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Review

Tumor-associated microenvironment, PD-L1 expression and their relationship with immunotherapy in glioblastoma, IDH-wild type: A comprehensive review with emphasis on the implications for neuropathologists

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ABSTRACT

Although novel knowledge has been acquired on the molecular landscape of glioblastoma (GBM), a relatively few steps forward have been made regarding its therapy. With the increasing use of novel immunotherapeutic drugs capable of stimulating the antitumor inflammatory response, in the last decades numerous studies aimed to characterize the tumor-associated microenvironment (TME) and its relationship with the immunogenicity of GBM. In this regard, although the tumor-associated microglia and macrophages (TAMs) and PD-L1/PD-1 axis have been emerged as one of the most relevant components of the GBM TME and one of the potential molecular pathways targetable with immunotherapy, respectively. It has been supposed that TAMs may acquire different phenotypes, switching from M1 to M2 phenotypes, with tumor-suppressive and tumor-stimulating role depending on the different surrounding conditions. PD-L1 is a type 1 transmembrane protein ligand expressed by T-cells, B-cells and antigen-presenting cells, with a main inhibitory checkpoint role on tumor immune regulation. While PD-L1 immunohistochemical expression has been extensively investigated in many cancers, its usefulness in the evaluation of GBM response rates to immunotherapy and its standardized evaluation by immunohistochemistry are still debated.

The present review paper focuses on the current "state of the art" about the relationship between TME, PD-L1/PD-1 pathway and immunotherapy in GBM, also providing neuropathologists with an updated guide about the clinical trials conducted with PD-L1 and PD-1 inhibitors.

1. Introduction

Glioblastoma (GBM), IDH-wild type, is defined by the World Health Organization (WHO) as a grade 4 malignant adult-type diffuse glioma lacking isocitrate dehydrogenase 1 or 2 (*IDH1/2*) mutations [1–3]. In the present review we utilized the term "GBM" to indicate the GBM, IDH-wild type category. The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) and the 2021 WHO Classification of Central Nervous System (CNS) Tumors introduced some molecular criteria to diagnose this entity even in the absence of the conventional histological criteria (necrosis and/or microvascular proliferation) [4–7]: i) combined chromosome 7 gain and chromosome 10 loss and/or ii) Epidermal Growth Factor Receptor (*EGFR*) amplification and/or iii) Telomerase Reverse Transcriptase (*TERT*) promoter mutation. Accordingly, in the last decade, an increasing attention to the molecular landscape of this tumor has been paid also with the aim of identifying potential targetable genes.

Despite the currently available treatments, GBM exhibits an

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invariably poor prognosis characterized by median survival times of approximately 14 months after the histological diagnosis [8,9]. Those patients harboring O-6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation have a slight better outcome since they are more susceptible to alkylating agents (temozolomide) due to the silencing of this gene [10]. GBM exhibits exceptional intratumoral heterogeneity both morphologically and molecularly and this explains the frequent onset of resistance to the conventional therapies and its poor outcome [11,12]. The recent incorporation of molecular data into the classification and prognosis of GBMs led to the identification of the following four molecular subtypes [13,14], the classical, mesenchymal, neural and proneural ones, characterized by epidermal growth factor receptor (*EGFR*) mutation/amplification/overexpression, neurofibromin-1 (*NF1*) deletion, EGFR amplification/overexpression and platelet-derived growth factor receptor alpha (*PDGFRa*) activation, respectively.

Despite the numerous acquisitions in terms of knowledge of the molecular mechanisms characteristic of this tumor, in the last decades a relatively few steps forward have been made in the field of GBM management. The conventional therapeutic approach includes surgical resection, followed by radiotherapy and adjuvant temozolamide chemotherapy [15–18]. Regarding other potential therapeutic options, some anti-angiogenic drugs (bevacizumab) have been approved by the U.S. Food and Drug Administration (FDA) and are available in the clinical practice [19–21].

