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Current advances in PD-1/PD-L1 axis-related tumour-infiltrating immune cells and therapeutic regimens in glioblastoma

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ABSTRACT

Glioblastoma (GBM) is the most common malignant tumour in the brain, and current treatments are not curative and cannot control recurrence. This limitation indirectly places immunotherapy at the focus of translational GBM research. Many studies on the PD-1/PD-L1 axis in GBM are ongoing, and the immunosuppressive mechanism of PD-1/PD-L1 in GBM is different from that in other solid tumours. This review focuses on the effect of the PD-1/PD-L1 axis on infiltrating immune cells in the suppressive GBM immune microenvironment and summarizes the recent progress in PD-1/PD-L1 axis-related therapies reported in preclinical and clinical GBM studies, providing a reference for the systematic study of PD-1/PD-L1 axis-related anti-GBM immunity.

1. Introduction

Glioblastoma (GBM) is the most common and uniformly fatal malignancy of the central nervous system (CNS) in adults, with a median age at diagnosis of 64.0 years and a median overall survival (OS) time of approximately 14 − 17 months. The standard of care (SOC) for the treatment of GBM according to the Chinese Glioma Cooperative Group (CGCG) clinical treatment guidelines includes maximal safe surgical resection and the Stupp chemoradiotherapy regimen (Jiang et al., 2016). Unfortunately, all surviving patients ultimately experience relapse (Weller et al., 2014). Although newer approaches, including nanomedicine and therapies that exploit tumour-specific signalling, have emerged, none of the current approaches can effectively prolong survival after relapse (Ozdemir-Kaynak et al., 2018). Due to the intracranial localization, molecular heterogeneity, high recurrence rates, and overall resistance to therapy of GBM tumours, it is difficult to develop an effective GBM treatment. Increasing evidence supports the existence of a dynamic interaction between the CNS and the systemic immune system, so the traditional principle of the CNS being an immune-privileged site has been abandoned. PD-1 and its ligand PD-L1 have important impacts on antitumour immunity by regulating the formation, survival and function of immune cells in many solid tumours, which we call the immune PD-1/PD-L1 axis. To date, increasing numbers of explorations of the PD-1/PD-L1 axis have been conducted in GBM (Litak et al., 2019; Wang et al., 2019a). These results suggest that the PD-1/PD-L1 axis tightly links GBM cells with tumour-infiltrating immune cells, which can be treated as a potentially valid mechanism by which immunogenic tumours escape host immune system attack, and blocking the interaction between PD-1 and PD-L1 may lead to an effective immunotherapeutic strategy for GBM. In GBM, tumour-infiltrating immune cells are not equivalent to tumour-infiltrating lymphocytes (TILs; T cells, B cells and NK cells), as this population contains tumour-infiltrating macrophages and myeloid-derived suppressor cells (MDSCs) (Gabrilovich, 2017; Gordon et al., 2017).

At present, in some related PD-1/PD-L1 reviews, the relevant cited mechanisms are often pan-neoplastic in nature, but GBM is different from other solid tumours in terms of location and immune characteristics. As a supplement to the current research system, this paper reviews the multi-dimensional nature of the PD-1/PD-L1 axis regarding immune cell infiltration into the GBM tumour microenvironment (TME), as well as the recent progress in preclinical and clinical research.

2. PD-1/PD-L1 expression in GBM patients

In the setting of GBM, PD-1 is expressed on T cells, B cells, tumour-associated macrophages (TAMs), MDSCs, and NK cells (Agata et al., 1996; Nam et al., 2019; Pesce et al., 2017; Vibhakar et al., 1997; Wang et al., 2019b), suggesting that PD-1 expression is a universal phenomenon involved in the innate and adaptive arms of the immune systems that takes part in forming the suppressive tumour environment. PD-L1 is located on the surface of glioma cells and is simultaneously expressed

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in TAMs, B cells and MDSCs (Kohanbash and Okada, 2012; Lee-Chang et al., 2019; Pardoll, 2012). Thus, constitutive PD-L1 expression on GBM tumour cells can induce widespread immunosuppression and alter the TME. The proneural and glioma-CpG island methylator phenotype (G-CIMP) subtypes of GBM always present with low PD-L1 expression, while the mesenchymal subtype is more prone to high PD-L1 expression. Currently, detection of the expression level of PD-L1 by immunohistochemistry is still the most commonly used method to determine which patients are most likely to benefit from anti-PD-1/PD-L1 therapy. For the proportion of cells with positive PD-L1 expression in GBM, the results from different research centres vary widely, ranging from 7.8%–88% (Berghoff et al., 2015; Peng et al., 2019). Part of the reason for the large differences in expression is because different studies use different definitions of the cut-off value for positive PD-L1 expression; another reason is related to sample differences in the selected patients, such as the proportions of patients with different pathological molecular subtypes in the study arm. Given the reasons mentioned above, a consensus has not yet been reached on the prognostic value of PD-L1 expression in GBM, and the results of different health centres are inconsistent (Berghoff et al., 2015; Han et al., 2017; Nduom et al., 2016). The key molecules and signalling pathways related to the expression level of PD-L1 in GBM tumour cells are summarized in Table 1.

3. PD-1/PD-L1 axis-related tumour-infiltrating immune cell events in GBM

The PD-1/PD-L1 axis has important effects on GBM tumour-infiltrating immune cells. Recently, many new concepts in this field have emerged (Fig. 1).

3.1. GBM-infiltrating T cells

Currently, the most recognized immunosuppressive effect of the PD-1/PD-L1 axis on GBM is the induction of functional exhaustion, apoptosis and anergy in tumour-infiltrating CD8 + T cells (cytotoxic T lymphocytes (CTLs)). This mechanism of action has been demonstrated in a number of different in vivo and in vitro assays. During GBM tumourigenesis, CTLs can be recruited to the intracranial location of tumourigenesis in an invasive manner; however, the invasive T cells that have tumour-killing functions often develop a functional exhaustion or anergy phenotype in the TME. This phenotypic defect should be closely related to TCR-mediated signalling changes in the infiltrating T cells from the perspective of tumour-specific antigen recognition (Morford et al., 1997). The PD-1/PD-L1 signalling axis can be used as a driving trigger of the cascade reaction generated after TCR signalling is activated in CTLs. Once the TCR binds to the cognate tumour-specific antigen, if there is binding of PD-1 on the corresponding T cells with PD-L1 expressed on the surface of the tumour cells, then the intracellular tyrosine of PD-1 is phosphorylated and activated. PD-1 then recruits SHP-1 and SHP-2 to the C-terminal immunoreceptor tyrosine-based switch motif (ITSM), which dephosphorylates the TCR-activated signalling molecules ZAP70 and CD-3ζ, resulting in downstream PI3K/AKT suppression. Inactivation of PI3K/AKT further downregulates the expression of Bcl-2 family genes and promotes CTL apoptosis while inhibiting CTL secretion of cytokines such as IFN-γ, TNF-α and IL-2 (Hofmeyer et al., 2011). The transforming growth factor beta (TGF-β)-Smad3/Satb1 (Stephen et al., 2017), TCR-GSK3 (Taylor et al., 2016) and TCR-Kyn-AhR (Liu et al., 2018) signalling pathways participate in regulating PD-1 expression in CTLs. In the GBM TME, in addition to CTLs, CD4 + T cells and regulatory T cells (Tregs) are also present among the infiltrating T cells. For CD4 + T cells, the PD-1/PD-L1 axis not only plays a role similar to its role in CTLs, which results in the loss of normal functions and dysregulation of cytokine secretion (Goods et al., 2017), but also can induce the transformation of CD4 + T cells into CD4+ CD25+ Foxp3+ Tregs (Francisco et al., 2009), which mediate the regulation of immunosuppressive factors and are also an area of interest in the GBM immune evasion mechanism (Dunn et al., 2004). PD-1 and CCR4 on the cell membrane of Tregs bind to their corresponding ligands PD-L1 and CCL2 on the cell membrane of GBM cells and together promote the immunoregulatory function of Tregs and induce the recruitment of Tregs to the TME (Chang et al., 2016). Tregs inhibit the killing effect of effector T cells on tumours by secreting IL-10, IL-35, other immunosuppressive cytokines and TGF-β (Sundstedt et al., 2003). Preclinical studies have shown that depleting Tregs in the TME enhances the antitumour immune response (Woroniecka et al., 2018).

3.2. GBM-infiltrating B cells

B cells can participate in the immune response by producing antibodies for specific humoural immunity and are also important antigen-presenting cells (APCs) that participate in cellular immunity regulation to promote T cell activation and proliferation (LeBien and Tedder, 2008). Currently, less is known about the mechanism involving infiltrating B cells than about that involving CTLs in antitumour immunity in the GBM microenvironment. The main role of GBM-infiltrating B cells in the construction of the TME is not to produce tumour-specific antibodies by differentiating into plasma cells but to act as APCs to enhance tumour antigen-specific CTL proliferation and T cell-dependent intracranial tumour cell clearance (Candolfi et al., 2011). Further research on the phenotype and function of GBM-infiltrating B cells shows that these B cells harbour an immunosuppressive phenotype characterized by the presence of the inhibitory molecules PD-L1 and CD155 and the production of the immunoregulatory cytokines TGF-β and IL10; these cells are known as GBM regulatory B cells (Bregs) (Lee-Chang et al., 2019). Bregs expressing MHC I (Rafei et al., 2009) exert immunosuppressive effects on activated CTLs, as shown by the inhibition of CD8 + T cell proliferation and further acquisition of an effector phenotype. One characteristic of GBM-associated Bregs is overexpression of TGF-β and IL-10 upon interaction with activated CTLs. This phenomenon may reflect an attempt by Bregs to maintain the immunosuppressive environment to prevent further CD8 + T cell activation upon initial inhibitory contact. In the GBM microenvironment, MDSCs play a fundamental role in supporting Bregs. MDSCs

Table 1
The potential mechanisms related to PD-L1 expression in GBM.

Factors affecting expression	Potential mechanisms in GBM
IFN-γ	IFN-γ binds with its receptor and subsequently activates the JAK/STAT signalling pathway, which leads to the downstream expression and activation of IRF-1, further inducing PD-L1 expression in tumour cells (Qian et al., 2018).
Hypoxia	HIF-1α regulates the expression of PD-L1 by binding directly to a hypoxia response element in the PD-L1 proximal promoter (Noman et al., 2015).
PTEN loss	Activation of the PI3K-Akt-mTOR-S6K1 pathway increases PD-1 expression (Parsa et al., 2007).
DNA promoter methylation	Increased promoter methylation is negatively associated with PD-L1 gene expression (Berghoff et al., 2017).
NF1 loss-of-function	NF1 loss-of-function was positively connected with PD-L1 expression (Heiland et al., 2017).
Non-coding RNAs	miR-34a attenuates glioma cell progression and chemoresistance by targeting PD-L1 (Wang and Wang, 2017).
Growth differentiation factors (GDFs)	GDF15 enhances PD-L1 expression via the Smad2/3 pathway in GBM (Peng et al., 2019).

