Immunotherapy in Glioblastoma: Why has Progress Been so Difficult?

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Abstract

Glioblastoma remains one of the most aggressive and difficult-to-treat brain tumors. Although immunotherapy has transformed the management of several cancers, its clinical impact in glioblastoma has been limited. This review summarizes the main immunotherapeutic approaches currently investigated, including immune checkpoint inhibitors, CAR-T cell therapy, cancer vaccines, and oncolytic viruses. It also examines the key biological factors responsible for treatment resistance. Major challenges include the highly immunosuppressive tumor microenvironment, tumor heterogeneity, antigen escape, low immunogenicity, and the presence of the blood–brain barrier. These factors restrict effective antitumor immune responses and reduce the efficacy of current therapies. Emerging strategies focusing on the tumor microenvironment, cytokine-based treatments, personalized medicine, and rational combination approaches may improve future outcomes. A deeper understanding of glioblastoma biology will be essential for the development of effective and individualized immunotherapeutic strategies.

Keywords: glioblastoma; immunotherapy; immune checkpoint inhibitors; CAR-T cells; cancer vaccines; oncolytic viruses; tumor microenvironment; immunosuppression; treatment resistance; personalized medicine

1. Introduction

Glioblastoma (GBM) remains the most aggressive primary malignant brain tumor in adults and continues to be associated with poor clinical outcomes despite multimodal treatment strategies. Standard therapy, consisting of maximal safe surgical resection followed by radiotherapy and temozolomide-based chemotherapy, has improved survival only modestly, and most patients develop tumor recurrence [1]. The ability of GBM to infiltrate surrounding brain tissue, together with its biological heterogeneity, makes effective treatment extremly challenging [1].

The impressive clinical success of immunotherapy in several malignancies, including melanoma, renal cell carcinoma, and non-small-cell lung cancer, has stimulated considerable interest in its potential application to glioblastoma [2]. Therapeutic approaches such as immune checkpoint blockade, adoptive cellular therapies, cancer vaccines, and oncolytic viruses have demonstrated the capacity to generate durable antitumor responses in selected patient populations and have fundamentally changed the management of several advanced cancers. These achievements raised expectations that similar strategies might also improve outcomes in patients with GBM [2].

Unlike melanoma, renal cell carcinoma, or non-small-cell lung cancer, glioblastoma possesses a unique combination of anatomical, molecular, and immunological characteristics that profoundly limit the efficacy of immunotherapy. GBM behaves as a highly dynamic and heterogeneous ecosystem in which malignant cells continuously interact with resident microglia, infiltrating macrophages, reactive astrocytes, endothelial cells, neurons, and extracellular matrix components [3,4].These interactions establish a immunosuppressive niche that promotes immune escape, therapeutic resistance, and rapid tumor recurrence despite aggressive multimodal treatment [4].

Unlike highly immunogenic malignancies, glioblastoma exhibits a relatively low tumor mutational burden and consequently generates only a limited number of neoantigens capable of eliciting effective T-cell responses [4]. Moreover, pronounced intra- and intertumoral heterogeneity results in the coexistence of multiple genetically and phenotypically distinct

cellular subpopulations within the same lesion [5]. This remarkable diversity allows continuous clonal evolution under therapeutic pressure and facilitates immune escape through antigen loss and phenotypic plasticity, representing one of the principal reasons for treatment failure [6].

Nevertheless, the clinical experience accumulated over the last decade has been less encouraging. While numerous preclinical studies have demonstrated promising antitumor activity, these findings have not translated into comparable clinical benefit. Most clinical trials evaluating immunotherapeutic agents in glioblastoma have reported limited efficacy, with only sporadic durable responses observed in selected cases [5]. This discrepancy between preclinical success and clinical outcome suggests that glioblastoma possesses unique biological characteristics that restrict the development of effective antitumor immune responses. Recent advances in single-cell RNA sequencing and spatial transcriptomic analyses have further demonstrated that these resistance mechanisms are not uniformly distributed throughout the tumor. Instead, different anatomical regions display distinct immune landscapes, cellular compositions, and metabolic programs that evolve during disease progression and under therapeutic pressure. Consequently, glioblastoma should be regarded as a spatially organized and temporally evolving disease rather than a biologically homogeneous entity [4,7].

Another therapeutic challenge arises from the unique anatomical and immunological characteristics of the central nervous system. The blood–brain barrier and the glioma blood–tumor barrier continue to restrict immune-cell trafficking and limit the delivery of many therapeutic agents to infiltrative tumor regions, thereby reducing the effectiveness of systemic immunotherapy [8]. Advances in molecular profiling, single-cell sequencing, and spatial transcriptomic technologies have further revealed the extraordinary complexity of glioblastoma biology, emphasizing the multifactorial nature of therapeutic resistance and highlighting the limitations of single-target treatment approaches [8].