The introduction of immunotherapy as potential therapeutic option of human solid tumors has opened novel research frontiers for adult high-grade gliomas [22]. It is largely known that the blood-derived immune cells can infiltrate the CNS particularly when a disruption of the blood–brain barrier (BBB) occurs. Since this damage is promptly demonstrable in GBMs by contrast-enhanced brain magnetic resonance imaging (MRI), the tumor-associated microenvironment (TME) is frequently enriched with both resident and infiltrating immune cells, mainly composed of suppressive tumor-associated macrophages (TAMs) and T regulatory lymphocytes with an overall suppressive effect on immune system [22,23]. Although these features confer to GBM an overall “cold tumor” phenotype, there is literature evidence that the immunotherapy may represent a promising tool capable of enhancing the anti-tumor response at the TME level [22,23]. As a result, an increasing number of clinical trials have been introduced to test the efficiency of immunotherapy in GBM. A relevant role in negatively regulating the immune system in several conditions, including cancer, is played by the PD-1/PD-L1 pathway [24]. The inflammatory cell component, including activated B-lymphocytes, T-lymphocytes, dendritic cells and macrophages, expresses PD-L1 ligand that binds to the PD-1 receptor expressed by T-lymphocytes, leading to the inhibition of the immune response. In addition, tumor cells may also express PD-L1 as an “adaptive immune mechanism” to be unrecognized by the immune system [24–26].

We herein present an update review of the current “state of the art” of the immune landscape of GBM, focusing on the cross-talk between neoplastic cells and TME, the PD-L1 expression and the various modalities by which the immunotherapy may affect GBM tumor immunity and prognosis.

2. GBM tumor-associated microenvironment

The marked intratumoral heterogeneity along with the crosstalk between neoplastic cells and TME are probably the main reasons why GBM exhibits an invariably poor outcome and frequent resistance to the therapies [23]. Multiple mechanisms of interaction are adopted by tumor cells to communicate with each other and with the surrounding TME, including extracellular vesicles, nano- and microtubules, gap junctions and neuron-to-brain tumor synaptic communications [23]. The latter represent an interesting mechanism mediated by specific neurotransmitter receptors on GBM cells and glutamate receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtype at postsynaptic level [27–29]. It has been supposed that the activation of neuron-to-GBM synapses could enhance the tumor invasiveness and progression and that GBM contained a complex cell network composed by both interconnected tumor cells and other unconnected components resembling neuronal- like cells at transcriptional level, that are thought to receive neuronal synaptic activation and to mediate GBM invasiveness [27–29].

Tumor-associated microglia and macrophages (TAMs) represent the most investigated cells of the TME, which the tumor is supposed to communicate with, accounting for approximately 30–50 % of the whole GBM mass [23,30,31]. Several studies have correlated the levels of TAMs with higher tumor grading and poorer prognosis [23]. Infiltrating TAMs mainly consist of resident microglia in the early stages of tumor growth and of peripheral, bone marrow-derived, monocytes and macrophages in more advanced stages [23,30–32]. The blood-brain barrier damage and the production of cytokines and chemokines by the tumor itself, including CC-chemokine ligand 7 (CCL7), CCL2, colony-stimulating factor-1 (CSF-1), glial cell line-derived neurotrophic factor (GDNF), stroma cell-derived factor 1 (SDF-1), granulocyte-macrophage colony-stimulating factor (GM-CSF) and hepatocyte growth factor (HGF), induce the recruitment of peripheral immune cells into CNS. Different phenotypes can be acquired by TAMs, ranging from M1 and M2 phenotypes, with tumor-suppressive and tumor-stimulating function, respectively [23,30–32]. M1-shifted TAMs stimulate an interferon gamma (IFγ)-mediated production of immune-stimulating factors such as tumor necrosis factor-α (TNF-α), while M2-shifted TAMs play the opposite function of producing angiogenic growth factors involved in chronic inflammation, tumor progression, invasion, and immune-suppression [23,30–32]. The complex interaction between tumor cells and TME and the production of immune-suppressive cytokines and chemokines lead to an overall inhibition of the immune response versus the neoplastic cells and to the acquisition of an immune-suppressive and a tumor-stimulating phenotype by the surrounding immune cells [23,30–32]. Accordingly, TAMs, along with the recruited neutrophils and mast cells, tend to stimulate tumor growth and progression [23,30–32].

