Breaking Barriers in Glioblastoma: Checkpoint Inhibition as a Therapeutic Frontier

ABSTRACT

Glioblastoma is the most aggressive and common malignant brain tumor in adults, with poor prognosis despite improvements in standard treatments such as surgery, radiation, and chemotherapy with temozolomide. Immune checkpoint inhibitors have emerged as a promising approach to strengthen the immune system's ability to fight GBM by targeting the mechanisms that allow the tumor to evade immune detection. ICIs, such as PD-1, PD-L1, and CTLA-4 inhibitors, work by reactivating T cells that are suppressed in the tumor's microenvironment. However, GBM presents significant challenges to the effectiveness of ICIs. These include its highly immunosuppressive environment, the protective barrier of the blood-brain barrier, and a low rate of genetic mutations, which makes it harder for the immune system to recognize the tumor as a threat. This review discusses the current knowledge on ICIs in treating GBM, focusing on both the successes and limitations seen in clinical trials. While ICIs as standalone treatments have shown limited success, combining them with other therapies like radiation, vaccines, or gene therapy has shown promise in enhancing the immune response and improving patient outcomes. The development of reliable biomarkers and innovative combination therapies is crucial to overcoming GBM's resistance to immunotherapy. The review emphasizes the urgent need for personalized treatment strategies and further research to fully realize the potential of ICIs in managing this challenging disease.

Keywords: Glioblastoma, Immune checkpoint inhibitors, Tumor microenvironment, Combination therapy, PD-1/PD-L1 inhibitors

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1. INTRODUCTION

The World Health Organization (WHO) has classified glioblastoma (GBM), the most prevalent and aggressive malignant primary brain tumor in adults, as a grade IV glioma[1]. With an annual incidence of 3–4 occurrences per 100,000, it makes up around 15% of all brain tumors and 45% of malignant gliomas[2]. People between the ages of 45 and 70 are usually affected by GBM, which has a small male predominance[3]. Rare familial disorders, past cranial radiation, and genetic predisposition (e.g., Li-Fraumeni syndrome, neurofibromatosis) have been identified as risk factors, however most cases are random[4]. Because of its extremely aggressive activity, GBM contributes

disproportionately to cancer-related mortality even though its incidence is relatively modest when compared to other malignancies[5].

Rapid growth, angiogenesis, necrosis, and widespread infiltration into adjacent brain tissue are characteristics of GBM[6]. The clinical course of this tumor, which is characterized by a sudden onset of neurological impairments, seizures, and elevated intracranial pressure, reflects its aggressiveness[7]. A heterogeneously enhancing tumor with surrounding edema and necrotic areas is usually visible on imaging. Poor results are often caused by their intrusive nature, which frequently precludes total surgical resection[8].

Although treatment for GBM has advanced, the prognosis is still poor[9]. With standard-of-care treatment, which consists of concurrent chemotherapy with temozolomide (TMZ), radiation, and maximal safe surgical resection, the median survival is roughly 12 to 15 months[10]. Survival is barely 3–4 months on average without treatment[11]. About 25% of people survive after two years, and less than 7% do so after five. Age, performance status, degree of resection, and molecular markers such as IDH1/IDH2 mutation and MGMT promoter methylation are some of the prognostic factors that affect results[10]. Despite being less frequent, IDH-mutant GBM is linked to a better prognosis than IDH-wildtype GBM[12].

Immune checkpoint inhibitors (ICIs) are a type of immunotherapy designed to boost the body's ability to fight cancer by targeting the mechanisms cancer cells use to escape detection by the immune system[13]. These mechanisms involve checkpoint proteins like CTLA-4, PD-1, and its partner PD-L1, which normally help keep the immune system in balance and prevent it from attacking healthy tissues[14]. While these proteins are important for preventing overactive immune responses and autoimmunity, cancer cells often exploit them to block the immune system from attacking tumors[15]. ICIs work by blocking these checkpoint proteins, reactivating immune cells, particularly cytotoxic T cells, and enabling them to mount a stronger attack against cancer cells[16].

The most studied immune checkpoint inhibitors (ICIs) include drugs that target PD-1 (like pembrolizumab and nivolumab), PD-L1 (such as atezolizumab and durvalumab), and CTLA-4 (like ipilimumab)[17]. PD-1 and PD-L1 inhibitors work by blocking the interaction between these proteins, which cancer cells often use to "turn off" T cells and avoid being attacked by the immune

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system[18]. On the other hand, CTLA-4 inhibitors boost the immune response by preventing CTLA-4 from binding to its partners (CD80/CD86), which normally act to suppress T-cell activation in the immune system. These treatments essentially "take the brakes off" the immune system, allowing it to recognize and attack cancer cells more effectively[19].

