a minority of patients but encouraging durability was observed in some patients. In general, toxicity was manageable despite relevant initial reservation\textsuperscript{97} and comparable to that observed with these agents for other cancer indications, although robust inflammatory reactions within the intracranial tumor bed

### Table 3 Vaccine trials for glioblastoma

<table>
<thead>
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<th>Phase</th>
<th>Indication</th>
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Abbreviations: APVAC, actively personalized vaccine; DC, dendritic cell; EGFRvIII, variant III of the epidermal growth factor receptor; GAPVAC, Glioma Actively Personalized Vaccine Consortium; GM-CSF, granulocyte-macrophage colony-stimulating factor; HSPPC-96, heat-shock protein peptide complex 96; IDH, isocitrate dehydrogenase; KLH, keyhole limpet hemocyanin; ND, newly diagnosed; PBMC, peripheral blood mononuclear cells; Rec, recurrent.
have been observed. Such radiographic changes have proven challenging for clinicians trying to distinguish true progression of underlying tumor from pseudoprogressive inflammatory reactions and led to the development of response assessment guidelines for neuro-oncology patients undergoing immunotherapy.98

In addition, a large randomized phase III study (CheckMate 143) evaluating nivolumab, a fully human IgG4 anti-PD-1 antibody versus bevacizumab for glioblastoma patients at first recurrence, was recently reported.99 This study confirmed an acceptable toxicity profile associated with anti-PD-1 therapy but nivolumab failed to demonstrate a survival advantage. Median progression free survival favored the bevacizumab arm (3.5 vs 1.5 months; p < 0.0001), while median overall survival was approximately 10 months for both treatment arms. Despite these overall disappointing results, patients who achieved a response with nivolumab had longer therapeutic benefit. Specifically, the median duration of radiographic response was 11.1 months among nivolumab responders compared with only 5.3 months for those on bevacizumab. This result should be interpreted cautiously due to the small number of nivolumab responding patients (8%). Of note, two recent case reports also highlight dramatic response to anti-PD-1 therapy among recurrent glioblastoma patients with hypermutated tumors associated with germline DNA repair defects.100,101

Importantly, two large randomized clinical trials are currently ongoing to evaluate the therapeutic benefit associated with anti-PD-1 therapy for newly diagnosed glioblastoma patients stratified by MGMT promoter methylation status (NCT02617589 and NCT02667587).

**Combination Strategies**

Excitement regarding the growing number of cancers deriving benefit from immunotherapy reagents, particularly checkpoint targeting agents, has been tempered by the realization that only a minority of patients respond. On the one hand, the fact that some patients benefit validates the potential of immune responses to impact cancer. On the other hand, the fact that only a minority respond indicates that the biology is complex and variable between patients. Mechanisms associated with response and lack of response are beginning to emerge but further research is critically needed. Better understanding of factors capable of impeding effective antitumor immune responses may lead to the identification of rationally designed combinatorial regimens capable of overcoming these factors.

High-grade gliomas including glioblastoma are relatively “cold” tumors immunologically and are therefore expected to respond poorly to immunotherapy. A recent analysis of 171 glioblastomas revealed that 54% demonstrated a complete absence of TILs, while TILs could be identified in less than 50% of the tumor in 35%.102 As described previously, several factors contribute to a “cold” glioblastoma microenvironment including (1) a prominence of immunosuppressive microglia, macrophages, Tregs, and MDSC;103 (2) high-level expression of myriad immunosuppressive molecules and cytokines;104-110 and (3) hostile conditions that are toxic to T cells in the microenvironment including hypoxia,111 nutrient deprivation,112 and acidosis.113 The net effect of these factors is the generation of a “perfect storm” capable of impairing entry, proliferation, and activation of immune effector cells in the glioblastoma microenvironment.

Furthermore, administration of corticosteroids for the treatment of tumor-associated cerebral edema likely exerts a significant iatrogenic detrimental effect both quantitatively and qualitatively on TILs in the glioblastoma microenvironment.114 Careful and conscientious administration of systemic corticosteroids must be considered by clinicians particularly for brain tumor patients undergoing immunotherapy treatment, and the historical practice of routinely prescribing corticosteroids prophylactically for brain cancer patients is no longer felt to be appropriate.115

A major focus of combinatorial therapy therefore evaluates regimens that may transform a “cold” tumor microenvironment to a “hot” one by inducing increased TIL penetration into the tumor. Radiation therapy,116 oncolytic virus administration,117 and possibly chemotherapy may induce immunogenic cell death,118 leading to an increase in TILs. Tumor vaccine strategies119,120 as well as administration of CTLA-4 blockade117,121 are additional potential strategies to increase TILs in the tumor microenvironment.

Another area of focus includes targeting immunosuppressive factors in the tumor microenvironment in combination with immunotherapeutics. Several clinical trials are now underway for glioblastoma patients evaluating immune checkpoint inhibitors combined with inhibitors targeting immunosuppressive factors including inhibitors of TGF-β (NCT02423343), vascular endothelial growth factor (NCT02336165; NCT02337491), and CSF-1 (NCT02526017).

Overcoming the hostile tumor microenvironment typically associated with high-grade gliomas is challenging, but ongoing clinical trials are evaluating low dose bevacizumab schedules as a strategy to normalize tumor vasculature that decrease hypoxia, acidosis, and nutrient depletion (NCT02336165). Preclinical studies demonstrate that low-dose antiangiogenic therapy can successfully convert an immunosuppressive tumor microenvironment to a more immunosupportive one.122,123

**Conclusion**

Immunotherapy has emerged as a bona fide modality to effectively contribute to the treatment of multiple cancers, although its role for glial tumors remains undefined. Although nuances exist regarding immune responses in the CNS, effective and dynamic immune responses are capable of being achieved in the brain and should not dampen enthusiasm for immunotherapy approaches for neuro-oncology. High-grade gliomas remain a major challenge in modern oncology. They are relatively “cold” tumors from an immunologic perspective and exploit multiple mechanisms of immunosuppression to evade antitumor immune responses. Furthermore, systemic corticosteroids used routinely to treat tumor-associated cerebral edema may antagonize immunotherapy responses.
Extensive preclinical and clinical research is underway seeking to optimize immunotherapeutic approaches including vaccines, adoptive T cell therapies, and immune checkpoint molecules for brain tumor patients. Rationally designed combinatorial regimens aimed at enhancing tumor antigen immunogenicity and overcoming mediators of tumor immunosuppression will likely be required to achieve optimal benefit using immunotherapy for brain cancer patients.

Conflict of Interest
David A. Reardon: Advisory board—Abbvie, Amgen, BMS, Cavion, Celldex, EMD Serono, Genentech/Roche, Inovio, Juno Pharmaceuticals, Merck, Midatech, Momenta Pharmaceuticals, Novartis, Novocure, Oxigene, Regeneron, Stemline Therapeutics; Research Support—Celldex Therapeutics, Incyte, Midatech; Speaker—Genentech/Roche, Merck.

Michael Platten: Patents: IDH1 vaccine, Tryptophan metabolites; Advisory board—Genentech/Roche, Merck, Bayer, Novartis; Research Support—Bayer, Pfizer; Speaker—Bayer, Merck, Medac, Novartis, Teva, Genentech/Roche.

Acknowledgments
None.

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