Immune Escape in Glioblastoma Multiforme and the Adaptation of Immunotherapies for Treatment

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Glioblastoma multiforme (GBM) is the most frequently occurring primary brain tumor and has a very poor prognosis, with only around 5% of patients surviving for a period of 5 years or more after diagnosis. Despite aggressive multimodal therapy, consisting mostly of a combination of surgery, radiotherapy, and temozolomide chemotherapy, tumors nearly always recur close to the site of resection. For the past 15 years, very little progress has been made with regards to improving patient survival. Although immunotherapy represents an attractive therapy modality due to the promising pre-clinical results observed, many of these potential immunotherapeutic approaches fail during clinical trials, and to date no immunotherapeutic treatments for GBM have been approved. As for many other difficult to treat cancers, GBM combines a lack of immunogenicity with few mutations and a highly immunosuppressive tumor microenvironment (TME). Unfortunately, both tumor and immune cells have been shown to contribute towards this immunosuppressive phenotype. In addition, current therapeutics also exacerbate this immunosuppression which might explain the failure of immunotherapy-based clinical trials in the GBM setting. Understanding how these mechanisms interact with one another, as well as how one can increase the anti-tumor immune response by addressing local immunosuppression will lead to better clinical results for immune-based therapeutics. Improving therapeutic delivery across the blood brain barrier also presents a challenge for immunotherapy and future therapies will need to consider this. This review highlights the immunosuppressive mechanisms employed by GBM cancers and examines potential immunotherapeutic treatments that can overcome these significant immunosuppressive hurdles.

Keywords: GBM - Glioblastoma multiforme, immune escape, immunotherapy, combinatorial therapy, treatment, overview
INTRODUCTION

Glioblastoma multiforme (GBM, WHO grade 4) is the most frequently occurring primary brain tumor. Although primarily a disease associated with old age, it can also occur in children. The prognosis for GBM patients is poor and the disease is almost uniformly fatal with only around 5% of patients surviving for a period of 5 years after diagnosis (1). The current course of therapy for GBM patients is surgical resection of the tumor (where possible) followed by concomitant radiotherapy and temozolomide chemotherapy, followed by adjuvant temozolomide. Despite aggressive multimodal therapy, GBM tumors nearly always recur, the majority close to the site of resection (2–4). This recurrence is most likely, and most often, due to the infiltrative nature of GBM making complete resection of tumor cells incredibly difficult. Although progress to improve the surgical removal of tumor cells has been made, such as the use of 5-aminolevulinic acid (5-ALA) which is approved for the surgical removal of tumor cells has been made, such as the use of 5-aminolevulinic acid (5-ALA) which is approved for intraoperative imaging of GBM cells increasing their removal, it is not possible to visualize all individual cancer cells that have migrated further into healthy areas of the brain (5). GBM tumors are histopathologically characterized by an abundance of poorly differentiated and pleomorphic astrocytes with nuclear atypia and high mitotic activity. GBM tumors are highly vascular and necrosis is often evident within these tumors (6). Metastasis is rarely seen in GBM tumors; however, they are highly invasive, and these tumors employ a plethora of mechanisms to avoid immune detection.

THE BRAIN AS A UNIQUE IMMUNE ENVIRONMENT

In order to understand the complexity of the brain’s interaction with the immune system, the presence of the blood-brain barrier (BBB) needs to be considered and understood. The endothelial cells of the brain vasculature are connected by tight junctions that control the permeability of the endothelium. Although these tight junctions under normal physiological conditions are highly regulated, under inflammatory conditions (such as those in GBM) these junctions are not as tightly connected making the endothelium ‘leaky’ (7). The BBB, a multi-component structure found in the wall of cerebral blood vessels, selectively restricts passage of cells and molecules into the brain from the circulation. The major, but not sole, players in this defense are the endothelial cells, and the FasL expressed on these cells is linked to a reduced T cell infiltration, most likely due to the FasL-induced death of T cells (18). There are very few immune cells normally present within the brain, however the microglia can act as antigen presenting cells (APCs). The brain traditionally has low major histocompatibility complex (MHC) class I and class II expression meaning that antigen expression is reduced when compared to other tissues (19). It is important to note, however, that GBM cells themselves have been shown to express MHC class I and II molecules meaning that these cells present antigens to antigen specific CD8+ and CD4+ T cells (20).

Not only does the unique physiology of the brain create an unusual immune environment but it is important to note that the tumors themselves create their own microenvironment. Tumor cells can co-opt stromal cells in order to support their growth and survival (21). The brain extracellular matrix is comprised of...
proteoglycans, glycoproteins and glycosaminoglycans. In the GBM setting, significant increases in heparan sulphate proteoglycans (HSPG) have been seen in the tumor microenvironment (TME) (22). The increase of HSPGs in the TME leads to greater retention of growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), thereby supporting tumor nutrition and growth. The increased local concentration of VEGF within the GBM TME results in upregulation of periostin and tenascin C within blood vessels trapping T cells and preventing tumor penetrance (23).

In the case of GBM, as with many cancers T cells are frequently exhausted and dysfunctional and therefore are inadequate at exerting an anti-tumor immune response. Persistent stimulation of T cells by tumor cells results in T cell senescence as indicated by the presence of CD57 on the surface of T cells (24). CD57 positive T cells can secrete cytokines when stimulated by their cognate peptides however they do not proliferate when stimulated (25). Tumor resident senescent T cells have also been shown to down regulate the co-stimulatory molecules CD27 and CD28 contributing to immune dysfunction, causing changes in APC phenotype such as a down regulation of CD80 and CD86 reducing their ability to stimulate T cells further exacerbating the local immune dysfunction (26). When compared to healthy donors GBM patients have a lower number of circulating CD3+ T cells in their peripheral blood mononuclear cells (PBMCs) further indicating a disease related immune dysfunction (27). Glioblastoma multiforme is more frequent in the older population with most cases occurring between the ages of 55 and 60 (28). Increased age is linked to T cell dysfunction; with elderly patients having a higher number of senescent T cells and thymic shrinkage being apparent (24, 29). The chronic stimulation of T cells by tumor cells also leads to the exhaustion of these cells, rendering them ineffective at tumor control. This exhaustion leads to an upregulation of immune checkpoint markers such as PD-1, LAG-3, TIGIT, and CD39 on GBM infiltrating CD8+ T cells (30). TILs isolated from murine GBM tumors show impaired cytokine production compared to peripheral T cells, with reduced levels of interferon gamma, tumor necrosis factor alpha and interleukin 2 being detected via flow cytometry when cells are stimulated in vitro (30). Transformed tumor cells also compete with other cells within the TME for glucose, GBM cells have an increased rate of glucose uptake when compared to non-transformed cells. T cells within the TME require glucose in order to perform effector functions and therefore the depletion of glucose by tumor cells results in impaired T cell function and exhaustion (31).

## STANDARD OF CARE AND IMMUNOSUPPRESSION

The current standard of care for GBM is maximal surgical resection (where possible) followed by concomitant radiotherapy and temozolomide chemotherapy (32). Patients are also given anti-inflammatory steroids such as dexamethasone to help control peritumoral edema (33). The US Food and Drug Administration (FDA) has also approved the use of tumor treating fields (TTFs) to treat GBMs. This involves using alternating electric fields administered via scalp electrodes to disrupt GBM tumor cell division (34).

Dexamethasone has been shown to lead to the upregulation of the immunosuppressive checkpoint cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) on the surface of T cells, thereby reducing their anti-tumor activity. Dexamethasone has also been shown to lead to a reduction of T cell proliferation (35). Dexamethasone has also been shown to dampen patients’ immune responses to immune checkpoint blockade (36).

As previously mentioned, the standard of care involves the use of the chemotherapeutic drug temozolomide (TMZ), which is known to influence the immune system. High dose temozolomide induces lymphopenia, an issue that is exacerbated when TMZ is combined with radiotherapy (37). TMZ has also been shown to result in T and B cell dysfunction in a murine model of GBM (38).

In the GBM setting, radiotherapy can be administered in a variety of ways such as whole brain radiotherapy, stereotactic radiosurgery, image guided radiotherapy and hypofractionated radiotherapy (39). Radiotherapy is known to have a number immune modulating effects (40–42), importantly brain tumor exposure to radiotherapy has been shown to upregulate MHC class I expression by brain tumors, and this improves the antigen presentation capability of these cells. Radiotherapy also increases the repertoire of peptides presented by tumor cells and the phenomenon of antigen spreading can occur – i.e. tumor cells die, and their antigens are taken up by nearby immune cells (43). Research has shown that radiotherapy is less efficient in mice lacking T cells, thereby highlighting the additive effect that radiotherapy has in immune cell-mediated control of cancer (44). Radiotherapy is often thought of as an in-situ vaccination that makes tumors susceptible to immune attack (44–46). Although a large amount of evidence points towards radiotherapy stimulating an anti-tumor immune response, radiotherapy can also unfortunately result in the secretion of immunosuppressive cytokines such as IL-6 and IL-10 from treated tumor cells (47, 48).

Combined TMZ, radiotherapy and dexamethasone therapy in GBM patients has been shown to induce a persistent lowering of CD4+ cell counts which is associated with increased rates of infection and poorer survival (49).

## IMMUNE INHIBITORY PROTEINS EXPRESSED BY GBM TUMORS

GBM cells secrete many immunosuppressive proteins and express many cell surface and cytoplasmic immune inhibitory proteins (as summarized in Figure 1). Intracellular adhesion molecule 1 (ICAM-1), a key regulator of cell-cell interactions, is commonly upregulated within GBM tumors, when compared to immunohistochemically stained normal brain (50). ICAM-1 interacts with lymphocyte function-associated antigen 1 (LFA-1) expressed on myeloid cells to promote migration of these cells into tumors, thereby enhancing intratumoral immune...
suppression (51). Myeloid derived suppressor cell (MDSC) accumulation in GBM tumors further contributes to local immune suppression (52). The presence of MDSCs circulating in the blood of GBM patients is also elevated when compared to non-diseased individuals (53). These MDSCs express many immunosuppressive molecules that suppress anti-tumor T cells such as TGF-β and arginase (52). GBM cells have been shown to overexpress galectin-1 (Gal-1), another protein important for the maintenance of cell-cell interactions. Expression of Gal-1 by GBM cells promotes the proliferation and migration of tumor cells (54, 55). Gal-1 expressing GBM cells have also been shown to induce T cell death when the two types of cells are co-cultured (55). Gal-1 interacts with CD45 and CD43 on T cells resulting in their clustering. Gal-1 also binds to CD7 on the T cells and these interactions result in T cell death (56–58).

GBM cells have also been shown to express non-classical MHC class I molecules on their surface which enables them to evade immune cell mediated killing. HLA-G is one such non-classical MHC class I molecule that is involved in immunogenic tolerance of trophoblasts and prevents immune response to the developing semi-allogeneic fetus. In the adult, HLA-G is expressed in thymic epithelial cells, nail matrix and cornea (59). Although HLA-G expression is tightly controlled in the human body, it appears that GBM cells can express HLA-G (59). HLA-G is not just expressed on the cell surface - a soluble isoform that is secreted has been detected in plasma, cerebrospinal fluid and seminal plasma. GBM tumors are frequently infected with cytomegalovirus (hCMV), and hCMV infection has been associated with high levels of HLA-G expression (60). Cytomegalovirus infection is prevalent in the population and infection is lifelong. The immunosuppression linked with GBM results in reduced control of hCMV and this results in reactivation of the virus (60). HLA-G can bind to several receptors, namely the inhibitory receptors Ig-Like Transcript 2 (ILT2) and Ig-Like Transcript 4 (ILT4) (61). HLA-G can also bind the non-inhibitory receptors CD8, CD160, and KIR2DL4. Binding of soluble HLA-G to CD8 on T cells induces a signaling cascade that results in Fas-FasL mediated apoptosis of CD8+ T cells (61). HLA-G binding to ILT2 on natural killer (NK) cells inhibits the polarization of lytic granules and the microtubule-organizing center at the contact zone, ultimately preventing NK cell-mediated lysis (61). HLA-E is another non-classical MHC class I molecule; it is a ligand for both NKG2A and NKG2C expressed on NK cells, CD8+ and γδ T cells. Binding of HLA-E to NKG2C can lead to immune cell activation, and its binding to NKG2A leads to immune cell inhibition. HLA-E, much like HLA-G, is believed to play a role in maternal tolerance of the fetus (62). HLA-E has been shown to be expressed on GBM cells and this HLA-E expression has been shown to prevent NK cell mediated lysis of these tumor cells. Blockade of the NKG2A – HLA-E interaction has been shown to improve NK cell mediated killing of GBM tumor cells (62).

GBM tumors have also been shown to express Fas ligand (CD95L) on their surface, the binding of which to Fas (CD95/CD95L)
APO-1) on T cells leads to apoptosis of the T cells, thereby enabling GBM cells to evade lysis by Fas-expressing T cells (63). GBMs can also induce T cell death via their expression of CD70. CD70 on GBM cells binds to CD27 on T cells inducing death of activated T cells, and blockade of this interaction has been shown to partially protect T cells from GBM cell induced death (64). GBMs have also been shown to express the immune dampening checkpoint ligand programmed death ligand 1 (PD-L1). PD-L1 binds to its cognate receptor programmed death 1 (PD-1) expressed on activated T cells, and this leads to inhibition of T cell responses to PD-L1 expressing GBM cells. It has been reported that as many as 88% of patient GBM samples express PD-L1 (65). This high level of PD-L1 expression has been shown to be linked with poorer patient survival (66).

Herpes virus entry mediator (HVEM) is an example of another immune checkpoint molecule that has been proven to be expressed in the GBM microenvironment (67). HVEM is usually expressed on T cells, it can have both co-stimulatory and inhibitory effects, depending upon its binding partner (67). HVEM exerts an immune inhibitory effect when bound to B and T lymphocyte attenuator (BTLA) or CD160 expressed by other immune cells (67). High expression of HVEM in GBM tumors has been linked to regulatory T cell differentiation, negatively associated with the regulation of T cell mediated cytotoxicity and with a decreased survival time (67).

Indoleamine 2,3-dioxygenase (IDO) is another protein involved in immunoregulation and prevention of fetal rejection. IDO catalyzes tryptophan into immune-regulatory kynurenines. IDO expression can be induced by a variety of receptors such as the toll like receptors (TLRs), tumor necrosis factor receptor superfamily members (TNFRs), interferon gamma receptors (IFNGRs), transforming growth factor beta receptors (TGFBRs) and aryl hydrocarbon receptors (AhRs) (68). The depletion of tryptophan by IDO activity inhibits immune cell function and prevents dendritic cell (DC) maturation (68). IDO expression is upregulated in recurrent GBMs, with 100% of patients being studied expressing IDO at the time of the second surgery (69). The expression of IDO within GBM tumors is associated with an increased infiltration of CD4+ regulatory T cells, immune escape and a poorer prognosis (70). Increased kynurenine production driven by IDO activity induces the differentiation of naive CD4+ T cells into immunosuppressive regulatory CD4+ T cells triggered by the binding of kynurenine to the aryl hydrocarbon receptor (AHR) on naive CD4+ T cells (71). Tryptophan 2,3-dioxygenase (TDO), another enzyme involved in the degradation of tryptophan into kynurenine, can also contribute to an immunosuppressive microenvironment high in kynurenine. TDO is expressed in brain tumors and represents a druggable target for reversing the immunosuppressive microenvironment (72).

