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Suppressive immune microenvironment and CART therapy for glioblastoma: Future prospects and challenges

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ABSTRACT

Glioblastoma, a highly malignant intracranial tumor, has acquired slow progress in treatment. Previous clinical trials involving targeted therapy and immune checkpoint inhibitors have shown no significant benefits in treating glioblastoma. This ineffectiveness is largely due to the complex immunosuppressive environment of glioblastoma. Glioblastoma cells exhibit low immunogenicity and strong heterogeneity and the immune microenvironment is replete with inhibitory cytokines, numerous immunosuppressive cells, and insufficient effective T cells. Fortunately, recent Phase I clinical trials of CART therapy for glioblastoma have confirmed its safety, with a small subset of patients achieving survival benefits. However, CART therapy continues to face challenges, including blood-brain barrier obstruction, antigen loss, and an immunosuppressive tumor microenvironment (TME). This article provides a detailed examination of glioblastoma's immune microenvironment, both from intrinsic and extrinsic tumor cell factors, reviews current clinical and basic research on multi-targets CART treatment, and concludes by outlining the key challenges in using CART cells for glioblastoma therapy.

1. Introduction

Glioblastoma (GB) is the most prevalent primary high-grade brain tumor. Glioblastoma has an incidence rate of 0.59–3.69 per 100,000 in worldwide and 3.21 per 100,000 in the United States, typically manifesting around the median age of 65 years [1–3]. Owing to its highly invasive and aggressive nature, complete surgical removal of the tumor is challenging. Despite aggressive treatment including surgery, postoperative concurrent chemoradiotherapy, and adjuvant chemotherapy, the 5-year survival rate for glioblastoma patients remains at only 9.8 %, with a median survival time of 12–15 months [4–6]. In 2018, the National Comprehensive Cancer Network (NCCN) included Tumor Treating Fields (TTFields) as a first-line treatment for newly diagnosed glioblastoma, extending the median survival time to approximately 20 months [7]. Tumor recurrence remains the primary cause of mortality, with glioblastoma typically recurring within 8–9 months [8]. For recurrent glioblastoma, bevacizumab (BEV), a humanized monoclonal antibody targeting VEGF, is approved by the NCCN as a first-line treatment to reduce tumor blood flow and volume, thereby extending progression-free survival (PFS) [9]. Even with timely administration of bevacizumab (BEV), the median overall survival (OS) for recurrent glioblastoma patients is only about 6 months [10,11]. Moreover, BEV can remodel glioblastoma blood vessels, rendering the tumor more tolerant to hypoxia and resistant to treatment [12]. Recent clinical trials demonstrated that vorasidenib significantly improves progression-free survival (PFS) in patients with IDH-mutant low-grade glioma, marking it as the first exclusive targeted therapy for glioma in two decades [13]. However, targeted therapies have made little progress in glioblastoma.

Chimeric antigen receptor (CAR) T cells, engineered from peripheral blood, are genetically modified to recognize specific tumor-associated antigens (TAAs) [14]. These cells are amplified in vitro and then reinfused into patients to target and kill tumor cells. The CAR structure

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primarily includes an extracellular domain, which comprises a single-chain variable fragment (scFv) for recognizing and binding tumor antigens, a hinge for connection, a transmembrane domain, and an intracellular domain that includes costimulatory and signaling domains [15,16]. The scFv component endows T cells with the capability to specifically recognize and bind tumor-associated antigens (TAAs), independent of MHC antigen presentation [17]. Additionally, sometimes cytokine cDNA can also be used for recognizing tumor antigens [18]. The hinge connects the scFv and transmembrane domains and its length is crucial for the activation of CAR-T cells. It positions the CAR-T cells optimally from tumor cells, thus avoiding CAR signal inhibition by phosphatases during antigen-antibody binding [19,20]. The transmembrane domain links CAR's extracellular domain with the intracellular signal domain and anchors the receptor to the T cell membrane. This domain commonly derives from CD8, CD8α, CD28, among others [21]. The costimulatory domain, typically derived from the CD28 receptor family (e.g., CD28, ICOS) or the tumor necrosis factor receptor family (e.g., 4-1BB, OX40, CD27), activates T cells to proliferate and release cytokines, enhancing their anti-tumor capabilities [15]. The signaling domain, derived from TCR/CD3ζ or the FcεRI γ chain, contains immunoreceptor tyrosine-based activation motifs (ITAMs) [15].