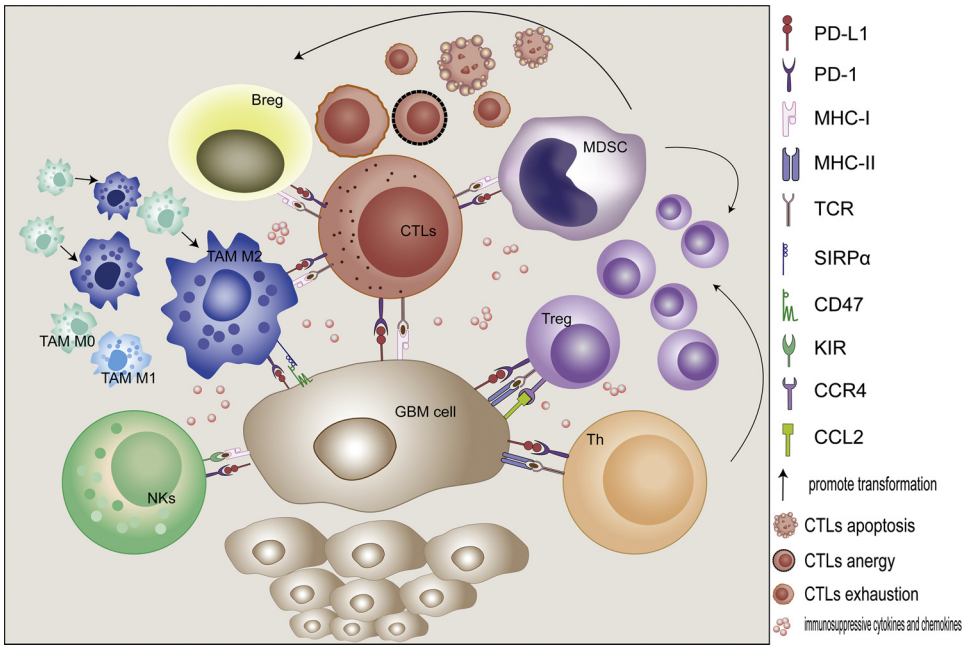

Fig. 1. The immunosuppressive GBM microenvironment-related PD-1/PD-L1 axis is mainly composed of tumour cells, CTLs, CD4 + T cells, Tregs, Bregs, TAMs, MDSCs, NK cells and the cytokines and chemokines secreted in response to PD-1/PD-L1 axis-mediated cell-cell interactions. After tumour cells bind to immune cells that recognize MHC class I molecules or MHC class II molecules (CTLs, CD4 + T cells, Tregs, and TAMs), PD-L1 expressed on the tumour cell surface binds to PD-1 expressed on the surface of these immune cells. These cells were "tamed" to become immune cell types suitable for supporting tumour proliferation and invasion. This interaction causes functional exhaustion, anergy and apoptosis in CTLs that initially had tumour-specific killing effects. Infiltrating MDSCs regulate the immune activity of Tregs and Bregs through the combination of PD-L1 and PD-1 on the surface of CTLs, which promotes the formation of a suppressive tumour immune microenvironment. At the same time, Tregs bind to PD-L1 and CCL2 on the tumour cell surface through PD-1 and CCR4, respectively, on the Treg surface, and Bregs bind to PD-1 on CTLs through PD-L1 on the Breg surface, which further promotes the production of immunosuppressive cytokines and chemokines (IL-6, IL-10, TGF-β, etc.).

mediate the transfer of membrane-bound PD-L1 to B cells, resulting in the promotion of B cell-mediated immunosuppression. Further research should be performed to explore the mechanisms and signalling pathway underlying the expression of PD-L1 on GBM Bregs.

3.3. GBM-infiltrating macrophages

TAMs are the predominant infiltrating immune cells in GBM, accounting for approximately 30 % of the cells in a GBM tumour, and have functional plasticity depending on their activated phenotype. Current studies show that TAMs in GBM are predominantly of the immunosuppressive M2 subtype (Li and Graeber, 2012) and play an immunomodulatory role via the secretion of the cytokines IL-6, IL-10 and TGF-β, reducing the phagocytic killing ability of TAMs and upregulating the expression of the cell-surface antigens FasL and PD-L1 during antigen presentation, which allows binding with Fas and PD-1, respectively, on the CTL membrane to initiate programmed cell death in CTLs (Dong et al., 2002). The expression of PD-L1 in tumour-infiltrating macrophages in GBM patients is significantly higher than that in normal controls (Bloch et al., 2013); via the interaction of PD-L1 with PD-1 on CTLs, TAMs transmit an inhibitory signal to T cells to weaken the proliferation of T cells and inhibit the targeted killing of GBM cells (Chen and Hambardzumyan, 2018). TAM PD-L1 expression is regulated by locally produced TNF-α and IL-10 (Bloch et al., 2013; Hartley et al., 2017). Regulation of the mTOR pathway may be one of the main mechanisms by which PD-L1 regulates macrophage functions (Hartley et al., 2018). GBM-infiltrating macrophages also express PD-1. The expression of PD-1 on TAMs is negatively correlated with phagocytosis and the ability to kill tumour cells. Blocking PD-1 in vivo can enhance the phagocytic function of macrophages towards tumour cells and control tumour growth, which was useful as an independent prognostic factor for survival in a mouse GBM model (Gordon et al., 2017). A recent study confirmed co-expression of the CD47 and PD-L1 genes in GBM tumourigenesis (Lian et al., 2019). Although the SIRPα/CD47 axis may be the most important regulatory checkpoint for macrophages (Zhang et al., 2018), the PD-1/PD-L1 axis as a co-stimulatory and co-inhibitory pathway plays an important role in communication between GBM cells and adaptive immune cells.

3.4. GBM-infiltrating MDSCs

MDSCs are a heterogeneous type of cells originating from the myeloid lineage with immunosuppressive properties in GBM. In the TME, cells with a monocytic (M)-MDSC–like phenotype can differentiate into macrophages, suggesting a potential lineage relationship between MDSCs and TAMs (Gabrilovich, 2017). Interestingly, granulocytic (G)-MDSCs are the most common subtype in the blood of patients with GBM; however, in the GBM TME, lineage-negative MDSCs are the most common type of infiltrating MDSC, followed by G-MDSCs and then M-MDSCs (Raychaudhuri et al., 2015). MDSCs account for a high proportion of cells, up to 40 %, in the GBM TME (Kamran et al., 2017), and they are efficient inhibitors of tumour antigen-specific T cell activation and proliferation. The expression of PD-L1 on tumour-infiltrating MDSCs can inhibit the initiation of the antitumour immune response of CTLs via binding of the MDSC-expressed PD-L1 to PD-1 on the surface of each CTL, resulting in functional inactivation of CTLs (Kim et al., 2016). GBM-associated MDSCs promote Breg and Treg functions by delivering microvesicles containing membrane-bound PD-L1, which can be taken up by Bregs and Tregs. The transfer of functional PD-L1 via microvesicles confers the potential for Bregs and Tregs to suppress CD8 + T cell activation and effector phenotype acquisition. At present, research on the regulation of MDSC PD-L1 expression tends to separate into two theories. The first is that in the TME, the expression of PD-L1 in MDSCs is controlled by HIF-1α. HIF-1α enters the nucleus and binds directly to the hypoxia-response element in the proximal promoter of PD-L1 and thus regulates the expression of PD-L1 (Noman et al., 2014). The second is that the expression of PD-L1 in MDSCs is controlled by INF-γ. INF-γ activates pSTAT1 and directly regulates the transcription of IRF1, which regulates the expression of PD-L1 (Lu et al., 2016). Neutrophils, with similar phenotype and morphology as g-MDSCs, are the most abundant white blood cells in the blood (Veglia et al., 2018). Tumour-induced neutrophils have been reported to inhibit T cell proliferation through PD-L1/PD-1 signal transduction in hepatoma (He et al., 2015). Neutrophils are considered to be non-professional APCs. Recent studies have shown that PD-L1 is expressed on the surface of neutrophils and increased after exposure to cytokines (Bankey et al., 2010) in vitro or after production of reactive oxygen

species in vivo (Bowers et al., 2014). However, whether there is an increased expression of PD-L1 on infiltrating neutrophils in patients with GBM has not been reported.

3.5. GBM-infiltrating NK cells

Infiltrating NK cells are an important part of the lymphocytic population in the GBM TME. Because NK cells do not need to recognize MHC-1-presented antigens and there is no process mediating activation, NK cells are considered to be the cell type least likely to be subverted by tumour cells to participate in the immunosuppressive TME (Golan et al., 2018). As research has progressed, NK cells have been found to exhibit a negative immunomodulatory mechanism. Normal human cells express MHC I molecules, which interact with the NK cell inhibitory receptor KIR to prevent self-cells from being recognized as target cells of NK cells and subsequently attacked and killed (Thielens et al., 2012). The MHC I molecules expressed on the surface of GBM cells can also interact with KIR on infiltrating NK cells, which prevents the recognition and destruction of the tumour cells by the infiltrating NK cells to some extent (Boussiotis and Charest, 2018). Recent studies have shown that PD-1 is expressed on the membrane of human NK cells (Pesce et al., 2017) and have identified a correlation between the expression of KIR and the expression of PD-1 on the membrane of NK cells. Based on these findings, some scholars have proposed that there may be a co-expression relationship between the MHC-1/KIR axis and the PD-1/PD-L1 axis in the interaction between NK cells and tumour cells, which mediates tumour cell immune escape by co-activation of these two molecular events (He et al., 2018). A glioma stem-like cell (GSC) study showed that interference with the PD-1/PD-L1 axis enhanced cytotoxicity to GSCs mediated by activated NK cells in mice (Huang et al., 2015). The study of immune suppression in the GBM TME mediated by the combined effects of the MHC-I/KIR and PD-1/PD-L1 axes may lead to paradigm shifts in immunotherapy, but these features require further research.

4. PD-1/PD-L1 axis-related therapeutic regimens in GBM

Among all cancers, GBM is considered to be one of the most immunologically “cold" tumours (Martikainen and Essand, 2019). The PD-1/PD-L1 axis, as one of the major molecular signalling pathways in the inhibition of GBM TME cellular immunity (Pardoll, 2012), represents a potential direction for developing interventions that can convert the "cold" immune environment into a "hot" environment to improve the response rate to antitumour immunotherapy. In GBM, approximately 81 clinical studies on the PD-1/PD-L1 axis were conducted between 2010 and 2020, as documented on the ClinicalTrials.gov website. In terms of the distribution of clinical research, the studies between 2010 and 2014 represent the initial stage of clinical regimen research (Fig. 2a). The first clinical study of PD-1/PD-L1 axis-related therapeutic regimens with GBM as an independent research object began in 2013 (NCT02430363). The PD-1/PD-L1 axis-related therapeutic regimens for GBM involved in a 2010 study represented only a very small part component of the study, which examined the effectiveness of tumour-specific cellular immune responses to pembrolizumab in various metastatic solid tumours (NCT01174121). Since 2015, this type of clinical research has increased significantly. In 2018, there were 21 studies. To date in 2020 (February 2020), 9 studies have been launched (Fig. 2a), indicating that the GBM-related PD-1/PD-L1 axis is currently a popular topic for clinical trials, and as this research increases, these trials will definitely provide important and substantial contributions. In 2014, the first large phase III trial (NCT02017717) was launched to study the efficacy and safety of nivolumab in GBM patients. Unfortunately, the primary outcome of this study showed that compared with bevacizumab, nivolumab did not prolong the OS of patients with primary recurrent GBM; thus, the trial was terminated prematurely. The summary of the experience of this abortive trial has been analysed in detail in many reviews (Kurz and Wen, 2018; Filley et al., 2017), providing valuable experience for future research. The results of this study, such as the differences in the tolerance and toxicity of nivolumab in different subtypes of GBM, are worthy of further study. In 2016, another large phase III trial (NCT02617589) was launched to study the efficacy of nivolumab in patients with the MGMT promoter-unmethylated GBM subtype, and the results are eagerly awaited. Generally, most clinical trials have evaluated the use of antibodies specific for components of the PD-1/PD-L1 axis and have been conducted predominantly in patients with recurrent GBM (Fig. 2b), and most of the results have not provided substantial progress or decisive conclusions (Table 2). The reason for this shortcoming may be that most clinical trials are in either the recruiting or the active, not recruiting status (Table 2), and most of these are still generally in phase 1 or 2 (Fig. 2a) with no available data. Collectively, the results shown in Table 2 prompt us to speculate that PD-1/PD-L1 axis-related therapeutic regimens for GBM still require proof of evidence. Regarding the completed clinical trials, even if the research results are inadequate, these trials provide important references for improving their deficiencies in future research. Regarding the ongoing clinical trials, in the foreseeable future, the results will provide us with an attainable approach to achieve GBM immunotherapy. Therefore, the following part of our review is to analyse completed or ongoing clinical trials of PD-1/PD-L1 axis-related therapeutic regimens for GBM and to discuss treatment options related to the PD-1/PD-L1 axis in anticipation of new potential specific breakthroughs in PD-1/PD-L1 axis-related clinical treatment for GBM in future.