GBM tumors also secrete numerous other immunosuppressive factors that shape the immune TME and enable immune evasion. GBM tumors secrete IL-6 (73, 74) and their expression of the IL-6 receptor is upregulated (75). IL-6 mediates signaling via the transcription factor STAT3. Upon activation, STAT3 is phosphorylated and persistent phosphorylation is linked with brain tumor grade; with GBM showing the highest levels of STAT3 phosphorylation. Knockdown of STAT3 in GBM cell lines slows in vitro and in vivo tumor cell growth (76). Human GBM cells isolated from tumors were shown to secrete the chemokine CCL22 (77) which attracts regulatory CD4+ CD25+ FoxP3+ T cells to the TME (78). GBM tumor cells also secrete the immunosuppressive cytokine TGF-β which reduces ICAM-1 and VCAM-1 expression on GBM endothelial cells and thereby T cell infiltration (79, 80). The active form of TGF-β secreted by GBM cells increases the activity of MMP2 and MMP9 on the surface of GBM cells which in turn increases cell motility and promotes the invasion of GBM cells into the surrounding brain (81). GBM tumor cells also secrete the anti-inflammatory cytokine IL-10 which, in the normal setting prevents excessive inflammation and reduces tissue damage by suppressing the activity of Th1 and CD8+ T cells. Immune cells such as regulatory T cells secrete IL-10 to quell the immune response (82). IL-10 mRNA is highly expressed in GBM tissues (83). More concerning is that IL-10 not only suppresses the immune system, but also increases the proliferation and migration of GBM cells. Intratumoral microglia/macrophages are major contributors to the IL-10 production within GBM tumors (84).

Human cytomegalovirus (hCMV) is a herpes virus that has been shown to persistently infect 50% to 90% of the adult population. Analysis of GBM tumors has revealed that a large proportion of tumors express hCMV proteins indicating the presence of hCMV within these tumors (85, 86). Human cytomegalovirus secretes a homolog of IL-10, known as cmvIL-10 which has the same immunoinhibitory properties as human IL-10 (87). The attenuation of the immune response by cmvIL-10 prevents eradication of the tumor as well as the virus itself. The secretion of cmvIL-10 leads to the differentiation of CD14+ monocytes to macrophages, thereby further supporting hCMV infection (88). In vitro studies have revealed that cmvIL-10 affects the maturation and life span of DCs, in that although monocyctic DCs exposed to cmvIL-10 reach maturation, their cytokine production is impaired in a non-reversible manner (88). The presence of IL-10 and TGF-β in GBM tumors is believed to downregulate the expression of MHC class I in the TME (89). GBM cells express macrophage migration inhibitory factor (MIF) which renders GBM cells resistant to NK cell mediated killing (90). VEGF secretion by GBM cells stimulates the growth of new blood vessels supplying oxygen and nutrients to rapidly dividing and often hypoxic tumor cells (91, 92). As well as increasing tumor vasculature, VEGF also upregulates expression of the macromolecules tenascin C (TNC) and peristin. TNC blocks the migration of T cells across the blood tumor barrier thereby preventing them from penetrating the tumor parenchyma (23). Peristin also recruits circulating immunoinhibitory M2 macrophages into the tumor parenchyma (93). GBM stem cells secrete the macrophage attracting cytokine peristin. These macrophages support tumor growth and result in a poorer prognosis (93). GBM cells exposed to radiotherapy and chemotherapy have been shown to display increased immunosuppression. This phenomenon has been shown to be due to increased prostaglandin E2 secretion by cells. Blockade of this secreted PGE2 reverses the immunosuppressive capacity of treated cells (47). Colony stimulating factor 1 (CSF-1) is a growth
factor that has been shown to be expressed in GBM tumors and by GBM cell lines (94). CSF-1 can either be secreted by cells or expressed as a transmembrane variant on the cell surface. CSF-1 is secreted by astrocytes within the brain during acute inflammatory responses. CSF-1 can bind to its receptor (CSF-1R) on the surface of macrophages and microglia within the brain promoting their switch to the immunosuppressive M2 phenotype (94, 95). GBM cells also secrete interleukin-1α and -1β (IL-1α and β) (96). The down regulation of HLA class II expression on the U-105 MG GBM cell line by IL-1β suggests that this could be another mechanism which reduces immune recognition by CD4+ T cells (97).

THE CONTRIBUTION OF IMMUNE CELLS WITHIN GBM TUMORS TO THE IMMUNE INHIBITORY PHENOTYPE

Whilst GBM tumor cells contribute to immunosuppression, the immune cells recruited to the tumor can also exacerbate the immune evasive properties of these tumors. Although immune cells can contribute to tumor control, immunosuppressive populations can also contribute to the immune escape of GBM tumors. Indeed, many of the anti-tumor immune cells recruited to the TME adopt an immunosuppressive phenotype due to the cytokines secreted by the GBM tumors and the unique microenvironment which these tumors create.

Myeloid-derived suppressor cells (MDSCs) can be found within GBM tumors, and these cells contribute to the immunosuppressive phenotype of GBMs (98). MDSCs can be divided into two main types, monocytic and granulocytic. Granulocytic MDSCs are rarely found in GBM tumors, whereas the monocytic subtype are more prevalent (99). Monocytic MDSCs support tumor growth by increasing the recruitment of CD4+ regulatory T cells via chemokine release in the TME (100). CD4+ regulatory T cells are well known immunosuppressive immune cells that dampen the immune response. When compared to healthy controls, the prevalence of regulatory T cells in the peripheral blood is higher in GBM patients. Of even more relevance is that the prevalence of regulatory T cells in lymphocyte populations infiltrating GBM tumors is significantly greater than that in lymphocyte populations from ‘normal’ brain tissue obtained from seizure patients (101, 102). Although immune cell infiltration is often viewed as a positive prognostic marker, it can also contribute to the pathology of GBM. Lymphocytes entering the tumor have been shown to downregulate costimulatory molecules such as CD28 and CD62L (103). The presence of immunosuppressive regulatory T cells within GBM tumors has been correlated with shorter recurrence-free survival. GBM associated microglia/macrophages, which constitute up to 30% of the GBM tumor bulk are of the immunosuppressive M2 phenotype (103, 104). The expression of PD-L1 by these immunosuppressive M2 cells further contributes to local immunosuppression, as does their secretion of CCL22 which recruits regulatory T cells and MDSCs into the TME (103, 104).

OVERCOMING GBM-DRIVEN IMMUNOSUPPRESSION

Active Immunotherapy via Vaccination

Vaccination presents an attractive method for immunotherapeutically targeting GBMs (ongoing trials are detailed in Tables 1–3). One issue that can arise with peptide vaccinations is that immune escape variants can develop, and tumors can overcome the immune pressure applied to them. This phenomenon has been seen in the case of Rindopepimut, an EGFRvIII-keyhole limpet hemocyanin peptide conjugate. When Rindopepimut was used to treat GBM patients with EGFRvIII positive tumors, their median overall survival was 26 months compared to the 15 months of matched controls. Although vaccination prolonged the overall survival of patients, tumors recurred in a large proportion of these patients. When the recurrent tumors were analyzed immunohistochemically for EGFRvIII expression, 82% of the tumors examined had lost expression of EGFRvIII and the other 18% only displayed EGFRvIII expression in less than 1% of their tumor cells (114). These data suggest that the targeting of a single antigen can lead to the generation of immune escape variants, as a consequence of which multiple antigens need to be employed in the formulation of such vaccines.

IMA950 is one such multi-peptide vaccine that is being investigated in GBM. IMA950 is made up of 9 CD8 specific peptides derived from BCAN, CSPG4, FABP7, IGF2BP3, NRCAM, NLGN4X, PTPRZ1, and TNC as well as two CD4 specific peptides derived from survivin and c-met (150). This multi-peptide vaccine was given in conjunction with the immune boosting adjuvant poly-ICLC to GBM patients in a phase I/II clinical trial (111). This vaccination was well tolerated by patients and induced antigen specific CD8+ and CD4+ T cell responses (111). The level of response seen in patients varied, and analysis of five tumor samples revealed that no vaccine-specific T cells were present in the TIL population, meaning that there may be issues with the homing of vaccine-induced T cells (111). When samples from the recurrent tumors were tested for expression of the target antigens, no change in the levels of these antigens compared to the pre-vaccination tumor samples was observed, further suggesting that the issues are with T cells not trafficking to the tumor site (111). The ability of tumor cells to present immunogenic epitopes at their surface may also explain the failure of peptide vaccine treatments. Whilst tumors may express the target antigen, they may not present the target epitope on the surface meaning that vaccine generated T cells will not target these tumors. Although vaccination with these peptides generates antigen-specific T cells, there appears to be an issue with the immune TME of these tumors. As a result, the combination of IMA950 vaccination with other modalities, such as immune activating anti-CD27 and anti-PD-1 are being explored in the clinic (111).

A ‘personalized’ peptide vaccination approach has also been explored in the GBM setting. In a phase I clinical trial, GBM patients were treated with a cocktail of manufactured peptides...
TABLE 1 | Peptide vaccine trials for glioblastoma.

| Trial name | ClinicalTrial.gov identifier | Phase | Immune targets | Control arm treatments in active arm | Sample size | T cell response(CD4/CD8 response details) | Humoral response | Median PFS(months) | Median OS(months) | Primary endpoint | Results |
|------------|------------------------------|-------|---------------|-------------------------------------|-------------|---------------------------------------|---------------|-------------------|------------------|-----------------|---------|
| IMA-950    | NCT02122221                  | I     | BCAN, CSPG4, FABP7, IGF2BP3, NLGN4X, NRCAM, PTPR21, TNC, MET, BIRC5, HbAcAg | None       | 40 | Yes (Up to 1.1% specific CD8) | NA            | NA (PFS6 = 74.4%) | 15.3             | Safety and immunological response | Positive |
| NOA-16     | NCT02454634                  | I     | None          | None                                | 32 | Results pending | Results pending | Results pending | Results pending | Safety | Safe vaccine |
| GAPVAC     | NCT02149225                  | I     | Personalized vaccine | None | 15 | Yes (Up to 0.02% specific CD8) | NA            | 14.2             | 29               | Safety and immunological response | Safe vaccine |
| SuVaxM     | NCT01250470                  | I     | Sunvivin (SVNS3-67/M57-KLH peptide) | None | 9 | Yes (CD8 response in 78% patients; at least 1% specific CD8) | Yes (88% patients) | 17.6             | 86.6             | Safety | Safe vaccine |
| NCT01621542| I     | WT2725        | None          | None                                | 21 | Results pending | Results pending | Results pending | Results pending | Safety and immunological response | Safe vaccine |
| UMNN000003506| I                  | I     | Cocktail of WT1 HLA class I and II peptides | None | 14 | Yes (CD8 response in 64% patients; median specific CD8 = 6% of total CD8) | NA            | 4               | 6.2             | (r-GBM) | Safe vaccine |
| PERFORMANCE| NCT02864368                  | I     | CMV peptide   | Temozolomide plus Pembrolizumab TTF | None | 70 | Results pending | Results pending | Results pending | Results pending | Safety and immunological response | Results pending |
| NeoVax     | NCT02287428                  | la/lb/ | Personalized neoantigen vaccine | None | 56 | Results pending | Results pending | Results pending | Results pending | Results pending | Feasibility and safety | Results pending |
| NCT02233103| I     | Personalized mutation-derived tumor antigens | Pembrolizumab | None | 20 | Ongoing | Ongoing | Ongoing | Ongoing | Safety | Ongoing |
| IMA-950    | NCT01920191                  | I     | BCAN, CSPG4, FABP7, IGF2BP3, NLGN4X, NRCA, PTPR21, TNC, MET, BIRC5, HbAcAg | IL-710 | 13 | Results pending (interim results: CD8 response in 63.2% patients) | Results pending | Results pending | Results pending | Safety and immunological response | Results pending |
| NCT02078648| II     | None          | Stage 1: irinotecan; Stage 2: Bevacizumab | None | 74 | Results pending (interim results: response in stage 2 patients) | Results pending | Results pending | Results pending | Safety, ORR, OS12 | Results pending |
| IMA90-106  | NCT02493846                  | I     | BCAN, CSPG4, FABP7, IGF2BP3, NLGN4X, NRCA, PTPR21, TNC, MET, BIRC5, HbAcAg | None | 24 | Ongoing | Ongoing | Ongoing | Ongoing | Safety | Ongoing |
| UCPVax-Glio| NCT02493846                  | II     | Telomerase (TERT) | None | 28 | Ongoing | Ongoing | Ongoing | Ongoing | Immunological response | Ongoing |
| VBI-1901   | NCT02382977                  | I     | CMV (pp65 and gB antigens) | None | 38 | Ongoing | Ongoing | Ongoing | Ongoing | Safety | Ongoing |
| ROSALIE    | NCT04116658                  | II     | TAA and microbiome-derived peptides (EC2401) | Nivolub +/- Bevacizumab Temozolomide | None | 32 | Ongoing (not yet recruiting) | Ongoing (not yet recruiting) | Ongoing (not recruiting) | Safety | Ongoing (not recruiting) |
| ACTIVAtE  | NCT00643097                  | II     | EGFR-viii     | Temozolomide | None | 22 | NA | Yes (33% patients) | 26.0             | PFS and immunological response | Positive |
| ACT II     | NCT00643097                  | II     | EGFR-viii     | Temozolomide | None | 18 | NA | Yes (43% patients) | 14.2             | PFS and OS | Positive |
| ACT III    | NCT00643097                  | II     | EGFR-viii     | Temozolomide | None | 65 | NA | Yes (85% patients) | 9.2              | PFS5.6 | Positive (PFS5.5 = 66%) |
| ReACT      | NCT01492328                  | II     | EGFR-viii     | Bevacizumab KLH and GM-CSF plus Bevacizumab | None | 36 | NA | Pending results | Pending results | PFS6 | Positive (trend) |
| SuVaxM     | NCT02455557                  | II     | Sunvivin: SVNS3-67/M57-KLH peptide | Temozolomide | 60 | Pending results | Pending results | Pending results | Pending results | OS12=93.4% | PFS6 | Positive |

(Continued)
derived from known GBM antigens followed by a vaccination that targeted neoepitopes derived from analysis of the patients’ tumor immunopeptidome and transcriptome (107). Each patient received a vaccine that was tailored to their tumor antigen expression profile. Vaccines were administered with the adjuvants Poly-ICLC and GM-CSF. The cocktail of ‘off the shelf’ peptides known as APVAC1 generated CD8+ T cell responses in twelve out of the thirteen patients studied and CD4+ T cell responses were found in nine of the thirteen patients studied (107). The neoepitope vaccine known as APVAC2 generated a predominantly CD4+ T cell response in eight out of the ten patients evaluated. The overall median overall survival of patients receiving this vaccination regime was 29 months (107). Although these findings are promising, these peptide vaccinations are far from curative. Whilst CD4+ and CD8+ responses were detected, these were at a relatively low level, with the frequency of antigen specific T cells being below 4 percent for CD4+ T cells and 1 percent for CD8+ T cells (107). The low frequency of target specific T cells may explain the failure of this therapy to act in a curative manner. Targeting of multiple antigens helps prevent the development of antigen escape variants, however combinatorial methods that enable vaccine-induced T cells to penetrate tumors and overcome the immunosuppressive microenvironment need to be explored.

