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REVIEW ARTICLE

Mechanisms underlying the failure of PD-1/PD-L1 blockade in glioblastoma: Therapeutic challenges and opportunities

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Abstract

Glioblastoma (GBM) is a highly aggressive primary brain tumor with a poor prognosis and limited therapeutic options. Although programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) blockade has transformed the treatment of several malignancies, its clinical benefit in GBM remains limited, and the mechanisms underlying this resistance remain poorly understood. This review summarizes the biological roles of the PD-1/PD-L1 axis in the GBM microenvironment, evaluates clinical evidence for immune checkpoint inhibitors, and discusses mechanisms of resistance and strategies to overcome therapeutic resistance. It integrates current preclinical and clinical evidence on PD-1/PD-L1 signaling, the cellular sources of PD-L1, clinical trial outcomes, resistance mechanisms, and emerging combination strategies. We propose a dual-source PD-L1 framework to discuss the potential contributions of tumor cell-derived and immune cell-derived PD-L1 to GBM-mediated immunosuppression and therapeutic response. However, whether these two PD-L1 sources have distinct functional specializations remains unproven and requires further validation through spatial omics analyses and cell-type-specific functional studies. This framework may help explain the limited value of total PD-L1 expression as a single predictive biomarker in GBM. We further outline a multilayer resistance model involving the blood–brain barrier, an immunologically cold tumor microenvironment, and compensatory immunosuppressive networks. Emerging strategies, including optimized treatment timing, microenvironmental modulation, and rational immunotherapy combinations, are also discussed. A deeper understanding of PD-1/PD-L1 biology and multilayered immune resistance may guide the development of more effective immunotherapeutic strategies for GBM.

Keywords: Glioblastoma; Programmed cell death protein 1; Programmed death-ligand 1; Immune checkpoint blockade

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1. Introduction

Gliomas are among the most common primary tumors of the central nervous system and are characterized by marked molecular heterogeneity and a highly immunosuppressive tumor microenvironment. Among them, glioblastoma (GBM) represents the most malignant subtype. The current standard treatment for newly diagnosed GBM remains maximal safe surgical resection, followed by concurrent radiotherapy and temozolomide (TMZ) chemotherapy, and subsequent adjuvant TMZ therapy.[1-7] In recent years, advances

such as fluorescence-guided surgery, intraoperative magnetic resonance imaging, neuronavigation, and functional mapping have contributed to improving the extent of tumor resection while reducing the risk of new neurological deficits. The extent of resection is also considered an important clinical factor influencing patient prognosis.[8,9] However, owing to the diffuse infiltrative growth, substantial heterogeneity, and a high propensity for recurrence of GBM, improvements in overall survival (OS) remain limited despite standard multimodal treatment. Most patients ultimately develop tumor recurrence, and recurrent GBM still lacks a standard therapeutic strategy capable of consistently improving OS; long-term survival remains uncommon.

In recent years, immune checkpoint inhibitors targeting programmed cell death protein 1 (PD-1) and its ligand, programmed death-ligand 1 (PD-L1), have revolutionized the treatment of multiple malignancies. However, in contrast to their success in other solid tumors, clinical trials in GBM have consistently failed to demonstrate significant survival benefits. This discrepancy highlights fundamental differences in tumor biology and immune regulation between GBM and other cancers; however, the underlying mechanisms remain incompletely understood.

In this review, we summarize the biological functions of the PD-1/PD-L1 axis in the glioma microenvironment and critically evaluate key clinical studies. We further propose a dual-source model of PD-L1 to explain its heterogeneous roles and limited predictive value as a biomarker in GBM. In addition, we integrate current evidence to outline a multilayered resistance framework and discuss emerging therapeutic strategies aimed at overcoming these barriers.

2. PD-1/PD-L1 axis and the immunological microenvironment of glioma

2.1. Biological functions of the PD-1/PD-L1 axis

Programmed cell death protein 1 is a key inhibitory receptor that is upregulated on activated T cells and other immune cells following chronic antigen exposure. Upon binding to its ligand, PD-L1, which is expressed on tumor cells and antigen-presenting cells, PD-1 transduces inhibitory signals that suppress T-cell activation, proliferation, and effector function. This pathway represents a central mechanism of immune evasion in cancer.[10-12]

In the glioma microenvironment, PD-L1 is frequently overexpressed not only by tumor cells but also by tumor-associated macrophages (TAMs). Persistent engagement of PD-1 on infiltrating T cells leads to T-cell exhaustion and impaired antitumor immunity. This sustained inhibitory signaling contributes to the establishment of an immunosuppressive niche and represents a critical pathway by which gliomas evade immune surveillance (Figure 1).

2.2. A dual-origin model of PD-L1 in glioma

Current evidence suggests that PD-L1 in the glioma

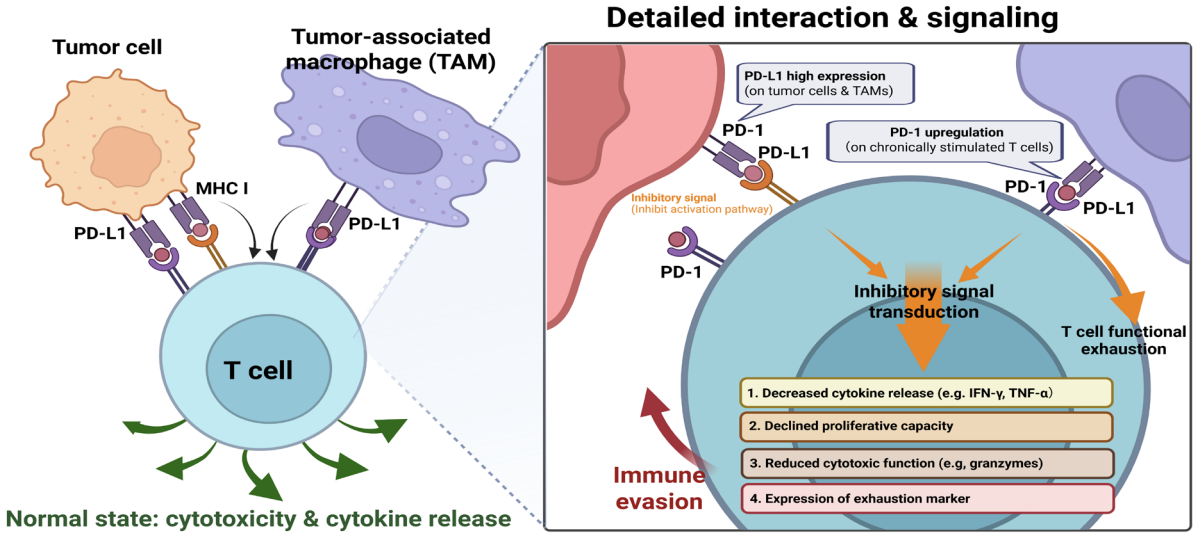

Figure 1. Mechanism diagram of T cell exhaustion mediated by the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) pathway in tumor cells and TAMs. The schematic summarizes how PD-L1 expressed by malignant glioma cells and TAMs engages PD-1 on effector T cells. Under normal immune activation, T cells release cytokines and maintain cytotoxic function; sustained PD-1/PD-L1 signaling suppresses T-cell receptor-related activation, reduces cytokine release and proliferative capacity, weakens cytotoxic effector function, and promotes exhaustion markers, thereby enabling immune escape. Created with BioRender. Lu, J. (2026) https://BioRender.com/ucjtifx

microenvironment is not expressed exclusively by tumor cells but can also be expressed by TAMs, microglia, and other infiltrating immune cells.[13-18] Therefore, in this review, we conceptualize PD-L1 expression as a "dual-source" pattern, in which tumor cell-derived PD-L1 and immune cell-derived PD-L1 jointly contribute to the formation of the immunosuppressive microenvironment in GBM (Figure 2). It should be emphasized that this pattern is currently best regarded as a conceptual framework or working hypothesis rather than a fully established mechanistic model.

Tumor cell-derived PD-L1 is mainly localized on the surface of malignant glioma cells and, in theory, may bind to PD-1 on infiltrating T cells, thereby suppressing T-cell receptor signaling, cytokine secretion, proliferation, and cytotoxic effector function. This process may promote T-cell exhaustion and tumor immune evasion.[11,13,14,17,18] Previous studies have shown that PD-L1 expression is increased in high-grade gliomas and may be associated with poor prognosis, an immunosuppressive state, and uncertainty regarding the efficacy of immune checkpoint blockade.[13,14,17,18] However, PD-L1 expression in GBM exhibits marked spatial heterogeneity and dynamic changes, and its biological significance cannot be adequately determined solely by total PD-L1 levels.[17,19]

Immune cell-derived PD-L1 is mainly associated with TAMs and microglia. The study by Zhu *et al.*,[13] together with analyses of the Chinese Glioma Genome Atlas (CGGA) and The Cancer Genome Atlas (TCGA) databases, suggests that PD-L1 expression correlates with M2-like macrophage markers and immunosuppression-related signaling, indicating that TAM-derived PD-L1 may participate in shaping an immunosuppressive microenvironment. Recent studies further support this view: TAM-targeted strategies are considered an important direction for glioma immunotherapy;[16] the arachidonate 5-lipoxygenase (ALOX5)/5-hydroxyeicosatetraenoic acid (5-HETE) axis can promote M2-like polarization and PD-L1 expression in glioma-associated microglia/macrophages;[20] and GBM-derived extracellular vesicles after radiotherapy can induce increased PD-L1 expression in supportive macrophages, thereby exacerbating local immunosuppression.[21] Nevertheless, this body of evidence remains largely focused on correlations, pathway induction, or treatment-associated changes in expression and does not directly demonstrate that TAM-derived PD-L1 has an independent function distinct from that of tumor cell-derived PD-L1.

Accurately distinguishing the cellular source of PD-L1 in clinical GBM samples remains methodologically challenging. Conventional immunohistochemistry can detect PD-L1 positivity, but the results are influenced by antibody clone, positivity thresholds, sampling region, and intratumoral heterogeneity. Moreover, this approach often cannot reliably determine whether PD-L1 originates from malignant glioma cells, TAMs, microglia, myeloid-derived suppressor cells (MDSCs), or other immune cells.[13,17] GBM tissue architecture is highly heterogeneous, and tumor cells and myeloid cells are often spatially intermingled; therefore, relying solely on morphology and a single marker may lead to misclassification of cellular origin. Single-cell RNA sequencing can improve cellular resolution, but messenger RNA (mRNA) expression does not necessarily correspond to protein expression, and tissue dissociation may alter immune-cell states or result in the loss of certain cell populations. By contrast, multiplex immunofluorescence, spatial transcriptomics, and spatial proteomics are better suited to simultaneously resolve the cellular identity of PD-L1-expressing cells and their spatial relationship with PD-1-positive T cells. Recent single-cell and spatial transcriptomic studies have demonstrated that tumor cell–TAM interactions, immune cell distribution, and tissue niches in glioma are highly spatially dependent.[19,22] However, evidence directly validating the functional distinction of PD-L1 derived from different cellular sources in GBM remains limited.