Increasing evidence also indicates that therapeutic failure is not solely determined by the tumor core but is strongly influenced by the infiltrative peritumoral zone, frequently referred to as the glioma penumbra. This region contains scattered infiltrating tumor cells, glioma stem-like cells, activated astrocytes, immunosuppressive myeloid populations, and structurally altered vasculature that collectively facilitate tumor recurrence [3]. Since most glioblastomas recur within this infiltrative margin despite apparently complete surgical resection, the glioma penumbra has recently emerged as an attractive therapeutic target for future pharmacological and immunotherapeutic interventions [9].

The present review provides a comprehensive overview of current immunotherapeutic strategies for glioblastoma while emphasizing the unique biological features that distinguish this malignancy from other solid tumors. Particular attention is devoted to the mechanisms responsible for therapeutic resistance, including tumor heterogeneity, glioma stem-like cells, metabolic adaptation, immune escape, and the highly immunosuppressive tumor microenvironment. Furthermore, emerging therapeutic concepts targeting the myeloid compartment, the glioma penumbra, and other components of the tumor ecosystem are discussed as promising strategies to overcome the current limitations of immunotherapy and improve future clinical outcomes.

2. Material and Methods

For this review, we examined the recent literature on immunotherapy in glioblastoma with the aim of identifying the major therapeutic strategies currently under investigation and the factors that may explain their limited clinical success. Relevant articles were retrieved primarily from the PubMed database, with additional studies identified through the reference lists of selected review papers and clinical trial reports. Attention was given to publications addressing immune checkpoint inhibitors, CAR-T cell therapy, cancer vaccines, oncolytic viral therapies, mechanisms of immune resistance, and the role of the tumor microenvironment. The available studies are diverse and new evidence continues to emerge. Therefore, a narrative review approach was used to summarize the current state of glioblastoma immunotherapy and the main challenges facing the field.

ChatGPT (OpenAI) was used solely to assist with English language editing, grammar correction, and improvement of the manuscript's readability. No AI tool was used to

generate scientific content, identify or interpret the literature, perform data analysis, or draw scientific conclusions. In addition, an AI-based image generation tool (OpenAI DALL·E) was used exclusively to create the schematic illustration presented in Figure 1 based on concepts defined by the authors. The figure was subsequently reviewed, verified, and approved by the authors to ensure its scientific accuracy and consistency with the manuscript. All literature selection, scientific interpretation, critical analysis, and final manuscript revisions were performed independently by the authors, who take full responsibility for the content of the manuscript.

3. Current Immunotherapy State in Glioblastoma

3.1. Immune Checkpoint Inhibitors

Immune checkpoint inhibitors were among the first immunotherapeutic strategies evaluated in glioblastoma because of their success in several other solid tumors. Most clinical experience has been gained with PD-1 inhibitors such as nivolumab and pembrolizumab, as well as the CTLA-4 inhibitor ipilimumab [10]. Although these agents have shown the ability to increase immune-cell infiltration and activate antitumor immune responses, their overall clinical benefit in glioblastoma has remained limited.

Large randomized clinical trials investigating nivolumab in both newly diagnosed and recurrent glioblastoma have consistently failed to demonstrate a significant survival advantage compared with standard therapy or bevacizumab-based treatment. In recurrent glioblastoma, the phase III CheckMate-143 trial showed no improvement in overall survival with nivolumab compared with bevacizumab, despite a favorable safety profile [11]. Similarly, the phase III CheckMate-498 [12] trial in patients with newly diagnosed MGMT-unmethylated glioblastoma and the CheckMate-548 trial in patients with MGMT-methylated tumors failed to demonstrate a significant survival benefit when nivolumab was added to standard chemoradiotherapy [13]. Collectively, these studies indicate that immune checkpoint inhibition alone is insufficient to overcome the multiple mechanisms of immune resistance that characterize glioblastoma.

Evidence supporting the use of pembrolizumab remains limited and is derived primarily from small prospective studies rather than large randomized trials. The most encouraging results have been reported with neoadjuvant PD-1 blockade, which appears to induce a more robust antitumor immune response than postoperative treatment alone. In a landmark clinical study, neoadjuvant pembrolizumab increased T-cell receptor clonality, enhanced dendritic-cell activation, promoted intratumoral cytotoxic T-cell infiltration, and was associated with prolonged overall survival compared with adjuvant treatment alone [14]. More recent prospective data have confirmed sustained immune activation and favorable biological effects following neoadjuvant PD-1 blockade; however, the magnitude of clinical benefit remains modest, highlighting the need for larger randomized studies to validate these findings [15].

To further enhance therapeutic efficacy, increasing attention is being directed toward combination strategies integrating immune checkpoint inhibitors with laser interstitial thermal therapy (LITT), tumor-treating fields (TTF), oncolytic viruses, therapeutic vaccines, CAR-T cell therapies, or anti-angiogenic agents [5].