We divide PD-1/PD-L1 axis-related therapeutic regimens into two categories. The first category is direct antibody therapies specifically targeting the PD-1/PD-L1 axis, i.e., directly targeting the PD-1 (most trials) or PD-L1 (fewer trials) protein to prevent ligand-receptor binding

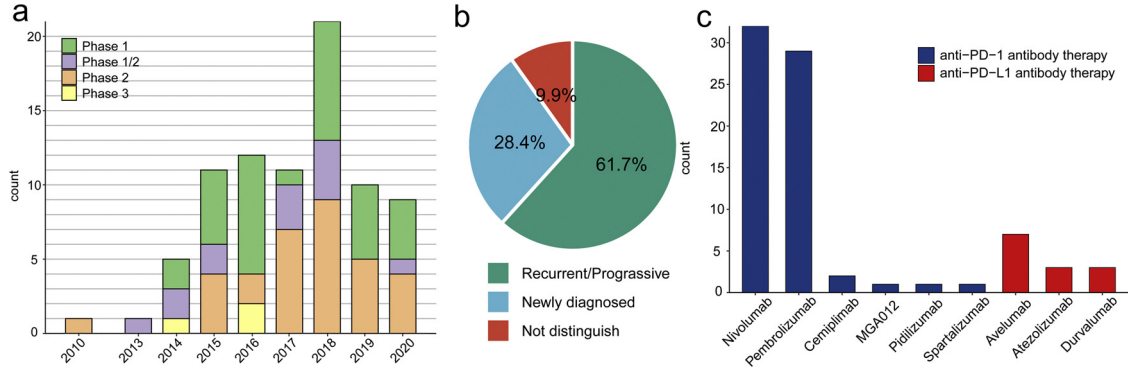

Fig. 2. a. The distribution of the phases of clinical trials related to the PD-1/PD-L1 axis reported on the ClinicalTrials.gov website between 2013 and 2020. b. The website published the different percentages of GBM patients in clinical trials related to the PD-1/PD-L1 axis. c. The distribution of direct therapeutic regimens related to the PD-1/PD-L1 axis reported on the ClinicalTrials.gov website between 2013 and 2020.

Table 2
PD-1/PD-L1 axis-related clinical trials in GBM (The clinical trial information used in this article was retrieved from the clinicaltrials.gov website (the last search was conducted on February 18, 2020).).

Trials	Status	Trial title	No.	Intervention	Phase	Year S/E	Available conclusions
NCT02017717	Active, not recruiting	A Study of the Effectiveness and Safety of Nivolumab Compared to Bevacizumab and of Nivolumab With or Without Ipilimumab in Glioblastoma Patients (CheckMate 143)	626	Nivolumab, Bevacizumab, Ipilimumab	Phase 3	January 27, 2014/ June 17, 2019	The interval analysis results showed that compared with bevacizumab, nivolumab failed to prolong OS in patients with recurrent GBM. The trial was terminated prematurely.
NCT02054806	Active, not recruiting	Study of Pembrolizumab (MK-3475) in Participants With Advanced Solid Tumors (MK-3475-028/KEYNOTE-28)	477	Pembrolizumab	Phase 1	February 17, 2014/ December 18, 2020	Treatment with pembrolizumab monotherapy was associated with a manageable safety profile, promising antitumor activity in patients with recurrent GBM.
NCT02287428	Active, not recruiting	Personalized NeoAntigen Cancer Vaccine w RT Plus Pembrolizumab for Patients With MGMT Unmethylated, Newly Diagnosed GBM	46	Pembrolizumab, Radiation Therapy, Personalized NeoAntigen Vaccine	Phase 1	November 2014/ August 2020	not available
NCT02311920	Active, not recruiting	Ipilimumab and/or Nivolumab in Combination With Temozolomide in Treating Patients With Newly Diagnosed Glioblastoma or Gliosarcoma	32	Nivolumab, Ipilimumab, Temozolomide, Laboratory Biomarker Analysis	Phase 1	April 16, 2015/ December 31, 2017	Nivolumab is safe and tolerable with similar toxicity profiles noted with other cancers when given with adjuavant TMZ for newly diagnosed GBM.
NCT02313272	Active, not recruiting	Hypofractionated Stereotactic Irradiation (HFSRT) With Pembrolizumab and Bevacizumab for Recurrent High Grade Gliomas	32	Pembrolizumab,Hypofractionated Stereotactic Irradiation (HFSRT), Bevacizumab	Phase 1	May 5, 2015/ September 13, 2018	Combination of HFSRT with pembrolizumab (200 mg every 3 weeks) and bevacizumab is safe.
NCT02327078	Active, not recruiting	A Study of the Safety, Tolerability, and Efficacy of Epacadostat Administered in Combination With Nivolumab in Select Advanced Cancers (ECHO-204)	307	Nivolumab, Epacadostat, Chemotherapy	Phase 1/2	November 2014/ February 1, 2019	Nivolumab + Epacadostat was generally well tolerated up to the maximum Epacadostat 300-mg dose.
NCT02336165	Active, not recruiting	Phase 2 Study of Durvalumab (MEDI4736) in Patients With Glioblastoma	159	Durvalumab, Standard radiotherapy, Bevacizumab	Phase 2	February 26, 2015/ November 2018	Durvalumab was well tolerated when combined with RT and seemed to have efficacy among patients with new unmeth GBM. Further studies may be warranted.
NCT02337686	Active, not recruiting	Pembrolizumab in Treating Patients With Recurrent Glioblastoma	20	Pembrolizumab, Therapeutic Conventional Surgery, Laboratory Biomarker Analysis, Pharmacological Study	Phase 2	April 28, 2015/ December 31, 2020	Although pembrolizumab was well tolerated, PFS6 data and immune analysis indicates that anti-PD-1 monotherapy is insufficient for a response in the majority of GBM patients.
NCT02526017	Active, not recruiting	Study of Cabiralizumab in Combination With Nivolumab in Patients With Selected Advanced Cancers (FPA008-003)	295	Nivolumab, Cabiralizumab	Phase 1	September 2015/ March 2020	not available
NCT02529072	Active, not recruiting	Nivolumab With DC Vaccines for Recurrent Brain Tumors (AVERT)	7	nivolumab,DC	Phase 1	January 2016/ September 15, 2017	Safety of nivolumab + DC vaccination in recurrent HGG is similar to nivolumab alone.
NCT02530502	Active, not recruiting	Radiation Therapy With Temozolomide and Pembrolizumab in Treating Patients With Newly Diagnosed Glioblastoma	4	Pembrolizumab, Temozolomide, Radiation Therapy, Laboratory Biomarker Analysis	Phase 1	September 30, 2015/ May 10, 2016	not available
NCT02617589	Active, not recruiting	An Investigational Immuno-therapy Study of Nivolumab Compared to Temozolomide, Each Given With Radiation Therapy, for Newly-diagnosed Patients With Glioblastoma (GBM, a Malignant Brain Cancer) (CheckMate 498)	550	Nivolumab,Temozolomide, Radiotherapy	Phase 3	January 30, 2016/ January 17, 2019	not available
NCT02794883	Active, not recruiting	Tremelimumab and Durvalumab in Combination or Alone in Treating Patients With Recurrent Malignant Glioma	36	Durvalumab, Tremelimumab, Surgical Procedure, Laboratory Biomarker Analysis	Phase 2	September 2016/ December 2019	not available
NCT02798406	Active, not recruiting	Combination Adenovirus + Pembrolizumab to Trigger Immune Virus Effects (CAPTIVE)	49	pembrolizumab, DNX-2401	Phase 2	June 2016/ December 2020	not available
NCT02852655	Active, not recruiting	A Pilot Surgical Trial To Evaluate Early Immunologic Pharmacodynamic Parameters For The PD-1 Checkpoint Inhibitor, Pembrolizumab (MK-3475), In Patients With Surgically Accessible Recurrent/Progressive Glioblastoma	35	Pembrolizumab	Phase 1	September 21, 2016/ March 28, 2018	not available

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Table 2 (*continued*)

Trials	Status	Trial title	No.	Intervention	Phase	Year S/E	Available conclusions
NCT02968940	Active, not recruiting	Avelumab With Hypofractionated Radiation Therapy in Adults With Isocitrate Dehydrogenase (IDH) Mutant Glioblastoma	43	Avelumab, Hypofractionated radiation therapy (HFRT)	Phase 2	March 17, 2017/ April 2020	not available
NCT03047473	Active, not recruiting	Avelumab in Patients With Newly Diagnosed Glioblastoma Multiforme (SEJ)	30	avelumab	Phase 2	March 10, 2017/ September 2022	The preliminary results suggest that the addition of avelumab to standard combination therapy early on, in patients with GBM is safe.
NCT03452579	Active, not recruiting	Nivolumab Plus Standard Dose Bevacizumab Versus Nivolumab Plus Low Dose Bevacizumab in GBM	90	Nivolumab, Standard Dose Bevacizumab, Reduced Dose Bevacizumab	Phase 2	May 10, 2018/ December 30, 2020	not available
NCT03491683	Active, not recruiting	INO-5401 and INO-9012 Delivered by Electroporation (EP) in Combination With Cemiplimab (REGN2810) in Newly-Diagnosed Glioblastoma (GBM)	52	Cemiplimab, Radiation Therapy, Temozolomide, INO-5401, INO-9012	Phase 1/2	May 31, 2018/ January 18, 2021	not available
NCT03636477	Active, not recruiting	A Study of Ad-RTS-hIL-12 With Veledimex in Combination With Nivolumab in Subjects With Glioblastoma; a Substudy to ATI001-102	21	Nivolumab, veledimex, Ad-RTS-hIL-12	Phase 1	June 18, 2018/ December 2020	Controlled IL-12 production using Ad-RTS-hIL-12 + veledimex with Nivolumab is a rational combination with initial data consistent with immune-mediated anti-tumor effects with a favorable safety profile
NCT03722342	Active, not recruiting	TTAC-0001 and Pembrolizumab Combination phase1b Trial in Recurrent Glioblastoma	9	pembrolizumab, TTAC-0001	Phase 1	January 16, 2019/ November 4, 2019	not available
NCT02335918	Completed	A Dose Escalation and Cohort Expansion Study of Anti-CD27 (Varlilumab) and Anti-PD-1 (Nivolumab) in Advanced Refractory Solid Tumors	175	Combination of varlilumab and nivolumab	Phase 1/2	January 2015/ December 12, 2018	The combination of nivolumab and varlilumab was well tolerated, associated with strong biological signals, and has evidence of clinical activity in subsets of patients with tumor types that are typically resistant to PD-1 inhibitor monotherapy
NCT02337491	Completed	Pembrolizumab +/- Bevacizumab for Recurrent GBM	80	Pembrolizumab, Bevacizumab	Phase 2	February 9, 2015/ December 31, 2018	Pembrolizumab is well tolerated +/- Bevacizumab but has limited monotherapy activity for rGBM. The anti-tumor activity of Pembrolizumab + standard-dosed Bevacizumab was comparable to historical Bevacizumab monotherapy data.
NCT02550249	Completed	Neoadjuvant Nivolumab in Glioblastoma (Neo-nivo)	29	Nivolumab	Phase 2	June 2015/ March 2017	Pirmary evidence showed that nivolumab does not seem to result in a marked delay or prevention of disease relapse following salvage surgery.
NCT03291314	Completed	Clinical Trial on the Combination of Avelumab and Axitinib for the Treatment of Patients With Recurrent Glioblastoma (GliAvAx)	52	Axitinib, Avelumab	Phase 2	May 3, 2017/ January 1, 2019	The combination of Avelumab plus Axitinib is sufficiently well tolerated but did not meet the threshold for activity justifying further investigation in an unselected population of patients with rGBM.
NCT03532295	Not yet recruiting	Epacadostat in Combination With Radiation Therapy and Avelumab in Patients With Recurrent Gliomas	55	Avelumab, Epacadostat, Bevacizumab, Radiation therapy, Peripheral blood draw	Phase 1/2	January 31, 2020/ October 31, 2022	not available
NCT03899857	Not yet recruiting	Pembrolizumab for Newly Diagnosed Glioblastoma (PERGOLA)	56	Pembrolizumab,stand of care	Phase 2	May 2019/ December 2022	not available
NCT03961971	Not yet recruiting	Trial of Anti-Tim-3 in Combination With Anti-PD-1 and SRS in Recurrent GBM	15	Spartalizumab, MBG453, Stereotactic radiosurgery (SRS)	Phase 1	February 2020/ June 2021	not available
NCT04013672	Not yet recruiting	Study of Pembrolizumab Plus SurVaxM for Glioblastoma at First Recurrence	51	Pembrolizumab, SurVaxM, Sargramostim, Montanide ISA 51	Phase 2	March 1, 2020/ December 31, 2020	not available
NCT04118036	Not yet recruiting	Abemaciclib + Pembrolizumab In Glioblastoma	47	Pembrolizumab, Abemaciclib	Phase 2	May 1, 2020/ August 1, 2022	not available