Traditionally, CAR-T cells are classified into three generations based on the presence of costimulatory domains. The first-generation CAR lacks a costimulatory domain, which leads to rapid exhaustion and a short lifespan [21]. The second-generation CAR includes a costimulatory domain from CD28 or 4-1BB, enabling continuous T cell proliferation and cytokine release, thus enhancing antitumor activity [22]. The superiority of specific domains remains a topic of debate among researchers. CD28 exhibits faster and stronger signaling activity, whereas 4-1BB is slower and milder. However, 4-1BB potentially extends T cell lifespan, reduces cell exhaustion, and sustains the anticancer effects of CART cells [23–26]. The third-generation CAR integrates both advantages by featuring two costimulatory domains, derived from the CD28 and 4-1BB families. Some preclinical studies suggest that third-generation CAR-T cells surpass the second generation in cell proliferation and antitumor activity, however, many clinical trials have not shown improved outcomes with the third generation, sometimes performing even worse than the second generation [27–29]. The fourth-generation CAR incorporates a cytokine receptor domain or cytokine gene (e.g., IL-12) along with a costimulatory domain, enabling the secretion of cytokines upon activation. This promotes enhanced T cell proliferation and cytotoxic activity, leading to more potent therapeutic effects [30,31]. Recently, the development of the fifth-generation CAR-T has included the addition of a cytoplasmic IL-2Rβ domain, which possesses a binding site for STAT3/5. Upon activation, this configuration triggers TCR costimulatory signals and cytokines concurrently, providing three synergistic signals that enhance T cell proliferation, survival, and antitumor efficacy [32]. (Fig. 1 and Supplementary Table 1).

CART therapy has achieved significant breakthroughs in treating hematological malignancies, particularly in cases of recurrent or refractory acute B-cell leukemia, diffuse large B-cell lymphoma, and chronic B-cell leukemia [21,33]. Numerous clinical trials have validated the effectiveness of anti-CD19 CAR-T cells in treating relapsed/refractory B-cell malignancies [34]. A long-term phase 1–2 trial of axicabtagene ciloleucel, an autologous anti-CD19 CART cell therapy, demonstrated that 83 % (84 out of 101) of patients achieved an objective response, with 58 % (59 out of 101) experiencing a complete response [35]. In a global study, tisagenlecleucel—an anti-CD19 CART cell therapy—achieved durable remission in children and young adults with relapsed/refractory B-cell lymphoblastic leukemia, with long-term persistence [36]. Additionally, tisagenlecleucel elicited satisfactory durable responses in adults with relapsed/refractory diffuse large B-cell lymphoma [37]. In a clinical trial, CD19 CAR-T cells treated relapsed/refractory chronic lymphoblastic leukemia (CLL) and small lymphoblastic leukemia (SLL) in 22 evaluable patients, with 82 % achieving overall responses and 45 % achieving complete responses [38]. However, CART therapy has not yielded satisfactory results in the treatment of solid tumors [39,40].

Recent years have seen slow progress in glioblastoma treatment, primarily limited to physical therapy and chemotherapy. Previous clinical trials have demonstrated that targeted therapy and immune checkpoint blockade for recurrent glioblastoma have not yielded positive outcomes [41]. CART therapy has shown promising results in small samples of recurrent glioblastoma [42]. Consequently, this review will examine the complex suppressive immune microenvironment of glioblastoma, as well as the current status, limitations, and future prospects of CART therapy for glioblastoma.