Tumor-infiltrating lymphocytes (TILs) join the TAMs in GBM TME, but, while they have been demonstrated to have favorable effect on the outcome of patients affected by other cancer types, including colorectal, ovarian and breast cancers [33–36], their role in diffuse gliomas is still controversial. Since CD4+ or CD8+ T-lymphocytes account for approximately <1 % of all cells of TME [37], their quantity is probably insufficient to guarantee adequate anti-tumor response [37]. At histopathological level, TIL amount and distribution have been reported to be usually mild and sparse, mainly aggregated around vessels rather than interspersed between neoplastic cells, suggesting that TILs might not be able to promptly migrate into the GBM immunosuppressive TME, and remain confined to the perivascular spaces [38,39]. Alsafwani et al. demonstrated that CD5+ T-lymphocytes had not a uniform distribution across the tumor tissue, but they were preferentially seen in non-hypoxic carbonic anhydrase IX (CAIX)-negative tumor areas [40]. These findings were present in approximately 70 % of the cases from their cohort and in almost all cases with prominent TIL enrichment [40].

Some colleagues evaluated the spatial distribution, the composition and the evolution upon treatment of immune cells in primary and recurrent GBM [41]. Their findings demonstrated that the evolution of the immune landscape between matched primary and recurrent GBM is highly heterogeneous: in more detail, a global increase in macrophages and differential localization of B7-H3 and HLA-DR were found [41].

Despite the presence of abundant immune infiltrate into the GBM TME, there have been described multiple immune escape pathways that contribute to produce the above-mentioned “cold” phenotype, including the TGFβ2-mediated T-cell suppression, the upregulation of co-inhibitory receptors on both CD4+ helper T-cells and CD8+ cytotoxic T-cells, and the well-known expression of inhibitor membrane-bound factors such as Fas antigen ligand (FAS-L) and PD-L1 [42].

3. PD-L1 pathway general considerations

PD-L1 is a type 1 transmembrane protein ligand that blocks the activation of T cells, leading to tumor progression [43–45] (Fig. 1). This protein not only inhibits T cell proliferation and cytokine production but enhances even T cell activation [43–45]. The explanation of this contradictory phenomenon is unknown.

In normal tissues, PD-1, which represents a PD-L1 receptor, is expressed on CD4+CD8+ T cells during thymic development and it is selectively expressed on $CD4^+$ and $CD8^+$ T cells, monocytes, natural killer T cells, B cells, and dendritic cells upon induction by TCR and cytokine arousal; its function is to decrease tissue damage and to promote tolerance to self-antigens [43–45]. Moreover, PD-L1 receptor is expressed on the surface of $CD4^+Foxp3^+$ regulatory T cells (Tregs), a subset of $CD4^+$ T cells that play a critical role in maintaining immune tolerance and weakening immune responses, and in promoting the development, the maintenance, and the immunosuppressive function of Tregs, by inhibiting mTOR and AKT phosphorylation (Fig. 1) [43–45]. An overexpression of PD-L1 in patients with gastric cancer, hepatocellular carcinoma, renal cell carcinoma, esophageal cancer, pancreatic cancer, ovarian cancer and bladder cancer is associated with poor clinical outcomes [43–45]. However, in breast cancer PD-L1 expression is related to a better clinical outcome [44].

By blocking antibodies that target PD-1 and PD-L1, such as pembrolizumab, nivolumab and atezolizumab, remarkable response rates have been achieved in cancer patients who have PD-L1-overexpressing tumors [45–47].

However, using PD-L1 as an exclusive predictive biomarker for cancer immunotherapy is questionable due to the low accuracy of PD-L1 immunohistochemistry staining [45–47]. In fact, factors that affect the accuracy of PD-L1 immunohistochemistry staining are multiples, such as the fact that different antibodies used in various studies have different sensitivity [45–47]. On the other hand, the cut-off value of PD-L1 staining positivity is different; finally, PD-L1 expression in tumors is not uniform, and sampling time and location may affect the results of PD-L1 staining [45–47].