Targeting Immune Inhibitory Cells and Cytokines

The contribution of macrophages/microglia to the immunosuppressive TME of GBM and their prevalence within the tumor bulk suggest them to be attractive therapeutic targets for the immunotherapeutic targeting of GBM. As mentioned previously, microglia and macrophages in the TME adopt an immunosuppressive M2 phenotype (103, 104). As also previously mentioned, microglia/macrophages express the CSF-1R and GBM cells secrete CSF-1 resulting in the switching of GBM macrophages/microglia to the immune inhibitory M2 phenotype. The blockade of this CSF-1/CSF-1R interaction presents an attractive approach for preventing the switching of tumor resident macrophages/microglia to the immunoinhibitory M2 phenotype. In this regard, blockade of the CSF-1R with the chemical BLZ945 has been shown to improve survival and reduce tumor development in GBM bearing mice without any visible deleterious side-effects. BLZ945 treatment did not alter macrophage numbers within the implanted tumors but reduced the polarization of these macrophages to the M2 phenotype (95). As a result, combining BLZ945 with active immunotherapy represents an exciting therapeutic option for GBM.

As previously discussed GBM cells are known to overexpress MIF, making them resistant to NK cell mediated killing (90). Not only does MIF protect GBM cells from NK cell mediated killing it also exerts effects on macrophages/microglia within the tumors. MIF has been shown to interact with CD74 on microglia resulting in the adoption of the immunosuppressive M2 phenotype. Disruption of the CD74/MIF pathway prevents this M2 phenotype switch and prolongs the survival of GBM tumor bearing mice (151). Immunotoxins have also been used to target
| Trial name | ClinicalTrial.gov identifier | Phase | Immune targets | Associated treatments in active arm | Control | Sample size | T cell response (CD4/CD8 response details) | Median PFS (months) | Median OS (months) | Primary endpoint | Results |
|------------|-----------------------------|-------|----------------|------------------------------------|---------|-------------|-----------------------------------------------|------------------|------------------|-----------------|---------|
| PERCELLVAC | NCT02709616, NCT02808364  | I     | Personalized TAA | None                               | None    | 5           | Yes (CD4 and CD8 response in 80% patients: up to 3.5% specific CD8) | NA               | NA               | Safety          | Positive |
| ATTAC      | NCT00369639, NCT00615404  | I     | CMV pp65        | None                               | None    | 11          | Yes (up to 4.5% specific CD8 in 55% patients) | NA               | 25.3             | Safety and feasibility | Safe vaccine |
|            |                            | I     | CMV RNA         | Tg + GM-CSF + Di-TMZ               | None    | 10          | Ongoing                                      | Ongoing          | Ongoing          | Safety and feasibility | Ongoing |
| Rudnick    | NCT00639639, NCT03615404  | I     | Autologous glioma lysate | None                             | None    | 34          | NA                                           | NA               | 34.4             | Safety and feasibility | Safe vaccine |
| Rudnick    | NCT00612001                | I     | Autologous glioma lysate | None                             | None    | 12          | Yes (CTL response in 50% patients)            | NA               | 15.5             | Safety and feasibility | Safe vaccine |
| Rudnick    | NCT00068510                | I     | Autologous glioma lysate | None                             | None    | 28          | Yes (CD8 response in 25% patients, no details in %specific CD8) | NA               | 32.1             | Safety and clinical outcome | Positive |
| MC1272     | NCT01957956                | I     | Autologous glioma lysate | Temozolomide                      | None    | 20          | Results pending                              | Results pending  | Results pending | Safety and feasibility | Safe vaccine |
| ICT-107    | NCT01171469                | I     | AIM-2, MAGE1, TRP-2, gp100, HER2, IL-13Ra2 | None                               | None    | 16          | Yes (specific CD6 increase in 31% patients)   | NA               | 16.9             | Immunological response | Positive (trend) |
| ICT-121    | NCT00809022                | I     | BTSC mRNA       | None                               | None    | 50          | Ongoing                                      | Ongoing          | Ongoing          | Safety, Feasibility and immune response | Ongoing |
| ICT-121    | NCT008914768               | I     | BTSC mRNA       | None                               | None    | 10          | Ongoing                                      | Ongoing          | Ongoing          | Safety, Feasibility and immune response | Ongoing |
| DENDR-STEM | NCT028203584, NCT02049489  | I     | Autologous BTSC | None                               | None    | 8           | Increase in IL-17 expressing CD4 (Th17) cells in stable patients compared to non-stable patients | None             | None             | MTD and immune response | Vaccine well tolerated with not MTD reached |
|            | NCT00846456                | I     | Autologous glioma stem cells lysate | None                             | None    | 7           | Yes (100% patients, defined via proliferation assay) | NA               | 23.1             | Safety          | Safe vaccine |
| 16-184-4412 | NCT028203584, NCT02049489  | I     | Autologous glioma stem cells | None                             | None    | 32          | Yes (CD8 response in 13% patients: up to 5.5% specific CD8 of total CD8 T cells) | NA               | 10.3             | Safety, feasibility, immunological response | Positive for safety and feasibility |
|            | NCT04388633                | I     | Autologous glioma stem cells | Temozolomide                      | None    | 10          | Ongoing                                      | Ongoing          | 18.0             | Safety and PFS6 | Ongoing |
|            | NCT052548571               | I     | Autologous glioma stem cells | Temozolomide                      | None    | 60          | Ongoing                                      | Ongoing          | 30.5             | Safety and PFS6 | Ongoing |

(Continued)
### TABLE 2 | Continued

| Trial name | ClinicalTrial.gov identifier | Phase | Immune targets | Associated treatments in active arm | Control | Sample size | T cell response (CD4/CD8 response details) | Humoral response | Median PFS (months) | Median OS (months) | Primary endpoint | Results |
|------------|-------------------------------|-------|----------------|-------------------------------------|---------|-------------|------------------------------------------|-----------------|---------------------|---------------------|------------------|---------|
| ADDIT-GLIO | NCT02649582                   | I/I   | WT1            | Temozolomide                        | None    | 20          | Ongoing (interim results: CD4 response correlated with OS) Ongoing | Results pending | Results pending | Results pending | OS               | Ongoing |
| NCT03879512 |                              | I/I   | Autologous tumor lysisate | Metronomic cyclophosphamide Temozolomide | TMZ     | 81          | Yes (CD8 response in 50% patients)         | NA              | 11.2               | 17.0               | Safety and Feasibility | Ongoing |
| NCT01280552 |                              | I/II  | AIM-2, MAGE1, TRP-2, gp100, HER2 and IL-13Ra2 | Radiotherapy-Temozolomide + fluorescence-guided surgery | NA     | 27          | Yes (11/27 patients displayed tumor specific responses with increased serum cytokine levels) | NA              | 12.7               | 23.4               | PFS              | Safe vaccine |
| NCT03879512 |                              | I/II  | Autologous tumor lysisate | None | None | 22          | No | NA | 10.5 | 20.1 | PFS12 | Positive (PFS12 = 41%) |
| NCT03879512 |                              | I/II  | Autologous tumor lysisate | None | None | 10          | Trends (CD8 and CD4) | NA | 9.5 | 28 | Immunological response | Positive trend |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | None | None | 22 (vs. control 21) | NA | NA | 7.7 | 13.7 | OS and PFS | Positive (trend for PFS) (Second phase of trial in IDH1wt TERTmt subgroups of GBM patients ongoing) |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | None | None | 60          | Results pending | Results pending | Results pending | Immunological response | Ongoing |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | None | None | 55          | Results pending | Results pending | Results pending | OS | Results pending |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | None | None | 100         | Results pending | Results pending | Results pending | OS and DC migration | Results pending |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | TAA-pulsed DC vaccine plus GM-CSF | None | 48          | Ongoing | Ongoing | Ongoing | OS | Ongoing |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | Unpulsed PBMC and saline Unpulsed DCs | None | 120         | Ongoing | Ongoing | Ongoing | OS | Ongoing |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | Temozolomide | None | 112         | Ongoing | Ongoing | Ongoing | OS, Safety and T reg depletion | Ongoing |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | DC vaccine plus TMZ | None | 136         | Ongoing | Ongoing | Ongoing | OS | Ongoing |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | Temozolomide | None | 24          | Ongoing | Ongoing | Ongoing | PFS12 | Ongoing |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | None | None | 50          | Ongoing | Ongoing | Ongoing | Safety and feasibility | Ongoing |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | TMZ + Radiotherapy | None | 42          | NA | NA | 22.9 (median for this trial and Taiwan DOH/MA911072504) | OS and safety | Positive |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | TMZ + radiotherapy | None | 28          | Ongoing | Ongoing | Ongoing | PFS | Ongoing |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | None | None | 331         | NA | NA | NA | Overall survival | Suspended |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | Autologous PBMCs | None | Estimated 414 but suspended | NA | NA | NA | PFS | Results pending |
| NCT03879512 |                              | I/II  | Autologous glioma stem-like lysisate | Brevizumab | None | 118         | Ongoing | Ongoing | Ongoing | OS | Ongoing |

Other considerations:

- **Military Malignant**: 
  - **CD4 response**: 
    - 20 Ongoing (interim results: CD4 response correlated with OS) Ongoing
    - 11.2 17.0 OS
    - Positive (trend for OS and significant for PFS)
  - **CD8 response**: 
    - 43.7
  - **OS**: 
    - 23.4
  - **PFS**: 
    - 41%
    - 10.5 20.1 PFS12
    - 9.5 28
    - 7.7 13.7

- **Trials with Additional Information**:
  - **Interim results**: 
    - CD4 response pending
    - CD8 response in 50% patients
    - 43.7 (interim results: CD4 response correlated with OS)
    - 17.0 (OS)
    - Positive (trend for OS and significant for PFS)
  - **Safety and Feasibility**: 
    - 10.5 20.1 PFS12
  - **Results pending**: 
    - Results pending

- **Control arms**:
  - **CD4 response**: 
    - 20
  - **CD8 response**: 
    - 43.7
  - **OS**: 
    - 23.4
  - **PFS**: 
    - 41%
  - **Safety and Feasibility**: 
    - 10.5 20.1 PFS12
  - **Results pending**: 
    - Results pending

- **Additional Considerations**:
  - **GM-CSF**, Granulocyte Macrophage-Colony Stimulating Factor
  - **Temozolomide**, BTSC, Brain Tumor Stem Cells
  - **CMV**, Cytomegalovirus
  - **PFS**, Progression Free Survival
  - **OS**, Overall Survival
  - **Td**, Tetanus Toxoid
  - **MTD**, Maximum Tolerated Dose

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**KLH**, keyhole limpet haemocyanin; **TTF**, Tumor Treating Fields; **nd-GBM**, newly-diagnosed glioblastoma; **r-GBM**, recurrent glioblastoma; **TAA**, Tumor Associated Antigen; **PFS**, progression free survival; **OS**, overall survival; **Td**, Tetanus toxoid; **GM-CSF**, Granulocyte Macrophage-Colony Stimulating Factor; **DI-TMZ**, Dose-Intensiﬁed Temozolomide; **BTSC**, Brain Tumor Stem Cells; **CMV**, Cytomegalovirus; **MTD**, maximum tolerated dose.
TABLE 3 | Other types of vaccine trials for glioblastoma.

| Trial | Type of vaccine | Immune targets | Associated treatments in active arm | Control | Sample size (evaluable patients) | Median PFS (months) | Median OS (months) | Primary endpoint | Other notes |
|-------|----------------|---------------|-----------------------------------|---------|-------------------------------|-------------------|-------------------|------------------|------------|
| NCT04015700 | DNA | Personalized neoantigen | None | None | 6 | Ongoing | Ongoing | Ongoing | Safety | (not yet recruiting) |
| NCT03750071 | I/II | DNA | VEGFR2 | Avelumab | None | 30 | Ongoing | Ongoing | Ongoing | Safety | Ongoing |
| Gliovac | – | HSPPC-96 | – | Bevacizumab | None | 46 | Results pending | Results pending | Results pending | Safety | NA |
| HSPPC-96 | II | HSPPC-96 | autologous tumor-derived –peptides | Temozolomide | None | 12 | NA | NA | Safety, Results | 36.0 |
| ALLIANCE | NCT01814813 (149) | HSPPC-96 | autologous tumor-derived –peptides | Bevacizumab | None | 90 | Results pending | Results pending | Results pending | Safety | Ongoing |
| NCT03018288 | II | HSPPC-96 | HSPPC-96 | Bevacizumab | None | 90 | Results pending | Results pending | Results pending | Safety | Ongoing |
| NCT03650257 | II | HSPPC-96 | HSPPC-96 | Temozolomide | Temozolomide | 150 | OS | 12 | Ongoing | Ongoing |

Tumor associated macrophages (TAMs); Nagai et al. (2009) utilized this methodology to selectively target TAMs. Activated TAMs were shown to express the antibody receptor beta (FRB), thereby providing a macrophage-specific target. The heavy and light chains of an anti-FRB antibody were conjugated to the toxin Pseudomonas exotoxin (152). The abundance of macrophages within the tumor allows delivery of the toxin to the tumor resulting in the death of tumor cells and the potentially immunosuppressive macrophages. Administration of this immunotoxin intratumorally to a subcutaneous rat C6 glioma tumor reduced tumor growth and the number of TAMs in these tumors (152). It is important to note that this treatment was injected directly into subcutaneous tumors which reduces the potential for any deleterious off-target effects. Although FRB was not detected in the normal brain, it was detected on macrophages resident in the heart and liver (152). This presents a potential hurdle to the systemic delivery of this immunoconjugate. The ability of this drug to cross the blood brain barrier is also unknown since this study utilized a subcutaneous model. In patients, this immunoconjugate could be administered intratumorally during surgery, or intravenicularly utilizing an Ommaya reservoir (an intracranial catheter device that allows direct delivery of drugs to the ventricles), thereby bypassing the blood brain barrier. However, this method is highly invasive and not without risks (153, 154).

Propentofylline (PPF) is a synthetic methylxanthine drug that is known to reduce the proliferation (155) and expression of inflammatory cytokines (155) by microglia in response to lipopolysaccharide. PPF could therefore be a novel therapeutic for targeting microglia within GBM tumors. In a rat model of GBM utilizing the CNS-1 cell line, a cell line which recapitulates the features of human GBM with minimal immunogenicity, systemic PPF administration reduced the volume of intracranial CNS-1 tumors (156). In vitro analysis revealed that PPF did not exert its effects on the CSF-1 cell line, rather its anti-tumor effects were attributed to its effect on microglial migration and the contribution of microglia to tumor cell migration (156). Rather than trying to remove microglia/macrophages from the TME, switching immunosuppressive M2 cells to the immune activating M1 phenotype also represents an attractive therapeutic option.