Beyond PD-1 and CTLA-4, increasing attention is being directed toward alternative inhibitory immune checkpoints that contribute to persistent T-cell dysfunction within glioblastoma. Molecules such as LAG-3, TIM-3, TIGIT, VISTA, CD39, and CD73 are frequently co-expressed on exhausted tumor-infiltrating lymphocytes and participate in redundant immunosuppressive pathways. Simultaneous inhibition of multiple checkpoint molecules may therefore prove more effective than targeting PD-1 alone [16]. Several early-phase clinical trials investigating dual or triple checkpoint blockade are currently underway and may help identify novel therapeutic combinations capable of overcoming immune resistance in glioblastoma.

3.2. CAR-T Cell Therapy

Adoptive cellular therapies, particularly chimeric antigen receptor (CAR)-T cells, represent one of the most innovative approaches currently being explored in glioblastoma. Unlike conventional T-cell responses, CAR-T cells are genetically engineered to recognize tumor-associated antigens independently of major histocompatibility complex (MHC) presentation. This allows them to target tumor cells that have developed mechanisms of immune escape [17].

Several targets have been investigated in glioblastoma, including EGFRvIII, IL13Rα2, and HER2 [2]. IL13Rα2 has attracted considerable attention because it is overexpressed in a substantial proportion of GBM cases while showing limited expression in normal brain tissue. Early clinical studies demonstrated that locoregional administration of IL13Rα2-directed CAR-T cells is feasible and generally well tolerated. Although durable radiological responses have been reported in individual patients, most responses have been transient, and long-term disease control remains uncommon. Recent studies suggest that intraventricular delivery and the use of memory-enriched CAR-T cell products may improve treatment efficacy [2].

Despite the remarkable cytotoxic potential of CAR-T cells demonstrated in hematological malignancies, translating these results to glioblastoma has proven considerably more challenging. Unlike leukemias or lymphomas, GBM exhibits pronounced antigenic heterogeneity, allowing tumor subclones lacking the targeted antigen to survive therapeutic pressure and ultimately drive tumor recurrence.
Moreover, CAR-T cells entering the glioblastoma microenvironment rapidly encounter profound immunosuppression mediated by tumor-associated macrophages, regulatory T cells, myeloid-derived suppressor cells, inhibitory cytokines, and metabolic stress, all of which contribute to progressive functional exhaustion of transferred lymphocytes [18].

Another major obstacle is the limited trafficking and persistence of CAR-T cells within the central nervous system. Although disruption of the blood–brain barrier may facilitate partial immune-cell infiltration, many infiltrative tumor cells remain protected within regions where the barrier is relatively preserved. Furthermore, the dense extracellular matrix, elevated interstitial pressure, abnormal tumor vasculature, and extensive hypoxia collectively restrict CAR-T migration throughout the tumor.
Consequently, sufficient numbers of functional effector cells frequently fail to reach infiltrating glioblastoma cells located beyond the contrast-enhancing tumor margin [6]. To overcome antigen escape resulting from the marked intratumoral heterogeneity of glioblastoma, recent research has increasingly focused on multispecific CAR-T cell platforms capable of simultaneously recognizing multiple tumor-associated antigens. Tandem and trispecific CAR-T constructs targeting combinations of EGFRvIII, IL13Rα2, HER2, EphA2, GD2, and B7-H3 have demonstrated improved tumor recognition in preclinical studies and may reduce the likelihood of clonal escape compared with single-antigen approaches [19].
Among these emerging targets, B7-H3 has attracted particular attention

because of its high and relatively homogeneous expression in glioblastoma, limited distribution in normal brain tissue, and association with aggressive tumor biology, making it an attractive candidate for next-generation CAR-T cell therapies [20]. Although these approaches remain in early clinical development, they represent promising strategies to overcome one of the major limitations of current CAR-T therapy in glioblastoma.

Novel generations of engineered CAR-T cells are also being developed to resist the immunosuppressive glioblastoma microenvironment. So-called "armored" CAR-T cells have been genetically modified to secrete pro-inflammatory cytokines such as IL-12 or IL-18, express dominant-negative TGF-β receptors, or locally release immune checkpoint inhibitors within the tumor microenvironment [18]. These modifications aim to enhance CAR-T persistence, reverse local immunosuppression, and simultaneously activate endogenous immune responses, thereby transforming CAR-T cells from simple cytotoxic effectors into multifunctional immune modulators [18].

Regional delivery strategies have likewise emerged as an important area of investigation. Instead of conventional intravenous infusion, CAR-T cells can be administered directly into the tumor cavity or ventricular system, thereby bypassing the blood–brain barrier and improving local immune-cell concentrations. Intraventricular administration has demonstrated encouraging preliminary results, including prolonged radiographic responses in selected patients, and may represent one of the most promising approaches for overcoming the anatomical limitations associated with systemic CAR-T delivery [21, 22].

Beyond conventional CAR-T cells, additional adoptive cellular therapies are currently entering clinical development. CAR-natural killer (CAR-NK) cells may offer several theoretical advantages, including lower risk of cytokine release syndrome, reduced neurotoxicity, and the possibility of developing "off-the-shelf" allogeneic cellular products. Although clinical experience remains limited, CAR-NK therapy represents an attractive complementary strategy that may help overcome several limitations associated with autologous CAR-T treatment [23].