The mode of action of TAM-derived PD-L1 also requires further investigation, particularly regarding the relative contributions of cis- and trans-mediated mechanisms. According to the classical trans-acting model, PD-L1 on the surface of TAMs can bind to PD-1 on neighboring $CD8^+$ T cells or other effector T cells, directly suppressing T-cell activation, proliferation, cytokine release, and cytotoxic function. This model emphasizes the role of TAMs as immunosuppressive antigen-presenting cells or myeloid cells that exert cross-cellular inhibitory effects on adjacent T cells.[16,18] In contrast, the cis-acting model proposes that PD-L1 may interact with PD-1, CD80, or other molecules on the same TAM membrane, thereby affecting PD-L1 ligand availability, the activation state of TAMs, myeloid cell polarization, or local immunoregulatory circuits. Recent studies on PD-L1/CD80 interactions suggest that cis- and trans-mediated mechanisms may have distinct, or even opposing, immunological consequences.[23] However, this mechanistic evidence is mainly derived from non-GBM models, and direct experimental evidence supporting a defined cis-regulatory role of TAM-derived PD-L1 in GBM remains lacking.

Taken together, tumor cell-derived PD-L1 and immune cell-derived PD-L1 may differ in their spatial distribution, cellular targets, and modes of immune regulation; however, such functional differences remain

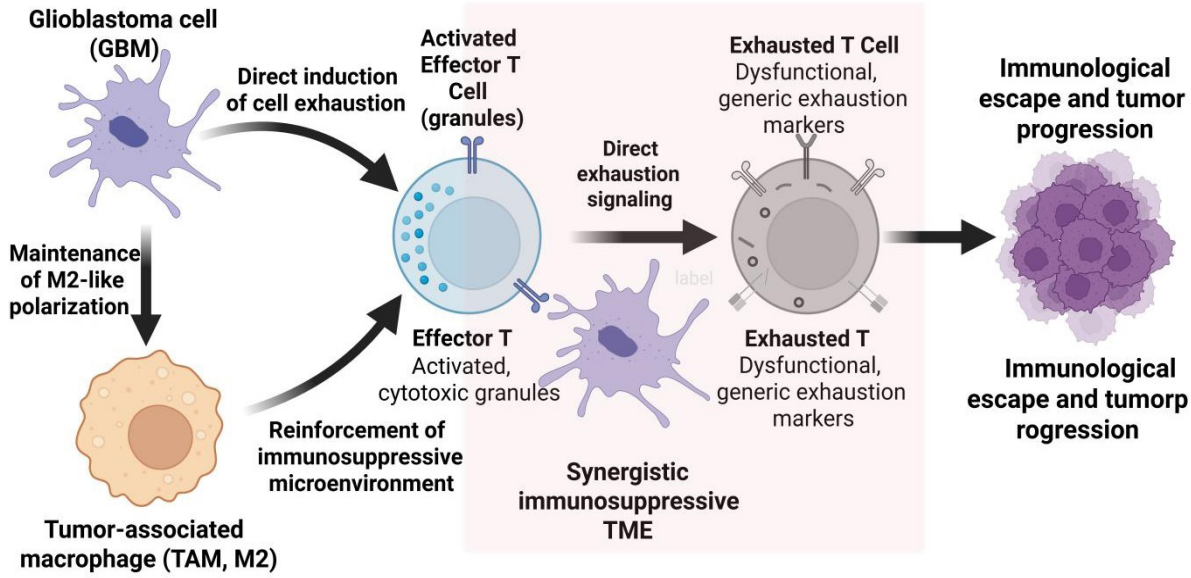

Figure 2. Synergistic mechanism of GBM immune escape: cooperative immunosuppression by tumor cells and TAMs. This schematic illustrates the proposed dual-source programmed death-ligand 1 (PD-L1) framework in which glioblastoma cells and M2-like TAMs jointly reinforce T-cell dysfunction. GBM cells may directly induce exhaustion signals, whereas TAMs maintain an immunosuppressive macrophage state and suppress effector T-cell activity, forming a TAM–tumor cell–T cell circuit that favors immune escape and tumor progression. Created with BioRender. Lu, J. (2026) https://BioRender.com/2qy946q
Abbreviation: TME: Tumor microenvironment.

speculative. A more rigorous interpretation is that the dual-source model provides a testable explanatory framework for understanding the heterogeneity of PD-L1 expression and the limited predictive value of PD-L1 in GBM, rather than a fully proven mechanistic conclusion. Future studies integrating spatial omics, single-cell multi-omics, cell-type-specific *CD274* (PD-L1 gene) knockout or silencing models, TAM–T-cell co-culture systems, and in vivo functional validation are needed to define the true contributions of PD-L1 from different sources to immune escape and the failure of PD-1/PD-L1 blockade therapy in GBM.[19-22]

2.3. Glioblastoma as an immunologically "cold" tumor

The clinical heterogeneity of PD-L1 across tumor types is largely influenced by differences in the tumor immune microenvironment, particularly the degree of effector T-cell infiltration.[24] Tumors can be broadly classified as "hot" or "cold" based on immune activity. "Hot" tumors are characterized by abundant T-cell infiltration and elevated expression of cytotoxic and interferon (IFN)-γ-related genes, and they tend to respond more favorably to immune checkpoint blockade.[25,26] In contrast, "cold" tumors exhibit limited T-cell infiltration and a highly immunosuppressive microenvironment, leading to primary resistance.

Glioblastoma represents a prototypical "cold" tumor, with markedly reduced infiltration of $CD8^+$ effector T cells.[27] Bioinformatic analyses further suggest that PD-L1 expression is inversely correlated with $CD8^+$ T- cell infiltration,[14] indicating its role in reinforcing immune exclusion. PD-L1 not only directly inhibits T-cell activation and proliferation but also promotes the recruitment of regulatory T cells (Tregs) and TAMs, thereby establishing an immunosuppressive network.

At the molecular level, PD-L1 expression is tightly regulated by the IFN-γ–Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signaling pathway, which also plays a critical role in antigen presentation and immune activation.[28] In GBM, dysregulation of this pathway, including mutations and reduced expression, impairs antigen presentation and further reinforces the "cold" tumor phenotype. These alterations contribute to both the heterogeneity of PD-L1 expression and the limited efficacy of PD-1/PD-L1 blockade (Figure 3).

2.4. Dynamic regulation of PD-L1 expression

The expression of PD-L1 is not static but is dynamically regulated by the tumor microenvironment, therapeutic interventions, and tumor grade. These dynamic changes have important implications for immunotherapy response

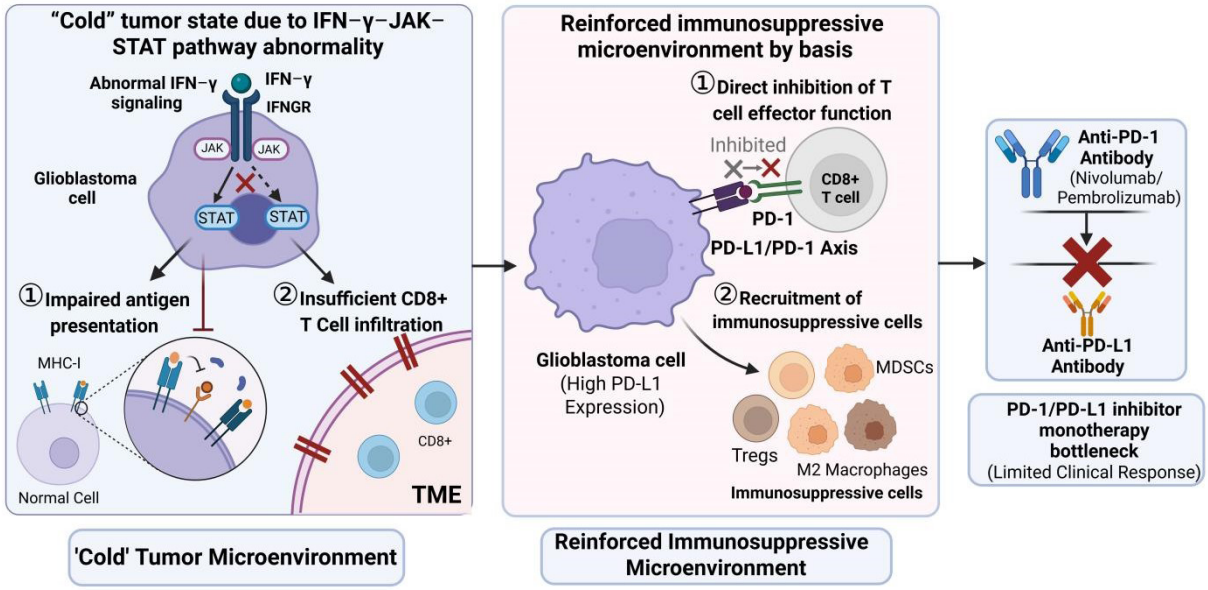

Figure 3. Mechanisms of programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitor resistance in glioblastoma: "Cold tumor state and reinforced immunosuppression. The left panel depicts the immunologically "cold" tumor state driven by interferon-gamma (IFN-γ)/Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway abnormalities, impaired antigen presentation, and insufficient $CD8^+$ T-cell infiltration. The middle panel shows reinforcement by an immunosuppressive microenvironment, including high PD-L1 expression, direct inhibition of effector T cells, and recruitment of myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and M2 macrophages. The right panel summarizes the clinical consequences: anti-PD-1 or anti-PD-L1 antibodies may be present, but the response remains limited because multiple resistance layers persist. Created with BioRender. Lu, J. (2026) https://BioRender.com/w77ldi5
Abbreviations: IFNGR: Interferon gamma receptor; MHC-I: Major histocompatibility complex class I; TME: Tumor microenvironment.

and clinical outcomes.

In glioma, PD-L1 expression varies across tumor grades and disease stages. For example, following bevacizumab-based treatment, levels of soluble PD-L1 (sPD-L1), a systemic inflammatory marker, are lower in recurrent low-grade glioma than those in recurrent GBM, suggesting differences in immune regulation between tumor subtypes. Notably, GBM is generally characterized by elevated PD-L1 expression,[29,30] which would theoretically favor a response to PD-1/PD-L1 blockade. However, therapeutic interventions, such as radiotherapy and chemotherapy, can further induce PD-L1 upregulation, contributing to adaptive immune resistance.