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Table 2 (*continued*)

Trials	Status	Trial title	No.	Intervention	Phase	Year S/E	Available conclusions
NCT04145115	Not yet recruiting	A Study Testing the Effect of Immunotherapy (Ipilimumab and Nivolumab) in Patients With Recurrent Glioblastoma With Elevated Mutational Burden	37	Nivolumab, Ipilimumab	Phase 2	April 4, 2020/ May 31, 2023	not available
NCT04160494	Not yet recruiting	D2C7-IT With Atezolizumab for Recurrent Gliomas	18	Atezolizumab, D2C7-IT	Phase 1	February 2020/ January 2021	not available
NCT04220892	Not yet recruiting	Pilot Study of Pembrolizumab Combined With Pemetrexed or Abemaciclib for High Grade Glioma	22	Pembrolizumab, Pemetrexed, Abemaciclib	Phase 1	January 6, 2020/ January 6, 2023	not available
NCT04225039	Not yet recruiting	Anti-GITR/Anti-PD1/Stereotactic Radiosurgery, in Recurrent Glioblastoma	32	PD-1 Inhibitor INCMGA00012, Anti-GITR Agonist INCAGN1876, stereotactic radiosurgery (SRS), Brain surgery	Phase 2	January 2020/ December 2024	not available
NCT01174121	Recruiting	Immunotherapy Using Tumor Infiltrating Lymphocytes for Patients With Metastatic Cancer	332	Pembrolizumab, Young TIL, Aldesleukin, Cyclophosphamide, Fludarabine	Phase 2	August 26, 2010/ December 29, 2023	not available
NCT02311582	Recruiting	MK-3475 in Combination With MRI-guided Laser Ablation in Recurrent Malignant Gliomas	58	Pembrolizumab, MRI-guided laser ablation, Surgical resection/debulking, Biopsy,	Phase 1/2	August 5, 2015/ February 28, 2021	not available
NCT02359565	Recruiting	Pembrolizumab in Treating Younger Patients With Recurrent, Progressive, or Refractory High-Grade Gliomas, Diffuse Intrinsic Pontine Gliomas, Hypermutated Brain Tumors, Ependymoma or Medulloblastoma	110	Pembrolizumab,MRI Procedure, Laboratory Biomarker Analysis	Phase 1	May 22, 2015/ April 1, 2020	not available
NCT02658279	Recruiting	Pembrolizumab (MK-3475) in Patients With Recurrent Malignant Glioma With a Hypermutator Phenotype	44	Pembrolizumab	phase1	January 22, 2016/ January 2021	not available
NCT02658981	Recruiting	Anti-LAG-3 Alone & in Combination w/ Nivolumab Treating Patients w/ Recurrent GBM (Anti-CD137 Arm Closed 10/16/18)	100	Nivolumab,BMS 986016, urelumab, Pharmacological Study, Laboratory Biomarker Analysis	Phase 1	August 2016/ March 2020	The trial is ongoing. RP2D (Recommended Phase II Dose) has been initially determined.
NCT02667587	Recruiting	An Investigational Immuno-therapy Study of Temozolomide Plus Radiation Therapy With Nivolumab or Placebo, for Newly Diagnosed Patients With Glioblastoma (GBM, a Malignant Brain Cancer) (CheckMate548)	693	Nivolumab, Temozolomide, Radiotherapy, Nivolumab Placebo	Phase 3	May 5, 2016/ February 11, 2022	not available
NCT02829931	Recruiting	Hypofractionated Stereotactic Irradiation With Nivolumab, Ipilimumab and Bevacizumab in Patients With Recurrent High Grade Gliomas	26	Nivolumab, Bevacizumab, Ipilimumab, Hypofractionated Stereotactic Irradiation	Phase 1	August 22, 2016/ April 2020	The trial is ongoing. 5 patients have been treated on this study
NCT03018288	Recruiting	Radiation Therapy Plus Temozolomide and Pembrolizumab With and Without HSPPC-96 in Newly Diagnosed Glioblastoma (GBM)	108	Pembrolizumab, Temozolomide, HSPPC-96, Placebo	Phase 2	September 21, 2017/ January 9, 2021	not available
NCT03058289	Recruiting	A Phase 1/2 Safety Study of Intratumorally Dosed INT230-6 (IT-01)	110	pembrolizumab, anti-CTLA-4 antibody, INT230-6	Phase 1/2	February 9, 2017/ July 2022	not available
NCT03173950	Recruiting	Immune Checkpoint Inhibitor Nivolumab in People With Select Rare CNS Cancers	180	Nivolumab	Phase 2	July 13, 2017/ December 31, 2020	not available
NCT03174197	Recruiting	Atezolizumab in Combination With Temozolomide and Radiation Therapy in Treating Patients With Newly Diagnosed Glioblastoma	60	Atezolizumab, Radiation Therapy, Temozolomide	Phase 1/2	June 30, 2017/ June 30, 2020	not available
NCT03197506	Recruiting	Pembrolizumab and Standard Therapy in Treating Patients With Glioblastoma	90	Pembrolizumab, External Beam Radiation Therapy, Radiation Therapy, Temozolomide, Laboratory Biomarker Analysis, Therapeutic Conventional Surgery	Phase 2	September 15, 2017/July 31, 2022	not available
NCT03233152	Recruiting	Intra-tumoral Ipilimumab Plus Intravenous Nivolumab Following the Resection of Recurrent Glioblastoma (GlitIpNi)	6	Nivolumab, Ipilimumab	Phase 1	November 17, 2016/November 17, 2019	not available
NCT03341806	Recruiting	Avelumab With Laser Interstitial Therapy for Recurrent Glioblastoma	30	Avelumab, MRI-guided LITT therapy	Phase 1	June 28, 2018/ September 2020	not available
NCT03347097	Recruiting	Adoptive Cell Therapy of Autologous TIL and PD1-TIL Cells for Patients With Glioblastoma Multiforme	40	Tumor-infiltrating T Lymphocyte(TIL),PD1-TIL(transgenic modified TIL cells, stablely express a high-level full-length PD1 antibody)	Phase 1	January 1, 2017/ March 2020	not available

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Trials	Status	Trial title	No.	Intervention	Phase	Year S/E	Available conclusions
NCT03347617	Recruiting	Ferumoxytol MRI in Assessing Response to Pembrolizumab in Patients With Brain Tumors From Melanoma and Glioblastoma	45	Pembrolizumab, Ferumoxytol, Magnetic Resonance Imaging, Laboratory Biomarker Analysis	Phase 2	December 20, 2017November 1, 2019/	not available
NCT03367715	Recruiting	Nivolumab, Ipilimumab, and Short-course Radiotherapy in Adults With Newly Diagnosed, MGMT Unmethylated Glioblastoma	24	Nivolumab, Ipilimumab, Radiation Therapy (RT)	Phase 2	February 7, 2018/ January 2020	not available
NCT03405792	Recruiting	Study Testing The Safety and Efficacy of Adjuvant Temozolomide Plus TTFields (Optune®) Plus Pembrolizumab in Patients With Newly Diagnosed Glioblastoma (2-THE-TOP)	29	Pembrolizumab, Temozolomide (TMZ), Optune System	Phase 2	February 23, 2018/ February 2023	not available
NCT03425292	Recruiting	A Longitudinal Assessment of Tumor Evolution in Patients With Brain Cancer	90	Nivolumab,Temozolomide,conformal brain radiation therapy,Ipilimumab,Bevacizumab,Metronomic Temozolomide	Phase 1	March 1, 2018/ February 1, 2021	not available
NCT03426891	Recruiting	Pembrolizumab and Vorinostat Combined With Temozolomide for Newly Diagnosed Glioblastoma	32	Pembrolizumab, Vorinostat, Temozolomide, Radiotherapy	Phase 1	March 16, 2018/ April 2021	As of June 4, 2018, enrollment to dose level 1 has been completed. So far no does limiting adverse event has been observed.
NCT03430791	Recruiting	Trial of Combination TTF(Optune), Nivolumab Plus/Minus Ipilimumab for Recurrent Glioblastoma	60	Nivolumab, Ipilimumab, NovoTTF200A (Optune)	Phase 2	November 5, 2018/ August 2020	not available
NCT03493932	Recruiting	Cytokine Microdialysis for Real-Time Immune Monitoring in Glioblastoma Patients Undergoing Checkpoint Blockade	20	Nivolumab, BMS-986016	Phase 1	September 24, 2018/ June 1, 2021	not available
NCT03557359	Recruiting	Nivolumab for Recurrent or Progressive IDH Mutant Gliomas	37	Nivolumab	Phase 2	June 12, 2018/ June 2020	not available
NCT03576612	Recruiting	GMCI, Nivolumab, and Radiation Therapy in Treating Patients With Newly Diagnosed High-Grade Gliomas (GMCI)	36	Nivolumab, AdV-tk, Valacyclovir, Radiation, Temozolomide, Laboratory Biomarker Analysis	Phase 1	February 27, 2018/ February 28, 2021	not available
NCT03661723	Recruiting	Pembrolizumab and Reirradiation in Bevacizumab Naïve and Bevacizumab Resistant Recurrent Glioblastoma	60	Pembrolizumab, Bevacizumab, Re-irradiation	Phase 2	September 28, 2018/ August 31, 2020	not available
NCT03665545	Recruiting	Pembrolizumab in Association With the IMA950/ Poly-ICLC for Relapsing Glioblastoma (IMA950-106)	24	pembrolizumab, IMA950/Poly-ICLC(vaccine)	Phase 1/2	October 25, 2018/ October 30, 2021	not available
NCT03673787	Recruiting	A Trial of Ipatasertib in Combination With Atezolizumab (IceCAP)	51	Atezolizumab, ipatasertib	Phase 1/2	August 13, 2018/ July 2020	not available
NCT03707457	Recruiting	Biomarker-Driven Therapy Using Immune Activators With Nivolumab in Patients With First Recurrence of Glioblastoma	30	Nivolumab, Ipilimumab, Anti-GITR Monoclonal Antibody MK-4166, IDO1 inhibitor INCB024360	Phase 1	March 22, 2019/ February 2022	not available
NCT03718767	Recruiting	Nivolumab in People With IDH-Mutant Gliomas With and Without Hypermutator Phenotype	95	Nivolumab	Phase 2	March 27, 2019/ December 31, 2022	not available
NCT03726515	Recruiting	CART-EGFRvIII + Pembrolizumab in GBM	7	Pembrolizumab, CART-EGFRvIII T cells	Phase 1	March 11, 2019/ December 2020	not available
NCT03743662	Recruiting	Nivolumab With Radiation Therapy and Bevacizumab for Recurrent MGMT Methylated Glioblastoma	94	Nivolumab, Bevacizumab, Re-irradiation (RT), Re-resection	Phase 2	November 12, 2018/ November 2021	not available
NCT03750071	Recruiting	VXM01 Plus Avelumab Combination Study in Progressive Glioblastoma	30	Avelumab, VXM01	Phase 1/2	November 21, 2018/ December 31, 2020	not available
NCT03797326	Recruiting	Efficacy and Safety of Pembrolizumab (MK-3475) Plus Lenvatinib (E7080/MK-7902) in Previously Treated Participants With Select Solid Tumors (MK-7902-005/E7080-G000-224/LEAP-005)	180	Pembrolizumab, Lenvatinib	Phase 2	February 12, 2019/ April 11, 2022	not available
NCT03890952	Recruiting	Translational Study of Nivolumab in Combination With Bevacizumab for Recurrent Glioblastoma	40	Nivolumab, Bevacizumab	Phase 2	October 1, 2018/ February 1, 2022	not available