The idea of using immune checkpoint inhibitors (ICIs) to treat glioblastoma (GBM) is based on the fact that GBM creates a highly immunosuppressive environment[20]. This environment includes regulatory T cells, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages, all of which weaken the immune system's ability to fight the tumor and reduce the effectiveness of standard treatments. GBM cells also often produce high levels of PD-L1, a protein that helps them hide from the immune system[21]. ICIs have the potential to counteract these suppressive effects, allowing the immune system to mount a stronger attack on the tumor. However, GBM poses unique challenges, such as the blood-brain barrier (BBB), which limits immune cell access to the brain, and a low level of mutations in GBM cells, which makes it harder for the immune system to recognize the tumor[22]. Despite these hurdles, early research and trials suggest that ICIs, especially when combined with other treatments like radiation or cancer vaccines, could help overcome these obstacles and provide new treatment options for GBM[23]. Scientists are actively exploring these strategies to improve outcomes for patients with this aggressive brain cancer

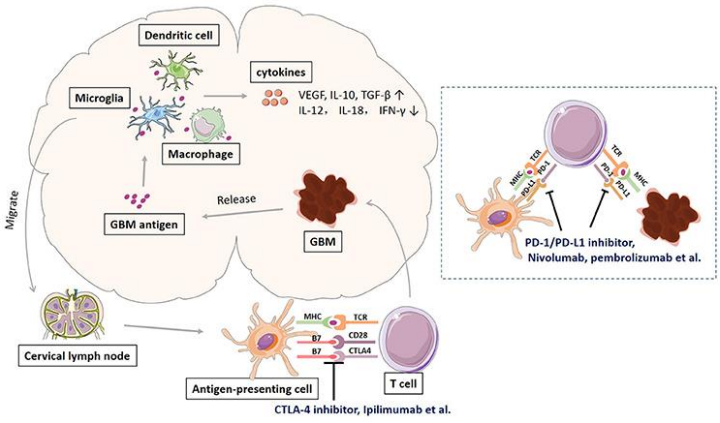

Figure 1. Diagrammatic Overview of Immune Responses and Checkpoint Mechanisms in Glioblastoma Immunotherapy. Glioblastoma immunotherapy focuses on modulating immune responses by targeting immune checkpoints like PD-1/PD-L1 and CTLA-4, which suppress T-cell activity in the tumor microenvironment. These therapies aim to overcome glioblastoma's immunosuppressive barriers, enhancing the body's anti-tumor immune response [24].

2. MATERIAL AND METHODS)

Researchers conduct a review of articles aligned with the research topic by formulating a search strategy based on the PI[E]COT framework, which defines the key components of the research question: P (patient/problem), I/E (intervention/exposure or implementation), C

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(control or comparison), O (outcome), and T (time). This approach refines the scope of the review and guides an effective literature search strategy. Articles are sourced from international journal databases, specifically PubMed, focusing on clinical trials published between 2020 and 2024. The inclusion criteria specify that studies must involve immune checkpoint inhibitors as a therapeutic intervention for glioblastoma, be clinical trials published within the specified timeframe, appear in PubMed, and be written in English, with a focus on treatment efficacy, safety, or immune-related mechanisms. Exclusion criteria eliminate studies that do not focus on glioblastoma or immune checkpoint inhibitors, are non-clinical trials (e.g., preclinical studies, reviews, or case reports), are published outside the 2020–2024 timeframe, are not in English, or do not address relevant outcomes. These criteria ensure a focused and systematic review of high-quality, relevant clinical evidence.