Moreover, the tumor microenvironment differs significantly between primary and recurrent GBM. Recurrent tumors often exhibit a more immunosuppressive milieu and more complex PD-L1 expression patterns due to prior treatments. These dynamic alterations may partially explain the limited efficacy of PD-1/PD-L1 inhibitors in GBM.[31,32]

Taken together, these findings indicate that PD-L1 is not the sole determinant of glioma progression or therapeutic response. Its dynamic and context-dependent expression underscores the need for combination strategies targeting multiple components of the tumor microenvironment.[31]

2.5. Molecular subtype-dependent PD-L1 expression and response to immunotherapy

In addition to *IDH* mutational status and *MGMT* promoter methylation, the transcriptional subtypes of GBM are important determinants of PD-L1 expression, immune-cell infiltration, and responses to immunotherapy. Traditionally, GBM has been classified into proneural, classical, and mesenchymal subtypes based on transcriptional profiles, with substantial differences in driver alterations, cellular states, immune-cell infiltration, and therapeutic sensitivity. It should be noted that these subtypes are not completely fixed or mutually exclusive entities; rather, they are better regarded as cellular states that can transition during tumor evolution and under therapeutic pressure. Recent studies further suggest that the proneural–mesenchymal axis represents one of the major trajectories of phenotypic heterogeneity in GBM and is associated with cell-cycle regulation, metabolic reprogramming, and immune evasion.[33,34]

With respect to the tumor immune microenvironment, mesenchymal GBM is generally characterized by a higher degree of immune-cell infiltration, particularly

enrichment of myeloid cells, macrophage/microglia-related populations, dendritic cell-associated signatures, and inflammatory pathways. Compared with proneural and classical tumors, mesenchymal tumors often exhibit a more pronounced immune-activated phenotype and higher expression of immune checkpoint-related molecules, including those associated with the PD-1/PD-L1 axis. Therefore, the mesenchymal subtype may provide a stronger biological rationale for PD-1/PD-L1 blockade.[35]

However, greater immune infiltration in mesenchymal GBM does not necessarily translate into an improved response to immunotherapy. A substantial proportion of infiltrating cells comprises immunosuppressive myeloid populations, such as TAMs and MDSCs, whereas effector T cells may be exhausted or functionally impaired. Thus, mesenchymal GBM is more accurately described as an "immune-enriched but highly immunosuppressed" state rather than simply an "immune-hot tumor." This may explain why elevated PD-L1 expression does not consistently predict benefit from PD-1/PD-L1 blockade, highlighting the need to concurrently assess the cellular source of PD-L1, T-cell functional status, and myeloid-cell composition.[35,36]

Clinical and translational studies also support an important role for molecular subtypes in shaping responses to immunotherapy. A prospective study of patients with newly diagnosed GBM treated with anti-PD-L1 therapy combined with standard chemoradiotherapy showed that a higher tumor immune-enrichment score was associated with longer survival. Immune enrichment was also strongly associated with the pretreatment mesenchymal subtype, and patients with mesenchymal tumors had a longer median OS than those with proneural or classical tumors.[37] Conversely, single-cell studies have shown that, following nivolumab treatment, some GBMs may transition toward mesenchymal-like tumor-cell states, accompanied by increased TAM infiltration, T-cell exhaustion, and expansion of proliferative T cells. This "latent immune" phenotype was associated with poorer survival after immune checkpoint therapy.[36] These findings suggest that mesenchymal features may have a dual significance: they may reflect an inflammatory state that could potentially be exploited by immunotherapy, but they may also indicate a treatment-induced state of adaptive resistance.

By comparison, the proneural subtype is generally characterized by neurodevelopmental and stem cell-like transcriptional programs, relatively limited immune-cell infiltration, and a possible lack of the pre-existing effector T-cell activity required for effective PD-1/PD-L1 blockade. The classical subtype is frequently associated with *EGFR* alterations, and some studies suggest that *EGFR* mutation or amplification may be associated with poor responses to immunotherapy.

In the same anti-PD-L1 cohort, *EGFR* mutations were associated with poorer OS, whereas *PTEN* mutations showed a trend toward relatively longer survival.[37] Therefore, patient selection for immune checkpoint inhibition should not be based solely on total PD-L1 expression. Instead, molecular subtype, driver gene alterations, the quality of immune-cell infiltration, and treatment-induced changes in cellular states should be considered collectively.

Glioblastoma immunotherapy research should shift from a single-biomarker model toward a multidimensional stratification framework. An optimal patient-selection strategy should incorporate *IDH* status, *MGMT* promoter methylation status, key driver alterations such as *EGFR* and *PTEN*, proneural/classical/mesenchymal transcriptional subtypes, the cellular source of PD-L1 expression, and the spatial distribution of TAMs and $CD8^+$ T cells. In particular, for mesenchymal GBM, future combination strategies should extend beyond PD-1/PD-L1 blockade to include approaches such as myeloid-cell reprogramming, *ITGA5* targeting, stimulator of interferon genes (STING) activation, and anti-angiogenic therapy. Such strategies may help overcome the paradoxical state of high immune-cell infiltration accompanied by profound immunosuppression, which may otherwise limit therapeutic efficacy.[35,36,38,39]

3. Clinical translation of PD-1/PD-L1 blockade in glioma

3.1. Clinical trial evidence and lessons learned

The inhibitors of PD-1/PD-L1 have demonstrated durable responses and clinical benefit in multiple malignancies.[40-45] However, these successes do not directly predict efficacy in GBM, which arises within the central nervous system and is characterized by blood–brain barrier (BBB)-related restrictions, limited effector T-cell infiltration, myeloid-cell enrichment, and marked spatial and cell-state heterogeneity.[18,36] Therefore, experience from other cancers should be regarded as a mechanistic rationale and guide for trial design, whereas clinical conclusions in GBM should rely on disease-specific prospective studies.

Early clinical trials have demonstrated the limitations of immune checkpoint inhibitor (ICI) monotherapy in GBM. In the phase III CheckMate 143 trial, nivolumab failed to improve OS compared with bevacizumab in recurrent GBM (median OS: 9.8 vs. 10.0 months; hazard ratio [HR] = 1.04; $p = 0.76$).[46] Although bevacizumab showed a higher objective response rate and longer progression-free survival

(PFS), nivolumab produced more durable responses and had a favorable safety profile. Subgroup analyses suggested potential benefit in patients with *MGMT* promoter methylation and no baseline corticosteroid use, whereas PD-L1 expression was not associated with OS.

Similarly, the KEYNOTE-028 phase Ib trial showed only modest efficacy of pembrolizumab monotherapy in recurrent GBM, with an objective response rate of 8% and a median PFS of 2.8 months.[47] No correlation was observed between PD-L1 expression and clinical benefit. These findings are consistent with the immunologically "cold" nature of GBM, in which limited T-cell infiltration restricts the efficacy of PD-1 blockade. Nevertheless, the manageable safety profile supports further exploration of combination strategies.

Compared with postoperative administration alone, neoadjuvant PD-1 blockade may initiate both local and systemic immune responses while tumor antigens remain present. A 2019 multicenter randomized pilot study of 35 patients with surgically resectable recurrent GBM reported longer median OS and PFS in the neoadjuvant pembrolizumab group compared with the adjuvant-only group.[48] Pembrolizumab was generally well tolerated, and preoperative treatment did not introduce new toxicity signals.

Earlier studies have further shown that neoadjuvant therapy upregulated intratumoral T-cell- and IFN-γ-associated gene expression, downregulated cell-cycle-related genes, induced focal PD-L1 expression, and expanded T-cell receptor clones.[48,49] Peripheral blood analyses also demonstrated decreased PD-1 expression on T cells and reduced monocyte populations, indicating measurable local and systemic pharmacodynamic effects of preoperative PD-1 blockade in GBM.

However, a subsequent single-arm expansion cohort of 25 patients confirmed downregulation of cell-cycle- and tumor-proliferation-related genes and upregulation of T-cell/IFN-related genes, but failed to reproduce the OS benefit observed in the initial study. Pembrolizumab remained generally well tolerated, with no unexpected toxicities; six Common Terminology Criteria for Adverse Events (CTCAE) grade ≥3 events were reported, including cerebral edema, headache, fatigue, adrenal insufficiency, and hyperthyroidism.[50] Treatment also did not significantly increase tumor-infiltrating lymphocyte density, suggesting that transcriptional immune activation may not translate into sufficient T-cell infiltration or durable clinical benefit.

Thus, neoadjuvant pembrolizumab should currently be regarded as an investigational strategy that induces measurable pharmacodynamic changes with a manageable safety profile, rather than as a treatment with definitive survival benefit. Existing systematic reviews consider the evidence for neoadjuvant plus adjuvant PD-1 blockade to be of low certainty. Future studies should include larger, adequately powered, well-stratified randomized trials with prespecified biomarker analyses, including cell-cycle signatures, IFN/T-cell features, myeloid inflammatory signals, and corticosteroid exposure.[51,52]

In newly diagnosed GBM, the phase I NRG-BN002 trial evaluated the safety of ipilimumab, nivolumab, and their combination administered with adjuvant TMZ in 32 patients after resection and standard chemoradiotherapy. Grade 4 adverse events occurred in 16% of patients, with no grade 5 events. One dose-limiting toxicity occurred in each monotherapy cohort, whereas none occurred in the combination cohort. Dual checkpoint blockade combined with adjuvant TMZ demonstrated an acceptable safety profile, with median OS and PFS of 20.7 and 16.1 months, respectively, in the combination group.[53] However, because NRG-BN002 was a nonrandomized phase I safety study without a concurrent control group, these survival outcomes should be interpreted only as exploratory findings.

The subsequent randomized phase II/III NRG-BN007 trial further evaluated dual immune checkpoint blockade in newly diagnosed *MGMT*-unmethylated GBM. Radiotherapy combined with ipilimumab and nivolumab did not improve PFS compared with radiotherapy plus TMZ (7.7 vs. 8.5 months; HR = 1.47), and no OS difference was observed.[54] The trial therefore discontinued enrollment and did not proceed to phase III. These results indicate that the favorable safety profile and preliminary survival signals observed in NRG-BN002 did not translate into efficacy in a randomized setting, suggesting that dual checkpoint blockade in GBM remains limited by the cold tumor microenvironment, patient selection, and treatment sequencing[52,55] (Table 1).

Taken together, these clinical studies indicate that PD-1/PD-L1 inhibitor monotherapy provides limited benefit in GBM, whereas neoadjuvant and dual-checkpoint strategies remain investigational. The limited efficacy is attributable to the intrinsic biology of GBM, including a "cold" immune microenvironment and complex immunosuppressive networks, rather than inadequate drug activity alone.