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Trials	Status	Trial title	No.	Intervention	Phase	Year S/E	Available conclusions
NCT03893903	Recruiting	AMPLIFYing NEOepitope-specific VACcine Responses in Progressive Diffuse Glioma (AMPLIFY-NEOVAC)	60	Avelumab, IDH1R132H peptide vaccine	Phase 1	October 19, 2018/ August 2021	not available
NCT03925246	Recruiting	Efficacy of Nivolumab for Recurrent IDH Mutated High-Grade Gliomas (REVOLUMAB)	39	Nivolumab	Phase 2	July 30, 2019/ December 2021	not available
NCT04003649	Recruiting	IL13Ralpha2-Targeted Chimeric Antigen Receptor (CAR) T Cells With or Without Nivolumab and Ipilimumab in Treating Patients With Recurrent or Refractory Glioblastoma	60	Nivolumab, Ipilimumab, IL13Ralpha2-specific Hinge-optimized 4−1BB-co-stimulatory CAR/Truncated CD19-expressing Autologous TN/MEM Cells, Quality-of-Life Assessment, Questionnaire Administration	Phase 1	December 2, 2019/ January 22, 2022	not available
NCT04006119	Recruiting	Study of Ad-RTS-hIL-12 + Veledimex in Combination With Cemiplimab in Subjects With Recurrent or Progressive Glioblastoma	30	Cemiplimab-Rwlc, Veledimex, Ad-RTS-hIL-12	Phase 2	August 1, 2019/ June 2022	not available
NCT04047706	Recruiting	Nivolumab, BMS-986205, and Radiation Therapy With or Without Temozolomide in Treating Patients With Newly Diagnosed Glioblastoma	30	Nivolumab, IDO1 Inhibitor BMS-986205, Radiation Therapy, Temozolomide	Phase 1	August 13, 2019/ June 9, 2022	not available
NCT04195139	Recruiting	Nivolumab and Temozolomide Versus Temozolomide Alone in Newly Diagnosed Elderly Patients With GBM (NUTMEG)	102	Nivolumab, Temozolomide	Phase 2	February 22, 2018/ December 31, 2021	not available
NCT04201873	Recruiting	Pembrolizumab and a Vaccine (ATL-DC) for the Treatment of Surgically Accessible Recurrent Glioblastoma	40	Pembrolizumab, Dendritic Cell Tumor Cell Lysate Vaccine, Placebo Administration, Poly ICLC	Phase 1	January 8, 2020/ August 1, 2024	not available
NCT02866747	Suspended	A Study Evaluating the Association of Hypofractionated Stereotactic Radiation Therapy and Durvalumab for Patients With Recurrent Glioblastoma (STERIMGLI)	62	Durvalumab, Hypofractionated stereotactic radiation therapy	Phase 1/2	January 17, 2017/ April 2020	Combining three 8 Gy fractions of hFSRT with 1500 mg Durvalumab on the 3rd fraction hFSRT and every 4 weeks for recurrent GBM is well tolerated justifying exploration of its efficacy in the phase II component of the study.
NCT03422094	Suspended	Neoantigen-based Personalized Vaccine Combined With Immune Checkpoint Blockade Therapy in Patients With Newly Diagnosed, Unmethylated Glioblastoma	30	Nivolumab, Ipilimumab, NeoVax, Research blood draw, Leukapheresis for research	Phase 1	October 31, 2018/ July 31, 2021	not available
NCT02648633	Terminated	Stereotactic Radiosurgery With Nivolumab and Valproate in Patients With Recurrent Glioblastoma	4	Nivolumab, Stereotactic Radiosurgery, Valproate	Phase 1	May 24, 2016/ February 21, 2017	not available
NCT01952769	Unknown	Anti PD1 Antibody in Diffuse Intrinsic Pontine Glioma	50	pidilizumab	Phase 1/2	February 2014/ November 2018	not available
NCT02430363	Unknown	Evaluation Of The Treatment Effectiveness Of Glioblastoma / Gliosarcoma Through The Suppression Of The PI3K/Akt Pathway In Compared With MK-3475	58	Pembrolizumab, Suppressor of the PI3K/Akt pathways	Phase 1/2	March 2013/ January 2016	not available
NCT02937844	Unknown	Pilot Study of Autologous Chimeric Switch Receptor Modified T Cells in Recurrent Glioblastoma Multiforme	20	Anti-PD-L1 CSR T cells, Cyclophosphamide, Fludarabine	Phase 1	July 2016/ July 2018	not available

and rescue TILs from exhaustion (Fig. 2c). The second category is indirect PD-1/PD-L1 axis blockade therapy, which indirectly uses PD-1/PD-L1 axis-targeted therapies to strengthen the antitumour immune effect by modifying infiltrating lymphocytes or via an oncolytic virus or a vaccine.

4.1. Direct PD-1/PD-L1 axis-targeted antibody therapies

Direct PD-1/PD-L1 axis-targeted antibody therapies in GBM are concentrated in two areas. One approach is combination with mature treatment regimens that have been applied in standard clinical practice, and the other strategy is combination with other targeted therapeutic agents.

Current SOC treatment has profound impacts on the immune system in patients with GBM (Lim et al., 2018; McGranahan et al., 2019). Determining how to successfully integrate direct PD-1/PD-L1 axis-targeted antibody therapies with the SOC treatment to maximize the benefit of patients is the focus of the current research. Neoadjuvant treatment is conventionally used to shrink tumours before surgery. Neoadjuvant immunotherapy targeting the PD-1/PD-L1 axis actually serves as a primer to launch an antitumour immune response; then, the bulk of the immunosuppressive tumour can be resected, and additional continued immunotherapy can be given to enhance the functions of TILs. A pilot study and phase 2 clinical trials have been initiated to study the preoperative application of the PD-1 checkpoint inhibitors pembrolizumab and nivolumab in GBM patients (NCT02550249). Temozolomide (TMZ), which is a chemotherapeutic agent responsible for inducing lymphopenia and myelosuppression (Friedman et al., 2000), works in different ways than an anti-PD-1 antibody to stop the growth of tumour cells, specifically by killing the cells, stopping them from dividing, or stopping them from spreading. However, recent preclinical evidence has shown that there are cross-linking relationships between TMZ therapy and PD-L1 downregulation at the GBM cell level and tissue level that involve JAK/STAT pathway regulation (Heynckes et al., 2019). An initial clinical trial on the safety and optimal dose of nivolumab in combination with TMZ in newly diagnosed GBM patients was conducted in 2015 (NCT02311920). For patients grouped according to the hypermethylation status of the MGMT promoter region, clinical trials with or without TMZ in the context of nivolumab plus radiotherapy are currently recruiting patients (NCT02617589 and NCT02667587). Clinical studies are also underway to analyse the efficacy of TMZ maintenance therapy in patients receiving anti-PD-1 therapy (NCT02530502 and NCT02311920). The results of these clinical trials combining these two kinds of agents are eagerly awaited. Radiotherapy not only causes leukopenia but also induces the upregulation of PD-L1 and PD-1 expression in tumour and immune cells, potentially evoking resistance to SOC treatment in GBM patients (Song et al., 2018; Osuka et al., 2013). Ordinary radiotherapy has a higher probability of leukopenia than hypofractionated treatment and stereotactic radiosurgery (SRS). Increasingly, clinical studies are focused on the feasibility and efficacy of combining direct PD-1/PD-L1 axis-targeted antibody therapy with hypofractionated stereotactic radiotherapy (HFSRT) or SRS. Two phase 1 clinical trials are being conducted to evaluate the safety and efficacy of anti-PD-1 therapy (pembrolizumab and nivolumab) combined with HFSRT (NCT02829931 and NCT02313272). A phase 1 clinical trial (NCT02648633) designed to evaluate the safety and efficacy of nivolumab and sodium valproate after stereotactic gamma knife radiotherapy of the head has also been completed. In addition, the abscopal effect, which is an antitumour response of the immune system after radiotherapy that reflects pathological changes in unirradiated sites of disease after radiotherapy, should be considered in the evaluation of the efficacy of radiotherapy. Because of its lack of capsule and its invasive growth in the brain, GBM tumour tissue cannot be completely separated from the surrounding normal brain tissue, which is one of the reasons why traditional SOC treatments are not curative. Using targeted anti-PD-1/PD-L1 antibody therapy to increase the number of activated tumour-specific T cells beyond the edge of the tumour after surgical resection and improve the efficacy of the abscopal effect produced by the systemic immune response after radiotherapy is anticipated to solve this problem (Liu et al., 2016). In September 2018, the latest PD-1 antibody agent, cemiplimab, was approved by the US Food and Drug Administration (FDA) for the treatment of cutaneous squamous cell carcinoma patients. Accordingly, the NCT03174197 clinical trial was initiated to evaluate the feasibility of cemiplimab combined with radiation and chemotherapy in newly diagnosed GBM.

At present, many preclinical studies have found that simultaneous inhibition of multiple immune checkpoints can produce a positive synergistic effect and enhance the efficacy of immunotherapy (Kim et al., 2017; Reardon et al., 2016; Wainwright et al., 2014). PD-1/PD-L1 axis-targeting drugs, in terms of the selectivity of their antitumour effects and their effectiveness and safety, are expected to be the baseline drugs that will be combined with other inhibitory immune checkpoint-targeting drugs. The response rates to long-term anti-CTLA-4 and anti-PD-1 drugs in a murine GBM model were as high as 75 %, significantly higher than that of either drug alone (Reardon et al., 2016). The mixture of an anti-PD-1 antibody and IDO1 inhibitor was shown to significantly improve the median survival time of GBM tumour-bearing mice via a tumour-specific T cell-dependent antitumour mechanism (Ladomersky et al., 2018). In recent years, many clinical trials, such as NCT03430791 and NCT03367715, have been designed to explore whether there is a synergistic effect produced by the combined application of ipilimumab and nivolumab and whether combined application can reduce immunotherapy-related adverse events. Thus, the combined application of these two targeted immunosuppressive checkpoint drugs may achieve better control of the progression of GBM. NCT03707457 was started in 2019 to determine the safety of an IDO1 inhibitor, ipilimumab and nivolumab in patients experiencing their first recurrence of GBM. Clinical studies of TIM-3 in combination with anti-PD-1 antibodies, as well as LAG-3 in combination with anti-PD-1 antibodies, are currently underway (NCT03961971, NCT03493932); however, the final results have not yet been published. In addition to combinations with immune checkpoint inhibitors, the combination of anti-PD-1/PD-L1 antibody therapies with targeting of key GBM-related immune signalling pathway molecules is another high-interest research area. A phase I clinical trial of an anti-glucocorticoid-induced tumour necrosis factor receptor (GITR) antibody combined with nivolumab (NCT03707457) is being conducted to study the safety and efficacy of this combination. The binding of colony stimulating factor-1 (CSF-1) to its receptor (CSF-1R) is one of the events involved in the differentiation of TAMs into the M2-like phenotype. At present, the combined application of CSF-1/CSF-1R axis- and PD-1/PD-L1 axis-targeted drugs has been carried out in clinical trials specifically for GBM with anti-CSF-1R and anti-PD-1 antibodies.

For PD-1/PD-L1 axis-related targeted therapies, in addition to the use of anti-PD-1 antibodies to block PD-1, the development of antibodies against PD-L1 is another strategy to block the effective expression of PD-L1 in GBM tumour tissue. At present, there are many related clinical trials evaluating PD-L1-targeted therapeutic antibodies in GBM. The safety and efficacy of an anti-PD-L1 antibody combined with HFSRT will be evaluated in recurrent GBM patients in the NCT02866747 (durvalumab) and NCT02968940 (avelumab) clinical trials. Phase 1/2 clinical trials have also been carried out to study the efficacy of SOC therapy for GBM combined with atezolizumab in patients with primary GBM (NCT03174197). There are also corresponding clinical trials for the combined application of an anti-PD-L1 antibody and other targeted immune checkpoint inhibitors. NCT02794883 is a phase 2 clinical trial focusing on the combination of durvalumab and tremelimumab for the treatment of recurrent GBM. NCT03291314 and NCT02336165 are two clinical studies on the combined use of anti-PD-L1 antibodies and drugs targeting GBM-related signal transduction molecules. These two clinical trials studied the effects of axitinib (a

VEGFR-specific tyrosine kinase inhibitor) combined with avelumab and durvalumab combined with bevacizumab, respectively, on the survival prognosis of GBM patients.