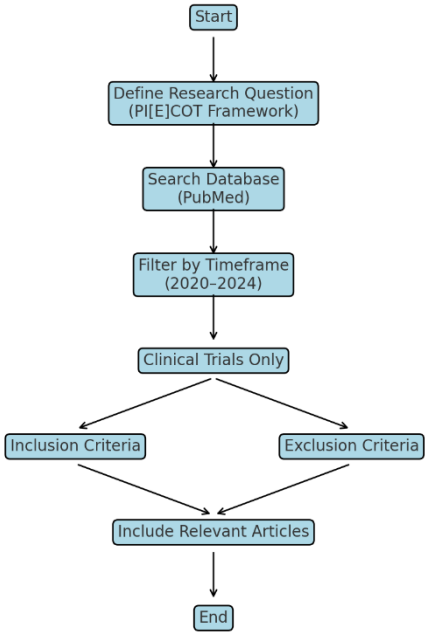

Figure 2. Flowchart of Study Selection Process

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3. RESULTS AND DISCUSSION

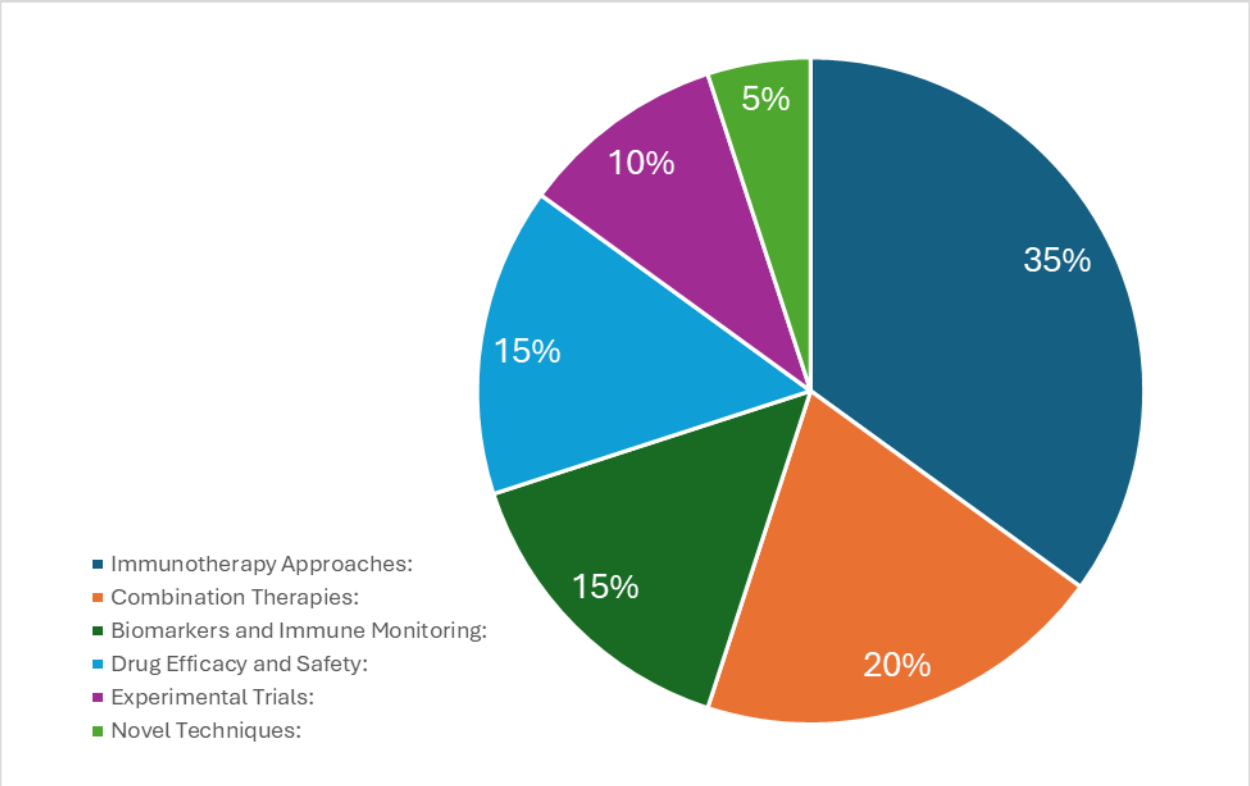

Figure 3. Analyzing the distribution of glioblastoma studies across various categories