3.2. Multilayered resistance mechanisms underlying the limited efficacy of PD-1/PD-L1 blockade in gliomas

The limited efficacy of PD-1/PD-L1 blockade in gliomas, particularly GBM, is not attributable to a single factor but rather to a multilayered resistance system involving

Table 1. Key clinical trials of PD-1/PD-L1 inhibitors in glioblastoma

Trial (Phase)	Treatment regimen	Patient population	Key data	Core conclusions	Reference
CheckMate 143 (Phase III, RCT)	Nivolumab monotherapy vs. Bevacizumab monotherapy	Recurrent GBM patients	(i) Median OS: 9.8 vs. 10.0 months (HR = 1.04, p = 0.76). (ii) Bevacizumab ORR: 23.1%, median PFS: 3.5 months. (iii) Nivolumab response duration: 11.1 months.	(i) PD-1 monotherapy did not improve OS in recurrent GBM; primary endpoint not met. (ii) Bevacizumab superior in ORR and PFS, but nivolumab responses more durable. (iii) Subgroup: Patients with *MGMT* promoter methylation and no baseline steroid use may benefit.	[46]
KEYNOTE-028 (Phase Ib, exploratory)	Pembrolizumab monotherapy	Recurrent GBM patients	(i) ORR: 8% (95% CI: 1%–26%). (ii) Median PFS: 2.8 months. (iii) Safety manageable, mostly low-grade AEs; no grade 5 toxicity.	(i) Overall efficacy limited, consistent with the immunologically "cold" nature of GBM. (ii) No correlation between PD-L1 expression and clinical benefit. (iii) Manageable safety profile supports combination strategies.	[47]
Neoadjuvant pembrolizumab (Phase II RCT & expansion cohort)	Neoadjuvant vs. adjuvant pembrolizumab	Recurrent surgically resectable GBM (RCT: n = 35; Expansion: n = 25)	(i) RCT: Median OS and PFS significantly improved in the neoadjuvant group. (ii) Expansion: Confirmed cell-cycle gene downregulation but failed to reproduce OS benefit. (iii) Reported 6 grade ≥3 events in expansion (cerebral edema, headache, etc.).	(i) Preoperative PD-1 blockade induces clear local and systemic pharmacodynamic effects. (ii) Transcriptional immune activation does not necessarily translate into sufficient T-cell infiltration or durable clinical benefit. (iii) Should be regarded as an investigational strategy rather than a definitive survival advantage.	[48-52]
NRG-BN002 (Phase I)	Ipilimumab, nivolumab, or combination + adjuvant TMZ	Newly diagnosed GBM after resection and chemoradiotherapy (n = 32)	(i) Grade 4 AEs: 16%; no grade 5 AEs. (ii) DLT: 1 in each monotherapy cohort; 0 in combination cohort. (iii) Exploratory OS: 20.7 months; median PFS: 16.1 months.	(i) Dual checkpoint blockade + adjuvant TMZ has an acceptable safety profile with no overlapping toxicities. (ii) Non-randomized phase I safety study lacking concurrent control; survival outcomes are exploratory and do not establish definitive prognosis benefit.	[53]
NRG-BN007 (Randomized phase II/III)	RT + Ipilimumab + Nivolumab vs. RT + TMZ	Newly diagnosed *MGMT*-unmethylated GBM patients (n = 159)	(i) Median PFS: 7.7 vs. 8.5 months (HR = 1.47). (ii) No difference in OS was observed between groups.	(i) Dual checkpoint blockade + RT failed to improve PFS or OS compared to standard care in *MGMT*-unmethylated patients. (ii) Acceptable safety/survival signals from NRG-BN002 did not translate into randomized trials, showing constraints from the cold tumor microenvironment. Trial discontinued.	[52,54,55]

Abbreviations: AE: Adverse event; DLT: Dose-limiting toxicity; GBM: Glioblastoma; HR: Hazard ratio; ORR: Objective response rate; OS: Overall survival; PD-1: Programmed cell death protein 1; PD-L1: Programmed death ligand 1; PFS: Progression-free survival; RCT: Randomized controlled trial; RT: Radiotherapy; TMZ: Temozolomide.

anatomical barriers, tumor-intrinsic immune evasion, an immunosuppressive microenvironment, and challenges in clinical response assessment. Current evidence suggests that the limited sensitivity of GBM does not indicate that the PD-1/PD-L1 axis is irrelevant, but rather that it operates within a complex immune ecosystem characterized by myeloid-cell enrichment, insufficient T-cell infiltration, vascular abnormalities, hypoxic adaptation, and pronounced spatial heterogeneity.[14,18,56-58]

First, the BBB and blood–brain tumor barrier (BBTB) impose major constraints on intracranial immunotherapy. Tight junctions, efflux transporters, and specialized brain vasculature restrict macromolecular drug entry into the brain parenchyma and tumor core. Although GBM can locally disrupt these barriers, permeability remains spatially heterogeneous, with relatively intact barrier function often persisting at infiltrative margins and microscopic lesions. Consequently, exposure to PD-1/PD-L1 antibodies may vary across tumor regions. The barrier also limits the entry of peripherally activated T cells into the central nervous system, contributing to insufficient immune-cell infiltration before treatment.[59-63] Studies investigating focused ultrasound-mediated barrier opening indirectly support this concept, showing that enhanced intracranial drug exposure and tumor perfusion may improve antitumor effects while highlighting inadequate drug delivery as a key translational limitation.[64-66]

Second, hypoxia, chronic inflammation, and abnormal vascularization within the GBM microenvironment promote adaptive immune resistance. Hypoxia regulates immune checkpoint expression, myeloid-cell recruitment, and effector immune-cell function through hypoxia-inducible factor (HIF)-related pathways and is closely linked to angiogenesis and metabolic reprogramming.[67-71] These factors may help explain why GBM can remain resistant to PD-1/PD-L1 blockade even when PD-L1 expression is increased.

Third, the GBM immune microenvironment contains a highly redundant immunosuppressive network dominated by myeloid cells. TAMs and microglia constitute the predominant immune-cell populations in GBM and often display immunosuppressive phenotypes. They restrict T-cell activation by expressing inhibitory ligands, secreting interleukin (IL)-10 and transforming growth factor-β (TGF-β), and impairing antigen presentation and costimulatory signaling.[18,57,58] MDSCs and Tregs further suppress effector T-cell function and reinforce these immunosuppressive circuits.[18,56,72] Myeloid-cell-specific inhibition of KDM6B can enhance GBM sensitivity to PD-1 blockade, indicating that myeloid-cell states may directly regulate checkpoint inhibitor responses.[56] Therefore, PD-1/PD-L1 blockade alone is often insufficient to overcome this multicellular, multipathway immunosuppressive network.

Fourth, impaired tumor antigen presentation and limited T-cell infiltration further weaken the biological basis for immune checkpoint inhibition. PD-1/PD-L1 blockade requires sufficient numbers of tumor-reactive T cells with recoverable function. However, GBM typically behaves as an immunologically "cold" tumor, with low $CD8^+$ effector T-cell infiltration, T-cell exhaustion, defective antigen presentation, and abnormal IFN-γ-related signaling.[14,18] Treg depletion can promote myeloid-cell activation and enhance GBM responses to anti-PD-1 therapy and tumor-targeted antibodies, suggesting that impaired T-cell function is continuously shaped by Treg–myeloid interaction networks.[72]

Differences among GBM molecular subtypes also contribute to the inconsistent efficacy of PD-1/PD-L1 blockade. *IDH* mutations, *MGMT* promoter methylation, and transcriptional subtypes such as proneural, classical, and mesenchymal tumors can influence the tumor immune ecosystem. Mesenchymal tumors often exhibit greater immune infiltration and PD-L1-related signaling, which may theoretically enhance sensitivity to checkpoint blockade; however, this infiltration is frequently myeloid-dominant and immunosuppressive, potentially generating stronger adaptive resistance. In contrast, proneural and some classical tumors may respond poorly because of insufficient T-cell infiltration, inadequate antigen presentation, or *EGFR*-related tumor-intrinsic mechanisms.[33,35-37] Thus, molecular subtypes link PD-L1 expression with immune microenvironment composition and therapeutic heterogeneity.

Finally, response assessment remains a major challenge for the clinical translation of immunotherapy, particularly because pseudoprogression can mimic true progression on conventional magnetic resonance imaging (MRI). Misclassifying pseudoprogression as treatment failure may lead to premature treatment discontinuation, whereas misclassifying true progression as treatment-related inflammation may delay effective therapy.[73,74] Available data suggest that the incidence of pseudoprogression after ICI therapy in GBM remains uncertain. Studies involving mixed brain tumor cohorts reported pseudoprogression rates of 9.8%, but these data cannot be directly interpreted as GBM-specific.[74,75] In contrast, a study of 55 GBM patients treated with ICIs found no pseudoprogression clearly attributable to ICI monotherapy.[76] Therefore, response assessment should integrate treatment timing, neurological symptoms, corticosteroid use, serial imaging changes, and, when needed, advanced imaging modalities or pathological confirmation, according to the Response

Assessment in Neuro-Oncology (RANO) 2.0 and immunotherapy-related response evaluation principles.[73]

Therefore, the therapeutic failure of PD-1/PD-L1 blockade in GBM should not be attributed simply to the ineffectiveness of PD-L1 as a therapeutic target. Rather, the PD-1/PD-L1 axis operates within a complex resistance network shaped by anatomical barriers, hypoxic adaptation, myeloid-mediated immunosuppression, insufficient T-cell infiltration, molecular heterogeneity, and response-assessment challenges. Accordingly, future strategies should be rationally designed to address specific resistance layers, including inadequate immune priming, abnormal vasculature, hypoxia, compensatory checkpoint suppression, and insufficient delivery of therapeutic agents or immune effector cells to intracranial tumors.

Collectively, resistance to PD-1/PD-L1 blockade in GBM arises from interconnected anatomical, vascular, cellular, molecular, and functional barriers. Table 2 summarizes these resistance mechanisms and links each to the therapeutic strategies discussed in the following section.

4. Strategies to overcome therapeutic resistance: Current frontiers and directions

Combination therapy is an important strategy for overcoming immunotherapy resistance in GBM, but the supporting evidence differs markedly across approaches. Evaluation should consider not only mechanistic complementarity but also clinical evidence, toxicity, treatment sequencing, and patient selection. Based on current evidence, these strategies can be divided into three categories: approaches with early signals of survival benefit or durable response that require randomized confirmation, such as oncolytic viruses plus PD-1 blockade and reirradiation plus PD-1 blockade in selected recurrent GBM patients; strategies with demonstrated feasibility or safety but unproven efficacy, such as dual immune checkpoint blockade and local intracranial delivery; and mainly preclinical approaches, including STING agonists, selected nanodelivery platforms, and chimeric antigen

Table 2. Multilayer resistance mechanisms to PD-1/PD-L1 blockade in glioblastoma and corresponding therapeutic strategies