IL-12 represents an excellent immunotherapeutic candidate due to its ability to activate T-cells and NK cells and provoke antigen-specific immunity (157). As systemic administration of recombinant IL-12 was associated with adverse effects (such as damage to vital organs), gene transfer of IL-12 was achieved by the intracranial administration of an adeno-associated virus (AAV) encoding IL-12 to rats, after which they were challenged by intracranial injection of rat RG2 GBM cells. Treatment improved the survival of tumor challenged mice when compared to PBS injected control mice. Analysis of treated tumors revealed an increase in the microglial activation markers ED1 and TNF-related apoptosis-inducing ligand (TRAIL), and this was accompanied by a downregulation of the proliferation marker Ki67 and an increase in TUNEL staining - an indicator of apoptosis (157).
The blockade of TGF-β presents an attractive adjunct for active immunotherapy, due to its immunoregulating and tumor promoting effects. Trabedersen is an anti-sense RNA for human TGF-β2 mRNA that has been administered via convection-enhanced delivery to patients with recurrent GBM. Although Trabedersen improved the median survival compared to chemotherapy alone, this difference was not of statistical significance (158). In a pre-clinical murine model of metastatic pancreatic cancer, active vaccination was combined with antibody blockade of TGF-β. Soares et al. (2015) treated a murine model of pancreatic cancer using a vaccine comprised of GM-CSF secreting irradiated pancreatic cancer cells known as GVAX. This vaccine was used to treat two models of pancreatic cancer, the Panc02 model and KPC model. When GVAX vaccination was combined with TGF-β blockade, the cure rate of tumor bearing mice was improved in both models when compared to mice given GVAX with an IgG isotype antibody. The anti-tumor effects of GVAX were even further improved when the vaccine was combined with both an anti-TGF-β and anti-PD-1 antibody. This blockade of TGF-β in combination with GVAX reduced the regulatory T cell infiltrate into these tumors, a trend not seen when either therapy was used alone (159).

**Immune Checkpoint Blockade**

Due to the expression of numerous immunosuppressive checkpoints within the GBM TME, many checkpoint blockade antibodies have been tested in the GBM setting. Immune checkpoint blockade also represents a method for rescuing exhausted T cells. As monotherapies, immune checkpoints have provided lukewarm results (160–162). One interesting method for altering the responsiveness to immune checkpoint blockade is to administer these immune checkpoint blocking antibodies in a neoadjuvant setting, as opposed to an adjuvant setting. Neoadjuvant administration of checkpoint blockade involves the dosing of the patient prior to tumor resection and standard therapy as opposed to after surgery and alongside standard therapy. In the GBM setting, neoadjuvant PD-1 blockade has been explored patients with recurrent disease – these patients received neoadjuvant PD-1 and therapy was then continued in the adjuvant setting post-surgery. Neoadjuvant treatment prolonged the overall survival when compared to adjuvant PD-1 blockade, and increased CD8+ T cell infiltration into tumors. An upregulation in the expression of interferon gamma related genes was also seen in the tumors of these patients (163).

Combining checkpoint blockade modalities or combining active immunotherapy with checkpoint blockade are also attractive methods for enhancing protective anti-GBM immunity. In a pre-clinical murine model of GBM, PD-1 blockade was combined with DC vaccination to great effect. Mice bearing intracranial GL261 tumors were vaccinated with DCs loaded with murine GL261 tumor cell lysate. Although this approach increased the infiltration of tumor cells into these intracranial tumors, this did not lead to improved survival in mice with an elevated tumor burden. It was hypothesized that local immune suppression within the TME was preventing tumor-specific lymphocytes from inducing tumor cell death. TILs were shown to have up-regulated their expression of PD-1, as a result of which it was decided to combine anti-PD-1 antibody therapy with DC vaccination. This combination increased the percentage of activated CD8+ T cells within the intracranial tumors and improved the survival of mice when compared to mice given vaccination alone (164).

As CSF-1R inhibition has been shown to reduce polarization of macrophages to the immunosuppressive M2 phenotype (95), combining CSF-1R inhibition with active vaccination and PD-1 blockade has been explored in the GBM setting. Myeloid derived cells recruited to the tumor were shown to express PD-L1 and contribute to the immunosuppressive environment seen in murine GL261 tumors. The presence of vaccine-induced TILs increased the recruitment of these immunosuppressive PD-L1 expressing myeloid cells. As a result, Antonios et al. (2017) combined PD-1 antibody and a CSF-1R inhibitor with active DC vaccination. CSF-1R inhibition increased the presence of TILs within tumors, whereas PD-1 blockade improved the activation of TILs. This triple therapy significantly increased the survival of GL261 tumor bearing mice when compared to non-treated, DC vaccinated and DC vaccinated mice with either CSF-1R or PD-1 blockade alone (165).

As detailed earlier, IDO and TDO expression within GBM tumors contributes to the immunosuppressive nature of these tumors. Targeting IDO alone or as part of a combinatorial strategy therefore also represents an attractive treatment avenue. The anti-viral drug acyclovir has been shown to inhibit both IDO and TDO and preventing the recruitment of regulatory T cells to the TME (166). In a pre-clinical murine model of GBM, the combined blockade of IDO, CTLA-4 and PD-1 reduced regulatory T cell infiltration into tumors and led to 100% long-term survival in mice harboring intracranial GL261 tumors (167).

**Engineered CAR T Cells**

Chimeric antigen receptor (CAR) T cells provide an avenue for generating tumor targeted T cells that can function in the defective tumor microenvironment. CAR T cells are generated by transfecting autologous T cells taken from patients with a construct combining a single chain variable fragment specific to a tumor cell target with costimulatory domains that enable T cell activation without the need for a secondary co-stimulatory signal (168). Numerous antigens have been targeted utilizing CAR T cells and the design of CAR T cells has been fine-tuned in order to optimize their anti-tumor activity. Traditionally CAR T cells’ intracellular signaling domain was derived from the CD3ζ chain of the T cell receptor (first generation), as progress has been made further costimulatory domains have been added to the intracellular region in order to improve the functionality of CAR T cells (second and third generation). These costimulatory domains are often derived from costimulatory CD28, OX-40, ICOS, and 4-1BB (169, 170). Whilst the design of the targeting domain of the CAR T cells has evolved so has the general design of these cells, with the knock in of other genes that enhance anti-
tumor function being explored (see Table 4). As mentioned previously, GBM tumors frequently upregulate their expression of FasL (63, 175). CAR T cells generated from patient derived T cells often express Fas, which makes these T cells susceptible to FasL mediated cytotoxicity when entering the TME (176). The development of CAR T cells expressing Fas dominant negative receptors by Yamamoto and colleagues resulted in the development of CAR T cells expressing Fas dominant negative receptors for TGF-β have also been developed for the treatment of prostate cancer. These CAR T cells target a prostate antigen known as prostate-specific membrane antigen (PSMA) and they also express the dominant negative TGF-βRII that blocks TGF-β signaling. These CAR T cells displayed improved anti-tumor function when compared to CAR T cells that did not have the dominant negative TGF-βRII transduced into them. These CAR T cells appeared to exhibit long-term persistence and resistance to exhaustion (178). CAR T cells have also been engineered to secrete a PD-1 blocking antibody single chain variable fragment (scFv) that binds to PD-1 on the surface of activated T cells (both CAR and bystander T cells), thereby preventing PD-L1 on tumor cells from dampening T cell anti-tumor responses (179). These CAR T cells enhance the survival of PD-L1 expressing tumor bearing mice when compared to CAR T cells that do not secrete the PD-1 scFv combined with an anti-PD-1 antibody. This is believed to be due to the increased amount of PD-L1 blockade within the TME when compared to systemic checkpoint blockade. These CAR T cells displayed efficacy against both hematologic and solid tumors (179). CAR T cells have also been modified to express the immunostimulatory molecule CD40L to improve the anti-tumor function of these cells (180). The interaction of CD40L on these T-cells with CD40 on DCs results in the secretion of the immunostimulatory cytokine IL-12 (180). CD19 directed CAR T cells armed with the CD40 ligand have been shown to lyse CD19 negative cells and prevent their expansion and the development of antigen negative variants that escape an immune response (180). In order to prevent the development of antigen escape variants, CAR T cells have also been developed to produce bi-specific T cell engagers (BiTEs) in the GBM setting. EGFRvIII targeting CAR T cells have been developed to secrete BiTEs that target the wild type epidermal growth factor receptor (EGFR). These BiTEs contain an anti-EGFR domain along with an anti-CD3 domain, homing T cells onto EGFR expressing tumor cells. The secretion of these BiTEs recruits bystander cells that target tumor cells, these CAR T cells can also eradicate tumors that do not express the EGFRvIII antigen, thereby highlighting the importance of the BiTEs produced by these CAR T cells (181). One study looked at utilizing CD123 (IL-3 Receptor α chain) directed CAR T cells to target Hodgkin lymphoma cells. The investigators also hypothesized that as CD123 is expressed on myeloid cells, these CAR T cells could also target these cells and overcome the local immune suppression induced by MDSCs and M2 macrophages. These CAR T cells targeted lymphoma cells in vitro and in vivo. What was even more interesting was that these CAR T cells were resistant to inhibition by M2 macrophages when compared to classical CD19 targeting CAR T cells (182).

**Oncolytic Virotherapy**

The design and delivery of immunotherapies must consider the pronounced immunosuppressive environment of the TME in GBM. The use of oncolytic viruses, which can selectively infect and kill tumor cells, is beginning to generate increased interest due to its tumor specificity and the ability of these viruses to turn an immunosuppressive microenvironment into an immune supporting environment (183). Oncolytic viruses are genetically altered to not infect non-transformed cells, and, in some cases, other genes may be knocked down or knocked in to enhance the immune stimulatory properties of these viruses. For example, the oncolytic herpes simplex virus T-VEC has been transfected with the human granulocyte-macrophage colony-stimulating factor (GM-CSF) gene. GM-CSF secreted by the virus increases the recruitment of DCs into the TME and thereby enhances antigen presentation and T cell activation (184). Tumor cell lysis by oncolytic viruses also triggers inflammatory immune responses involving the release of antigens, danger associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) within the TME (184). Several different types of viruses have been used in the oncolytic virotherapy of GBM, viruses such as the herpes simplex virus (HSV), Newcastle disease virus (NDV), poliovirus, reovirus, adenovirus, measles virus and H1 parvovirus (185) (see Table 5). Not only have viruses been used to directly induce the death of tumor cells they have also been used to transfer genes to tumor cells that enable these cells to be targeted. One such example of one of these viruses is Toca 511, a retroviral vector that delivers cytosine deaminase to rapidly dividing malignant cells (193). The transferred cytosine deaminase enzyme then converts the pro-drug 5-fluorocytosine to the active antineoplastic compound 5-fluorouracil resulting in the death of tumor cells (193). The use of this virus pro-drug combination has also been shown to result in an increase of immune cell activity within murine brain tumors, with a decrease in immunosuppressive cells and an increase in interferon gamma positive CD8 T cells within the tumor microenvironment (194). Whilst in treating preclinical models of GBM Toca 511 showed great promise recent however results from a phase II/III clinical trial revealed that Toca 511 in combination with 5-fluorocytosine did not improve overall survival when compared to standard therapy (195). Although oncolytic viral therapy represents an exciting avenue for GBM therapy, it is not without obstacles and as a result combinatorial therapy utilizing oncolytic viruses needs to be considered. Very little virus crosses the blood brain barrier when oncolytic viruses are delivered systemically, oncolytic viruses needs to be considered. Very little virus crosses the blood brain barrier when oncolytic viruses are delivered systemically, yet these therapies are still efficacious in brain tumor models. Oncolytic herpes simplex viruses (HSV) can be used in combination with various other therapeutics for the treatment of GBM. The virus can also be altered with immunomodulating transgenes to improve anti-tumor efficacy and enable modulation of the TME (196). ‘Arming’ an oncolytic HSV with the murine IL-4 gene has been shown to increase the survival of mice bearing intracranial GL-261 cells. Conversely, no survival benefit compared to sham treated animals was observed when immunosuppressive IL-10 was transfected into this oncolytic virus (197). Clinical testing of
| Trial name | ClinicalTrials.gov identifier | Phase | CAR generation | Targets | Associated treatments in active arm | Sample size (evaluable patients) | Median PFS (months) | MedianOS (months) | Primary endpoint | Results |
|------------|-------------------------------|-------|----------------|---------|-------------------------------------|-------------------------------|-------------------|------------------|------------------|---------|
| NCT01109095 | (171)                         | I     | Second         | HER2 and CMV pp65 | None                                        | 16                            | NA                | 24.8 for children and 30 months for adults | Safety and feasibility | Positive |
| NCT02442297 | I                             | Second | HER2           | None                     | None                                         | 28                            | Ongoing           | Ongoing          | Safety and feasibility | Ongoing |
| NCT02208362 | (172)                         | I     | Second         | IL13Rα2                  | None                                         | 92                            | Ongoing           | Ongoing          | Safety and feasibility | Ongoing |
| NCT02209376 | (173)                         | I     | Unknown        | EGFRvIII                 | None                                         | 10                            | Not evaluable     | 8 months | Safety and feasibility | CAR T cells seen to traffic to tumors, however adaptive changes in TME need to be accounted for |
| NCT02664363 | I                             | Third | EGFRvIII       | None                     | TMZ induced lymphodepletion                   | 3                             | Ongoing           | MTD              | Ongoing          |
| NCT04003849 | I                             | Second | IL-13Rα2       | Ipilimumab and Nivolumab | None                                         | 60                            | Ongoing           | Safety and feasibility | Ongoing |
| INTERCEPT  | NCT02853831                   | I     | Unknown        | EGFRvIII                 | None                                         | 24                            | Ongoing           | MTD              | Ongoing          |
| NCT04077866 | I                             | Unknown | EGFRvIII       | None                     | Pembrolizumab                                 | 20                            | Ongoing           | Safety and feasibility | Ongoing |
| NCT04045847 | I                             | Unknown | CD147          | TMZ                      | None                                         | 7                             | Ongoing           | Safety, feasibility, OS and PFS | Ongoing |
| NCT02937844 | I                             | Second | PD-L1 (PD-1 on CAR T cell linked to co-stimulatory CD28 cytoplasmic domain) | Cyclophosphamide and Fludarabine | None                                         | 31                            | Ongoing           | Safety and feasibility | Ongoing |
| NCT04270461 | I                             | Third | NG2D           | None                     | None                                         | 10                            | Ongoing           | Safety and feasibility | Ongoing |
| NCT043835137 | I                             | Unknown | B7-H3          | TMZ                      | None                                         | 12                            | Ongoing           | Safety, feasibility, OS and PFS | Ongoing |
| NCT01454596 | I/II                          | Third | EGFRvIII       | Chemotherapy induced lymphodepletion and aldesleukin | None                                         | 18                            | Ongoing           | Safety, feasibility and PFS | Ongoing |