A study by Cloughesy TF et al. [25] demonstrated that neoadjuvant anti-PD-1 immunotherapy offers significant survival benefits for patients with recurrent glioblastoma compared to adjuvant therapy administered post-surgery. Neoadjuvant therapy enhanced the tumor microenvironment by upregulating immune-related genes and promoting T-cell activation while simultaneously reducing immunosuppressive markers systemically. This dual action delayed tumor progression and improved overall survival. The findings highlighted that pre-surgical administration of anti-PD-1 therapy primes both local and systemic immune responses, optimizing the efficacy of immune checkpoint inhibitors. The study by Reardon DA et al.[26] part of the CheckMate 143 Phase 3 trial, evaluated the efficacy of nivolumab (an anti-PD-1 therapy) compared to bevacizumab (a VEGF inhibitor) in patients with recurrent glioblastoma. The findings showed that while nivolumab did not significantly improve overall survival compared to bevacizumab, it demonstrated a more durable response in a subset of patients. Bevacizumab provided better progression-free survival and symptom management, likely due to its anti-angiogenic effects, but these benefits were transient. Nivolumab, in contrast, showed promise in eliciting sustained immune responses, particularly in patients with specific biomarkers, such as high tumor mutational burden or PD-L1 expression. The study by Nayak L et al.[27] explored the efficacy of combining pembrolizumab (an anti-PD-1 therapy) with bevacizumab (a VEGF inhibitor) versus pembrolizumab alone in patients with recurrent glioblastoma. The findings revealed that the combination therapy did not significantly improve overall survival compared to pembrolizumab monotherapy, although it enhanced progression-free survival. Bevacizumab's anti-angiogenic properties contributed to better symptom management and reduced peritumoral edema, improving quality of life for some patients.

However, pembrolizumab alone showed a more durable immune response in certain patients, particularly those with favorable immune profiles. The study by Chiocca EA et al.[22] investigated the safety and efficacy of combining IL-12 gene therapy with immune checkpoint blockade in patients with recurrent glioblastoma. IL-12 gene therapy was designed to enhance immune activation by promoting pro-inflammatory cytokine production, while immune checkpoint inhibitors, such as anti-PD-1 antibodies, aimed to overcome T-cell exhaustion. The findings revealed that the combination therapy was well-tolerated and showed early signs of clinical efficacy, including increased intratumorally immune cell infiltration and improved systemic immune activation. Some patients experienced durable responses, suggesting that this combination could synergistically enhance antitumor immunity. The study by Nayak L et al.[27] evaluated the use of durvalumab, a PD-L1 checkpoint inhibitor, in patients with newly diagnosed and recurrent glioblastoma. The findings indicated that while durvalumab was generally well-tolerated, it had limited efficacy as a monotherapy, with modest improvements in progression-free survival and no significant impact on overall survival. Biomarker analysis revealed that patients with higher levels of PD-L1 expression and favorable immune profiles exhibited better responses, highlighting the importance of tumor microenvironment and patient selection. The study emphasized that glioblastoma's highly immunosuppressive environment poses significant challenges to PD-L1 blockade efficacy, underscoring the need for combination strategies and biomarker-driven approaches to improve outcomes in future trials. The study by Bagley SJ et al.[28] investigated the safety and efficacy of combining anti-EGFRvIII CAR T-cell therapy with pembrolizumab, an anti-PD-1 antibody, in patients with newly diagnosed EGFRvIII-positive glioblastoma. The phase 1 trial demonstrated that this combination was safe, with no dose-limiting toxicities observed. However, the treatment did not show significant efficacy, as indicated by a median progression-free survival of 5.2 months and a median overall survival of 11.8 months. Exploratory analyses revealed substantial changes in the tumor microenvironment post-treatment, including increased infiltration of exhausted and regulatory T cells, as well as interferon-stimulated T cells at relapse. These findings suggest that while the combination therapy is biologically active, it lacks clinical efficacy, highlighting the need for alternative strategies to enhance therapeutic outcomes in glioblastoma patients. The study by Hilf N et al.[29] investigated the efficacy of actively personalized neoantigen vaccination in patients with newly diagnosed glioblastoma. The trial utilized individualized vaccines tailored to each patient's tumor-specific mutations to stimulate an anti-tumor immune response. The findings demonstrated that the personalized vaccines were safe and well-tolerated, with no serious adverse events. Immunogenicity analysis revealed strong T-cell responses specific to the targeted neo-antigens, and some patients exhibited prolonged disease control. However, the overall impact on survival was modest, likely due to the immunosuppressive glioblastoma microenvironment. The authors concluded that while personalized vaccination shows promise in eliciting robust immune responses, its clinical efficacy may require combination with other therapies, such as immune checkpoint inhibitors, to overcome glioblastoma's inherent resistance to immunotherapy. The study by Nassiri F et al.[30] evaluated the safety and efficacy of combining the oncolytic virus DNX-2401 with pembrolizumab, an anti-PD-1 antibody, in patients with recurrent glioblastoma. The phase 1/2 trial involved 49 patients who received intratumorally DNX-2401 followed by intravenous pembrolizumab. The combination therapy was well-tolerated, with no dose-limiting toxicities observed. The objective response rate was 10.4%, which did not significantly exceed the prespecified control rate of 5%. However, the 12-month overall survival rate was 52.7%, surpassing the prespecified control rate of 20%, with a median overall survival of 12.5 months. Notably, three patients achieved durable responses and remained alive at 45, 48, and 60 months. Exploratory analyses suggested that the balance between immune cell infiltration and expression of checkpoint inhibitors may influence treatment response and resistance mechanisms. The study concluded that the combination of intratumoral DNX-2401 and pembrolizumab is safe and may offer survival benefits in select patients with recurrent glioblastoma. The study by Pouessel D et al.[31] evaluated the safety and efficacy of