4. Clinical and prognostic significance of PD-L1 expression in GBM

Several studies have recently focused on the correlations between PD-L1 expression and prognosis for gliomas and glioblastomas [48–55]. However, the role of PD-L1 in predicting glioma prognosis remains controversial.

Liu et al. reported for the first time in 2013 that PD-L1 expression may predict a poor clinical outcome [48]. Han et al. reported similar results in a cohort of 54 glioma patients where PD-L1 positivity was assessed by immunohistochemistry, with a cut-off of 5 % of cells showing membrane staining [52]. Similar results were observed by other studies [49–51,53–55].

However, several studies on large cohorts of GBM patients such as a TCGA database analysis evaluating 446 patients and a retrospective study of 117 patients failed to observe a significant correlation between PD-L1 and prognosis [50,56].

Despite these conflicting results, two recent meta-analyses evaluating all the available literature on the role of PD-L1 expression in predicting OS in GBM patients demonstrated a statistically significant association between PD-L1 expression and poor overall survival [57,58]. Based on these finding, PD-L1 expression might represent a negative prognostic predictor in GBM.

It is widely known that hotspot mutations in IDH-1/2 in diffuse astrocytic tumors are associated with a better prognosis compared with GBM, IDH-wild type [59,60]. Interestingly, several studies suggest that PD-L1 expression in diffuse gliomas might be influenced by IDH-1/2