2. Glioblastoma immunosuppressive microenvironment

Glioblastoma is known as a "cold tumor," notoriously insensitive to

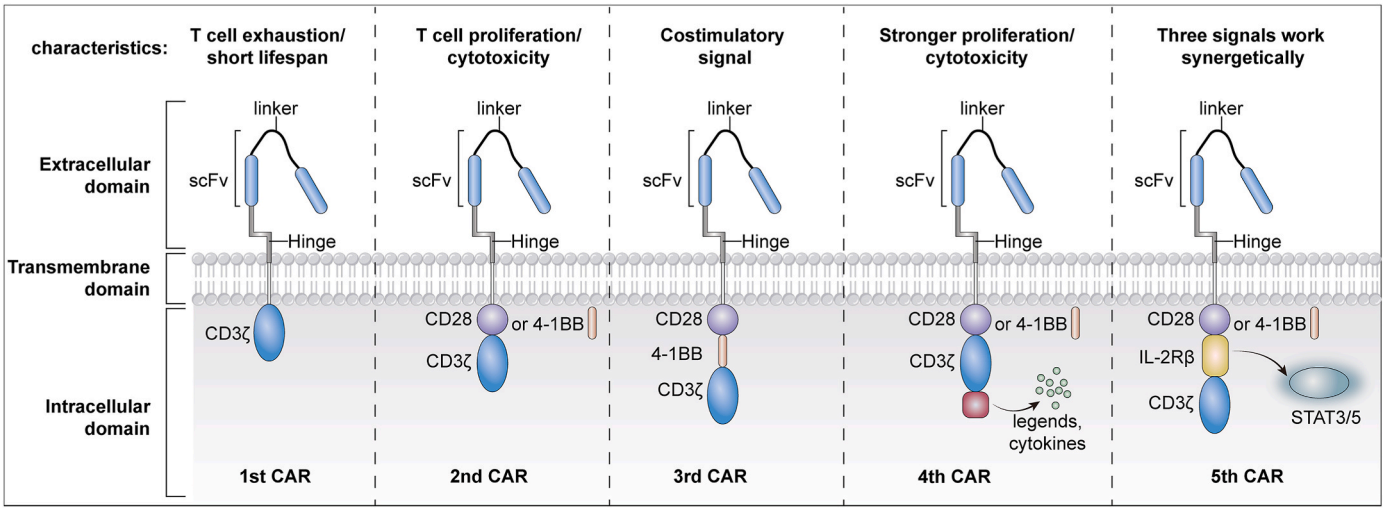

Fig. 1. The structure of the 5 generations of CART cells. The intracellular domain of the 1st generation of CART cells only contains CD3ζ, and T cells are prone to exhaustion and have a short lifespan; the 2nd generation of CART cells contains a CD28 or 4-1BB co-stimulatory domain, which enhances the cytotoxic and proliferative abilities of T cells; the 3rd generation of CART cells has two co-stimulatory domains, but its efficacy needs further verification compared to the 2nd generation; the 4th generation of CART cells can synthesize or secrete receptors or cytokines, which enhances the cytotoxic and proliferative abilities of T cells; the 5th generation of CART contains IL-2Rβ domain, and the three signals synergistically increase anti-tumor efficacy.

immunotherapy. The underlying reasons are multifaceted, generally stemming from both tumor-cell-intrinsic and tumor-cell-extrinsic factors, such as low tumor cell immunogenicity, suppressive cytokines, limited effective T cells, and extensive suppressive immune cell infiltration. Collectively, these factors contribute to the formation of an immunosuppressive microenvironment in glioblastoma.

2.1. Glioblastoma is characterized by a low tumor mutational load (TML), limited neoantigens, and few mismatch repair (MMR) mutations