Current evidence therefore suggests that successful adoptive cellular therapy for glioblastoma will probably require combination approaches rather than CAR-T monotherapy. Integration with immune checkpoint inhibitors, oncolytic viruses, dendritic-cell vaccines, radiotherapy, and myeloid-targeted therapies may

simultaneously enhance CAR-T expansion, improve intratumoral trafficking, and overcome multiple mechanisms of immune resistance.

3.3 **Oncolytic Viral Therapy**

Oncolytic viruses represent a particularly attractive immunotherapeutic strategy for glioblastoma because they combine direct tumor destruction with stimulation of antitumor immunity [2]. These genetically modified viruses selectively infect and replicate within tumor cells, leading to cell lysis and the release of tumor-associated antigens. That promotes the recruitment and activation of both innate and adaptive immune cells and may help convert the typically immunologically “cold” glioblastoma microenvironment into a more inflammatory and treatment-responsive state [16].

Several viral platforms have been investigated in glioblastoma, including modified herpes simplex viruses, adenoviruses, polioviruses, and viral vectors carrying immunostimulatory genes. Among them, the third-generation herpes simplex virus G47Δ has generated considerable interest. Clinical studies have demonstrated encouraging survival outcomes in selected patients, ultimately leading to its approval in Japan for the treatment of malignant gliomas [21].

DNX-2401, one of the most extensively studied oncolytic adenoviruses, has demonstrated acceptable safety and signs of clinical activity, particularly when combined with immune checkpoint inhibitors such as pembrolizumab [24]. Similarly, newer adenoviral platforms designed to enhance tumor selectivity and improve delivery across the blood-brain barrier have produced encouraging early results and continue to be evaluated in clinical trials [24].

Increasing evidence suggests that the therapeutic efficacy of oncolytic viruses extends beyond direct tumor cell lysis. Viral infection induces immunogenic cell death, resulting in the release of tumor-associated antigens, danger-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs), which promote dendritic-cell maturation and subsequent activation of tumor-specific T lymphocytes.
Consequently, oncolytic virotherapy may function as an in situ vaccination strategy capable of converting the typically immunologically "cold" glioblastoma microenvironment into a more inflamed and immunologically active state [25].

PVSRIPO is a genetically modified poliovirus that selectively targets tumor cells expressing CD155. Early clinical studies have reported prolonged survival in some patients with recurrent glioblastoma [26].

Viral-mediated inflammation may increase immune-cell infiltration, enhance antigen presentation, and upregulate PD-L1 expression within the tumor, thereby potentially sensitizing glioblastoma to immune checkpoint blockade. Early clinical studies combining DNX-2401 with pembrolizumab have demonstrated encouraging safety profiles and preliminary evidence of improved immune activation, although larger randomized trials are still required to establish clinical efficacy [16].

Viral vectors have also been used as delivery systems for therapeutic genes. Several experimental approaches have employed vectors encoding immunostimulatory cytokines, including IL-12, or genes designed to enhance dendritic-cell activation and antigen presentation [25].

Recent advances in genetic engineering have enabled the development of next-generation oncolytic viruses carrying multiple immunostimulatory transgenes. In addition to cytokines such as IL-12, experimental viral platforms are now being designed to express immune checkpoint inhibitors, bispecific T-cell engagers, co-stimulatory molecules, or chemokines capable of recruiting cytotoxic lymphocytes directly into the tumor microenvironment. These multifunctional viral constructs seek not only to destroy tumor cells but also to actively reshape the local immune landscape and overcome the profound immunosuppression characteristic of glioblastoma [21].

Nevertheless, several biological barriers continue to limit the efficacy of virotherapy. Viral replication may be restricted by heterogeneous receptor expression, incomplete intratumoral distribution, antiviral immune responses, and the extensive molecular heterogeneity of glioblastoma [3]. Infiltrative tumor cells located within the peritumoral brain tissue frequently remain inaccessible to locally administered viruses, allowing residual malignant clones to survive and eventually initiate tumor recurrence. These observations emphasize that oncolytic viruses alone are unlikely to eradicate glioblastoma and will probably achieve their greatest therapeutic potential as components of rational combination immunotherapy [3].

3.4. Cancer Vaccines

Therapeutic cancer vaccines aim to stimulate a tumor-specific immune response through the presentation of tumor-associated antigens to the patient's immune system. Several vaccine platforms have been explored, including peptide vaccines, dendritic cell vaccines, viral vector-based vaccines, and, more recently, personalized nucleic acid vaccines.