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combining hypofractionated stereotactic re-irradiation (hFSRT) with the anti-PD-L1 antibody durvalumab in patients with recurrent glioblastoma. In this phase I trial, six patients received hFSRT (24 Gy in three fractions) alongside durvalumab. The combination was generally well-tolerated, with one patient experiencing a dose-limiting toxicity of grade 3 vestibular neuritis related to durvalumab. The median progression-free interval was 2.3 months, and the median overall survival was 16.7 months. Notably, a multimodal deep learning analysis incorporating MRI, cytokine levels, and lymphocyte/neutrophil ratios identified patients with pseudoprogression and those with longer survival outcomes. These findings suggest that the combination of hFSRT and durvalumab is feasible and may offer clinical benefits, warranting further investigation in larger, randomized trials.

The study by Omuro A et al. [32]evaluated the efficacy of combining radiotherapy (RT) with either nivolumab, an anti-PD-1 antibody, or temozolomide (TMZ) in patients with newly diagnosed glioblastoma harboring an unmethylated MGMT promoter. This phase III trial randomized 560 patients to receive RT plus nivolumab or RT plus TMZ. The findings revealed that the median overall survival was 13.4 months for the nivolumab group and 14.9 months for the TMZ group, indicating that nivolumab did not improve survival compared to the standard TMZ regimen. Progression-free survival and response rates were also comparable between the two groups. Safety profiles were consistent with the known effects of each treatment, with no new safety signals observed. The authors concluded that nivolumab combined with RT does not offer a survival advantage over the standard RT plus TMZ therapy in this patient population, underscoring the continued role of TMZ in treating glioblastoma with unmethylated MGMT promoters. The study by Chiu D et al.[33] investigated the safety and efficacy of combining VEGF-A inhibition with PD-L1 blockade in patients with recurrent glioblastoma (GBM). The trial involved administering avelumab, a PD-L1 inhibitor, alone or in combination with bevacizumab, a VEGF-A inhibitor, following laser interstitial thermal therapy (LITT). The combination therapy was well-tolerated, with manageable side effects. Patients receiving the combined treatment exhibited enhanced immune modulation, including increased T-cell infiltration and activation within the tumor microenvironment. These immunological changes correlated with improved progression-free survival compared to avelumab monotherapy. The study by Simonelli M et al.[34] evaluated the safety and efficacy of combining isatuximab, an anti-CD38 antibody, with atezolizumab, an anti-PD-L1 antibody, in patients with advanced solid tumors, including epithelial ovarian cancer, glioblastoma, hepatocellular carcinoma, and squamous cell carcinoma of the head and neck. The phase I/II trial demonstrated that the combination therapy was generally well-tolerated, with no dose-limiting toxicities observed. However, the treatment did not meet the prespecified efficacy criteria to proceed to the next stage of the study. Pharmacodynamic analyses revealed a reduction in tumor-infiltrating CD38+ immune cells, indicating effective target engagement by isatuximab. Despite this, there was no significant modulation of regulatory T cells or PD-L1 expression in the tumor microenvironment. The authors concluded that while the combination of isatuximab and atezolizumab is safe, it does not provide a clinical benefit in these patient populations, suggesting that CD38 inhibition may not enhance responsiveness to PD-L1 blockade in advanced solid tumors. The study by Joerger M et al.[35] evaluated the safety and anti-tumor activity of lisavanbulin, a novel microtubule destabilizer, in patients with recurrent glioblastoma and ovarian cancer. Administered as a 48-hour intravenous infusion on days 1, 8, and 15 of a 28-day cycle, lisavanbulin was generally well-tolerated, with manageable side effects. In the glioblastoma cohort, the treatment demonstrated modest anti-tumor activity, with some patients achieving stable disease. Pharmacokinetic analyses indicated that lisavanbulin effectively penetrated the central nervous system, achieving therapeutic concentrations in the brain. The conclusion of this study lisavanbulin shows potential as a treatment for recurrent glioblastoma, warranting further investigation in larger clinical trials to better assess its efficacy and optimal dosing strategies.