TML, neoantigen presence, and MMR mutations may influence tumor immunogenicity and responsiveness to immunotherapy. Recent studies indicate that glioblastoma exhibits a lower tumor mutational load (TML) compared to NSCLC and melanoma, which typically display higher TML levels [43–45]. Hodges et al. conducted an analysis of TML in 198 glioblastoma patients using next-generation sequencing (NGS), finding that only 3.5 % (7 out of 198) exhibited high TML, defined as more than 20 mutations per 1.4 Mb, and 10 % (20 out of 198) displayed moderately elevated TML, defined as 10–20 mutations per 1.4 Mb. In contrast, low-grade gliomas (WHO I-II) exhibited even lower TML levels [43]. Neoantigens, defined as mutant proteins absent in normal human tissues and associated with TML abundance, are tumor-specific mutant antigens and could potentially indicate immunotherapy response [46]. However, unlike melanoma and lung squamous carcinoma, which have high neoantigen expression, glioblastoma typically shows only "occasional" neoantigen expression, defined as less than 1 mutation per 1 Mb [47,48]. Recent studies have confirmed that mismatch repair (MMR) functionality is correlated with clinical responses to immune checkpoint inhibitors. Le [49] observed that colorectal cancer patients proficient in mismatch repair exhibited poorer responses to PD-1 treatment in terms of both progression-free survival (PFS) and overall survival (OS) compared to those deficient in mismatch repair. Particularly, biallelic mismatch repair deficiency, associated with higher rates of exonic mutations and neoantigen presence in glioblastoma, can enhance the therapeutic response to PD-1 antibodies [50]. Additionally, the expression of mismatch repair proteins is evident in glioblastoma, although complete loss occurs infrequently: 6.7 % (2 out of 30) for MLH1, 10 % (3 out of 30) for MSH2, 13.3 % (4 out of 30) for MSH6, and 6.7 % (2 out of 30) for PMS2. Rare MMR mutations in glioblastoma are positively correlated with higher TML [43]. Further research suggests that mutations in polymerase epsilon (POLE) are indicative of mismatch repair deficiency status and are closely associated with the presence of neoantigens [51]. In glioblastoma, POLE mutations may lead to giant-cell glioblastomas, which generally have a favorable prognosis [52]. However, POLE mutations are rare in glioblastoma and exclusively occur in patients with high TML [43]. These findings demonstrate that glioblastoma is a malignancy with infrequent mutations, stable genomic features, and low immunogenicity, explaining its resistance to standard immunotherapy.

2.2. Molecular expressions in glioblastoma inhibit immune responses

2.2.1. IDH status

The IDH gene encodes isocitrate dehydrogenase, which normally catalyzes the conversion of isocitrate to α-ketoglutaric acid (α-KG). Upon mutation, it produces a significant amount of the carcinogenic metabolite D-2-hydroxyglutarate (D-2HG) [53]. IDH mutations serve as crucial prognostic markers for gliomas, influencing the tumor microenvironment (TME) through the regulation of the oncometabolite D-2HG within tumor cells [54]. Compared to mutant IDH cells, IDH wild-type tumors contain a greater number of immune cells [55], including activated $CD4^+$ and $CD8^+$ T lymphocytes [56,57], as well as inhibitory cells like Treg cells [54] and monocyte-derived macrophages (MDM) [58]. It is evident that IDH wild-type tumors exhibit higher proliferation and activation of $CD4^+$ and $CD8^+$ T lymphocytes compared to IDH mutant tumors [59]. Additionally, IDH mutations can increase microglia populations [60,61] and decrease dendritic cell infiltration [62]. IDH wild-type tumors show greater infiltration of immature NK cells, whereas IDH mutant tumors demonstrate more cytotoxic NC cell infiltration [60]. Previous studies have demonstrated that IDH-mutated tumors promote DNA hypermethylation of the CD274 promoter, resulting in reduced PD-L1 expression compared to IDH wild-type cells [55,63].

2.2.2. TP53

Inactivation of the TP53 pathway is common in glioblastoma, affecting approximately 90 % of patients. This often results from upstream ARF deletions, TP53 mutations/deletions, and MDM2/4 amplifications [64]. Cancer-associated fibroblasts (CAFs) harboring TP53 mutations exert pro-inflammatory and pro-tumorigenic effects, primarily through increased secretion of chemokines and cytokines, including CXCL12 [65]. In tumor cells, TP53 pathway inactivation primarily regulates chemokine and cytokine secretion, thereby exerting pro-inflammatory and carcinogenic effects on the tumor microenvironment (TME) [66]. Earlier research has shown that TP53 deficiency or mutation can recruit tumor-infiltrating myeloid populations, influence microglia and monocyte infiltration via CCL2 and TNFA [67], alter the function of tumor-associated macrophages (TAMs) through the CSF1 pathway [68,69], and attract tumor-related polymorphonuclear leukocytes (PMNs) through increased secretion of CXCL17 [70]. Furthermore, TP53 pathway inactivation can hinder the infiltration and functionality of $CD4^+$ and $CD8^+$ T cells by upregulating CXCR3/CCR2 expression, thus augmenting the suppressive role of Treg cells [71].