The blood-brain barrier (BBB) is another factor requiring consideration in the treatment of GBM with direct PD-1/PD-L1 axis-targeted antibodies. It is generally believed that the BBB can prevent targeted monoclonal antibodies (mAbs) from entering the brain parenchyma, thus affecting their therapeutic effect on intracranial tumours (van Tellingen et al., 2015). In GBM, peritumoral edema, aseptic inflammation, proliferation and invasion of tumour tissue, pathological microangiogenesis and BBB destruction caused by the tumour microenvironment can significantly increase the permeability of the BBB compared with that in normal brain neurovascular units (Arvanitis et al., 2020). Histopathological examination of GBM patients indicates a significant increase in peripheral blood immune cell infiltration compared with that in normal brain tissue, confirming the change in BBB permeability from another perspective. In addition, many minimally invasive methods have emerged to improve the transport of targeted drugs across the BBB, including molecular, cellular, and physical approaches; endothelial cell signalling pathways; radiation; and nanoparticles (Arvanitis et al., 2020). These approaches provide an appropriate condition for PD-1/PD-L1 antibody drugs to enter GBM tissue. Moreover, recent studies have confirmed that anti-PD-1 antibodies exhibit a concise mechanism by which they can penetrate the BBB more easily than other targeted drugs. They can irreversibly bind to circulating lymphocytes in peripheral blood and then enter the tumour microenvironment by avoiding the BBB through the cervical lymph node pathway and cerebrospinal fluid circulation pathway; this study also found that nivolumab belongs to the family of IgG monoclonal antibodies with FcRn binding, which can penetrate the BBB without producing pathological changes (van Bussel et al., 2019).

4.2. Indirect PD-1/PD-L1 axis blockade therapy

To enhance the potential of PD-1/PD-L1-targeted therapy, preclinical research is searching for “helpers” to develop a more effective indirect combination therapy. Among the many potential helpers identified, genetically engineered T cells, oncolytic viruses, and GBM vaccines are the most interesting new indirect therapeutic regimens to inhibit the PD-1/PD-L1 axis. These treatments as independent regimens have also been evaluated in combination with direct PD-1/PD-L1 axis-targeted antibody drugs, which can prompt the immune system to fight tumour cells (Wang et al., 2019a; Preusser et al., 2015), but we are more concerned with these agents as carriers to indirectly promote PD-1/PD-L1 axis-targeted therapy, which should be better summarized.

T cell genetic engineering mainly includes producing T cells that express chimeric antigen receptors (CARs), genetically engineered TCRs or other T cell surface receptors (Brown et al., 2019). These methods have been indicated to be successful in changing the specificity of T cells (Choi et al., 2019). Regarding indirect PD-1/PD-L1 axis blockade therapeutic regimens, PD-1-blocking single-chain variable fragment (scFv)-expressing CAR-T cells and pluripotent killer-programmed cell death 1 (PIK-PD-1) are currently being studied in GBM (Rafiq et al., 2018) (NCT03347097) (Fig. 3). Via specific binding of CAR-T cells to tumour cells, PD-1-blocking scFvs can be transported to sites that facilitate contact with the tumour, thereby reducing the adverse reactions caused by using direct PD-1/PD-L1 axis-targeted antibody therapies. These adverse events include gastrointestinal, hepatic, pulmonary, renal, endocrine, and skin adverse reactions and the newly proposed hyperprogressive disease (HPD) (Champiat et al., 2017). Recent studies have found that a PD-1-blocking scFv produced by CAR-T cells can be detected only in the TME. CAR-T cells with the ability to produce a PD-1-blocking scFv can enhance the survival of tumour-bearing mice with high expression of PD-L1 (Rafiq et al., 2018). PIK-PD-1 T cells are genetically engineered T cells that can secrete high levels of anti-PD-1 antibodies and have the potential to block PD-1/PD-L1 axis responses in the TME. The exact mechanism by which PIK-PD-1 cells work is not fully understood. Once these cells enter the TME, they continue to secrete antibodies against PD-1. Because PD-1 is ubiquitous in tumour-infiltrating immune cells, it is one of the key links in the immunosuppressive microenvironment of GBM. Thus, blocking PD-1-mediated signal transduction will rescue the immunosuppressive microenvironment to a certain extent. In the clinical trial NCT03347097, genetically modified tumour-infiltrating PIK-PD-1 T cells that stably and continuously expressed high levels of an anti-PD-1 antibody in GBM patients were constructed. The results of the safety and efficacy evaluation of this cell-based therapy have yet to be reported. In addition to research on the infection of tumour cells, the focus of oncolytic virus research has shifted to evaluate the immunostimulatory properties of activating the immunosuppressive GBM TME and viruses playing a role in transferring therapeutic payloads to tumours (Martikainen and Essand, 2019). An anti-PD-1 single-chain antibody expressed by an oncolytic herpes simplex virus has been studied in a GBM mouse model, and infection with this virus induced a durable antitumour response (Passaro et al., 2019). Tumour vaccines with the potential to produce anti-PD-1 antibodies have not been developed for GBM. However, in other solid tumours (e.g., colon cancer and melanoma tumours), tumour vaccines simultaneously containing anti-PD-1 monoclonal antibodies have been generated in murine models. In these studies, the treatments ameliorated the immunosuppressive microenvironment and thus rescued the specific antitumour immune response (Tian et al., 2016).

5. Conclusions

This paper reviews the PD-1/PD-L1 axis in GBM and the corresponding TME. It highlights two issues. One issue is the existence of extensive PD-1/PD-L1 axis-mediated immune events between tumour cells and tumour-infiltrating immune cells in the setting of GBM, which suggests that PD-1/PD-L1 axis-induced tumour immune suppression is a common molecular phenomenon in antitumour immunity. Through these findings, the importance of blocking this axis is explained. Furthermore, the other issue is how to block the PD-1/PD-L1 axis in GBM. We divided PD-1/PD-L1 axis-related therapeutic regimens into two categories. The first is direct PD-1/PD-L1 axis-targeted antibody therapy; the second is indirect PD-1/PD-L1 axis blockade therapy. Overall, this review allows us to understand that the antitumour immunosuppression mediated by the PD-1/PD-L1 axis is not directed only at CTLs; it also affects ubiquitous infiltrating immune cells. Although there is no conclusive evidence supporting the effectiveness of PD-1/PD-L1 axis-based therapies in patients with GBM, clinical studies on this axis have replicated the main results of basic research, indicating that translation is achievable. Given the unacceptable prognosis of GBM, anti-PD-1/PD-L1 axis-based therapies are worthy of further research.

Authors’ contributions

Shu Chang reviewed the literature, wrote the manuscript and prepared the figures and tables. Li Qingguo guided and assisted in the completion of the paper.

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Declaration of Competing Interest

No competing interests.

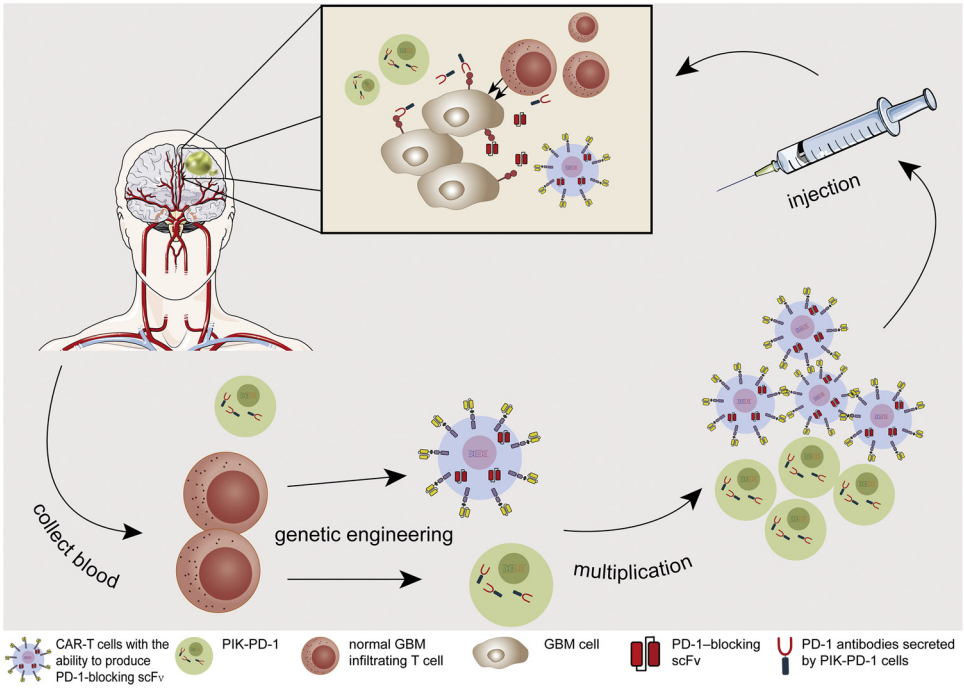

Fig. 3. Indirect PD-1/PD-L1 axis blockade therapies, including PD-1-blocking scFv-expressing CAR-T cells and PIK-PD-1 cells, are currently being studied in the context of GBM.

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References

Agata, Y., Kawasaki, A., Nishimura, H., Ishida, Y., Tsubata, T., Yagita, H., Honjo, T., 1996. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int. Immunol. 8 (5), 765–772. https://doi.org/10.1093/intimm/8.5.765.

Arvanitis, C.D., Ferraro, G.B., Jain, R.K., 2020. The blood-brain barrier and blood-tumour barrier in brain tumours and metastases. Nat. Rev. Cancer 20 (1), 26–41. https://doi.org/10.1038/s41568-019-0205-x.

Bankey, P.E., Banerjee, S., Zucchiatti, A., De, M., Sleem, R.W., Lin, C.F., Miller-Graziano, C.L., De, A.K., 2010. Cytokine induced expression of programmed death ligands in human neutrophils. Immunol. Lett. 129 (2), 100–107. https://doi.org/10.1016/j.imlet.2010.01.006.

Berghoff, A.S., Kiesel, B., Widhalm, G., Rajky, O., Ricken, G., Wohrer, A., Dieckmann, K., Filipits, M., Brandstetter, A., Weller, M., et al., 2015. Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. Neuro Oncol. 17 (8), 1064–1075. https://doi.org/10.1093/neuonc/nou307.

Berghoff, A.S., Kiesel, B., Widhalm, G., Wilhelm, D., Rajky, O., Kurscheid, S., Kresl, P., Wohrer, A., Marosi, C., Hegi, M.E., et al., 2017. Correlation of immune phenotype with IDH mutation in diffuse glioma. Neuro Oncol. 19 (11), 1460–1468. https://doi.org/10.1093/neuonc/nox054.

Bloch, O., Crane, C.A., Kaur, R., Safaee, M., Rutkowski, M.J., Parsa, A.T., 2013. Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages. Clin. Cancer Res. 19 (12), 3165–3175. https://doi.org/10.1158/1078-0432.CCR-12-3314.

Boussiotis, V.A., Charest, A., 2018. Immunotherapies for malignant glioma. Oncogene 37 (9), 1121–1141. https://doi.org/10.1038/s41388-017-0024-z.

Bowers, N.L., Helton, E.S., Huijbregts, R.P., Goepfert, P.A., Heath, S.L., Hel, Z., 2014. Immune suppression by neutrophils in HIV-1 infection: role of PD-L1/PD-1 pathway. PLoS Pathog. 10 (3), e1003993. https://doi.org/10.1371/journal.ppat.1003993.

Brown, M.P., Ebert, L.M., Gargett, T., 2019. Clinical chimeric antigen receptor-T cell therapy: a new and promising treatment modality for glioblastoma. Clin. Transl. Immunol. 8 (5), e1050. https://doi.org/10.1002/cti2.1050.

Candolfi, M., Curtin, J.F., Yagiz, K., Assi, H., Wibowo, M.K., Alzadeh, G.E., Foulad, D., Muhammad, A.K., Salehi, S., Keech, N., et al., 2011. B cells are critical to T-cell-mediated antitumor immunity induced by a combined immune-stimulatory/conditionally cytotoxic therapy for glioblastoma. Neoplasia 13 (10), 947–960. https://doi.org/10.1593/neo.11024.