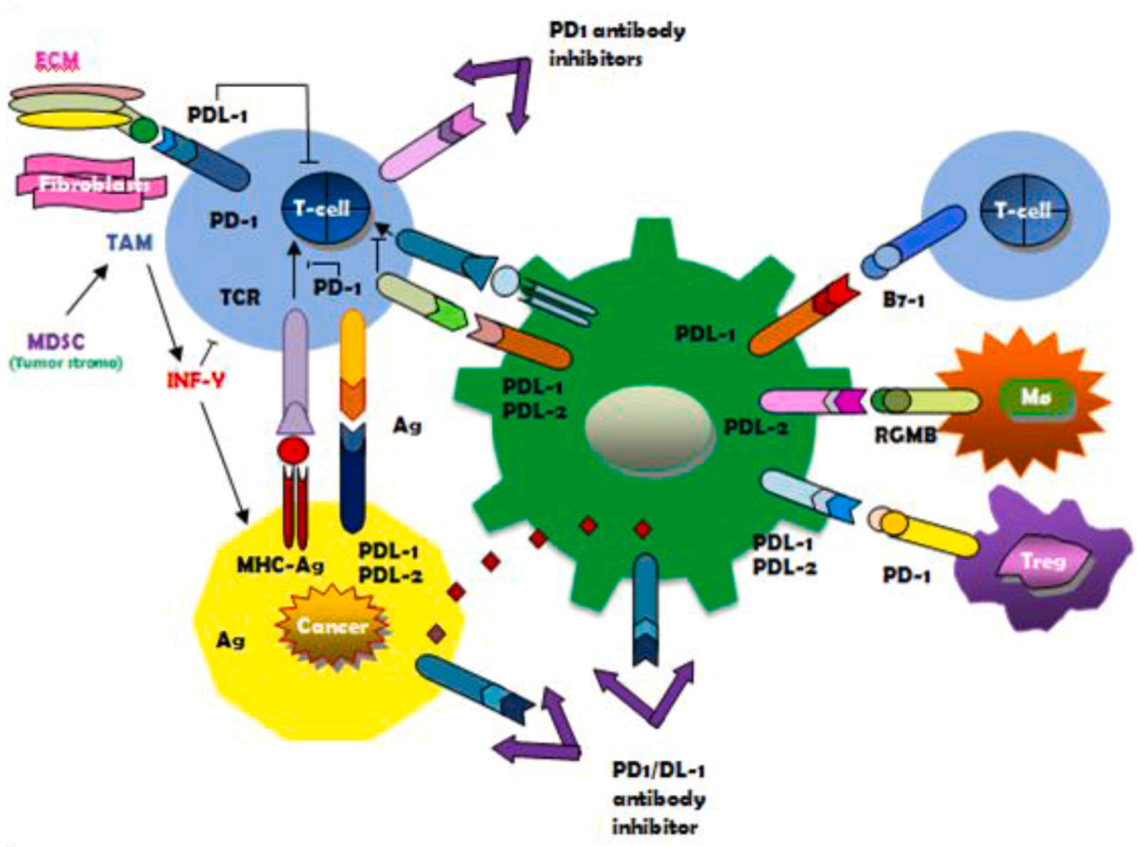

Fig. 1. A representative scheme of the mechanism of action of PD-L1/PD-1 binding in cancer.

mutational status [57,61,62]. A recent meta-analysis investigated the possible correlation between PD-L1 expression and IDH mutations [57]. In this study, authors demonstrated that PD-L1 positive GBMs were mainly IDH-wild type [57]. The possible explanation for this unexpected correlation is that IDH mutation may directly influence the down-regulation of PD-L1 throughout PD-L1 promoter hypermethylation [57]. Therefore, the available literature data suggest that IDH1-mutant gliomas may not benefit from PD-L1 immune checkpoint inhibitors therapy given their low PD-L1 expression levels.

Some authors investigated the characteristics of GBM TME related to response to PD-1 inhibitors and developed a response prediction model for selecting patients who might benefit from anti-PD-1 therapy [63]. The amount of M0 macrophages of responders was found to be lower, while they found a greater proportion of activated dendritic cells before PD-1 inhibitors therapy [63]; in addition, some genes, including *ITGAX*, *LRRFIP1* and *FMN1*, were identified as capable of reliably predict the response to PD-1 inhibitors and a good predictive and clinical value was shown by the response prediction model based on them [63].

5. PDL-1 immunohistochemistry in GBM

To date, the majority of published studies demonstrated high PD-L1 expression in GBM [50,64,65].

However, the positive rate of PD-L1 expression is largely variable across studies, ranging from 6.1 % to 100 % with a reported overall positive percentage of PD-L1 expression of 44.72 % [66].

To date, the main limitations of PD-L1 IHC that could explain this expression variability are related to the presence of different techniques and methods of preparing the tumor tissue (tissue microarray versus standard paraffin blocks), as well as different sample size across studies [24,50,64–66]. Additionally, multiple immunohistochemical assays for PD-L1 evaluation along with different immunohistochemical scoring systems and cut-offs greatly influence the reported prevalence of PD-L1 positivity in GBM [50,64–66].

To date, several PD-L1 IHC assays are utilized in clinical practice for predicting the response to anti-PD-L1 immunotherapy in solid tumors [24]. The most commonly utilized PD-L1 clones include SP142, 28–8, SP263, and 22C3 [24].

Differently from solid tumors of other anatomical regions, PD-L1 IHC evaluation in GBM has not been extensively investigated since a limited number of studies have been published so far.

However, the scoring system and cut-offs proposed in a study by Berghoff et al. in 2015 have been utilized in the majority subsequent studies of PD-L1 IHC in GBM patients [50]. In this regard, the tumor proportion score (TPS) is the most commonly utilized system for evaluating PD-L1 expression in GBM [50]. This score estimates the number of neoplastic cells showing PD-L1 cytoplasmic expression of any intensity over the total number of neoplastic cells. Based on this score, the following staining patterns can be observed: i) minimal/absent: staining in <1 % of tumor cells; ii) intermediate: staining in ≥ 1 % and <50 % of tumor cells (Fig. 2A); iii) high: staining in ≥ 50 % of tumor cells [49] (Fig. 2B).

6. Immunotherapy with anti PD-1/PD-L1 in glioblastoma

While the standard approach to first diagnosed GBM consists in the most extensive and safe as possible surgical resection and in adjuvant radio-chemotherapy, unfortunately there is no first line treatment options for recurrent disease and ones that are used are very scarce and their effects are limited [67,68]. In the last decade, molecularly targeted therapies against oncogenic alterations and immune checkpoint molecules (ICIs) have successfully been used in other types of cancer while GBM hasn't met the same reproducibility as other tumours due to its intrinsic low immunogenicity, the immune evasion created by GBM cells and the presence of blood-brain barrier [56,67,69].