PFS, progression free survival; OS, overall survival; MTD, maximum tolerated dose.
| Trial name ClinicalTrials.gov identifier | Phase | Virus used/mode of action | Associated treatments in active arm | Control | Sample size (evaluable patients) | Median PFS(months) | MedianOS(months) | Primary endpoint | Results |
|----------------------------------------|-------|---------------------------|-------------------------------------|---------|----------------------------------|-------------------|------------------|------------------|---------|
| NCT0390299 | I | Oncolytic carcinoembryonic antigen expressing measles virus (MV-CEA) | None | None | 23 | Ongoing | Ongoing | Safety, feasibility OS and PFS | Ongoing |
| NCT02444546 | I | Reovirus (REOLYSIN®) | Sargramostim (GM-CSF) | None | 6 | Ongoing | Ongoing | MTD and safety | Ongoing |
| NCT0031083 | I | Reovirus (REOLYSIN®) | None | None | 18 | Ongoing | Ongoing | MTD and safety | Ongoing |
| NCT03043391 | I | Adenoviral transfer of IFN-β gene | Poliovirus (PVSRIPO) | None | 35 | Suspended | Suspended | Safety and feasibility | Suspended |
| NCT03072134 | I | Neural stem cells loaded with adenovirus | None | None | 12 | Ongoing | Ongoing | Safety, feasibility and OS24 | Ongoing |
| NCT0911388 | I | HSV 0207 | +/- Single dose of 5 Gy radiation | None | 15 | Ongoing | Ongoing | Safety and feasibility | Ongoing |
| NCT01491893 | I | Poliovirus (PVSRIPO) | None | Historical controls | 15 | Results pending | Results pending | MTD, safety and feasibility | Positive |
| NCT02457845 | I | Poliovirus (PVSRIPO) | None | None | 5 | Results pending | Results pending | Safety and feasibility | Positive |
| D24GBM NCT01956734 | I | Adenovirus (DNX-2401) | TMZ | None | 31 | Pending | Pending | Safety PFS6 and OS12 | Pending |
| NCT01971699 | I | Adenovirus (DNX-2401) | +/- IFNγ | None | 27 | Results pending | Results pending | (interim OS12 = 33 %, interim OS18 = 22 %) | Safety and feasibility |
| NCT03657576 | I | C134-HSV | None | None | 24 | Ongoing | Ongoing | Safety and efficacy | Ongoing |
| NCT03152318 | I | HSV (RNestin34.5v.2) | +/- Cyclophosphamide | None | 108 | Ongoing | Ongoing | Safety and efficacy | Ongoing |
| NCT0206271 | I | Ad-RTS-hIL-12 | Veledimex | None | 31 | NA | 12.7 | Safety and feasibility | Positive |
| NCT03638477 | I | Ad-RTS-hIL-12 | Veledimex + Nivolumab | None | 21 | Ongoing (not recruiting) | Ongoing (not recruiting) | Safety and feasibility | Ongoing |
| NCT03896668 | I | Allogenic stem cells loaded with adenovirus (DNX-2401) | None | None | 36 | Ongoing | Ongoing | Safety, feasibility and MTD | Ongoing |
| NCT03679574 | I | Ad-RTS-hIL-12 | Veledimex | None | 36 | Ongoing | Ongoing | Safety and feasibility | Ongoing |
| NCT01811992 | I | Ad-hCMV-TK and Ad-hCMV-Flt3L | None | None | 19 | Ongoing (not recruiting) | Ongoing (not recruiting) | Safety and feasibility | Ongoing (not recruiting) |
| NCT03714334 | I | DNX-2440 | None | None | 24 | Ongoing | Ongoing | Safety, feasibility and OS | Ongoing |
| NCT02031965 | I | HSV-1716 | Dexamethasone + surgery | None | 2 | Results pending | Results pending | Safety and feasibility | Ongoing |
| NCT02062827 | I | HSV-1 | None | None | 36 | Ongoing | Ongoing | MTD | Ongoing |
| NCT04327011 | I | Toca 511/5-FC | None | None | 65 | Terminated | Terminated | Safety and OS | Terminated |
| NCT0028158 | II | HSV 0207 | None | None | 65 | Results pending | Results pending | Safety and feasibility | Results pending |
| NCT01301430 | II | Parovirus H-1 (ParOryx) | None | None | 18 | Results pending | Results pending | Safety and feasibility | Results pending |

(Continued)
TABLE 5

| Trial name (Clintrial.gov identifier) | Phase | Virus used/mode of action | Control | Associated treatments in active arm | Sample size (evaluable patients) | Median PFS(months) | Median OS(months) | Primary endpoint | Results | Results pending |
|-------------------------------------|-------|---------------------------|---------|-------------------------------------|---------------------------------|-------------------|------------------|----------------|---------|----------------|
| NCT01582516                         | I/II  | Adenovirus (Delta-24-rgd) | None    | Matched control                      | 20                              | Results pending    |                   | Safety, feasibility and efficacy | Positive | Results pending |
| NCT00589875 (191)                   | II    | AdV-tk                     | Valacyclovir + matched control | None                        | 47                              | 12.7              | 25.1             | for patients with maximal resection | Positive | Safety, feasibility and maximal OS |
| NCT04482933                         | II    | HSV G207                   | None    | Standard of care                     | 30                              | Ongoing (not yet recruiting) | Ongoing (not yet recruiting) | Objective response for PFS6 and OS | Positive | Ongoing (not yet recruiting) |
| NCT02798406                         | II    | Adenovirus (DNX-2401)      | Pembrolizumab | None                        | 49                              | 8.7               | 11.4             | for patients with standard of care | Positive | Ongoing (not recruiting) |
| NCT04406272                         | II    | VB-111                     | Bevacizumab | Standard of care                    | 45                              | Ongoing            | Ongoing          | TIL density and dose limiting toxicity | Positive |
| NCT04006119                         | II    | Ad-RTS-hIL-2               | Cemiplimab | None                                 | 36                              | Ongoing            | Ongoing          | Safety, feasibility and OS | Positive |
| NCT04105374                         | II/III| Toca 511/Toca FC           | TMZ + radiotherapy | None                        | Terminated | Terminated | Terminated | DFS, OS, maximal tolerable dose | Terminated |

PFS, progression free survival; OS, overall survival; MTD, maximum tolerated dose.

The lymphodepletion caused by high dose TMZ increased the survival of mice bearing established intracranial tumors when given CAR T cell therapy. This lymphodepletion to led to oncolytic viruses remains in its relative infancy, with several viral therapies undergoing phase I/II clinical trials (198). The prospect of genetically modifying these viruses provides great hope for the future treatment of GBM. Not only can viruses be genetically manipulated but they can also be combined with other immunotherapeutic modalities to help overcome the immunosuppressive TME. One such example is combination of oncolytic measles virus therapy with anti-PD-1 checkpoint blockade (199). This combination was shown to increase survival in C57BL/6 mice bearing intracranial GL261 tumors when compared to either monotherapy, as well as increasing survival this combinatorial therapy increased T cell infiltrate into these tumors (199). Checkpoint blockade has also been combined with the IL-12 expressing oncolytic HSV in a preclinical model of GBM to great effect (200). This IL-12 secreting HSV was combined with both anti-CTLA-4 and anti-PD-1 checkpoint blockade for the treatment of two murine GBM models, this triple therapy reduced the number of regulatory T cells present within tumors and increased the influx of immune supporting M1 macrophages resulting in the complete cure of these mice (200). As previously mentioned GBMs frequently overexpress PGE2 which promotes an immunosuppressive environment and provides an attractive target for therapy. An oncolytic vaccinia virus has been developed that expresses 15-(NAD)-hydroxyprostaglandin-inactivating enzyme (HPGD); an enzyme that inactivates PGE2 (201). This modified vaccinia virus was tested in a variety of mouse solid tumor models and it was found to reduce the number of MDSCs and regulatory T cells within these tumors increasing the response of these tumors to viral therapy and adoptive T cell transfer (201). Whilst viral therapy is in its relative infancy with regards to clinical approval these early findings provide great hope for the future of this treatment modality.

**Combining Immunotherapy With Standard Therapy**

Adapting current therapies also needs to be considered in the context of immunotherapy for GBM, especially given the likelihood that all new approaches will need to be delivered in the context of current ‘standard’ therapy. Both TMZ and radiotherapy have immune augmenting effects that can be capitalized upon when considering the immunotherapeutic treatment of GBM. As mentioned previously, TMZ can induce lymphodepletion in patients. This lymphodepletion can be capitalized on to potentially enhance the efficacy of CAR T cell therapy. In a murine model of GBM, EGFRvIII CAR T cells failed to confer a survival advantage for mice bearing intracranial EGFRvIII expressing tumors, despite that fact that these cells were shown to have anti-tumor cell activity in vitro. Lymphodepletion with radiotherapy administered prior to CAR T cell therapy was shown to improve the efficacy of CAR T cell therapy by resulting in long-term survival of mice (202). Similarly, TMZ was used to lymphodeplete prior to CAR T cell administration. TMZ was either used in a standard or high dose, with the higher dose inducing more marked lymphodepletion. The lymphodepletion caused by high dose TMZ increased the survival of mice bearing established intracranial tumors when given CAR T cell therapy. This lymphodepletion to led to
persistence of the injected CAR T cells within the blood of treated mice and this correlated with lower tumor burden (202). As well as using high dose TMZ to lymphodeplete, the dosing can also be given as low frequent doses, known as metronomic dosing. Ouyang and colleagues (2016) designed immune activating CpG carbon nanotube conjugates (SWCNT/CpG-2) that prolonged the survival of mice bearing intracranial GL261 tumors. This SWCNT/CpG-2 was used to treat a more invasive GBM model using the KR158B cell line, a model that more faithfully represents the characteristics of human GBM within a murine model. Although this intracranial SWCT/CpG-2 therapy was not curative for this KR158B model, as it was in the case of the GL261 model, when this SWCT/CpG-2 was combined with low dose daily TMZ, it significantly improved survival when compared to SWCT/CpG-2 monotherapy (203). Splenocytes taken from mice that had received metronomic TMZ in combination with SWCT/CpG-2 were more efficient at inducing in vitro KR158B tumor cell death than splenocytes from mice given either SWCT/CpG-2 or TMZ alone. This dual therapy did not reduce the number of regulatory T cells in the tumors. However, both SWCT/CpG-2 therapy and dual therapy induced an increased macrophage infiltrate into the tumors. The researchers hypothesized that the metronomic TMZ dosing increased the relative proportions of immune activating M1 macrophages to immune inhibitory M2 macrophages within the tumors (203). Radiotherapy can also be used as an adjunct to immunotherapy in order to boost the anti-tumor immune response. Weiss et al. (2018) generated an NKG2D expressing CAR T cell therapy that when systemically administered penetrated brain tumors in a murine GL261 GBM model. These NKG2D CAR T cells were shown to cure 22% of GL261 bearing mice treated. Radiotherapy upregulated the expression of NKG2D ligands on the surface of GBM cells and, as a result, it was decided to combine radiotherapy with NKG2D CAR T cells. Mice were given a single 4 gray (Gy) dose of radiotherapy on day 7 after tumor implant and CAR T cells were given on days 5, 7 and 10. The single radiotherapy dose alone did not alter the survival of tumor bearing mice compared to control mice, however it increased the survival of mice harboring intracranial GL261 cells when combined with the NKG2D CAR T cell therapy. This effect was also shown in mice bearing intracranial SMA-560 tumor cells (204). Another alternative that has been considered is the intratumoral administration of TMZ, as opposed to systemically administered alternative that has been considered is the intratumoral expression GM-CSF (205). This combined intracranial TMZ and immunotherapy increased CD8+ T cell infiltrate and decreased MDSCs (205).

**Overcoming the Blood Brain Barrier (BBB)**

The BBB can act as a significant barrier for systemically administered therapeutics, including immune checkpoint blocking antibodies. Several approaches can be used to address this issue. These include direct modification and masking of therapeutic agents, encapsulation of therapeutics within vesicle-based delivery systems, and targeted opening of the BBB/BBT by physical or biochemical disruption. Conceptually, the simplest route to bypass the BBB is direct administration to the brain parenchyma or the cerebrospinal fluid (CSF). Although this is a commonly used approach in pre-clinical, experimental work, it is clinically problematic. Direct intra-parenchymal injection is rarely performed outside of intensive care medicine due to the difficulties associated with infection risk and needle damage. Moreover, although GBM are rarely metastatic (206), direct administration to the tumor site is contra-indicated due to the slow rate of diffusion of therapeutic molecules through compact brain tissue, injected substances rarely travelling more than a few millimeters beyond the injection site (207–209). This route is therefore unlikely to be sufficient for treating GBM, given both the likely tumor size on diagnosis and accessibility issues. Intracerebroventricular or intrathecal injection, delivery to the CSF, has similarly poor distribution issues (210). Passage of drugs from the CSF to the parenchymal tissue is primarily diffusive, which, coupled with rapid removal from the ependymal surface via bulk flow and the glymphatic system, results in minimal transfer of therapeutic agents into the tissue (211, 212). These restrictions are even more relevant to the delivery of large molecules such as therapeutic antibodies (213).

Working on the principle that the simplest way to overcome the BBB/BBT is to remove it, a number of methods of disrupting barrier function have been investigated for their potential use in the treatment of GBM and other neurological disorders. Such techniques were first begun over 50 years ago, with studies employing hypertonic solutions of osmolytes such as mannitol to induce osmotic endothelial shrinkage and tight junction opening (214). Such targeted disruption, whilst effective in permitting increased therapeutic access to the brain, is also indiscriminate and enables the entry of pro-inflammatory and potentially toxic serum proteins such as albumin and complement factors (215), thereby rendering this non-specific approach unsuitable for clinical use. More targeted methods of inducing increased BBB/BBT permeability have used endogenous bioactive agents such as bradykinin or its synthetic analogues (216). Although such approaches have increased permeability of chemotherapeutic agents to the brain in preclinical models, they have not translated into clinical practice, possibly due to having too brief a duration of action (217).

Rather than chemical or osmotic-mediated disruption, another technique used to circumvent the BBB/BBT is the use of high-power focused ultrasound (218) to generate foci of
increased tissue permeability. Although this approach is effective in opening the barrier to therapeutic antibodies (219), it suffers from producing bystander tissue distortion and damage in experimental animals (220). In an attempt to overcome this issue, the technique has been refined to improve specificity and reduce energy transfer through the use of injected microbubbles (221, 222). In this case, lower frequency ultrasound is used to stimulate microbubble oscillation and cavitation, disrupting the endothelial wall through local shock wave production and permitting access of therapeutic agents to the brain. Although promising, it is not yet clear to what degree brain penetration can be enhanced as efflux transport systems remain active (223), and the long-term consequences of disruption have not yet been studied.