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The study by Lukas RV et al.[36] evaluated the safety and clinical activity of atezolizumab, an anti-PD-L1 antibody, in patients with recurrent glioblastoma. In this phase 1a trial, 16 patients received atezolizumab intravenously every three weeks until disease progression or unacceptable toxicity. The treatment was generally well-tolerated, with no grade 4 or 5 treatment-related adverse events reported. Efficacy outcomes were modest: one patient achieved a partial response lasting 5.3 months, and three patients experienced stable disease. The median overall survival was 4.2 months, ranging from 1.2 to over 18.8 months. Notably, patients with IDH1-mutant tumors or a hypermutated phenotype, such as those with POLE mutations, demonstrated longer survival, suggesting that specific genetic alterations may influence responsiveness to atezolizumab. The conclusion of this study is that while atezolizumab is safe for patients with recurrent glioblastoma, its clinical activity is limited. They emphasized the need for further research to identify biomarkers that could predict which patients might benefit from PD-L1 blockade, potentially guiding more personalized therapeutic approaches. Recent studies have explored innovative methods for monitoring glioblastoma treatment responses. Guo G et al.[37] demonstrated that analyzing circulating tumor DNA (ctDNA) from tumor in situ fluid (TISF) can serve as a biomarker for assessing the efficacy of combined low-dose bevacizumab and anti-PD-1 therapy in recurrent glioblastoma patients, offering a potential tool for real-time treatment monitoring. Similarly, Wang D et al.[38] found that changes in TISF-ctDNA dynamics correlate with treatment outcomes, suggesting that ctDNA levels could predict the effectiveness of immune checkpoint blockade combined with low-dose bevacizumab. Additionally, Lynes J et al.[39] utilized cytokine microdialysis to monitor immune responses in real-time, providing insights into the tumor microenvironment during checkpoint blockade therapy. Collectively, these studies highlight the potential of ctDNA analysis and cytokine monitoring as non-invasive strategies to evaluate and predict treatment responses in glioblastoma. The NRG-BN002 study by Sloan AE et al.[40] evaluated ipilimumab (anti-CTLA-4), nivolumab (anti-PD-1), and their combination in newly diagnosed glioblastoma. The combination therapy showed modest immune activation but limited survival benefits, emphasizing the challenges of overcoming glioblastoma's immunosuppressive microenvironment. Similarly, the Ipi-Glio trial by Brown NF et al.[41] compared ipilimumab plus temozolomide versus temozolomide alone in newly diagnosed glioblastoma, finding no significant improvement in survival with the combination. Both studies highlight the need for further optimization and biomarker-driven approaches to improve immunotherapy outcomes in glioblastoma patients. The study by Krol I et al.[42] offers significant insights into glioblastoma biology by identifying circulating tumor cell (CTC) clusters in the blood, challenging the long-held assumption that the blood-brain barrier prevents tumor cells from entering circulation. This breakthrough highlights the potential for CTC clusters to serve as biomarkers for aggressive tumor behavior and poor prognosis, which is crucial for advancing disease monitoring and personalized treatment strategies.

4. CONCLUSION

Glioblastoma remains one of the most challenging cancers to treat due to its aggressive behavior, ability to suppress the immune system, and resistance to conventional therapies. Immune checkpoint inhibitors (ICIs) have emerged as a promising option, working by reactivating the immune system and targeting the tumor's mechanisms for evading detection. However, ICIs alone have shown limited effectiveness. Combining them with other treatments, such as radiation, cancer vaccines, and immunomodulatory therapies, has shown potential in improving outcomes. Despite progress, significant hurdles remain, including the blood-brain barrier and GBM's low mutation rate, which make it harder for the immune system to recognize and attack the tumor. To address these challenges, developing reliable biomarkers and tailoring treatments to individual patients will be crucial. Continued research and clinical trials are essential for refining these therapies and discovering new combinations to effectively

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leverage the immune system. Incorporating ICIs into comprehensive treatment strategies offers hope for extending survival and improving the quality of life for patients battling this aggressive disease.

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