While there is preclinical evidence of promising therapeutic utility of anti PD-1/PD-L1 antibodies, most clinical trials results have showed a limited clinical benefit of PD-1/PD-L1 in glioma treatment [70,71]. In this regard, combination therapy protocols were ICIs have been utilized in combination with standard chemotherapy/ Temozolomide and/or radiotherapy showed the most promising results [70,71].

Based on the study by Awada et al., radiotherapy can be exploited to induce immunostimulatory effects on glioblastoma cells in order to improve tumour immunogenicity and to make immunotherapy a potential therapeutic option, especially on GBM relapse [72].

To date, several clinical trials utilizing anti PD-1/PD-L1 therapy in GBM have been conducted (Table 1.).

6.1. PD1 inhibitors trials

Checkmate- 143 (NCT02017717) was a randomized trial that investigated the combination of Ipilimumab/Nivolumab treatment compared to one with Nivolumab as monotherapy drug in patients with recurrent GBM: in the I Phase monotherapy with Nivolumab was found better tolerated than a combination therapy; the III Phase, where Bevacizumab treatment versus Nivolumab was investigated, led to trial failure, since there was no overall survival (OS) bettering for patients treated with Nivolumab as compared to those treated with Bevacizumab (9,77 vs. 10,02 months) [73]. Although responses were more durable in Nivolumab treatment arm of the trial, no data was available on long-term survivors.

CheckMate 498 (NCT02617589) is a randomized phase III clinical trial studying the efficacy of Nivolumab associated with radiotherapy alone compared with standard protocol based on temozolamide (TMZ) plus radiotherapy in patients with newly diagnosed GBM with unmethylated *MGMT* promoter [74], since it has been demonstrated that radiation could enhance the efficacy of PD-1 inhibitors, by exposing antigenic mutations leading to activated T cells. However, as in CheckMate 143, Nivolumab did not demonstrate any improvement in OS when compared to standard protocol.

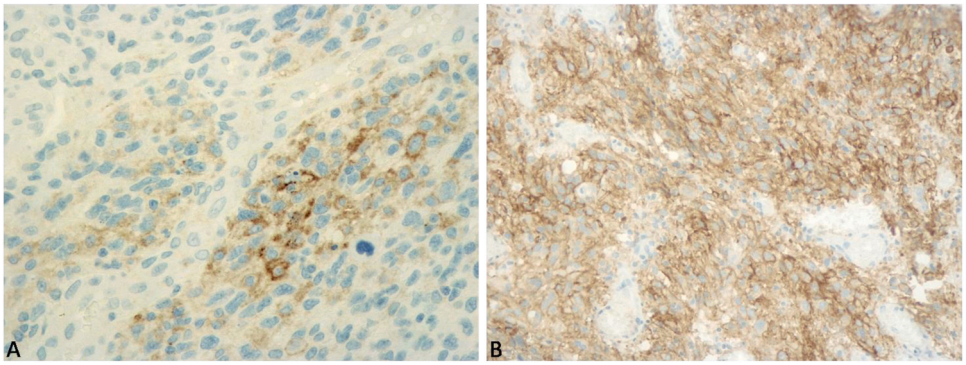

Fig. 2. Representative images of intermediate (**A**) and high (**B**) TPS of PD-L1 (SP263 clone) in GBM tissue (immunoperoxidase; original magnifications 200x).

Table 1
Clinical trials with anti PD-1/PD-L1 drugs in glioblastoma.