Resistance level	Key mechanisms	Consequences for PD-1/PD-L1 blockade	Potential therapeutic strategies
Physical and anatomical barriers	Heterogeneous BBB/BBTB permeability; limited drug penetration; restricted trafficking and intratumoral distribution of effector T cells	Suboptimal antibody exposure and insufficient access of effector T cells to tumor sites	Focused ultrasound-mediated BBB opening; nanoparticle-based delivery; local or intratumoral administration; convection-enhanced delivery
Hypoxia and abnormal vasculature	Hypoxia and HIF-1α signaling; aberrant angiogenesis; adaptive upregulation of PD-L1	Promotes immunosuppression, impairs immune-cell infiltration, and reduces responsiveness to checkpoint blockade	Anti-angiogenic therapy; vascular normalization; hypoxia-targeted or HIF-1α-modulating strategies
Immunosuppressive cellular network	TAMs, MDSCs, and Tregs; IL-10 and TGF-β signaling; suppressive myeloid reprogramming	Establishes redundant immunosuppressive circuits that are not adequately reversed by PD-1 blockade alone	Myeloid-cell reprogramming; STING agonists; dual-checkpoint blockade; cytokine- or pathway-directed interventions
Insufficient T-cell priming and effector function	Low $CD8^+$ T-cell infiltration; limited antigen presentation; T-cell exhaustion; antigen heterogeneity and escape	Leaves too few functional effector cells to be reinvigorated by PD-1/PD-L1 blockade	Radiotherapy; oncolytic viruses or tumor vaccines; CAR-T or other cellular therapies; dual-checkpoint blockade
Challenges in treatment-response assessment	Pseudoprogression; difficulty distinguishing treatment-related inflammation from true tumor progression	Complicates clinical endpoint interpretation and treatment decision-making	iRANO/RANO 2.0 criteria; serial and advanced imaging; liquid biopsy; multimodal longitudinal assessment
Tumor-intrinsic and molecular heterogeneity	*IDH* mutation, *MGMT* promoter methylation, transcriptional subtypes, antigen heterogeneity, and *EGFR*-related mechanisms	Produces heterogeneous immune infiltration, variable PD-L1 signaling, and inconsistent therapeutic responses	Molecular stratification, biomarker-guided patient selection, and subtype-adapted combination strategies

Abbreviations: BBB: Blood–brain barrier; BBTB: Blood–brain tumor barrier; CAR-T: Chimeric antigen receptor T-cell; HIF-1α: Hypoxia-inducible factor 1-alpha; IL-10: Interleukin-10; iRANO: Immunotherapy Response Assessment in Neuro-Oncology; MDSCs: Myeloid-derived suppressor cells; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; RANO 2.0: Response Assessment in Neuro-Oncology 2.0; STING: Stimulator of interferon genes; TAMs: Tumor-associated macrophages; TGF-β: Transforming growth factor-beta; Tregs: Regulatory T cells.

receptor T-cell (CAR-T)-based combinations.[32,53,77-84]

4.1. Combination with standard therapies and anti-angiogenic treatment

Combining radiotherapy with PD-1/PD-L1 blockade has a compelling biological rationale. Irradiation can induce tumor-cell death and antigen release, enhance dendritic-cell cross-presentation, enhance antigen presentation, and recruit T cells.[85-88] However, radiotherapy can also upregulate PD-L1 and other checkpoint molecules, leading to adaptive immune resistance. Studies using human GBM cell lines have shown that conventionally fractionated radiotherapy alters tumor immunogenicity and tumor-associated phenotypes, supporting the biological complementarity of checkpoint blockade, although immune phenotypes vary among different cellular backgrounds.[89] Therefore, clinical benefit cannot be inferred solely from animal survival data or from the assumption that radiotherapy increases tumor immunogenicity.

More specifically, radiotherapy may induce PD-L1 expression in both malignant GBM cells and non-malignant immune compartments. DNA damage responses and radiation-induced IFN/JAK/STAT signaling can increase PD-L1 on tumor cells, whereas radiotherapy-induced extracellular vesicles and inflammatory cues may increase PD-L1 in accessory macrophages. Thus, radiotherapy acts as a double-edged immune modulator: it may release antigens and promote T-cell priming, but it may also induce adaptive PD-1/PD-L1-mediated resistance, providing a stronger rationale for carefully timed checkpoint blockade rather than automatically administering both therapies concurrently.[21,89]

Clinically, a phase II study of reirradiation plus pembrolizumab in recurrent GBM suggested a potential survival benefit in selected patients, particularly those previously treated with bevacizumab, but not in bevacizumab-naive patients, although the lack of a randomized comparator remains a major limitation.[77] Thus, radiotherapy plus PD-1 blockade should be viewed as a promising option for selected recurrent GBM patients rather than an established treatment regimen. This combination may increase the risk of local inflammation, vascular permeability changes, cerebral edema, pseudoprogression, radiation necrosis, and corticosteroid requirements. Because corticosteroids suppress T-cell activity and may reduce the efficacy of immunotherapy, future trials should prospectively evaluate radiation dose, target volume, immunotherapy timing, acceptable corticosteroid exposure thresholds, and imaging criteria for distinguishing treatment-related inflammation from true progression. Stratification should include recurrence status, prior anti-angiogenic therapy, tumor burden, corticosteroid exposure, and the timing of radiographic assessments.

Combining immune checkpoint inhibitors with chemotherapy, particularly TMZ, also warrants cautious evaluation. In several extracranial solid malignancies, chemotherapy may act synergistically with PD-1/PD-L1 blockade by reducing tumor burden, inducing immunogenic cell death, and increasing antigen release.[90] Pembrolizumab plus chemotherapy improves survival in selected patients with non-small-cell lung cancer and triple-negative breast cancer,[91,92] but these findings cannot be directly extrapolated to GBM. Although TMZ may enhance immune recognition through cytoreduction and antigen release, it also induces peripheral lymphopenia. Its overall immunological effect depends on dose intensity, sequencing, radiation exposure, and corticosteroid use.[90-92] In GBM, low baseline effector T-cell abundance and dominant myeloid immunosuppression may limit the ability of chemotherapy-induced antigen release to generate effective antitumor immunity. Recent analyses of patients treated with the Stupp regimen also suggest that systemic inflammation and immune-cell status before and during treatment are associated with clinical outcome.[93]

Therefore, combining TMZ with PD-1/PD-L1 inhibition should be considered mechanistically plausible but clinically unproven. NRG-BN002 showed that nivolumab, ipilimumab, and their combination could be safely administered after standard chemoradiotherapy, with no unexpected fatal toxicity in the combination arm, although approximately 16% of patients experienced grade 4 treatment-related adverse events; as a phase I study, it was not designed to establish survival benefit.[53] The interaction between TMZ and immunotherapy is therefore better described as sequence-dependent and unconfirmed rather than inherently synergistic. Future strategies should test immune priming before severe lymphopenia, tailor treatment according to absolute lymphocyte count and $CD4^+$ T-cell status, and compare concurrent, sequential, and neoadjuvant schedules. Initiating checkpoint blockade after marked lymphocyte depletion may leave too few effector cells to generate a meaningful therapeutic response.

Current GBM-specific evidence does not support the conclusion that TMZ uniformly enhances the efficacy of PD-1/PD-L1 blockade. TMZ can promote tumor-cell death and antigen release, but it also produces dose- and schedule-dependent lymphopenia, particularly affecting $CD4^+$ T cells and the effector T-cell pool required for PD-1 blockade. Its effects on macrophages are also context-dependent: treatment-related tumor injury may recruit or reprogram myeloid cells, but the dominant

GBM microenvironment often remains TAM-rich and immunosuppressive. Evidence that TMZ directly regulates PD-L1 on GBM cells remains limited and inconsistent across experimental settings. Therefore, the proposed combination of TMZ and checkpoint inhibitors should be regarded as a testable, sequence-dependent hypothesis rather than an established synergistic regimen.[53,90-93]

Combining anti-angiogenic therapy with PD-1/PD-L1 blockade aims to address abnormal tumor vasculature, hypoxia, and inadequate drug delivery in GBM. Vascular endothelial growth factor (VEGF) inhibition may reduce pathological vascular permeability, alleviate hypoxia, promote vascular normalization, and facilitate infiltration of effector T cells, thereby enhancing checkpoint blockade.[94-99] Improved vascular function may also increase intratumoral distribution of therapeutic antibodies and cell-based therapies. In murine GBM models, anti-VEGF therapy enhanced the delivery and efficacy of epidermal growth factor receptor variant III (EGFRvIII)-directed CAR-T cells, supporting the concept that vascular normalization may enhance immunotherapy.[100]

However, clinical findings in GBM have not consistently confirmed these potential benefits. In a randomized phase II trial in recurrent GBM, pembrolizumab plus bevacizumab did not significantly improve OS and PFS compared with pembrolizumab alone.[101] A recent systematic review likewise found insufficient evidence for survival or objective response benefit with anti-PD-1 therapy plus bevacizumab.[51] In a small 2024 retrospective study, eight recurrent GBM patients received sintilimab, bevacizumab, and TMZ; although radiographic responses appeared relatively frequent, median PFS was only 3.3 months, and the small sample size and retrospective design preclude any change in the current interpretation of the evidence.[79]

These studies must also consider the direct imaging effects of anti-angiogenic agents. Bevacizumab can rapidly reduce vascular permeability, contrast enhancement, edema, and corticosteroid requirements; therefore, radiographic response or prolonged PFS does not necessarily indicate durable tumor control. In the randomized phase II NRG/RTOG 1205 trial without immunotherapy, bevacizumab plus reirradiation improved six-month PFS but not OS, illustrating the disconnect between imaging-based control and survival benefit.[102]

Anti-angiogenic agents and checkpoint blockade may also result in additive toxicities, including hypertension, proteinuria, thrombosis, hemorrhage, impaired wound healing, immune-related adverse events, intracranial hemorrhage, and perioperative complications. Because the vascular normalization window is transient, excessive or prolonged VEGF inhibition may cause excessive pruning of tumor vessels, worsen hypoxia, and reinforce immune-cell exclusion.

At present, anti-angiogenic therapy plus PD-1/PD-L1 blockade should be regarded as biologically rational but clinically unproven, rather than a high-priority treatment strategy. Further evaluation requires randomized controlled studies with prespecified stratification by prior bevacizumab exposure, corticosteroid use, *MGMT* status, tumor vascularity, immune-infiltration pattern, and molecular subtype. Perfusion imaging, hypoxia biomarkers, and dynamic measures of immune-cell infiltration should be incorporated to define the optimal dose and timing of anti-angiogenic therapy relative to PD-1/PD-L1 blockade.

4.2. Combination with other immunotherapies

A major limitation of PD-1/PD-L1 blockade in GBM is the presence of compensatory immune checkpoints and parallel immunosuppressive pathways. Combination strategies should therefore target immune-escape mechanisms beyond the PD-1/PD-L1 axis, rather than simply combining multiple immunotherapeutic agents. Dual-checkpoint blockade targets both T-cell priming and effector phases; T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT) blockade addresses compensatory exhaustion; STING agonists enhance innate immunity and antigen presentation; and CAR-T-based combinations are intended to overcome engineered T-cell exhaustion and antigen escape in the GBM microenvironment.[32,53,103-109]

Dual immune-checkpoint blockade is among the most direct combination strategies. Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) mainly regulates T-cell priming, whereas PD-1/PD-L1 primarily constrains effector-phase and local antitumor immunity, providing a rationale for combined inhibition.[103] However, efficacy in GBM cannot be inferred from results in extracranial malignancies. Early clinical data, including NRG-BN002, showed that nivolumab plus ipilimumab administered with adjuvant TMZ was feasible and had manageable safety, but did not demonstrate a survival benefit.[53] In contrast, the randomized phase II component of NRG-BN007 showed that ipilimumab plus nivolumab did not improve PFS compared with TMZ in newly diagnosed *MGMT*-unmethylated GBM, and the trial did not proceed to phase III.[54] Local intracranial administration of nivolumab and ipilimumab also demonstrated the feasibility of drug delivery and acceptable dose safety, but not efficacy.[78] Thus, dual-checkpoint blockade remains clinically feasible but of uncertain efficacy and investigational in GBM, requiring

refined immune phenotyping, rational sequencing, and disease-specific biomarkers.