2.2.3. NF1

NF1 gene inactivating mutations or deletions, which not only precipitate neurofibromatosis but are also prevalent in 23 % of glioblastoma cases, act as suppressor genes in human glioblastoma [64]. Recent studies have demonstrated that NF1 deletions recruit tumor-associated macrophages and microglia in glioblastoma, thereby facilitating tumor progression [72]. Additionally, it has been confirmed that low-grade NF1 gliomas are characterized by increased T cell infiltration and enhanced $CD8^+$ T cell activation [73].

2.2.4. PTEN

The PTEN gene, a recognized tumor suppressor, plays a critical role in activating the RTK/PI3K pathway when deleted, a phenomenon occurring in nearly 90 % of glioblastomas [64]. PTEN deletion contributes to a suppressive tumor microenvironment (TME). This involves complex mechanisms: PTEN deficiency activates Gal-9/Tim-3 and increases M2-type TAM infiltration [74]. Additionally, PTEN deletions reduce $CD8^+$ T-cell infiltration and cytotoxicity by inhibiting the c-Gas/STING pathway and upregulating PD-L1 expression [75,76]. In addition, research has also shown that PTEN deletion also promotes infiltration of Treg cells and M2 macrophages in the TME [77]. It triggers macrophage infiltration via YAP1/LOX overexpression, enhancing SPP1 secretion and angiogenesis [78]. Moreover, PTEN deletion increases HIF-1a expression, directly stimulating angiogenesis in the TME [79].

2.2.5. RB signaling

Inactivation of the RB signaling pathway is common in glioblastoma, affecting approximately 80 % of cases. RB pathway inactivation is primarily due to CDKN2A/B/C deletions, CDK4/CDK6/CCND2 amplification, and RB mutations or deletions [64]. The RB pathway primarily influences the cell cycle and significantly impacts cell proliferation. However, recent studies indicate that it also affects the tumor microenvironment (TME). Li et al. demonstrated that RB pathway inactivation promotes the formation of an inhibitory tumor microenvironment by increasing CCL2 secretion, which recruits TAMs, MDSCs, and Foxp3+ Treg cells [80]. Additionally, Orozco et al. reported that RB mutations and RAS overexpression in glioblastoma induce resistance to the cytotoxic effects of NK cells [81] (Fig. 2).