Clinical trial identification number	Study phase	Monoclonal Antibody
NCT02829931	I	Nivolumab
NCT02312272	I	Pembrolizumab
NCT02529072	I	Nivolumab
NCT02658981	I	Anti-LAG-3, Urelumab, Nivolumab
NCT02526017	I	Nivolumab
NCT03233152	I	Ipilimumab, Nivolumab
NCT02937844	I	Anti-PD-L1 CSR T cells
NCT03058289	I/II	Anti-PD-1
NCT02327078	I/II	Nivolumab, Epacadostat
NCT02311582	I/II	MK-3475, MRI-guided laser ablation
NCT02866747	I/II	Durvalumab
NCT02798406	II	Pembrolizumab
NCT02335918	II	Varlilumab, Nivolumab
NCT02968940	II	Avelumab
NCT02794883	II	Durvalumab, Tremelimumab
NCT02336165	II	Bevacizumab
NCT02337491	II	Pembrolizumab, Bevacizumab
NCT03014804	II	Nivolumab
NCT02550249	II	Nivolumab
NCT02852655	I	Pembrolizumab
NCT02617589	III	Nivolumab and radiotherapy
NCT02017717	III	Ipilimumab and Nivolumab
NCT02667587	III	Nivolumab and standard therapy
NCT03341806	I	Avelumab and LITT
NCT03233152	I	Ipilimumab and Nivolumab, intracranially
NCT03291314	II	Avelumab and Axitinib
NCT02054806	Ib	Pembrolizumab
NCT02337686	II	Pembrolizumab
NCT03047473	II	Avelumab
NCT03197506	II	Pembrolizumab
NCT03899857	II	Pembrolizumab
NCT03174197	I/II	Atezolizumab
NCT04729959	II	Tocilizumab and Atezolizumab

The more recent results from CheckMate 548 study (NCT02667587) in newly diagnosed GBM with methylated MGMT have also shown the addition of Nivolumab to standard protocol (TMZ + RT) does not improve OS compared with TMZ plus RT alone [75]. This clinical trial is a placebo (PBO)-controlled phase III study that showed median OS of combinatory Nivolumab plus TMZ plus RT was of 28,9 months vs. 32,1 months of PBO+TMZ+RT.

A single arm phase II trial (NCT02550249) tested how presurgical and postsurgical Nivolumab administration affected the tumour microenvironment. Nivolumab treatment in neoadjuvant setting demonstrated to enhance the expression of chemokines and to increase immune cell infiltration and TCR clonal diversity among tumour-infiltrating T lymphocytes. In terms of clinical benefits, none was observed after surgery, but two patients treated with Nivolumab showed long term survival remaining disease-free for more than 33.3 and 28.5 months [76]. Those patients also presented MGMT methylation and one of them was harbouring IDH1 mutation, associated with improved prognosis. Similar results were seen using Pembrolizumab in neoadjuvant setting. Cloughes et al., demonstrated that patients in the neoadjuvant arm of the study showed a significant increase in OS compared to the adjuvant-only group (13,7 months vs. 7,5 months) [77]. They also showed neoadjuvant treatment was associated with focal induction of PD-L1 expression, even though it was not as striking as expected, as similarly demonstrated by the phase II trial NCT02550249 [77].

Glitipni trial (NCT03233152) aimed to administer immunotherapy drugs locally, during surgical resection, in order to improve anti-tumour immune response and to overcome the intrinsic therapeutic resistance of the tumour and the blood-brain barrier which limits the systemic therapy [78]. Ipilimumab and Nivolumab were administered in a pre- and postoperative neoadjuvant setting, starting four weeks prior the surgical resection. Intracranial injections of Ipilimumab and Nivolumab were administered at the end of the surgical intervention and were followed by intravenous administration of Nivolumab at every two weeks for a maximum of five administrations. This trial demonstrated that the intracavitary administrations are safer and more tolerated than intravenous administrations, with a lower incidence of adverse effects [67, 78]. While there was no substantial difference in progression free survival (PFS) in the phase I of this trial compared to patients treated with Bevacizumab, there was a significant difference in terms of overall survivor (OS) [78]. Currently, changes in dosing, administered compounds, and methods of administration have been made following the first two cohorts and results will be published in the following year [67].

6.2. *PD-L1 inhibitors trials*

GliAvAx (NCT03291314) was a clinical trial conducted on recurrent glioblastoma patients that hypothesized the combination of anti-PD-L1 antibody Avelumab (fully human anti-PD-L1 IgG1 antibody) and Axitinib (a tyrosine kinase inhibitor selective for VEGF receptors). While the combined therapy was well tolerated, the trial did not meet the study threshold for activity justifying further investigation (PFS of 18 % in six months) [79].