Among these approaches, dendritic cell vaccines have produced some of the most encouraging clinical results. Dendritic cells are professional antigen-presenting cells capable of activating both CD4+ and CD8+ T lymphocytes [27]. Several vaccine platforms have been developed using patient-derived dendritic cells loaded with tumor lysates, glioma stem-cell antigens, synthetic peptides, or viral antigens. The most extensively studied example is DCVax-L, which utilizes autologous tumor lysate to generate a broad antitumor immune response. Unlike single-antigen vaccines, this strategy may partially overcome the marked heterogeneity of glioblastoma by targeting multiple tumor-associated antigens simultaneously. Clinical studies have reported prolonged survival in patients with both newly diagnosed and recurrent glioblastoma; however, interpretation of these findings remains cautious because of methodological limitations and the need for further randomized validation [28].

Peptide vaccines have also been evaluated extensively. Early efforts focused on single antigens, particularly EGFRvIII, a tumor-specific mutation found in a subset of glioblastomas. Initial studies demonstrated encouraging immunogenicity and early clinical activity, but larger randomized trials failed to demonstrate a significant survival benefit, highlighting the limitations of targeting a single tumor antigen in a highly heterogeneous disease [27].

More recently, attention has shifted toward broader antigen-targeting strategies. Vaccines directed against survivin and other molecules involved in tumor growth and glioma stem-cell maintenance have shown encouraging preliminary results and continue to be evaluated in ongoing clinical trials. Advances in molecular profiling have also enabled the development of personalized vaccine approaches based on the unique antigenic landscape of individual tumors. One of the most promising recent developments is the emergence of personalized mRNA vaccines [29]. These platforms allow rapid generation of vaccines encoding multiple patient-specific neoantigens and may offer greater flexibility than traditional peptide- or cell-based approaches. Early clinical studies suggest that mRNA vaccines can induce both

systemic and intratumoral immune responses, although clinical experience remains limited and their long-term clinical benefit remains to be established [29].

4. Why Has Progress Been So Difficult? Challenges and Mechanisms of Immunotherapy Resistance in Glioblastoma

The limited efficacy of immunotherapy in glioblastoma is not the result of a single resistance mechanism. It reflects a complex interaction between tumor biology, the local microenvironment, and the unique anatomical characteristics of the central nervous system. These factors create multiple barriers that reduce the effectiveness of both innate and adaptive immune responses.

4.1. Immunosuppressive Tumor Microenvironment

Glioblastoma is characterized with profoundly immunosuppressive microenvironment. Tumors are typically infiltrated by large numbers of tumor-associated macrophages, microglia, regulatory T cells, and myeloid-derived suppressor cells, while cytotoxic T lymphocytes are relatively scarce. Together, these cellular populations create conditions that favor tumor growth and suppress effective antitumor immunity [30].

Glioblastoma cells contribute to immune evasion through increased expression of inhibitory molecules such as PD-L1 and reduced expression of molecules involved in antigen presentation. As a result, T-cell activation becomes impaired and immune checkpoint blockade often fails to generate durable responses. The tumor produces a variety of immunosuppressive cytokines, including transforming growth factor-β (TGF-β) and interleukin-10 (IL-10). These mediators inhibit dendritic-cell maturation, suppress T-cell function, and promote the development of an immune-tolerant environment [31].

Tumor-associated macrophages appear to play a particularly important role. Depending on local signals within the tumor microenvironment, these cells can adopt immunosuppressive phenotypes that support angiogenesis, tumor invasion, and immune escape [32]. Consequently, therapies aimed at modifying macrophage function are increasingly being explored as potential adjuncts to immunotherapy.

The predominance of immunosuppressive myeloid cells is now recognized as one of the principal reasons for the limited efficacy of immune checkpoint inhibitors and

therapeutic vaccines in glioblastoma. Consequently, combination strategies targeting macrophages, myeloid-derived suppressor cells, or TGF-β signaling are being actively investigated to enhance antitumor immune responses [30].

4.2. Hypoxia and Metabolic Adaptation

Rapid tumor growth frequently leads to areas of hypoxia and metabolic stress. These conditions promote the activation of signaling pathways that support tumor survival while simultaneously impairing immune-cell function [33]. Accumulation of metabolites such as lactate contributes to local immune suppression by reducing the activity of cytotoxic T cells and natural killer cells [33].

Hypoxia also promotes the expression of immune checkpoint molecules and further impairs dendritic-cell maturation, thereby reducing the effectiveness of checkpoint blockade and vaccine-based immunotherapy.

4.3. Tumor Heterogeneity and Antigen Escape

Glioblastoma is characterized by remarkable genetic and phenotypic heterogeneity. Different cellular populations may coexist within the same tumor and express distinct molecular profiles [6].This diversity complicates the development of targeted immunotherapies and increases the likelihood of treatment resistance [6].

A major consequence of this heterogeneity is antigen escape. When therapies target a single antigen, tumor cells lacking that antigen may survive and eventually become the dominant population [4]. This phenomenon has been observed in both vaccine-based approaches and CAR-T cell therapies. The presence of glioma stem-like cells further contributes to this process by maintaining tumor plasticity and driving disease recurrence [4].