The principal limitation of dual-checkpoint blockade is increased immune-related toxicity. Concurrent CTLA-4 and PD-1 inhibition may increase dermatologic, gastrointestinal, hepatic, endocrine, and neurologic toxicities. In GBM, perioperative corticosteroid use, neurological symptoms, and impaired performance status further complicate the differentiation between immune-mediated neurotoxicity, tumor progression, cerebral edema, and pseudoprogression. Because toxicity management often requires corticosteroids that may suppress the intended immune response, the risk-benefit balance of dual-checkpoint blockade should be established before adding radiotherapy, chemotherapy, or anti-angiogenic therapy. Early-phase trials should preferably adopt staged escalation rather than simultaneous introduction of multiple treatment modules.

Beyond CTLA-4, TIGIT represents another important compensatory checkpoint in GBM. TIGIT is expressed mainly on activated or exhausted $CD8^+$ T cells, Tregs, and natural killer (NK) cells. Its principal ligand is CD155/poliovirus receptor (PVR), which is expressed on tumor cells, antigen-presenting cells, and myeloid cells; TIGIT can also bind CD112.[14,110-112] The functional activity of this pathway depends on competition among TIGIT, CD96, and the activating receptor CD226 for CD155. TIGIT–CD155 binding limits CD226-mediated costimulation, while phosphorylation of TIGIT intracellular immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based T-cell transactivation (ITT)-like motifs recruits SH2 domain-containing inositol polyphosphate 5-phosphatase 1 (SHIP1) through growth factor receptor-bound protein 2 (GRB2) or beta-arrestin 2, suppressing phosphoinositide 3-kinase (PI3K)-, mitogen-activated protein kinase (MAPK)-, and nuclear factor kappa B (NF-κB)-associated signaling and thereby reducing lymphocyte proliferation, IFN-γ secretion, degranulation, and cytotoxicity.[111,112]

PD-1 and TIGIT converge functionally on the CD226 costimulatory axis. PD-1 signaling reduces CD226 phosphorylation and downstream T-cell receptor signaling through Src homology region 2 domain-containing phosphatase 2 (SHP2), whereas TIGIT competes with CD226 for binding to CD155 and limits CD226 clustering.[112] Thus, PD-1 blockade may relieve only part of this inhibitory signaling, while persistent TIGIT–CD155 signaling can sustain T-cell exhaustion. GBM models showing increased TIGIT expression after PD-1 blockade and stronger antitumor immunity with combined PD-1/TIGIT inhibition support this mechanistic rationale.[104,105] Recent studies also indicate that GBM-associated myeloid cells include spatially and functionally distinct subsets, suggesting that future combination strategies should target defined myeloid states rather than indiscriminately depleting TAMs.[57,58]

The role of TIGIT in GBM may extend beyond T cells and NK cells. Several myeloid populations may express high levels of TIGIT, and GBM-derived extracellular vesicles can induce comparable immunosuppressive phenotypes in healthy monocytes. *TIGIT* knockdown reduced IL-13-associated immunosuppression and, in an NLR family pyrin domain-containing 3 (NLRP3)-dependent context, restored proinflammatory myeloid activity and improved T-cell function.[113] These findings suggest that TIGIT links lymphocyte exhaustion with myeloid reprogramming. Mechanism-driven strategies include combined PD-1/TIGIT blockade, bispecific antibodies targeting the CD155–PVR axis, preservation of CD226 costimulation, and TIGIT/CD155-recognition modules in engineered NK cells or other cellular platforms.[111,114] TIGIT immuno-positron emission tomography (PET) may facilitate mapping of intratumoral target distribution, but remains preclinical and requires optimization of signal intensity and specificity.[115] Patient selection should integrate CD155, TIGIT, and CD226 expression with immune-cell composition, and paired tissue and peripheral blood analyses should confirm restoration of effector T- and NK-cell function.

Oncolytic viruses combined with PD-1 blockade represent another biologically promising but clinically unproven strategy. In a phase I/II study, intratumoral DNX-2401 followed by pembrolizumab met the prespecified safety endpoint, and the 12-month survival rate and durable responses in a small subset provided encouraging preliminary evidence. However, the prespecified objective-response endpoint was not met, and the absence of a randomized control precludes definitive conclusions regarding efficacy.[80] The conceptual strength of this approach lies in sequencing: local viral therapy may first induce immunogenic cell death and antigen presentation, after which systemic PD-1 blockade may amplify the resulting immune response. Randomized trials with baseline immune-phenotype selection are needed to identify patients most likely to benefit.

Activation of innate immunity, including the use of STING agonists combined with PD-L1 blockade, may complement checkpoint inhibition by inducing type I IFN signaling, enhancing antigen presentation, and promoting effector T-cell infiltration.[32,106-108] This approach aims to convert an immunologically "cold" GBM microenvironment into a more inflamed phenotype.

Preclinical studies, including a dual PD-L1/CD47-targeted nanoplatform delivering a STING agonist with radiotherapy and the STING agonist 8803 across several GBM models, demonstrated immune remodeling and improved survival.[32,84] However, efficacy is model- and pathway-dependent; adding a STAT3 inhibitor to a STING agonist did not consistently improve outcomes and sometimes attenuated IFN signaling.[84] Dose optimization, intracranial delivery, systemic inflammation, neurotoxicity, and the therapeutic window remain major barriers to clinical translation.

Combining CAR-T-cell therapy with PD-1/PD-L1 blockade is intended to mitigate CAR-T-cell exhaustion in the GBM microenvironment. PD-1/PD-L1 blockade may partially restore CAR-T cell cytotoxicity under immunosuppressive conditions.[116-119] EGFRvIII-directed CAR-T cells engineered to secrete a signal regulatory protein gamma (SIRPγ)-derived CD47-blocking molecule may eliminate antigen-positive targets while locally enhancing myeloid phagocytic activity, thereby addressing antigen heterogeneity and myeloid suppression.[109] Anti-VEGF therapy may also improve EGFRvIII CAR-T delivery and activity in GBM models.[100] Clinically, however, EGFRvIII CAR-T cells plus pembrolizumab demonstrated biological activity and acceptable safety, but no clear clinical efficacy; recurrent tumors displayed increased exhausted, regulatory, and IFN-stimulated T-cell states and myeloid remodeling.[81] PD-1 blockade alone therefore does not overcome antigen heterogeneity, limited CAR-T persistence, poor infiltration, local-delivery constraints, neurotoxicity, or antigen escape. This strategy therefore remains exploratory and requires substantial optimization.

Overall, combinations of PD-1/PD-L1 inhibitors with oncolytic viruses, dual-checkpoint blockade, CAR-T cells, or innate immune activators have varying degrees of mechanistic support, but their clinical performance depends on the platform, route of administration, host immune status, and tumor molecular features. Most encouraging findings derive from small, single-arm, or early-phase studies, whereas randomized trials and expansion cohorts have not shown consistent survival benefit. Atezolizumab with radiotherapy and TMZ was feasible in newly diagnosed GBM and identified immune-enriched and mesenchymal features associated with longer survival outcomes, but OS remained comparable to historical outcomes with standard therapy.[37] An expansion cohort of neoadjuvant pembrolizumab confirmed pharmacodynamic immune changes but did not reproduce the initially reported survival advantage.[50] NRG-BN002 established safety for nivolumab and ipilimumab, whereas NRG-BN007 did not improve PFS with dual-checkpoint blockade compared with standard therapy.[53,54] Therefore, pharmacodynamic changes, occasional durable responses, and encouraging early survival signals cannot substitute for evidence from randomized clinical trials.

4.3. Delivery optimization

The BBB and BBTB limit effective intratumoral exposure to macromolecular drugs, nucleic-acid therapeutics, and immunomodulators within GBM. Enhancing the concentration and spatial distribution of immunotherapeutic or immunoregulatory agents within intracranial tumors is therefore a central translational challenge for combination therapy. Focused ultrasound, local administration, and nanomedicine-based delivery may improve intracranial exposure, but improved drug delivery alone does not constitute clinical benefit. Such strategies should be prioritized only if increased drug exposure can be linked to intratumoral immune activation and improvements in clinically meaningful outcomes.

Nanocarriers and peptide-modified delivery systems may help overcome limited intracranial drug exposure. Targeting and cell-penetrating peptides, such as angiopep-2, cyclic arginine–glycine–aspartic acid peptide (cRGD), IL-13, and transactivator of transcription peptide (TAT), can facilitate BBB transport of liposomes, polymeric nanoparticles, and nanogels and may improve targeting of glioma cells or the tumor microenvironment. Nanoplatforms have been developed to deliver PD-L1 small-interfering RNA (siRNA) alone or with chemotherapeutic agents, STING agonists, or radiosensitizers to reduce local checkpoint signaling while enhancing antitumor immunity.[32,120,121] Representative examples include iRGD-modified solid-lipid nanoparticles carrying PD-L1 siRNA and T7 peptide-modified, reactive oxygen species-responsive nanoparticles co-delivering doxorubicin and PD-L1 siRNA.[120] These studies support the preclinical feasibility of local PD-L1 silencing and enhanced antitumor activity, but they do not demonstrate reliable intratumoral drug exposure or improved survival in patients.

A small number of nanomedicine platforms have advanced to early clinical evaluation. The phase Ib NANO-GBM study evaluated intravenous Activation and Guidance of Irradiation by X-ray (AGuIX) nanoparticles combined with radiotherapy and TMZ in newly diagnosed GBM and demonstrated preliminary safety and broad intratumoral distribution.[122] Rhenium-186 nanoliposomes delivered by convection-enhanced delivery have also been investigated in recurrent GBM, with patient-specific imaging-based models showing that catheter placement can strongly influence tumor coverage and off-target

distribution.[123] These studies demonstrate the clinical feasibility of nanomedicine delivery and monitoring, but they remain limited by small sample sizes and endpoints focused mainly on safety, dose, biodistribution, or optimization of drug delivery. Clear OS or PFS benefit has not been established. Further development must address manufacturing reproducibility, long-term toxicity, drug clearance, heterogeneous tumor targeting, insufficient drug exposure within viable tumor tissue, and the lack of validated efficacy endpoints and companion biomarkers.[124-126]

Focused ultrasound combined with microbubbles can transiently and reversibly disrupt the BBB, thereby increasing delivery of macromolecular agents and immunomodulators to tumor tissue.[64-66,82] In animal models, focused-ultrasound-mediated BBB opening improved tumor perfusion and delivery of PD-1 inhibitors, enhancing antitumor immune responses in glioma.[64] Clinically, a phase I/II study of an implanted multi-emitter ultrasound device plus carboplatin in recurrent GBM demonstrated the feasibility of repeated, localized BBB opening.[65] Focused-ultrasound-assisted doxorubicin delivery also modulated myeloid-cell states and improved responses to PD-1 blockade in animal models.[82] In another preclinical study, an Fc-enhanced CTLA-4 antibody combined with PD-1 blockade, doxorubicin, and low-intensity pulsed ultrasound produced robust antitumor effects in mice and detectable pharmacodynamic changes within myeloid cells in limited patient samples.[83] The main translational advantage of focused ultrasound is its precise spatial and temporal control, allowing localized enhancement of drug delivery tailored to tumor location and monitored using imaging.