As an alternative to BBB/BTB disruption, numerous attempts have been made to modify the therapeutic agents themselves or their delivery systems to permit greater transfer across an intact BBB/BTB. Building on the rationale that more lipophilic agents are better able to cross the BBB/BTB, initial approaches aimed to improve therapeutic agent lipid solubility. Although such modifications do indeed improve CNS access, this was achieved at the cost of increased non-specific membrane permeability and a consequent rise in off-target effects (224, 225).

To overcome these difficulties, ongoing attempts at achieving effective drug delivery across the BBB/BTB have employed a wide range of different nanocarriers, also termed nanoparticles. These are diverse molecular structures, including lipid micelles, liposome composites of phospholipid and other molecules, and polymer-based particles, with the common property that they form a vesicle that can be loaded with therapeutic agents and which can then cross the BBB to enter the parenchyma (226). Once within the brain, variation in environmental pH at the tumor site, amongst other conditions destabilize the nanocarrier structure and trigger release of the cargo within the tissue (227). Although effective, these nanocarriers are indiscriminate and passively deliver their cargo widely across the brain, a drawback that has spurred the development of more effectively targeted nanocarrier delivery systems.

Targeting can be substantially enhanced by including molecular tags within the vesicle wall using proteins, peptides, nucleic acids or small molecules that specifically recognize tumor-associated receptors, thereby minimizing off-target actions. A wide range of different molecular tags have been exploited for this purpose in vivo, including for example, the interaction of nanocarrier borne transferrin with the transferrin receptor TIR-1 on GBM cells (228), the EGFP-EGF1 fusion protein on nanoparticles with tissue factor in tumor cells (229), and cholera toxin with tumor-expressed chloride channels and matrix metalloproteinase-2 (230). Such strategies hold significant promise as they allow for both the concentration of therapeutic agents at the tumor site and, by virtue of the encapsulation, protect therapeutic agents from hepatic metabolism (230). Although a number of nanocarrier-encapsulated small molecule approaches are currently undergoing clinical trial in GBM, as yet none have been approved for use (231). Questions about the efficiency of large molecule, i.e. therapeutic antibody, encapsulation efficiency remain.

As direct lipophilic modification of therapeutic agents and encapsulation strategies have relatively broad specificity, even with improved targeting strategies, interest has grown in the use of direct molecular tagging of the therapies themselves to permit recognition by specific endothelial transporters, e.g. the transferrin receptor, insulin receptor or low density lipoprotein receptor (232, 233), a process sometimes termed receptor mediated transcytosis.

This approach has proven to hold significant promise for the experimental delivery of protein agents, including therapeutic antibodies. Exposure of ‘normal’ CNS to circulating biologic agents is restricted to less than 0.5% of the concentrations that are present in serum (234, 235), a level at which target engagement is unlikely to occur (236). However, molecular engineering of therapeutic antibodies has enabled significant enhancements in uptake across the BBB. These approaches include the development of bispecific antibodies in which one F(ab) binds the target of interest and the other binds and is transported by an endothelial transporter (237), therapeutic antibodies in which a transporter recognition domain is linked to the immunoglobulin heavy or light chain (238, 239), or, more recently, molecules in which the Fc domain itself is directly recognized by an endothelial transporter (240, 241). Such an approach has not yet been tested directly for the clinical delivery of immunotherapies targeting GBM but does hold significant promise. BBB penetrating Nano immunoconjugates have been developed with the aim of crossing the BBB and penetrating intracranial tumors. Galstyan and colleagues (2019) developed anti-CTLA-4 and anti-PD-1 IgG antibodies conjugated to poly (β-L-malic acid), a natural biopolymer scaffold. These Nano immunoconjugates cross the BBB more efficiently than the anti-PD-1 and CTLA-4 IgG antibodies without polymer conjugation, increased the CD4+ and CD8+ T cell infiltrate into tumors and improved the survival of mice bearing intracranial GL261 tumors compared to those treated with the non-conjugated antibodies (242). Antibody delivery to intracranial tumors can also be improved by disrupting the BBB using focused ultrasound and microbubbles to physically disrupt the tight junctions enabling penetration of the brain parenchyma. The combination of focused ultrasound (FUS) with microbubbles improves entry of the anti-HER2 antibody Herceptin into brains (243). Similar results have also shown with FUS in combination with microbubbles increases the penetration of anti-amyloid beta antibodies into the brains of mice in two separate models of Alzheimer’s disease (244). FUS is an attractive option since the opening of BBB is transient (245), thereby minimizing the potential for damage to the brain. More interestingly, FUS itself can be used therapeutically to target intracranial tumors due to its immunomodulation action. Ultrasound waves can expand and contract air bubbles present within cells to generate heat and physically damage cells, inducing cell death, leading to the release of antigenic material and an up-regulation of immune activating molecules such as heat shock proteins (246).
CONCLUDING REMARKS

Glioblastoma multiforme (GBM) is the most frequently occurring primary brain tumor. It is uniformly fatal due to its highly invasive nature and resistance to standard therapies. GBM tumors employ several mechanisms to avoid being detected and killed by immune cells. These include the downregulation of important immune activating molecules such as MHC molecules, as well as upregulating expression of molecules that induce the death of immune cells such as Fas ligand, non-classical MHC molecules such as HLA-E and -G, and PD-L1. GBM cells also secrete numerous immunoinhibitory cytokines such as IL-10, TGF-β, Gal-1, IL-6 and PGE2, to name a few. These cytokines result in the inactivation/death of immune cells as well as the recruitment of inhibitory cells such as regulatory T cells and MDSCs to the TME. These cytokines also lead to a conversion of tumor resident macrophages from the immune activating M1 phenotype to the immunosuppressive M2 phenotype further dampening the anti-tumor immune response.

The plethora of immunosuppressive mechanisms that GBM tumors utilize, as well as their physiological location, make treating them with immunotherapy a daunting task. Although these tumors are immunosuppressive, this immunosuppression can be leveraged to try and boost the anti-tumor immune response. The concept of combining immune checkpoint blockade with active vaccination is one such method that can be used, or the use of genetically modified oncolytic viruses and CAR T cells that actively attack tumors whilst overcoming the local immunosuppression, either via the secretion of immune activating cytokines or immune blocking scFvs. Combinatorial immunotherapy along with improvement of BBB penetration represents an encouraging avenue for GBM therapy in the future. The only caveat to these combined therapies is the possibility of an overactive immune response and potential autoimmunity, this will have to be monitored when moving combinatorial immunotherapy forward.

AUTHOR CONTRIBUTIONS

JP wrote the initial draft with SC and SM making significant contributions. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Ostrom QT, Gittleman H, Liao P, Vecchione-Koval T, Wolinsky Y, Kruchko C, et al. CBTRUS Statistical Report: Primary brain and other central nervous system tumors diagnosed in the United States in 2010-2014. Neuro Oncol (2017) 19(suppl_5):v1–v88. doi: 10.1093/neuonc/nox158
2. Petrecca K, Guoet MC, Panet-Raymond V, Souharni L. Failure pattern following complete resection plus radiotherapy and temozolomide is at the resection margin in patients with glioblastoma. J Neurooncol (2013) 111 (1):19–23. doi: 10.1007/s11060-012-0983-4
3. Brandes AA, Tosoni A, Franceschi E, Sotti G, Frezza G, Amista P, et al. Recurrence pattern after temozolomide concomitant with and adjuvant to radiotherapy in newly diagnosed patients with glioblastoma: correlation With MGMT promoter methylation status. J Clin Oncol (2009) 27(8):1275–9. doi: 10.1200/JCO.2008.19.4969
4. Sherriff J, Tamangani J, Senthil L, Cruickshank G, Spooner D, Jones B, et al. Patterns of relapse in glioblastoma multiforme following concomitant chemoradiotherapy with temozolomide. Br J Radiol (2013) 86 (1022):20140414. doi: 10.1259/bjr.20120414
5. Hadipanayis CG, Stummer W, 5-ALA and FDA approval for glioma surgery. J Neurooncol (2019) 141(3):479–86. doi: 10.1007/s11060-019-03098-y
6. Young RM, Jamshidi A, Davis G, Shermahan J. Current trends in the surgical management and treatment of adult glioblastoma. Ann Transl Med (2015) 3 (9):121–5839. doi: 10.3978/j.issn.2305-8399.2015.05.10
7. Pachter JS, de Vries HE, Fabry Z. The blood-brain barrier and its role in immune privilege in the central nervous system. J Neuroimmunol Exp Neurol (2003) 62(6):593–604. doi: 10.1016/j.jenm.2006.2.593
8. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood-brain barrier. Neurobiol Dis (2010) 37(1):13–25. doi: 10.1016/j.nbd.2009.07.030
9. McArthur S, Loiola RA, Maggioni E, Errede M, Virgintino D, Solito E. The restorative role of annexin A1 at the blood-brain barrier. Fluids Barriers CNS (2016) 13(1):17–016-0043-0. doi: 10.1186/s12987-016-0043-0
10. Jain RK, di Tomaso E, Duda DG, Loeﬄer JS, Sorensen AG, Batchelor TT. Angiogenesis in brain tumours. Nat Rev Neurosci (2007) 8(8):610–22. doi: 10.1038/nrn2175
11. Anderson JC, McFarland BC, Gladson CL. New molecular targets in angiogenic vessels of glioblastoma tumours. Expert Rev Mol Med (2008) 10:e23. doi: 10.1017/S1462399408000768
12. Dhermain FG, Hau P, Lanfermann H, Jacobs AH, van den Bent MJ. Advanced MRI and PET imaging for assessment of treatment response in patients with gliomas. Lancet Neurol (2010) 9(9):906–20. doi: 10.1016/S1474-4422(10)70181-1
13. Henderson JT, Piquette-Miller M. Blood-brain barrier: an impediment to neuropharmaceuticals. Clin Pharmacol Ther (2013) 95(4):308–13. doi: 10.1002/cpt.77
14. Dantzer R. Neuroimmune Interactions: From the Brain to the Immune System and Vice Versa. Trends Immunol (2011) 32(1):3–13. doi: 10.1016/j.it.2010.08.004
15. Heimberger AB, Sampson JH. Immunotherapy coming of age: what will it take to make it standard of care for glioblastoma? Neuro Oncol (2011) 13 (1):3–13. doi: 10.1093/neuonc/noq169
16. Engelhardt B, Ransohoff RM. Capture, crawl, cross: the T cell code to breach the blood-brain barriers. Trends Immunol (2012) 33(12):579–89. doi: 10.1016/j.it.2012.07.004
17. Wilson EH, Weninger W, Hunter CA. Trafﬁcking of immune cells in the central nervous system. J Clin Invest (2010) 120(5):1368–79. doi: 10.1172/JCI41911
18. Ichinose M, Masuoka J, Shiraishi T, Mineta T, Tabuchi K. Fas ligand expression and depletion of T-cell inﬁltration in astrocytic tumors. Brain Tumor Pathol (2001) 18(1):37–42. doi: 10.1007/BF02789293
19. Vega EA, Graner MW, Sampson JH. Combating immunosuppression in glioma. Future Oncol (2008) 4(3):433–42. doi: 10.2217/14796994.3.4.433
20. Mullins C, Walter A, Schmitt M, Classen C, Linnebacher M. Tumor antigen and MHC expression in glioma cells for immunotherapeutic interventions. World J Immunol (2013) 11/27:3. doi: 10.5411/wji.v3.i3.62
21. Moserle L, Casanovas O. Anti-angiogenesis and metastasis: a tumour and stromal cell alliance. J Intern Med (2013) 273(2):128–37. doi: 10.1111/joim.12018
22. Steck PA, Moser RP, Bruner JM, Liang L, Freidman AN, Hwang TL, et al. Altered expression and distribution of heparan sulfate proteoglycans in human gliomas. Cancer Res (1989) 49(3):2906–10.

23. Quill DL, Joyce JA. The Microenvironmental Landscape of Brain Tumors. Cancer Cell (2017) 31(3):326–41. doi: 10.1016/j.ccell.2017.02.009

24. Woroniecka KI, Rhodin KE, Chongsthaidkiet P, Keith KA, Fecci PE, T-cell Dysfunction in Glioblastoma: Applying a New Framework. Clin Cancer Res (2018) 24(16):3792–802. doi: 10.1158/1078-0432.CCR-18-0047

25. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. Blood (2003) 101(7):2711–20. doi: 10.1182/blood-2002-07-2103

26. Huff WX, Kwon HJ, Henriquez M, Fetcbo K, Dey M. The Evolving Role of CDW+(+CD28(-)) Immunosenescent T Cells in Cancer Immunology. Int J Mol Sci (2019) 11(21):851. doi: 10.3390/ijms20112810

27. Chen PY, Wu CY, Fang H, Chen HC, Fong LY, Huang CY, et al. Functional Change of Effector Tumor-Infiltrating CCR5+(+CD38(-)+HLA-DR)-(+CD8(+) T Cells in Glioma Microenvironment. Front Immunol (2019) Oct 9:2395. doi: 10.3389/fimmu.2019.02395

28. Hanif F, Muzaffar K, Perven K, Malik SH, Simjee S. Glioblastoma Multiforme: A Review of its Epidemiology and Pathogenesis through Clinical Presentation and Treatment. Asian Pac J Cancer Prev (2017) 18(13):1–9. doi: 10.22034/APJCP.2017.18.13.1

29. Pongrazzi L, Weinberger B. T cells, aging and senescence. Exp Gerontol (2020) 134:110887. doi: 10.1016/j.exger.2020.110887

30. Woroniecka K, Chongsthaidkiet 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 Cancer Res (2018) 24(17):4715–86. doi: 10.1158/1078-0432.CCR-17-1846

31. Mirzaei R, Sarkar S, Yong VW. T Cell Exhaustion in Glioblastoma: Of Immune Escape in Glioblastoma Multiforme therapy and mechanisms of resistance. Pharmaceuticals (Basel) (2013) 6(2):1475–506. doi: 10.3390/ph6021475

32. Lee EQ, Wen PY. Corticosteroids for peritumoral edema: time to overcome our bias? J Neurosurg (2013) 123(3):459–64. doi: 10.1093/jnr/fet056

33. Ramirez YP, Weatherbee JL, Wheelhouse RT, Ross AH. Glioblastoma Multiforme: A Review of its Epidemiology and Pathogenesis through Clinical Presentation and Treatment. Asian Pac J Cancer Prev (2017) 18(13):1–9. doi: 10.22034/APJCP.2017.18.13.1

34. Chow KK, Hara W, Lim M, Li G. Combining immunotherapy with radiation for the treatment of glioblastoma. J Neurooncol (2015) 123(3):459–64. doi: 10.1007/s11060-015-1762-9

35. Formenti SC, Demaria S. Radiation therapy to convert the tumor into an in situ vaccine. Int J Radiat Oncol Biol Phys (2012) 84(4):879–80. doi: 10.1016/j.ijrobp.2012.06.020

36. Formenti SC, Demaria S. Radiation therapy to convert the tumor into an in situ vaccine. Int J Radiat Oncol Biol Phys (2012) 84(4):879–80. doi: 10.1016/j.ijrobp.2012.06.020