This biological complexity has been directly implicated in the limited efficacy of antigen-specific therapies, including EGFRvIII-targeted vaccines and CAR-T cells directed against single antigens such as EGFRvIII, IL13Rα2, or HER2, providing a strong rationale for the development of multi-antigen and personalized therapeutic approaches [6].

4.4. Low Immunogenicity

Compared with highly immunogenic malignancies such as melanoma or non-small-cell lung cancer, glioblastoma generally exhibits a relatively low tumor mutational burden [4]. Consequently, fewer neoantigens are generated, limiting immune recognition and reducing the likelihood of strong spontaneous antitumor responses.

This low immunogenicity may partially explain why immune checkpoint inhibitors, which have transformed the treatment of several other cancers, have produced only modest benefits in glioblastoma [7]. The low neoantigen burden also limits the efficacy of therapeutic cancer vaccines by reducing the number of immunogenic targets capable of eliciting durable T-cell responses.

4.5. Anatomical Barriers and Drug Delivery

The blood–brain barrier remains another important challenge. Although it is often disrupted within the tumor core, significant portions of infiltrating tumor cells reside in regions where barrier function is partially preserved. This limits the penetration of many therapeutic agents and restricts immune-cell trafficking to areas of active disease [7].

Novel delivery strategies, including convection-enhanced delivery, intratumoral administration, intranasal drug delivery, and cell-based transport systems, are currently being investigated to overcome these limitations and improve drug distribution within the brain [13]. These delivery limitations are particularly relevant for large biologics, including monoclonal antibodies, CAR-T cells, and oncolytic viruses, prompting the development of local administration strategies such as intratumoral injection and convection-enhanced delivery.

4.6. Treatment-Related Immunosuppression

Current standard therapies may themselves compromise antitumor immunity. Corticosteroids, frequently used to control cerebral edema, can suppress T-cell activation and reduce the efficacy of immunotherapeutic interventions [15]. Likewise, radiotherapy and temozolomide often induce lymphopenia, further limiting immune responses. These treatment-related effects may partially explain the discrepancy between encouraging preclinical findings and disappointing clinical outcomes [15]. Optimizing corticosteroid use and carefully

integrating immunotherapy with radiotherapy or chemotherapy have therefore become important considerations in the design of current clinical trials.

The principal biological barriers that limit the effectiveness of immunotherapy in glioblastoma are summarized in Figure 1.