Phase II study (NCT02336165) on Durvalumab (human IgG1 monoclonal Ab against PD-L1) association with radiotherapy followed by Durvalumab administration in monotherapy setting was performed in patients with new unmethylated MGMT glioblastoma. This study showed a modest increase in OS (15,1 months vs. 12,7 months of historical control) [80].

Recently, a phase I clinical trial (NCT03341806) of VEGF-A inhibition combined with PD-L1 blockade has been published were patients with recurrent glioblastoma were enrolled and divided into two groups: group A was treated with Avelumab while group B was treated with Avelumab within a week after laser interstitial thermal therapy (LITT) following the continuation of Avelumab. Bevacizumab was allowed to be combined in both groups, since its use is demonstrated to spare steroids administration [81]. While there was no significant response to Avelumab or LITT followed by Avelumab or improvement in PFS (median PFS 2,75 months), Kaplan- Meier analysis revealed the median OS for all patients was of 12,75 months and that six patients survived longer than 12 months (five out of six received a combination treatment with Bevacizumab). However, the combination of LITT followed by Avelumab resulted in increased toxicity due to cerebral formation [81].

KEYNOTE-028 trial (NCT02054806), a multicohort phase IB study, enrolled adult patients with PD-L1-positive tumours in the recurrent glioblastoma cohort [82]. Pembrolizumab was administered every two weeks for up to two years. PFS rate at 6 months was 37.7 % (median, 2.8 months; 95 % CI, 1.9–8.1 months), and the overall survival (median, 13.1 months; 95 % CI, 8.0–26.6 months) rate at 12 months was 58 %. Pembrolizumab used in a monotherapy setting demonstrated durable antitumor activity in a subset of patients with recurrent glioblastoma [82]. The response rate was similar to the one in CheckMate-143 study (8 %) that enrolled patients with no data available on PD-L1 expressions among the cohort.

STERIMGLI (NCT02866747) is a phase I/II trial is evaluating the association of Hypofractionated Stereotactic Radiation Therapy (hFSRT) and the anti-Programmed Death-Ligand 1 (PD-L1) Durvalumab in recurrent glioblastoma patients [83]. Up until now, the study has demonstrated that this combined treatment protocol is well tolerated, and phase II of the study has just began, enrolling 100 patients that will be randomized into two arms: Arm A (control arm) treated with radiation therapy alone and Arm B (Experimental arm) that will undergo combined treatment with anti-PD-L1 Durvalumab [83].

7. Conclusions

There are currently multiple clinical trials investigating the use of immunotherapy targeting co-inhibitors molecules in GBMs. Although these therapies have found success in treatment of other tumor types,

this has not been replicated yet in GBM. Neoadjuvant setting use of PD-1 blockers has proved to help with the removal of immune suppressing ability of GBM by enhancing anti-tumour immune response releasing PD-1/PD-L1 checkpoint and enabling T cell receptor modulation. Some promising results, mostly with combination therapy protocols, suggest the need to identify effective methods to discern subgroups of GBM patients who can be potentially treated with immunotherapy.

However, although in the last decade this topic has been of great interest and numerous steps forward have been made in understanding the immune landscape of GBM, further larger-scale prospective studies are still needed to validate the potential value of PD-L1 for the prognosis and treatment of GBM patients.

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Compliance with ethical standards

Not applicable.

Research data statement

No new data have been generated in the present research.

CRediT authorship contribution statement

Farina Jessica: Conceptualization. **Tinnirello Giordana:** Conceptualization. **Barresi Valeria:** Methodology. **Zanelli Magda:** Methodology. **Palicelli Andrea:** Methodology. **Certo Francesco:** Resources. **Barbagallo Giuseppe:** Resources. **Magro Gaetano:** Validation. **Caltabiano Rosario:** Writing – review & editing, Validation. **Broggi Giuseppe:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Angelico Giuseppe:** Writing – original draft, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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