37. Vanpouille-Box C, Pilones KA, Wennerberg E, Formenti SC, Demaria S. In situ vaccination by radiotherapy to improve responses to anti-CTLA-4 treatment. Vaccine (2015) 33(51):7415–22. doi: 10.1016/j.vaccine.2015.05.105

38. Authier A, Farrant KJ, Broadway KW, Ancelet LR, Hunn MK, Stone S, et al. Enhanced immunosuppression by therapy-exposed glioblastoma multiforme tumor cells. Int J Cancer (2015) 136(11):2566–78. doi: 10.1002/ijc.29309

39. Tabatabaei P, Visse E, Bergstrom P, Brannstrom T, Siesjo P, Benjersheim AT. Radiotherapy induces an immediate inflammatory reaction in malignant glcoma: a clinical microdialysis study. J Neurooncol (2017) 131(1):83–92. doi: 10.1007/s11060-016-2271-1

40. Grossman SA, Ye X, Lesser G, Sloan A, Carraway H, Desideri S, et al. Immunosuppression in patients with high-grade gliomas treated with radiation and temozolomide. Clin Cancer Res (2011) 17(16):5473–80. doi: 10.1158/1078-0432.CCR-11-0774

41. Ginzras MC, Roussel E, Bruner JM, Branch CD, Moser RP. Comparison of cell adhesion molecule expression between glioblastoma multiforme and autologous normal brain tissue. J Neuroimmunol (1995) 57(1-2):143–53. doi: 10.1016/0165-7287(94)00178-Q

42. Piao Y, Henry V, Tao N, Park SY, Martinez-Ledesma J, Dong JW, et al. Targeting intercellular adhesion molecule-1 prolongs survival in mice bearing bvacizumab-resistant glioblastoma. Oncotarget (2017) 8(57):96970–83. doi: 10.18632/oncotarget.18859

43. Kamran N, Kadiyala P, Saxena M, Candolli M, Li Y, Moreno-Ayala MA, et al. Immunosuppressive Myeloid Cells’ Blockade in the Glioma Microenvironment Enhances the Efficacy of Immune-Stimulatory Gene Therapy. Mol Ther (2017) 25(1):232–48. doi: 10.1016/j.ymthe.2016.10.003

44. Demaria S, Formenti SC. Role of T lymphocytes in tumor response to radiotherapy. Front Oncol (2012) 2:95. doi: 10.3389/fonc.2012.00095

45. Formenti SC, Demaria S. Radiation therapy to convert the tumor into an in situ vaccine. Int J Radiat Oncol Biol Phys (2012) 84(4):879–80. doi: 10.1016/j.ijrobp.2012.06.020

46. Stilman BN, Hu D, Pang M, Brewer CE, Johnson P, Liu FT, et al. Galectin-3 is a predictive biomarker for glioblastoma. J Pathol (2013) 229(2):540–9. doi: 10.1002/path.4342

47. Kovacs-Solyom F, Blasko A, Fajka-Boja R, Katona RL, Vegh L, Novak J, et al. Galectin-3 is a predictive biomarker for glioblastoma. J Pathol (2013) 229(2):540–9. doi: 10.1002/path.4342

48. Stillman BN, Hu D, Pang M, Brewer CE, Johnson P, Liu FT, et al. Galectin-3 is a predictive biomarker for glioblastoma. J Pathol (2013) 229(2):540–9. doi: 10.1002/path.4342

49. Sullivan MA, Wischhusen J, Friese MA, Mittelbronn M, Meyermann R, Weller M. HLA-E protects glioma cells from NKG2D-mediated immune responses in vitro: implications for immune escape in vivo. J Neuropathol Exp Neurol (2005) 64(6):523–8. doi: 10.1093/jnen/64.6.523
Pearson et al. Immune Escape in Glioblastoma Multiforme

Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Brad Mbongue JC, Nicholas DA, Torrez TW, Kim NS, Firek AF, Langridge WH.
Xue S, Song G, Yu J. The prognostic significance of PD-L1 expression in patients with glioma: A meta-analysis. Sci Rep (2017) 7(1):4231–017-04023-x. doi: 10.1038/s41598-017-04023-x.
Han MZ, Wang S, Zhao WB, Ni SL, Yang N, Kong Y, et al. Immune checkpoint molecule herpes virus entry mediator is overexpressed and associated with poor prognosis in human glioblastoma. ElBioMedicine (2019) 43:159–70. doi: 10.1016/j.ebiom.2019.04.002.
Mbungue JC, Nicholas DA, Torrez TW, Kim NS, Firek AF, Langridge WH. The Role of Indoleamine 2, 3-Dioxygenase in Immune Suppression and Autoimmunity. Vaccines (Basel) (2015) 3(1):703–29. doi: 10.3390/vaccines3030703.
Mitsui K, Watanuki T, Satoh E, Ashara T, Horikoshi T, Kinouchi H. Expression of indoleamine 2,3-dioxygenase and correlation with pathological malignancy in gliomas. Neurosurgery (2013) 72(6):1031–8; discussion 1038-9. doi: 10.1227/NEU.0b013e31828cf945.
Luwor RB, Stylli SS, Kaye AH. The role of Stat3 in glioblastoma multiforme. EBioMedicine (2015) 17(8):1064–70. doi: 10.1016/j.ebiom.2019.09.002.
Kudo M, Jono H, Shinriki S, Yano S, Nakamura H, Makino K, et al. IDO expression in brain tumors increases the infiltration of CD4+CD25+FOXP3+ regulatory T cells: implications for immunotherapy. Front Immunol (2011) 2:231–8. doi: 10.3389/fimmu.2011.00383-8.
Kotenko SV, Saccani S, Izotova LS, Mirochnitchenko OV, Pestka S. Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci USA (2000) 97(4):1695–700. doi: 10.1073/pnas.97.4.1695.
Chang WL, Barry PA. Attenuation of innate immunity by cytomegalovirus IL-10 establishes a long-term deficit of adaptive antiviral immunity. Proc Natl Acad Sci USA (2010) 107(52):22647–52. doi: 10.1073/pnas.101379410.
Brown NF, Carter TJ, Ottaviani D, Mulholland P. Harnessing the immune system in glioblastoma. Br J Cancer (2018) 119(10):1171–81. doi: 10.1038/s41416-018-0258-8.
Mittelbronn M, Platten M, Zeiner P, Dombrowsky W, Frank B, Zachskorn C, et al. Macrophage migration inhibitory factor (MIF) expression in human malignant gliomas contributes to immune escape and tumour progression. Acta Neuropathol (2011) 122(3):355–65. doi: 10.1007/s00401-011-0858-3.
Neurath KM, Kougou MP, Mikkelsen T, Claffey KP. AMP-dependent protein kinase alpha 2 isoform promotes hypoxia-induced VEGF expression in human glioblastoma. Glia (2006) 53(7):733–43. doi: 10.1002/glia.20326.
Oka N, Soeda A, Inagaki A, Onodera M, Maruyama H, Har a A, et al. VEGF promotes tumorigenesis and angiogenesis of human glioblastoma stem cells. Biochem Biophys Res Commun (2007) 360(3):533–9. doi: 10.1016/j.jbc.2006.09.094.
Wu T, Luo Q, Ouyang G. Periostin: a potent chemotactic factor for recruiting tumor-associated macrophage. Protein Cell (2015) 6(4):225–7. doi: 10.1007/s13238-015-0141-9.
Alterman RL, Stanley ER. Colony stimulating factor-1 expression in human gliomas. Mol Chem Neuropathol (1994) 21(2-3):177–88. doi: 10.1010/ f02815350.
Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Qual LF, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nat Med (2013) 19(10):1264–72. doi: 10.1038/nm.3337.
Lu T, Tian L, Han Y, Vogelbaum M, Stark GR. Dose-dependent cross-talk between the transforming growth factor-beta and interleukin-1 signaling pathways. Proc Natl Acad Sci USA (2007) 104(11):4365–70. doi: 10.1073/pnas.070118104.
Cowan EP, Pierce ML, Dhib-Jalbut S, Verrelle P, Villafranca EJ, Momma K, et al. Antitumor effect of humanized anti-interleukin-6 receptor antibody tocilizumab on glioma cell proliferation. Mol Med (2019) 25(6):584–95. doi: 10.1093/necds/nos014.
Lohr J, Ratiliff T, Huppertz A, Ge Y, Dicots C, Ahmadl R, et al. Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-beta. Clin Cancer Res (2011) 17(13):4296–308. doi: 10.1158/1078-0432.CCR-10-2557.
Bohdmer S, Strommer K, Frei K, Siegel C, de Tribollet 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(10):3229–36.
Platten M, Wick W, Weller M. Malignant glioma biology: role for TGF-beta in growth, motility, angiogenesis, and immune escape. Micros Res Tech (2001) 52(4):401–10. doi: 10.1002/1097-029X(20010215)52:4<401::AID-JEMT1025>3.0.CO;2-C.
Coppes KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. J Immunol (2008) 180(9):5771–7. doi: 10.4049/jimmunol.180.9.5771.
Huettner C, Paulus W, Roggendorf W. Messenger RNA expression of the immunosuppressive cytokine IL-10 in human gliomas. Am J Pathol (1995) 146(2):317–22.
Wagner S, Czub S, Greif M, Vinc H, Suss N, Kerka S, et al. Microglial/macrophage expression of interleukin 10 in human glioblastomas. Int J Cancer (1999) 82(1):12–6. doi: 10.1002/(SICI)1097-0215(19990702)82:1<12::AID-JICC3>3.0.CO;2-O.
Gobbs CB, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH, et al. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res (2002) 62(12):3347–50.
Lucas KG, Bao L, Bruggeman R, Dunham K, Specht C. The Detection of CMV pp65 and IE1 in glioblastoma multiforme. J Neurooncol (2011) 103 (2):231–8. doi: 10.1007/s11060-010-0386-3.
Pfisterer C, Marsault C, Audibert C, Dugoujon J-M, Desbois C, Deplanque D, et al. Glioblastoma cells release interleukin 6 in vivo and in vitro. Cancer Res (1990) 50(20):6683–8.
Tschirkov A, Rohlhorn C, Bertrand S, Doré JF, Dubost JJ, Verrelle P, et al. Soluble factors secreted by glioblastoma cell lines facilitate recruitment, survival, and expansion of regulatory T cells: implications for immunotherapy. Neuro Oncol (2013) 15(4):584–95. doi: 10.1093/neocep/014.
Lohr J, Ratiliff T, Huppertz A, Ge Y, Dicots C, Ahmadl R, et al. Effector T-cell infiltration positively impacts survival of glioblastoma patients and is impaired by tumor-derived TGF-beta. Clin Cancer Res (2011) 17(13):4296–308. doi: 10.1158/1078-0432.CCR-10-2557.
Bohdmer S, Strommer K, Frei K, Siegel C, de Tribollet 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(10):3229–36.
Platten M, Wick W, Weller M. Malignant glioma biology: role for TGF-beta in growth, motility, angiogenesis, and immune escape. Micros Res Tech (2001) 52(4):401–10. doi: 10.1002/1097-029X(20010215)52:4<401::AID-JEMT1025>3.0.CO;2-C.
Coppes KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. J Immunol (2008) 180(9):5771–7. doi: 10.4049/jimmunol.180.9.5771.
113. Rudnick JD, Fink KL, Landolfi JC, Markert J, Piccioni DE, Glantz MJ, et al. Immunological targeting of CD133 in recurrent glioblastoma: A multi-center phase I translational and clinical study of autologous CD133 dendritic cell immunotherapy. JCO (2017) 35(15):2059–2059. doi: 10.1200/JCO.2017.35.15_suppl.2059

114. Vik-Mo EO, Nyakas M, Mikkelsen BV, Moe MC, Due-Tennesen P, Suso EM, et al. Therapeutic vaccination against autologous cancer stem cells with mRNA-transfected dendritic cells in patients with glioblastoma. Cancer Immunol Immunother (2013) 62(9):1499–509. doi: 10.1007/s00262-013-1453-3

115. Akasaki Y, Kikuchi T, Homma S, Koido S, Ohkusa T, Tasaki T, et al. Phase I/II trial of combination of temozolomide chemotherapy and immunotherapy with fusions of dendritic and glioma cells in patients with glioblastoma. Cancer Immunol Immunother (2016) 65(12):1499–509. doi: 10.1007/s00262-016-1905-7

116. Berneman ZN, Anguille S, Willemen Y, de Velde AV, Germonpré P, Huizing M, et al. Vaccination of cancer patients with dendritic cells electroporated with mRNA encoding the wilms’ tumor 1 protein (WT1): correlation of clinical effect and overall survival with T-cell response. Cytotherapy (2019) 21(5, Supplement):S10. doi: 10.1046/j.jcct.2009.03.065

117. Wen PY, Reardon DA, Armstrong TS, Phuphanich S, Aiken RD, Landolfi JC, et al. A Randomized Double-Blind Placebo-Controlled Phase II Trial of Dendritic Cell Vaccine ICT-107 in Newly Diagnosed Patients with Glioblastoma. Clin Cancer Res (2019) 25(19):5797–809. doi: 10.1158/1078-0432.CCR-19-0261

118. Inoges S, Tejada S, de Cerio AL, Gallego Perez-Larraya J, Espinós I, Idoate MA, et al. A phase II trial of autologous dendritic cell vaccination and radiochemotherapy following fluorescence-guided surgery in newly diagnosed glioblastoma patients. J Transl Med (2017) 15(1):104–1202-z. doi: 10.1186/s12967-017-1202-z

119. Pellegratta S, Eoli M, Cuccarini V, Fudol P, Bessina S, et al. Survival gain in glioblastoma patients treated with dendritic cell immunotherapy is associated with increased NK but not CD6(+) T cell activation in the presence of adjuvant temozolomide. Onc immunology (2018) 7(4):e1412901. doi: 10.1080/2162402X.2017.1412901

120. Buchroithner J, Erhart F, Pichler J, Widhalm G, Preusser M, Stockhammer G, et al. Audient Immunotherapy Based on Dendritic Cells Has No Effect on Overall and Progression-Free Survival in Newly Diagnosed Glioblastoma: A Phase II Randomized Trial. Cancers (Basel) (2018) 10(10):372. doi: 10.3390/cancers10100372

121. Erhart F, Buchroithner J, Reitermaier R, Fischhuber K, Klingenbrunner S, Slama I, et al. Immunological analysis of phase II glioblastoma dendritic cell vaccine (Audient) trial: immune system characteristics influence outcome and Adjuvanted up-regulates Th1-related immunomarkers. Acta Neuropathol Commun (2018) 6(1):135–018-0621-2. doi: 10.1186/s40478-018-0621-2

122. Farol CE, Fisher JL, Hampton TH, Lallana EC, Li Z, Guy J, et al. Immune response in patients with newly diagnosed glioblastoma multiforme treated with intranodal autologous tumor lysate-dendritic cell vaccination after radiation chemotherapy. J Immunother (2011) 34(4):382–9. doi: 10.1097/01.jiol.0b013e318215e300