Champiat, S., Dercle, L., Ammari, S., Massard, C., Hollebecque, A., Postel-Vinay, S., Chaput, N., Eggermont, A., Marabelle, A., Soria, J.C., et al., 2017. Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1. Clin. Cancer Res. 23 (8), 1920–1928. https://doi.org/10.1158/1078-0432.CCR-16-1741.

Chang, A.L., Miska, J., Wainwright, D.A., Dey, M., Rivetta, C.V., Yu, D., Kanojia, D., Pituch, K.C., Qiao, J., Pytel, P., et al., 2016. CCL2 produced by the glioma microenvironment is essential for the recruitment of regulatory t cells and myeloid-derived suppressor cells. Cancer Res. 76 (19), 5671–5682. https://doi.org/10.1158/0008-5472.CAN-16-0144.

Chen, Z., Hambardzumyan, D., 2018. Immune microenvironment in glioblastoma subtypes. Front. Immunol. 9, 1004. https://doi.org/10.3389/fimmu.2018.01004.

Choi, B.D., Yu, X., Castano, A.P., Bouffard, A.A., Schmidts, A., Larson, R.C., Bailey, S.R., Boroughs, A.C., Frigault, M.J., Leick, M.B., et al., 2019. CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. Nat. Biotechnol. 37 (9), 1049–1058. https://doi.org/10.1038/s41587-019-0192-1.

Dong, H., Strome, S.E., Salomao, D.R., Tamura, H., Hirano, F., Flies, D.B., Roche, P.C., Lu, J., Zhu, G., Tamada, K., et al., 2002. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat. Med. 8 (8), 793–800. https://doi.org/10.1038/nm730.

Dunn, G.P., Old, L.J., Schreiber, R.D., 2004. The immunobiology of cancer immunosurveillance and immunoediting. Immunity 21 (2), 137–148. https://doi.org/10.1016/j.immuni.2004.07.017.

Filley, A.C., Henriquez, M., Dey, M., 2017. Recurrent glioma clinical trial, CheckMate-143: the game is not over yet. Oncotarget 8 (53), 91779–91794. https://doi.org/10.18632/oncotarget.21586.

Francisco, L.M., Salinas, V.H., Brown, K.E., Vanguri, V.K., Freeman, G.J., Kuchroo, V.K., Sharpe, A.H., 2009. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J. Exp. Med. 206 (13), 3015–3029. https://doi.org/10.1084/jem.20090847.

Friedman, H.S., Kerby, T., Calvert, H., 2000. Temozolomide and treatment of malignant glioma. Clin. Cancer Res. 6 (7), 2585–2597.

Gabrilovich, D.I., 2017. Myeloid-derived suppressor cells. Cancer Immunol. Res. 5 (1), 3–8. https://doi.org/10.1158/2326-6066.CIR-16-0297.

Golan, I., Rodriguez de la Fuente, L., Costoya, J.A., 2018. NK cell-based glioblastoma immunotherapy. Cancers (Basel) 10 (12). https://doi.org/10.3390/cancers10120522.

Goods, B.A., Hernandez, A.L., Lowther, D.E., Lucca, L.E., Lerner, B.A., Gunel, M., Raddassi, K., Coric, V., Hafler, D.A., Love, J.C., 2017. Functional differences between PD-1 + and PD-1- CD4 + effector T cells in healthy donors and patients with glioblastoma multiforme. PLoS One 12 (9), e0181538. https://doi.org/10.1371/journal.pone.0181538.

Gordon, S.R., Maute, R.L., Dulken, B.W., Hutter, G., George, B.M., McCracken, M.N., Gupta, R., Tsai, J.M., Sinha, R., Corey, D., et al., 2017. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature 545 (7655), 495–499. https://doi.org/10.1038/nature22396.

Han, J., Hong, Y., Lee, Y.S., 2017. PD-L1 expression and combined status of PD-L1/PD-1-positive tumor infiltrating mononuclear cell density predict prognosis in glioblastoma patients. J. Pathol. Transl. Med. 51 (1), 40–48. https://doi.org/10.4132/jptm.2016.08.31.

Hartley, G., Regan, D., Guth, A., Dow, S., 2017. Regulation of PD-L1 expression on murine tumor-associated monocytes and macrophages by locally produced TNF-alpha. Cancer Immunol. Immunother. 66 (4), 523–535. https://doi.org/10.1007/s00262-017-1955-5.

Hartley, G.P., Chow, L., Ammons, D.T., Wheat, W.H., Dow, S.W., 2018. Programmed cell

death ligand 1 (PD-L1) signaling regulates macrophage proliferation and activation. Cancer Immunol. Res. 6 (10), 1260–1273. https://doi.org/10.1158/2326-6066.CIR-17-0537.

He, G., Zhang, H., Zhou, J., Wang, B., Chen, Y., Kong, Y., Xie, X., Wang, X., Fei, R., Wei, L., et al., 2015. Peritumoural neutrophils negatively regulate adaptive immunity via the PD-L1/PD-1 signalling pathway in hepatocellular carcinoma. J. Exp. Clin. Cancer Res. 34, 141. https://doi.org/10.1186/s13046-015-0256-0.

He, Y., Liu, S., Mattei, J., Bunn Jr., P.A., Zhou, C., Chan, D., 2018. The combination of anti-KIR monoclonal antibodies with anti-PD-1/PD-L1 monoclonal antibodies could be a critical breakthrough in overcoming tumor immune escape in NSCLC. Drug Des. Dev. Ther. 12, 981–986. https://doi.org/10.2147/DDDT.S163304.

Heiland, D.H., Haaker, G., Delev, D., Mercas, B., Masalha, W., Heynckes, S., Gabelein, A., Pfeifer, D., Carro, M.S., Weyerbrock, A., et al., 2017. Comprehensive analysis of PD-L1 expression in glioblastoma multiforme. Oncotarget 8 (26), 42214–42225. https://doi.org/10.18632/oncotarget.15031.

Heynckes, S., Daka, K., Franco, P., Gaebelein, A., Frenking, J.H., Doria-Medina, R., Mader, I., Delev, D., Schnell, O., Heiland, D.H., 2019. Crosslink between temozolomide and PD-L1 immune-checkpoint inhibition in glioblastoma multiforme. BMC Cancer 19 (1), 117. https://doi.org/10.1186/s12885-019-5308-y.

Hofmeyer, K.A., Jeon, H., Zang, X., 2011. The PD-1/PD-L1 (B7-H1) pathway in chronic infection-induced cytotoxic T lymphocyte exhaustion. J. Biomed. Biotechnol. 2011, 451694. https://doi.org/10.1155/2011/451694.

Huang, B.Y., Zhan, Y.P., Zong, W.J., Yu CJ, Li J.F., Qu, Y.M., Han, S., 2015. The PD-1/B7-H1 pathway modulates the natural killer cells versus mouse glioma stem cells. PLoS One 10 (8), e0134715. https://doi.org/10.1371/journal.pone.0134715.

Jiang, T., Mao, Y., Ma, W., Mao, Q., You, Y., Yang, X., Jiang, C., Kang, C., Li, X., Chen, L., et al., 2016. CGCG clinical practice guidelines for the management of adult diffuse gliomas. Cancer Lett. 375 (2), 263–273. https://doi.org/10.1016/j.canlet.2016.01.024.

Kamran, N., Kadiyala, P., Saxena, M., Candolfi, M., Li, Y., Moreno-Ayala, M.A., Raja, N., Shah, D., Lowenstein, P.R., Castro, M.G., 2017. Immunosuppressive myeloid cells' blockade in the glioma microenvironment enhances the efficacy of immune-stimulatory gene therapy. Mol. Ther. 25 (1), 232–248. https://doi.org/10.1016/j.ymthe.2016.10.003.

Kim, E.S., Kim, J.E., Patel, M.A., Mangraviti, A., Ruzevick, J., Lim, M., 2016. Immune checkpoint modulators: an emerging antiglioma armamentarium. J. Immunol. Res. 2016, 4683607. https://doi.org/10.1155/2016/4683607.

Kim, J.E., Patel, M.A., Mangraviti, A., Kim, J.E.S., Theodros, D., Velarde, E., Liu, A., Sankey, E.W., Tam, A., Xu, H., et al., 2017. Combination therapy with anti-PD-1, Anti-TIM-3, and focal radiation results in regression of murine gliomas. Clin. Cancer Res. 23 (1), 124–136. https://doi.org/10.1158/1078-0432.CCR-15-1535.

Kohanbash, G., Okada, H., 2012. Myeloid-derived suppressor cells (MDSCs) in gliomas and glioma-development. Immunol. Invest. 41 (6-7), 658–679. https://doi.org/10.3109/08820139.2012.689591.

Kurz, S.C., Wen, P.Y., 2018. Quo Vadis-Do immunotherapies have a role in glioblastoma? Curr. Treat. Options Neurol. 20 (5), 14. https://doi.org/10.1007/s11940-018-0499-0.

Ladomersky, E., Zhai, L., Lenzen, A., Lauing, K.L., Qian, J., Scholtens, D.M., Gritsina, G., Sun, X., Liu, Y., Yu, F., et al., 2018. IDO1 inhibition synergizes with radiation and PD-1 blockade to durably increase survival against advanced glioblastoma. Clin. Cancer Res. 24 (11), 2559–2573. https://doi.org/10.1158/1078-0432.CCR-17-3573.

LeBien, T.W., Tedder, T.F., 2008. B lymphocytes: how they develop and function. Blood 112 (5), 1570–1580. https://doi.org/10.1182/blood-2008-02-078071.

Lee-Chang, C., Rashidi, A., Miska, J., Zhang, P., Pituch, K.C., Hou, D., Xiao, T., Fischietti, M., Kang, S.J., Appin, C.L., et al., 2019. Myeloid-derived suppressive cells promote B cell-mediated immunosuppression via transfer of PD-L1 in glioblastoma. Cancer Immunol. Res. https://doi.org/10.1158/2326-6066.CIR-19-0240.

Li, W., Graeber, M.B., 2012. The molecular profile of microglia under the influence of glioma. Neuro Oncol 14 (8), 958–978. https://doi.org/10.1093/neuonc/nos116.

Lian, S., Xie, R., Ye, Y., Xie, X., Li, S., Lu, Y., Li, B., Cheng, Y., Katanaev, V.L., Jia, L., 2019. Simultaneous blocking of CD47 and PD-L1 increases innate and adaptive cancer immune responses and cytokine release. EBioMedicine 42, 281–295. https://doi.org/10.1016/j.ebiom.2019.03.018.

Lim, M., Xia, Y., Bettegowda, C., Weller, M., 2018. Current state of immunotherapy for glioblastoma. Nat. Rev. Clin. Oncol. 15 (7), 422–442. https://doi.org/10.1038/s41571-018-0003-5.

Litak, J., Mazurek, M., Grochowski, C., Kamieniak, P., Rolinski, J., 2019. PD-L1/PD-1 axis in glioblastoma multiforme. Int. J. Mol. Sci. 20 (21). https://doi.org/10.3390/ijms20215347.

Liu, J., Blake, S.J., Yong, M.C., Harjunpaa, H., Ngiow, S.F., Takeda, K., Young, A., O'Donnell, J.S., Allen, S., Smyth, M.J., et al., 2016. Improved efficacy of neoadjuvant compared to adjuvant immunotherapy to eradicate metastatic disease. Cancer Discov. 6 (12), 1382–1399. https://doi.org/10.1158/2159-8290.CD-16-0577.

Liu, Y., Liang, X., Dong, W., Fang, Y., Lv, J., Zhang, T., Fiskesund, R., Xie, J., Liu, J., Yin, X., et al., 2018. Tumor-repopulating cells induce PD-1 expression in CD8(+) t cells by transferring kynurenine and AhR activation. Cancer Cell 33 (3), 480–494. https://doi.org/10.1016/j.ccell.2018.02.005. e487.

Lu, C., Redd, P.S., Lee, J.R., Savage, N., Liu, K., 2016. The expression profiles and regulation of PD-L1 in tumor-induced myeloid-derived suppressor cells. Oncoimmunology 5 (12), e1247135. https://doi.org/10.1080/2162402X.2016.1247135.

Martikainen, M., Essand, M., 2019. Virus-based immunotherapy of glioblastoma. Cancers (Basel) 11 (2). https://doi.org/10.3390/cancers11020186.