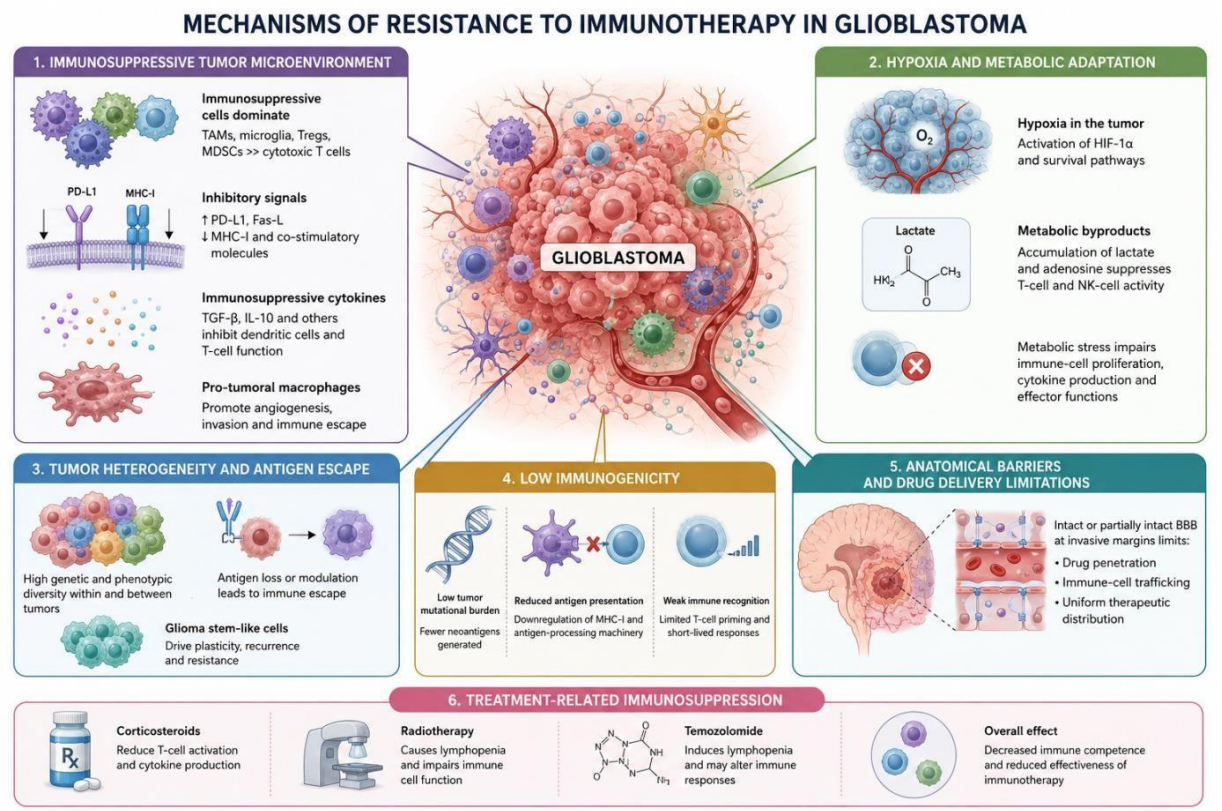

Figure 1. Mechanisms of Resistance to Immunotherapy in Glioblastoma

The principal immunotherapeutic approaches currently investigated in glioblastoma, together with their mechanisms of action, clinical experience, and major limitations, are summarized in Table 1.

Table 1. Major Immunotherapeutic Strategies in Glioblastoma: Mechanisms, Clinical Experience and Current Limitations

Therapeutic Approach	Main Mechanism of Action	Representative Therapies	Clinical Findings	Major Limitations
Immune Checkpoint Inhibitors	Restore antitumor T-cell activity by blocking inhibitory immune checkpoints	Nivolumab, Pembrolizumab, Ipilimumab, Relatlimab	Increased immune-cell infiltration has been observed in some studies;	Immunosuppressive tumor microenvironment, low tumor mutational burden, T-cell

			however, large clinical trials have demonstrated limited survival benefit	exhaustion, corticosteroid use, treatment-related lymphopenia
CAR-T Cell Therapy	Genetically engineered T cells recognize tumor antigens independently of MHC presentation	EGFRvIII CAR-T, IL13Rα2 CAR-T, HER2 CAR-T	Occasional durable responses and radiographic regressions have been reported; overall efficacy remains inconsistent	Antigen heterogeneity, antigen loss, limited CAR-T persistence, poor trafficking within the CNS, adaptive immune resistance
Dendritic Cell Vaccines	Presentation of multiple tumor antigens to stimulate adaptive immune responses	DCVax-L, ICT-107, GSC-DCV	Among the most encouraging vaccine-based approaches; prolonged survival reported in selected studies	Variable antigen expression, limited intratumoral T-cell activity, immunosuppressive microenvironment
Peptide and Personalized Vaccines	Induce tumor-specific immune responses against selected antigens or neoantigens	Rindopepimut, SurVaxM, personalized neoantigen vaccines, mRNA vaccines	Strong peripheral immune responses have been demonstrated; clinical benefit remains variable	Antigen escape, tumor heterogeneity, inadequate T-cell infiltration into tumor tissue
Oncolytic Viral Therapy	Selective viral infection and lysis of tumor cells combined with stimulation of local immune responses	G47Δ, DNX-2401, PVSRIPO	Promising survival outcomes reported in selected trials; G47Δ approved in Japan	Heterogeneous treatment responses, antiviral immunity, delivery challenges, difficulties in radiological response assessment
Cytokine-Based Approaches	Enhancement of local immune activation through cytokine delivery	IL-12-based therapies, Ad-RTS-hIL-12, M032	Early studies suggest immune activation and acceptable safety profiles	Risk of systemic toxicity, limited clinical data, uncertain long-term efficacy
Myeloid Cell-	Modulation of tumor-associated	Temferon, macrophage-	Emerging strategy with	Early-stage development, lack of

Targeted Therapies	macrophages and immunosuppressive myeloid cells	directed approaches, STING agonists	encouraging preliminary results	long-term clinical outcomes, complex interactions within the tumor microenvironment

5. Future Therapies

The limited success of current immunotherapeutic approaches has stimulated the development of novel treatment strategies aimed at overcoming the unique biological barriers of glioblastoma. Rather than targeting tumor cells alone, emerging therapies increasingly focus on the complex interactions within the tumor microenvironment that contribute to immune escape and therapeutic resistance.

One of the most promising areas of investigation involves modulation of tumor-associated macrophages and other immunosuppressive myeloid cell populations, which constitute a major component of the glioblastoma microenvironment. These cells promote tumor progression by suppressing antitumor immunity, supporting angiogenesis, and facilitating tumor invasion. Novel therapeutic approaches, including genetically engineered myeloid cells such as Temferon, macrophage-directed therapies, and stimulator of interferon genes (STING) agonists, aim to reprogram these cells toward an antitumor phenotype. Early clinical studies have demonstrated encouraging safety profiles and preliminary evidence of biological activity, although long-term clinical efficacy remains to be established [34].

Another rapidly evolving field is cytokine-based immunotherapy. Cytokines such as interleukin-12 (IL-12) have the capacity to activate both innate and adaptive immune responses while enhancing T-cell infiltration into the tumor microenvironment. To minimize systemic toxicity, several investigational approaches employ viral vectors, oncolytic viruses, or gene therapy platforms to achieve localized cytokine delivery within the central nervous system. Although these strategies remain experimental, early studies have reported encouraging immunological responses and acceptable safety profiles [35].