Focused ultrasound should not be regarded as a risk-free delivery strategy. Potential complications include vascular injury, cerebral edema, hemorrhage, inflammation, and heterogeneous barrier opening across tumor regions. When combined with immunotherapy, ultrasound parameters, microbubble dose, treatment frequency, imaging-monitoring criteria, and safety windows must be clearly defined.[64-66] Local intracranial delivery of PD-1 and CTLA-4 antibodies may reduce systemic exposure while increasing local drug concentrations,[78] but it carries risks of catheter-related infection, hemorrhage, local inflammation, uneven drug distribution, and repeated invasive procedures. Delivery-focused studies should therefore report intracranial drug concentrations, target occupancy, and immune pharmacodynamic markers, alongside procedure-related adverse events.

Other approaches, including direct local administration, resection-cavity delivery, convection-enhanced delivery, and integration with radiotherapy or immune modulators such as STING agonists, may further enhance local intracranial immunotherapy.[32,120,121] Early clinical evidence supports the technical feasibility of focused-ultrasound-mediated BBB opening for improving intracranial drug delivery,[82] whereas PD-L1-targeted nanoparticles combined with STING agonists remain supported mainly by preclinical data.[32] These strategies may improve drug delivery and enhance local immune activation, but their safety, efficacy, and effects on survival require validation in prospective clinical trials.

4.4. Neoadjuvant therapy and treatment sequencing

The success of a combination regimen depends not only on agent selection but also on treatment sequencing. In GBM, the timing of PD-1/PD-L1 inhibition may substantially influence immune pharmacodynamics. Neoadjuvant PD-1 blockade is administered while tumor antigens remain in situ and before treatment-induced lymphocyte depletion, thereby potentially promoting T-cell priming and clonal expansion.[48,127] An initial randomized pilot study suggested that administering pembrolizumab before surgery, followed by postoperative treatment, might improve clinical outcomes compared with postoperative treatment alone and was associated with activation of intratumoral T-cell/IFN-γ programs, suppression of cell cycle-related genes, and expansion of T-cell clones.[48] However, these findings should be interpreted cautiously. The study was small, and a 2024 single-arm expansion cohort confirmed pharmacodynamic effects and a manageable safety profile but did not reproduce an OS advantage.[50] Thus, the principal value of neoadjuvant immunotherapy lies in initiating antitumor immunity while the tumor remains present and in enabling paired tissue analyses of response and resistance. Its effects on survival, optimal preoperative window, and target population still require prospective randomized validation.[49,51,52]

In this review, the term "neoadjuvant" specifically refers to checkpoint blockade administered before planned surgical resection of the tumor, while tumor antigens remain in situ. It does not refer to treatment administered after resection but before the Stupp chemoradiotherapy protocol, or to treatment administered after concurrent radiotherapy plus TMZ but before adjuvant TMZ. The former should be described as early postoperative or peri-chemoradiotherapy checkpoint blockade, and the latter as post-chemoradiotherapy or adjuvant checkpoint blockade. At present, the optimal timing of checkpoint inhibition relative to radiotherapy–TMZ remains unknown. A biologically rational treatment schedule would evaluate checkpoint inhibition before severe TMZ-induced lymphopenia and during the radiotherapy-induced

window of antigen release and inflammation, while minimizing corticosteroid exposure; however, this strategy requires validation in prospective randomized trials.

This concept is consistent with sequential treatment strategies in which local immune priming precedes systemic checkpoint blockade. In the DNX-2401 study, intratumoral viral therapy was followed by pembrolizumab rather than being administered concurrently.[80] For radiotherapy plus immune-checkpoint blockade, checkpoint inhibition should ideally begin during the period of radiation-induced antigen release and inflammatory signaling, while avoiding severe lymphopenia, exposure to high-dose corticosteroids, and excessively large-field radiation. For TMZ plus checkpoint blockade, completion of intensive chemotherapy should not automatically precede immunotherapy; instead, eligibility and treatment-delay criteria should incorporate absolute lymphocyte count, $CD4^+$ T-cell level, and corticosteroid dose. For anti-angiogenic therapy, treatment should take advantage of the transient vascular-normalization window to improve drug delivery and immune-cell infiltration, rather than rely on prolonged high-intensity vascular suppression.

Future combination trials should treat sequencing as a prespecified experimental variable rather than a procedural detail. Key comparisons include neoadjuvant versus adjuvant treatment, concurrent versus sequential administration, short-course vascular normalization versus sustained anti-angiogenic therapy, and local immune priming followed by systemic checkpoint blockade. Longitudinal assessment of peripheral immune cells, tumor tissue, and imaging biomarkers should be incorporated to identify biologically optimal treatment windows.

Table 3 summarizes the current evidence, translational status, major safety concerns, and sequencing or delivery considerations for representative combination strategies involving PD-1/PD-L1 blockade in GBM.

4.5. Why combinations that succeed preclinically fail in clinical trials

The discrepancy between preclinical and clinical outcomes in GBM combination immunotherapy largely reflects the limitations of current preclinical models. Common syngeneic tumor implantation models are often highly immunogenic, relatively homogeneous, and established at a defined time, with treatment typically initiated at a low tumor burden. By contrast, human GBM is spatially and clonally heterogeneous. By the time immunotherapy is initiated, patients have often undergone surgery and received radiotherapy, TMZ, bevacizumab, and corticosteroids, and frequently exhibit lymphopenia, antigen loss, and dominant myeloid immunosuppression. Thus, young, immunocompetent laboratory hosts differ fundamentally from patients with recurrent GBM.

Preclinical studies also allow precise control of dose and timing and often permit direct intratumoral administration, whereas clinical treatment is limited by BBB heterogeneity, tumor volume, necrosis, poor perfusion, and systemic toxicity. Complete responses observed in animal models may therefore reflect model-specific antigenic or immune contexts. For example, STING agonists and multidrug immune combinations can demonstrate striking efficacy in selected mouse models,[32,83,84] but these effects may not be generalizable across the diverse molecular subtypes and immune states of GBM.

Mechanistic pharmacodynamic changes do not necessarily translate into meaningful clinical benefit. EGFRvIII CAR-T cells combined with pembrolizumab can reach the tumor and remodel its immune microenvironment, without improving survival.[81] Similarly, anti-angiogenic therapy may improve perfusion and reduce radiographic enhancement through reductions in edema and vascular permeability, without producing durable tumor control. Apparent responses observed in small single-arm studies may also reflect patient selection, corticosteroid reduction, or pseudoresponse.

To improve translational success, preclinical combinations should be validated across multiple orthotopic models representing distinct immune states and, where possible, incorporate standard chemoradiotherapy, recurrent disease, corticosteroid exposure, and clinically relevant tumor burdens. Clinical trials should incorporate mechanism-based biomarkers, pharmacodynamic endpoints, and prespecified treatment schedules. Complex multidrug regimens should not advance to clinical testing solely on the basis of improved survival in a single animal model.

5. Summary and future perspectives

5.1. Key findings

The PD-1/PD-L1 pathway is an important component of the immune-evasion network in GBM, but it does not independently determine the response to immunotherapy. PD-L1 expression is influenced by tumor-intrinsic oncogenic signaling, hypoxia, inflammatory mediators, and treatment-related pressure, and varies across patients, tumor regions, and time points. Beyond malignant cells, TAMs and other myeloid cell populations may also represent major sources of PD-L1. Thus, a single biopsy or isolated immunohistochemical assessment is unlikely to capture the functional status of the PD-1/PD-L1 axis

Table 3. Evidence landscape and translational considerations for combination strategies involving PD-1/PD-L1 blockade in glioblastoma

Combination strategy	Current evidence base	Suggested translational positioning	Major safety considerations	Key sequencing or delivery considerations	Key references
Oncolytic virus plus PD-1 blockade	Phase I/II evidence supports feasibility and an acceptable safety profile. Survival signals and a small number of durable responses have been reported, although the prespecified objective-response endpoint was not met.	Promising, but requires confirmation in randomized trials	Cerebral edema, local neuroinflammation, and procedure-related complications	Local viral administration should precede systemic ICB to promote immunogenic tumor-cell death, antigen release, and immune priming.	[80]
Reirradiation plus PD-1 blockade	Phase II studies have reported survival signals in selected patients, particularly those with prior bevacizumab-refractory disease; findings in bevacizumab-naive populations have been less consistent, and randomized evidence is lacking.	Potentially valuable in selected patients with recurrent GBM	Cerebral edema, radiation necrosis, pseudoprogression, and increased corticosteroid requirements	Radiation dose and target volume should be optimized, and ICB should be initiated within a biologically plausible window of antigen release and inflammatory signaling.	[77]
Dual-checkpoint blockade with or without TMZ	Early-phase studies demonstrate clinical feasibility, but efficacy has not been established in adequately powered randomized trials.	Intermediate priority; remains investigational	Overlapping immune-related adverse events and, when combined with TMZ, treatment-related lymphopenia	ICB should preferably be initiated before profound lymphocyte depletion. Stepwise treatment escalation may be more informative and safer than simultaneous multi-agent administration.	[53,78]
Anti-angiogenic therapy with or without ICB and TMZ	Randomized studies have generally not demonstrated a clear survival benefit. Small retrospective studies have reported radiographic signals, but these findings are vulnerable to selection bias and the permeability-reducing effects of anti-VEGF therapy.	Low-to-intermediate priority; biomarker-guided evaluation is needed	Hypertension, hemorrhage, thrombosis, impaired wound healing, and immune-related toxicity	Treatment should exploit the transient vascular-normalization window while avoiding prolonged or excessive vascular pruning that may aggravate hypoxia and immune exclusion.	[79,101]
CAR-T cells plus PD-1 blockade	The combination is supported by preclinical rationale. Phase I studies have shown acceptable safety and biological activity, but no clear clinical efficacy has been demonstrated.	Exploratory; substantial redesign is required	Neuroinflammation, antigen escape, T-cell exhaustion, and limited cellular persistence	Future strategies should incorporate multi-antigen targeting, local delivery, and microenvironmental remodeling, rather than relying solely on the addition of PD-1 antibodies.	[81,100]
STING agonists or nanoplatforms plus ICB	Evidence is derived predominantly from animal models, in which some platforms have produced marked immune remodeling and survival improvement.	Preclinical or proof-of-concept	Systemic inflammation, intracranial inflammatory toxicity, and uncertain dose and delivery windows	Local delivery, pharmacokinetics, target engagement, and immune pharmacodynamics should be established before escalation to complex multi-drug regimens.	[32,84]
Focused ultrasound or local delivery plus ICB	Preclinical studies and early feasibility trials support the technical potential of BBB/BBTB opening or direct intracranial administration, but therapeutic efficacy remains unproven.	Promising delivery platform; efficacy not yet established	Hemorrhage, edema, infection, catheter-related complications, and other procedure-associated risks	BBB/BBTB opening or local administration should be synchronized with systemic or regional drug dosing, with concurrent monitoring of tissue exposure and procedure-related toxicity.	[78,82,83]

Abbreviations: BBB: Blood–brain barrier; BBTB: Blood–brain tumor barrier; CAR-T: Chimeric antigen receptor T-cell; GBM: Glioblastoma; ICB: Immune checkpoint blockade; PD-1: Programmed cell death protein 1; PD-L1: Programmed death ligand 1; STING: Stimulator of interferon genes; TMZ: Temozolomide; VEGF: Vascular endothelial growth factor.

throughout the tumor, and PD-L1 expression alone cannot yet serve as a robust or reproducible predictor of therapeutic benefit.