McGranahan, T., Therkelsen, K.E., Ahmad, S., Nagpal, S., 2019. Current state of immunotherapy for treatment of glioblastoma. Curr. Treat. Options Oncol. 20 (3), 24. https://doi.org/10.1007/s11864-019-0619-4.

Morford, L.A., Elliott, L.H., Carlson, S.L., Brooks, W.H., Roszman, T.L., 1997. T cell receptor-mediated signaling is defective in T cells obtained from patients with primary intracranial tumors. J. Immunol. 159 (9), 4415–4425.

Nam, S., Lee, A., Lim, J., Lim, J.S., 2019. Analysis of the expression and regulation of PD-1 protein on the surface of myeloid-derived suppressor cells (MDSCs). Biomol. Ther. (Seoul) 27 (1), 63–70. https://doi.org/10.4062/biomolther.2018.201.

Nduom, E.K., Wei, J., Yaghi, N.K., Huang, N., Kong, L.Y., Gabrusiewicz, K., Ling, X., Zhou, S., Ivan, C., Chen, J.Q., et al., 2016. PD-L1 expression and prognostic impact in glioblastoma. Neuro Oncol 18 (2), 195–205. https://doi.org/10.1093/neuonc/nov172.

Noman, M.Z., Desantis, G., Janji, B., Hasmim, M., Karray, S., Dessen, P., Bronte, V., Chouaib, S., 2014. PD-L1 is a novel direct target of HIF-1alpha, and its blockade under hypoxia enhanced MDSC-mediated T cell activation. J. Exp. Med. 211 (5), 781–790. https://doi.org/10.1084/jem.20131916.

Noman, M.Z., Hasmim, M., Messai, Y., Terry, S., Kieda, C., Janji, B., Chouaib, S., 2015. Hypoxia: a key player in antitumor immune response. a review in the theme: cellular responses to hypoxia. Am. J. Physiol., Cell Physiol. 309 (9), C569–579. https://doi.org/10.1152/ajpcell.00207.2015.

Osuka, S., Sampetrean, O., Shimizu, T., Saga, I., Onishi, N., Sugihara, E., Okubo, J., Fujita, S., Takano, S., Matsumura, A., et al., 2013. IGF1 receptor signaling regulates adaptive radioprotection in glioma stem cells. Stem Cells 31 (4), 627–640. https://doi.org/10.1002/stem.1328.

Ozdemir-Kaynak, E., Qutub, A.A., Yesil-Celiktas, O., 2018. Advances in glioblastoma multiforme treatment: new models for nanoparticle therapy. Front. Physiol. 9, 170. https://doi.org/10.3389/fphys.2018.00170.

Pardoll, D.M., 2012. The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer 12 (4), 252–264. https://doi.org/10.1038/nrc3239.

Parsa, A.T., Waldron, J.S., Panner, A., Crane, C.A., Parney, I.F., Barry, J.J., Cachola, K.E., Murray, J.C., Tihan, T., Jensen, M.C., et al., 2007. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat. Med. 13 (1), 84–88. https://doi.org/10.1038/nm1517.

Passaro, C., Alayo, Q., De Laura, I., McNulty, J., Grauwet, K., Ito, H., Bhaskaran, V., Mineo, M., Lawler, S.E., Shah, K., et al., 2019. Arming an oncolytic herpes simplex virus type 1 with a single-chain fragment variable antibody against PD-1 for experimental glioblastoma therapy. Clin. Cancer Res. 25 (1), 290–299. https://doi.org/10.1158/1078-0432.CCR-18-2311.

Peng, H., Li, Z., Fu, J., Zhou, R., 2019. Growth and differentiation factor 15 regulates PD-L1 expression in glioblastoma. Cancer Manage. Res. 11, 2653–2661. https://doi.org/10.2147/CMAR.S192095.

Pesce, S., Greppi, M., Tabellini, G., Rampinelli, F., Parolini, S., Olive, D., Moretta, L., Moretta, A., Marcenaro, E., 2017. Identification of a subset of human natural killer cells expressing high levels of programmed death 1: a phenotypic and functional characterization. J. Allergy Clin. Immunol. 139 (1), 335–346. https://doi.org/10.1016/j.jaci.2016.04.025. e333.

Preusser, M., Lim, M., Hafler, D.A., Reardon, D.A., Sampson, J.H., 2015. Prospects of immune checkpoint modulators in the treatment of glioblastoma. Nat. Rev. Neurol. 11 (9), 504–514. https://doi.org/10.1038/nrneurol.2015.139.

Qian, J., Wang, C., Wang, B., Yang, J., Wang, Y., Luo, F., Xu, J., Zhao, C., Liu, R., Chu, Y., 2018. The IFN-gamma/PD-L1 axis between T cells and tumor microenvironment: hints for glioma anti-PD-1/PD-L1 therapy. J. Neuroinflammation 15 (1), 290. https://doi.org/10.1186/s12974-018-1330-2.

Rafei, M., Hsieh, J., Zehntner, S., Li, M., Forner, K., Birman, E., Boivin, M.N., Young, Y.K., Perreault, C., Galipeau, J., 2009. A granulocyte-macrophage colony-stimulating factor and interleukin-15 fusokine induces a regulatory B cell population with immune suppressive properties. Nat. Med. 15 (9), 1038–1045. https://doi.org/10.1038/nm.2003.

Rafiq, S., Yeku, O.O., Jackson, H.J., Purdon, T.J., van Leeuwen, D.G., Drakes, D.J., Song, M., Miele, M.M., Li, Z., Wang, P., et al., 2018. Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. Nat. Biotechnol. 36 (9), 847–856. https://doi.org/10.1038/nbt.4195.

Raychaudhuri, B., Rayman, P., Huang, P., Grabowski, M., Hambardzumyan, D., Finke, J.H., Vogelbaum, M.A., 2015. Myeloid derived suppressor cell infiltration of murine and human gliomas is associated with reduction of tumor infiltrating lymphocytes. J. Neurooncol. 122 (2), 293–301. https://doi.org/10.1007/s11060-015-1720-6.

Reardon, D.A., Gokhale, P.C., Klein, S.R., Ligon, K.L., Rodig, S.J., Ramkissoon, S.H., Jones, K.L., Conway, A.S., Liao, X., Zhou, J., et al., 2016. Glioblastoma eradication following immune checkpoint blockade in an orthotopic, immunocompetent model. Cancer Immunol. Res. 4 (2), 124–135. https://doi.org/10.1158/2326-6066.CIR-15-0151.

Song, X., Shao, Y., Jiang, T., Ding, Y., Xu, B., Zheng, X., Wang, Q., Chen, X., Gu, W., Wu, C., et al., 2018. Radiotherapy upregulates programmed death Ligand-1 through the pathways downstream of epidermal growth factor receptor in glioma. EBioMedicine 28, 105–113. https://doi.org/10.1016/j.ebiom.2018.01.027.

Stephen, T.L., Payne, K.K., Chaurio, R.A., Allegrezza, M.J., Zhu, H., Perez-Sanz, J., Perales-Puchalt, A., Nguyen, J.M., Vara-Ailor, A.E., Eruslanov, E.B., et al., 2017. SATB1 expression governs epigenetic repression of PD-1 in tumor-reactive T cells. Immunity 46 (1), 51–64. https://doi.org/10.1016/j.immuni.2016.12.015.

Sundstedt, A., O'Neill, E.J., Nicolson, K.S., Wraith, D.C., 2003. Role for IL-10 in suppression mediated by peptide-induced regulatory T cells in vivo. J. Immunol. 170 (3), 1240–1248. https://doi.org/10.4049/jimmunol.170.3.1240.

Taylor, A., Harker, J.A., Chanthong, K., Stevenson, P.G., Zuniga, E.I., Rudd, C.E., 2016. Glycogen synthase kinase 3 inactivation drives T-bet-mediated downregulation of Co-receptor PD-1 to enhance CD8(+) cytolytic t cell responses. Immunity 44 (2), 274–286. https://doi.org/10.1016/j.immuni.2016.01.018.

Thielens, A., Vivier, E., Romagne, F., 2012. NK cell MHC class I specific receptors (KIR): from biology to clinical intervention. Curr. Opin. Immunol. 24 (2), 239–245. https://

doi.org/10.1016/j.coi.2012.01.001.
Tian, H., Shi, G., Wang, Q., Li, Y., Yang, Q., Li, C., Yang, G., Wu, M., Xie, Q., Zhang, S., et al., 2016. A novel cancer vaccine with the ability to simultaneously produce anti-PD-1 antibody and GM-CSF in cancer cells and enhance Th1-biased antitumor immunity. Signal Transduct. Target. Ther. 1, 16025. https://doi.org/10.1038/sigtrans.2016.25.
van Bussel, M.T.J., Beijnen, J.H., Brandsma, D., 2019. Intracranial antitumor responses of nivolumab and ipilimumab: a pharmacodynamic and pharmacokinetic perspective, a scoping systematic review. BMC Cancer 19 (1), 519. https://doi.org/10.1186/s12885-019-5741-y.
van Tellingen, O., Yetkin-Arik, B., de Gooijer, M.C., Wesseling, P., Wurdinger, T., de Vries, H.E., 2015. Overcoming the blood-brain tumor barrier for effective glioblastoma treatment. Drug Resist. Updat. 19, 1–12. https://doi.org/10.1016/j.drup.2015.02.002.
Veglia, F., Perego, M., Gabrilovich, D., 2018. Myeloid-derived suppressor cells coming of age. Nat. Immunol. 19 (2), 108–119. https://doi.org/10.1038/s41590-017-0022-x.
Vibhakar, R., Juan, G., Traganos, F., Darzynkiewicz, Z., Finger, L.R., 1997. Activation-induced expression of human programmed death-1 gene in T-lymphocytes. Exp. Cell Res. 232 (1), 25–28. https://doi.org/10.1006/excr.1997.3493.
Wainwright, D.A., Chang, A.L., Dey, M., Balyasnikova, I.V., Kim, C.K., Tobias, A., Cheng, Y., Kim, J.W., Qiao, J., Zhang, L., et al., 2014. Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1 in mice with brain tumors. Clin. Cancer Res. 20 (20), 5290–5301. https://doi.org/10.1158/1078-0432.CCR-14-0514.
Wang, Y., Wang, L., 2017. miR-34a attenuates glioma cells progression and chemoresistance via targeting PD-L1. Biotechnol. Lett. 39 (10), 1485–1492. https://doi.org/10.1007/s10529-017-2397-z.
Wang, X., Guo, G., Guan, H., Yu, Y., Lu, J., Yu, J., 2019a. Challenges and potential of PD-1/PD-L1 checkpoint blockade immunotherapy for glioblastoma. J. Exp. Clin. Cancer Res. 38 (1), 87. https://doi.org/10.1186/s13046-019-1085-3.
Wang, X., Wang, G., Wang, Z., Liu, B., Han, N., Li, J., Lu, C., Liu, X., Zhang, Q., Yang, Q., et al., 2019b. PD-1-expressing B cells suppress CD4(+) and CD8(+) t cells via PD-1/PD-L1-dependent pathway. Mol. Immunol. 109, 20–26. https://doi.org/10.1016/j.molimm.2019.02.009.
Weller, M., van den Bent, M., Hopkins, K., Tonn, J.C., Stupp, R., Falini, A., Cohen-Jonathan-Moyal, E., Frappaz, D., Henriksson, R., Balana, C., et al., 2014. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. Lancet Oncol. 15 (9), e395–403. https://doi.org/10.1016/S1470-2045(14)70011-7.
Woroniecka, K.I., Rhodin, K.E., Chongsathidkiet, P., Keith, K.A., Fecci, P.E., 2018. T-cell dysfunction in glioblastoma: applying a new framework. Clin. Cancer Res. 24 (16), 3792–3802. https://doi.org/10.1158/1078-0432.CCR-18-0047.
Zhang, X., Chen, W., Fan, J., Wang, S., Xian, Z., Luan, J., Li, Y., Wang, Y., Nan, Y., Luo, M., et al., 2018. Disrupting CD47-SIRPalpha axis alone or combined with autophagy depletion for the therapy of glioblastoma. Carcinogenesis 39 (5), 689–699. https://doi.org/10.1093/carcin/bgy041.