Increasing attention is also being directed toward rational combination therapies designed to overcome multiple mechanisms of immune resistance simultaneously. Combinations of immune checkpoint inhibitors with oncolytic viruses, therapeutic vaccines, CAR-T cell

therapies, radiotherapy, or anti-angiogenic agents may produce synergistic effects by enhancing antigen presentation, promoting immune-cell infiltration, and reversing local immunosuppression. These multimodal strategies are expected to play an increasingly important role in future glioblastoma treatment [16].

Collectively, these emerging therapeutic approaches illustrate the ongoing transition from conventional immunotherapy toward mechanism-based precision immuno-oncology. Although most strategies remain in early clinical development, they provide promising opportunities to overcome several of the biological barriers that currently limit successful immunotherapy in glioblastoma.

6. Conclusion

Glioblastoma remains one of the most therapeutically challenging malignancies in oncology, with survival outcomes having improved only marginally despite considerable advances in cancer treatment. Although immunotherapy has transformed the management of several solid tumors, its clinical impact in glioblastoma has been substantially more limited, underscoring the unique biological complexity of this disease.

Current evidence indicates that therapeutic failure results not from a single dominant mechanism but from the interplay of multiple interconnected barriers. The blood–brain barrier, profound intratumoral heterogeneity, low tumor immunogenicity, extensive immune suppression, persistence of glioma stem-like cells, and dynamic remodeling of the tumor microenvironment collectively limit the effectiveness of currently available immunotherapeutic approaches. Furthermore, adaptive resistance mechanisms, including antigen loss and T-cell exhaustion, continue to compromise durable clinical responses.

Recent advances in molecular profiling, single-cell sequencing, spatial transcriptomics, and immune characterization have substantially expanded our understanding of glioblastoma biology. These technologies have demonstrated that successful treatment will require individualized therapeutic strategies based on the molecular and immunological characteristics of each patient's tumor rather than a uniform treatment approach.

Future progress will likely depend on biomarker-guided patient selection, rational combination therapies, and innovative clinical trial designs capable of evaluating personalized

treatment strategies. Integration of immunotherapy with complementary modalities targeting both tumor cells and the immunosuppressive microenvironment offers the greatest potential to overcome current resistance mechanisms and improve clinical outcomes.

Although major challenges remain, the rapidly evolving understanding of glioblastoma immunobiology provides genuine reasons for optimism. Continued advances in precision medicine, molecular diagnostics, and next-generation immunotherapeutic approaches may ultimately establish immunotherapy as an effective component of multimodal glioblastoma treatment and translate into meaningful improvements in patient survival and quality of life.

List of Abbreviations:

PD-L1 Programmed Death-Ligand 1
BBB Blood–Brain Barrier
CNS Central Nervous System
NK Natural Killer (cells)
CAR-NK Chimeric Antigen Receptor-Natural Killer (cells)
DAMPs Damage-Associated Molecular Patterns
PAMPs Pathogen-Associated Molecular Patterns
STING Stimulator of Interferon Genes
IL-12 Interleukin-12
IL-18 Interleukin-18
MDSCs Myeloid-Derived Suppressor Cells
Tregs Regulatory T Cells (or Regulatory T Lymphocytes)
TAMs Tumor-Associated Macrophages
VISTA V-domain Immunoglobulin Suppressor of T-cell Activation
CD39 Cluster of Differentiation 39 (also known as Ectonucleoside Triphosphate Diphosphohydrolase-1, ENTPD1)
CD73 Cluster of Differentiation 73 (also known as Ecto-5'-Nucleotidase, NT5E)
G47Δ Genetically Engineered Triple-Mutated Herpes Simplex Virus Type 1 (HSV-1) Oncolytic Virus G47 Delta (the "Δ" denotes the deletion mutation)
PVSRIPO Poliovirus–Rhinovirus Recombinant
GBM Glioblastoma
PD-1 Programmed Cell Death-1
CTLA-4 Cytotoxic T-lymphocyte-associated protein 4
ITT interstitial thermal therapy
TTF Tumor-treating fields
LAG-3 Lymphocyte Activation Gene 3
TIM-3 T-cell immunoglobulin and mucin-domain containing 3
TIGIT T-cell immunoreceptor with immunoglobulin and ITIM domain CD137 Cluster of Differentiation 137
IDO Indolamin-2,3-Dioxygenase
CAR-T Chimeric Antigen Receptor T-Cell

MHC	Major Histocompatibility complex
EGFRvIII	Epidermal Growth Factor Receptor Variant III
IL13Rα2	Interleukin 13 receptor alpha 2
HER2	Human epidermal growth factor receptor 2
DCVax-L	Dendritic Cell Vaccine - Lysate
TGF-β	Transforming growth factor-β
IL-10	Interleukin-10

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