Clinical studies consistently demonstrate limited activity of PD-1/PD-L1 inhibitor monotherapy in unselected patients with GBM. This limited efficacy cannot be explained solely by low PD-L1 expression; rather, it reflects multilayered immunosuppression, including sparse functional effector T-cell infiltration, T-cell exhaustion, low or heterogeneous tumor immunogenicity, and a microenvironment dominated by TAMs and other suppressive myeloid cells. Drug exposure is further constrained by the BBB and the heterogeneous BBTB, while hypoxia, abnormal vasculature, and antigenic diversity limit durable immune responses. In clinical practice, TMZ-associated lymphopenia, radiotherapy-induced immune alterations, and corticosteroid use may further diminish the efficacy of checkpoint blockade.

Together, these findings indicate that resistance to PD-1/PD-L1 blockade in GBM arises from interactions among malignant cells, immune compartments, vascular abnormalities, metabolic constraints, and treatment-related factors rather than from a single molecule or cell population. Even when PD-1-mediated inhibition is relieved, insufficient effector T cells, persistent myeloid-mediated immunosuppression, compensatory checkpoint activation, or antigen loss may prevent effective antitumor immunity from being initiated or sustained. Accordingly, target expression, T-cell activation, and IFN-related transcriptional programs should not be interpreted as evidence of durable clinical benefit. Neoadjuvant studies have reported T-cell clonal expansion and immune transcriptional changes, but subsequent expansion cohorts have not consistently reproduced the survival advantage suggested by earlier small studies, reinforcing the conclusion that pharmacodynamic activity cannot substitute for clinically meaningful endpoints.

Combination therapy provides a rational framework for overcoming the barriers to checkpoint inhibition in GBM. Radiotherapy may increase antigen release and local inflammation; anti-angiogenic therapy may transiently normalize vasculature, reduce hypoxia, and improve immune-cell access; dual-checkpoint blockade, TIGIT-directed therapy, and STING agonists may counteract compensatory immunosuppressive mechanisms, enhance innate immunity, and improve antigen presentation; and oncolytic viruses and CAR-T cells may promote local immune priming or antigen-specific cytotoxicity. Local drug delivery, focused ultrasound, and nanocarrier systems may further improve intracranial drug exposure. However, most of these strategies remain at the preclinical or early clinical stage. Although immune remodeling, radiographic responses, or encouraging survival signals have been observed in selected subgroups, randomized trials and later expansion cohorts have not demonstrated reproducible OS benefit.[37,50,51,53,54,80,81]

The efficacy of combination treatment is strongly influenced by treatment sequence, dose intensity, disease setting, and prior therapy. Radiotherapy-induced antigen release may create a therapeutic window for checkpoint blockade, whereas extensive radiation exposure, severe lymphopenia, and high-dose corticosteroids may counteract this effect. Anti-angiogenic therapy may transiently improve perfusion, but prolonged or excessive vascular inhibition can aggravate hypoxia and immune-cell exclusion. TMZ may promote tumor-cell death and antigen release while simultaneously compromising immune competence through lymphocyte depletion. Combination regimens should therefore not be designed solely on the basis of theoretical synergy; treatment order, biological timing, steroid exposure, lymphocyte status, and immune context should be predefined design variables.

Overall, the PD-1/PD-L1 axis remains an important entry point for understanding immune escape and developing immunotherapeutic strategies in GBM. However, current evidence does not support the routine use of PD-1/PD-L1 inhibitor monotherapy or related combination regimens in molecularly and immunologically unselected patients. Meaningful clinical benefit is more likely to occur in biologically defined subgroups and will require integrated assessment of tumor genotype, immune-cell composition, cellular sources of PD-L1, spatial immune organization, and prior treatment exposure. The field should move beyond single-marker selection and empirically assembled combinations toward multidimensional stratification, mechanism-based treatment design, and prospective, randomized validation in patients with GBM. The clinical role of PD-1/PD-L1-targeted therapy will remain uncertain until pharmacodynamic changes in tumor tissue, peripheral immunity, and imaging are consistently shown to translate into improvements in PFS or OS.

5.2. Future directions

Future research should focus on interconnected priorities that enhance the efficacy of PD-1/PD-L1 blockade in GBM and facilitate clinical translation. These priorities include validating strategies to improve intracranial drug delivery, defining the cellular sources and functional roles of PD-L1, refining combination regimens, and developing biologically informed patient-stratification strategies and clinical trial designs.

First, local delivery approaches, focused ultrasound, and

nanocarrier-based systems should be evaluated for their ability to increase effective intratumoral exposure through pharmacokinetic studies, paired tissue sampling, and prospective clinical trials. Nanocarriers may be particularly useful for gene-editing and immunomodulatory payloads with limited intracranial penetration. However, most nanoparticle platforms remain preclinical, and early human studies have mainly shown preliminary safety and favorable biodistribution rather than improved PFS or OS. Future studies should quantify drug concentrations across spatially distinct tumor regions, assess target engagement and immune pharmacodynamics, and determine whether improved drug delivery yields clinically meaningful benefit rather than simply increasing tissue accumulation.

A second priority is to define the cellular origin and functional significance of PD-L1 expression in GBM. PD-L1 may be expressed by both tumor cells and myeloid immune cells, but whether these cellular sources exert independent effects on immunosuppression remains unclear. Multiplex immunofluorescence, spatial transcriptomics, spatial proteomics, and single-cell multi-omics should be integrated to characterize spatial relationships and functional interactions between PD-L1-positive cells and PD-1-positive T cells. Tumor cell-specific and myeloid cell-specific PD-L1 perturbation models are also needed to determine the causal contributions to T-cell exhaustion, macrophage polarization, treatment response, and tumor progression.

Combination therapies should be developed on the basis of GBM-specific biology rather than on efficacy observed in other solid tumors. Rational combination strategies may include PD-1/PD-L1 inhibition combined with TIGIT blockade, STING agonists, HIF-1α-modulated chemotherapy, anti-angiogenic therapy, oncolytic viruses, CAR-T cells, or local delivery platforms. These approaches may enhance antigen release, improve drug delivery, activate innate immunity, or restore effector-cell function. Evidence from other malignancies should primarily serve to generate mechanistic hypotheses, whereas clinical recommendations should rely on prospective, randomized studies in patients with GBM that incorporate analyses of tumor tissue, peripheral blood, and imaging biomarkers to determine whether pharmacodynamic changes translate into meaningful survival benefits.

The development of personalized therapies will require molecular profiling, artificial intelligence, and longitudinal clinical data to improve patient selection and optimize treatment timing and dosing. Standardization of PD-L1 assessment, including antibody platforms, scoring methods, cellular compartments, and sampling time points, is essential. PD-L1 should be evaluated within a multimarker framework rather than as an isolated biomarker to better define its prognostic and predictive value.[128]

Future trials should incorporate molecular subtype-directed stratification. In addition to *IDH* status, *MGMT* promoter methylation, corticosteroid exposure, and PD-L1 expression, proneural, classical, and mesenchymal transcriptional subtypes, as well as tumor microenvironment subtypes, should be considered. Mesenchymal GBM often exhibits greater immune-cell infiltration and more prominent PD-L1-related signaling, but this infiltrate may be dominated by TAMs and other suppressive myeloid populations. Multiplex immunohistochemistry, spatial transcriptomics, single-cell sequencing, and RNA expression signatures should distinguish effective antitumor infiltration from suppressive inflammation. Such stratification may help identify patients who are more likely to benefit from PD-1/PD-L1 blockade and provide a rationale for combining immune checkpoint inhibition with myeloid-cell reprogramming, anti-angiogenic therapy, or innate immune activation.[35-39]

Clinical trial populations should also be expanded to include underrepresented groups, including older adults and patients with *MGMT*-unmethylated tumors, and patients with molecularly distinct *IDH*-mutant high-grade gliomas, analyzed separately where biologically appropriate. Neoadjuvant, perioperative, and palliative treatment settings warrant further investigation, but trials should distinguish biological activity from meaningful clinical benefit and evaluate whether treatment reduces postoperative recurrence. Treatment sequence, corticosteroid exposure, lymphocyte status, disease burden, and prior therapy should be carefully controlled or incorporated as stratification factors because they may substantially modify responses to checkpoint blockade.

Overall, combining PD-1/PD-L1 inhibitors with radiotherapy, anti-angiogenic therapy, chemotherapy, dual-checkpoint blockade, oncolytic viruses, CAR-T cells, or local delivery platforms may help overcome barriers related to antigen release, drug access, and immune-cell dysfunction. However, clinical evidence remains inconsistent. Although early studies have reported pharmacodynamic changes, objective responses, and encouraging survival signals in selected subgroups, randomized trials and subsequent expansion cohorts have not demonstrated a reproducible OS advantage.[37,50,51,53,54,80,81] Combination therapies should therefore remain investigational until validated in appropriately controlled clinical trials incorporating biologically informed stratification, predefined treatment sequencing, and longitudinal biomarker analyses.

6. Conclusion

Glioblastoma remains a formidable clinical challenge because of its invasiveness, immunosuppressive microenvironment, and marked heterogeneity. PD-L1-targeted immunotherapy remains a biologically relevant therapeutic strategy, but its clinical impact is limited by suboptimal drug delivery, suboptimal combination strategies, variability in PD-L1 assessment, and incompletely understood regulatory mechanisms. Multidisciplinary research integrating immunology, materials science, imaging, and artificial intelligence will likely be essential for refining future therapeutic strategies. Continued optimization of combination regimens, drug-delivery technologies, biomarker frameworks, and clinical trial designs will be essential to determine whether PD-L1-targeted therapy can provide durable survival benefits in patients with GBM.

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Conflict of interest

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Author contributions

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During the preparation of this manuscript, the authors used an AI-assisted language tool solely to improve the readability and language. The tool was not used to generate scientific insights, analyze or interpret data, draw scientific conclusions, or perform any substantive research activities. All AI-assisted language edits were reviewed, verified, and approved by the authors. The authors take full responsibility for the content of the manuscript.

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