123. Yao Y, Luo F, Tang G, Chen D, Qin Z, Hua W, et al. Molecular subgroups and B7-H4 expression levels predict responses to dendritic cell vaccines in glioblastoma: an exploratory phase II clinical trial. Cancer Immunol Immunother (2018) 67(11):1777–88. doi: 10.1007/s00262-018-2327-y

124. Rapp M, Grauer OM, Kamp M, Sevens N, Zott N, Sabel M, et al. A randomized controlled phase II trial of vaccination with lysate-loaded, mature dendritic cells integrated into standard radiochemotherapy of newly diagnosed glioblastoma (GlioVax): study protocol for a randomized controlled trial. Trials (2018) 19(1):293–018-2659-7. doi: 10.1186/s13063-018-2659-7

125. Huang YC, Chang CN, Wei KC, Lee HC, Chen CC, Cho DY, et al. Atimmune-42. Safety and Efficacy of Autologous Dendritic Cells/tumor Cell Antigen Adjuvant Therapy of Glioblastoma Multiforme: Results of 59 Cases. Neuro Oncol (2014) 20(Suppl 6):vii11. doi: 10.1093/neuonc/nou148.037

126. Liu LM, Ashkan K, Tran DD, Campion 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(1):142–018-1507-6. doi: 10.1186/s12967-018-1507-6

127. Bota DA, Chung J, Dandekar M, Carrillo JA, Kong XT, Fu BD, et al. Phase II study of ERC1671 plus bevacizumab versus bevacizumab plus placebo in recurrent glioblastoma: interim results and correlations with CD4(+) T-lymphocyte counts. CNS Oncol (2018) 7(3):CNS22–2018-0009. doi: 10.2217/ CNS-2018-0009

128. Crane CA, Han SJ, Ahn B, Oehlke J, Kivett V, Fedoroff A, et al. Individual patient-specific immunity against high-grade glioma after vaccination with autologous tumor derived peptides bound to the 96 KD chaperone protein. Clin Cancer Res (2013) 19(1):205–14. doi: 10.1158/1078-0432.CCR-11-3358

129. Bloch O, Raizer JJ, Lim M, Sughrue M, Komorat R, Abrahams J, et al. Newly diagnosed glioblastoma patients treated with an autologous heat shock protein peptide vaccine. PD-L1 expression and response to therapy. J Clin Oncol (2015) 33(15_suppl):2011–1. doi: 10.1200/jco.2015.33.15_suppl.2011

130. Bloch O, Crane CA, Fuku K, Kaur R, Aghik MG, Berger MS, et al. Heat-shock protein peptide complex-96 vaccination for recurrent glioblastoma: a phase II, single-arm trial. Neuro Oncol (2014) 16(2):274–9. doi: 10.1093/neuonc/not203

131. Blumenthal D, Yalon M, Vainer GW, Lossos A, Yust S, Tzach L, et al. Pembrolizumab: first experience with recurrent primary central nervous system (CNS) tumors. J Neurooncol (2016) 129(3):453–60. doi: 10.1007/s11060-016-2190-1
161. Chamberlain MC, Kim BT. Nivolumab for patients with recurrent glioblastoma progressing on bevacizumab: a retrospective case series. J Neurooncol (2017) 133(3):561–9. doi: 10.1007/s11060-017-2466-0

162. Filley AC, Henriquez M, Dey M. Recurrent glioma clinical trial, CheckMate-143: the game is not over yet. Oncotarget (2017) 8(53):91779–94. doi: 10.18632/oncotarget.21586

163. Cloughesy TF, Mochizuki AY, Orpilla JR, Hugo W, Lee AH, Davidson TB, et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. Nat Med (2019) 25(3):477–86. doi: 10.1038/s41591-018-0337-7

164. Antonios JP, Soto H, Everson RG, Orpilla J, Moughon D, Shin N, et al. PD-1 blockade enhances the vaccination-induced immune response in glioma. JCI Insight (2016) 1(10):e87059. doi: 10.1172/jci.insight.87059

165. Antonios JP, Soto H, Everson RG, Moughon D, Orpilla JR, Shin NP, et al. Immunosuppressive tumor-infiltrating myeloid cells mediate adaptive immune resistance via a PD-1/PD-L1 mechanism in glioblastoma. Neuro Oncol (2017) 19(6):796–807. doi: 10.1093/neuonc/now287

166. Soderlund J, Erhardt S, Kast RE. Acyclovir inhibition of IDO to decrease Tregs as a glioblastoma treatment adjunct. J Neuroinflammation (2010) 7:44–2094-7–44. doi: 10.1186/1742-2094-7-44

167. Wainwright DA, Chang AL, Dey M, Balyasnikova IV, Kim CK, Tobias A, et al. Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1 in mice with brain tumors. Clin Cancer Res (2014) 20(20):5290–301. doi: 10.1158/1078-0432.CCR-14-0514

168. Migliorini D, Dietrich PY, Stupp R, Linette GP, Posey AD, June CH. CART cells are prone to Fas- and DR5-mediated cell death. J Immunother Cancer (2015) 3(Suppl 2):S11-42. doi: 10.1186/2051-1426-3-S2-O11

169. Brown CE, Alizadeh D, Starr R, Weng L, Wagner JR, Naranjo A, et al. Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy. N Engl J Med (2016) 375(26):2561–9. doi: 10.1056/NEJMoa1610497

170. Hyrenius-Wittsten A, Roybal KT. Paving New Roads for CARs. Neuro Oncol (2019) 21(7):1426–3-S2-O11. doi: 10.1186/2051-1426-3-S2-O11

171. Ostertag D, Amundson KK, Lopez Espinoza F, Martin B, Buckley T, Galvão EF, et al. Toca 511 gene transfer and treatment with the prodrug, 5-fluorocytosine, promotes durable antitumor immunity in a mouse glioma model. J Immunother Cancer (2019) 7:4462. doi: 10.1158/2159-8290.CD-19-0192-1

172. Friedan G, Bag A, Madan-Sawin LI, Li R, Chakravarthi K, Orsorio D, et al. IMM08-0008. Phase 1 TRIAL (NCT02457845) SAFETY, TOLERABILITY AND PRELIMINARY EFFICACY OF IMMUNOVIROTHERAPY WITH HSV G207 IN CHILDREN WITH PROGRESSIVE MALIGNANT SUPRATENTORIAL BRAIN TUMORS. Neuro Oncol (2018) 20(10):1137–40. doi: 10.1093/neuonc/nopy59.324

173. Geletyeky S, Huesing J, Rommelaere J, Schlehofer JR, Leuchs B, Dahm M, et al. Phase II/a study of intratumoral/intracerebral or intravenous/intracranial administration of Parvovirus H-1 (ParvOryx) against WHO grade IV malignant glioma (MG) clinical trial compared to historical controls. JCO (2016) 34(15):2061–6. doi: 10.1200/JCO.2016.34.15_suppl.2061

174. Marchini A, Daefler L, Pozdevze VI, Angelova A, Rommelaere J. Immune Conversion of Tumor Microenvironment by Oncolytic Viruses: The Protoparvovirus H-1PV Case Study. Front Immunol (2019) 10:1848. doi: 10.3389/fimmu.2019.01848

175. Wollmann G, Ozduman K, van den Pol AN. Oncolytic virus therapy for glioblastoma multiforme: concepts and candidates. Cancer J (2012) 18(1):69–89. doi: 10.1097/PPO.0b013e3182671c9

176. Desjardins A, Sampson JH, Peters KB, Vlahovic G, Randazzo D, Threart S, et al. Patient survival on the dose escalation phase of the Oncolytic Polio/ Rhinovirus Recombinant (PVSRIPO) against WHO grade IV malignant glioma (MG) clinical trial compared to historical controls. JCO (2016) 34(15):2061–6. doi: 10.1200/JCO.2016.34.15_suppl.2061

177. Hyrenius-Wittsten A, Roybal KT. Paving New Roads for CARs. Neuro Oncol (2019) 21(7):1426–3-S2-O11. doi: 10.1186/2051-1426-3-S2-O11

178. O’Rourke DM, Nasrallah MP, Desai A, Melenhorst JJ, Mansfield K, Morrisette JF, et al. A single dose of peripherally infused EGFRVIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Trans Med (2017) 9(599):eaao9884. doi: 10.1126/scitranslmed.aao9884

179. Bagley S, Desai A, Binder Z, Nasrallah M, Hwang W, Maloney-W lensky E, et al. RTBT-12. A PHASE 1 STUDY OF EGFRVIII-DIRECTED CAR T CELLS COMBINED WITH PD-1 INHIBITION IN PATIENTS WITH NEWLY, DIAGNOSED, MGMT-UNMETHYLATED GliOBlastoma: TRIAL IN PROGRESS. Neuro-oncology (2019) 21(1):221–1. doi: 10.1093/neuonc/noz175.923

180. Jansen T, Tyler B, Mankowski JL, Recinos VR, Pradilla G, Legnani F, et al. Fasl gene knock-down strategy enhances the antiangiogenic immune response. Neuro Oncol (2010) 12(5):482–9. doi: 10.1093/neuonc/nop032

181. Thucui BO, Dumaouthier N, Marti B, Zhang L, Lanitis E, Irving M, et al. CAR-T cells are prone to Fas- and DR5-mediated cell death. J Immunother Cancer (2018) 6(1):71–018-0358-z. doi: 10.1186/s40425-018-0410-2

182. Yamamoto TN, Lee PH, Vodnala SK, Gurusamy D, Kishon RJ, Yu Z, et al. T cells genetically engineered to overcome death signaling enhance adoptive cancer immunotherapy. J Clin Invest (2019) 129(4):1551–65. doi: 10.1172/JCI121491

183. Kloss CC, Lee J, Zhang A, Chen F, Melenhorst JJ, Lacey SF, et al. Dominant-Negative TGF-beta Receptor Enhances PSMA-Targeted Human CAR T Cell Proliferation And Augments Prostate Cancer Eradication. Mol Ther (2019) 27(6):1855–66. doi: 10.1038/s41394-019-0235-0

184. Rafiq S, Yeku OO, Jackson HJ, Purdon TJ, van Leeuwen DG, Drakes DJ, et al. Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. Nat Biotechnol (2018) 36(9):847–56. doi: 10.1038/nbt.4195

185. Kothari NE, Purdon TJ, van Leeuwen DG, Lopez AV, Curran KJ, Danyian AF, et al. CD40 Ligand-Modified Chimeric Antigen Receptor T Cells Enhance Antitumor Function by Eliciting an Endogenous Antitumor Response. Cancer Cell (2019) 35(3):473–488.e6. doi: 10.1016/j.ccell.2019.02.006

186. Choi BD, Yu X, Castano AP, Bouaff A, Schmidt A, Larson RC, et al. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. Nat Biotechnol (2019) 37(9):1049–58. doi: 10.1038/s41587-019-0192-1

187. Ruella M, Klichinsky M, Kenderian SS, Shestova O, Zobel A, Kraft DO, et al. Overcoming the Immunosuppressive Tumor Microenvironment of Hodgkin Lymphoma Using Chimeric Antigen Receptor T Cells. Cancer Discovery (2019) 7(10):1154–67. doi: 10.1158/2326-066X.CD-18-0850

188. Pearson et al. Immune Escape in Glioblastoma Multiforme

Frontiers in Immunology | www.frontiersin.org 24 October 2020 | Volume 11 | Article 582106
235. St-Amour I, Paré I, Alata W, Coulombe K, Ringuette-Goulet C, Drouin-Ouellet J, et al. Brain bioavailability of human intravenous immunoglobulin and its transport through the murine blood-brain barrier. *J Cereb Blood Flow Metab* (2013) 33(12):1983–92. doi: 10.1038/jcbfm.2013.160

236. Yu YJ, Watts RJ. Developing therapeutic antibodies for neurodegenerative disease. *Neurotherapeutics* (2013) 10(3):459–72. doi: 10.1007/s13311-013-0187-4

237. Yu YJ, Atwal JK, Zhang Y, Tong RK, Wildsmith KR, Tan C, et al. Therapeutic bispecific antibodies cross the blood–brain barrier in nonhuman primates. *Sci Transl Med* (2014) 6(261):261ra154. doi: 10.1126/scitranslmed.3009835

238. Boado RJ, Zhang Y, Zhang Y, Pardridge WM. Humanization of anti-human insulin receptor antibody for drug targeting across the human blood-brain barrier. *Biotechnol Bioeng* (2007) 96(2):381–91. doi: 10.1002/bit.21120

239. Niewoehner J, Bohrmann B, Collin L, Urich E, Sade H, Maier P, et al. Increased brain penetration and potency of a therapeutic antibody using a monovalent molecular shuttle. *Neuron* (2014) 81(1):49–60. doi: 10.1016/j.neuron.2013.10.061

240. Ullman JC, Arguello A, Getz JA, Bhalla A, Mahon CS, Wang J, et al. Brain delivery and activity of a lysosomal enzyme using a blood-brain barrier transport vehicle in mice. *Sci Transl Med* (2020) 12(545):eaay1163. doi: 10.1126/scitranslmed.aay1163

241. Kariolis MS, Wells RC, Getz JA, Kwan W, Mahon CS, Tong R, et al. Brain delivery of therapeutic proteins using an Fc fragment blood-brain barrier transport vehicle in mice and monkeys. *Sci Transl Med* (2020) 12(545):eaay1359. doi: 10.1126/scitranslmed.aay1359

242. Galstyan A, Markman JL, Shatalova ES, Chiuchi A, Korman AJ, Patil R, et al. Blood-brain barrier permeable nano immunoconjugates induce local immune responses for glioma therapy. *Nat Commun* (2019) 10(1):3850–019-11719-3. doi: 10.1038/s41467-019-11719-3

243. Kinoshita M, McDannold N, Jolesz FA, Hynynen K. Noninvasive localized delivery of Herceptin to the mouse brain by MRI-guided focused ultrasound-induced blood-brain barrier disruption. *Proc Natl Acad Sci USA* (2006) 103 (31):11719–23. doi: 10.1073/pnas.0604318103

244. Raymond SR, Treat LH, Dewey JD, McDannold NJ, Hynynen K, Bacskai BJ. Ultrasound enhanced delivery of molecular imaging and therapeutic agents in Alzheimer’s disease mouse models. *PloS One* (2008) 3(5):e2175. doi: 10.1371/journal.pone.0002175

245. Burgess A, Shah K, Hough O, Hynynen K. Focused ultrasound-mediated drug delivery through the blood-brain barrier. *Expert Rev Neurother* (2015) 15(5):477–91. doi: 10.1586/14737175.2015.1028369

246. Cohen-Inbar O, Xu Z, Sheehan JP. Focused ultrasound-aided immunomodulation in glioblastoma multiforme: a therapeutic concept. *J Ther Ultrasound* (2016) 4:2–016-0046-y. doi: 10.1186/s40349-016-0046-y

Conflict of Interest: Author LD is a director and shareholder of Scancell Ltd and is a named inventor on the ImmunoBody patents.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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