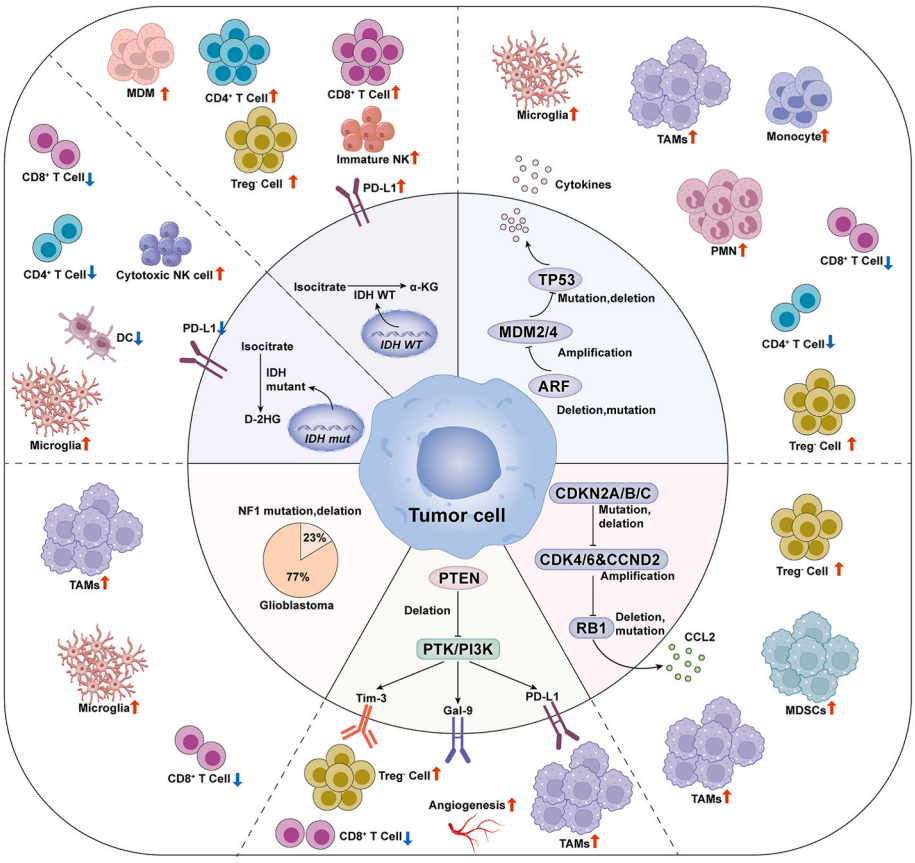

Fig. 2. The molecular characteristics of glioblastoma change the tumor microenvironment. IDH wt can recruit more $CD8^+$ T cells, $CD4^+$ T cells, monocyte-derived macrophages (MDMs), Treg cells, and immature NK cells compared to IDH mutations. Inactivation of the TP53 pathway recruits more tumor-associated macrophages (TAMs), monocytes, T lymphocytes, and tumor-related polymorphonuclear leukocytes (PMNs) through section of cytokines. Deletion of NF1 leads to increased TAMs in the TME and decreased $CD8^+$ T cell infiltration. PTEN gene deletion can increase the expression of tumor cell surface receptors, thus recruiting TAMs, Treg cells, and angiogenesis; reducing $CD8^+$ T cell infiltration. Inactivation of the RB pathway can recruit TAMs, MDSCs, and Treg cells.

Other dysregulated pathways in tumor cells also suppress the TME. For example, the over-activated JAK/STAT pathway in glioblastoma can lead to a suppressive microenvironment and induce the recruitment of dysfunctional $CD8^+$ T cells, MDSCs, immature DCs, and macrophages, thus promoting tumor progression [82,83].

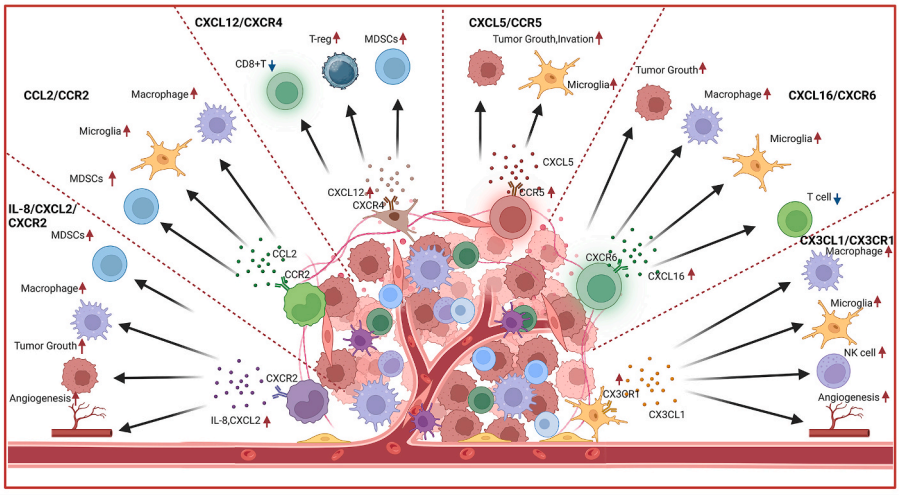

Fig. 3. The role of cytokines in the TME. The IL-8/CXCL2/CXCR2 axis recruits macrophages, MDSCs, and promotes tumor proliferation and angiogenesis; the CCL2/CCR2 axis recruits macrophages, microglia, and MDSCs; CXCL12/CXCR4 can recruit MDSCs, Treg, and reduce $CD8^+$ T cell infiltration. CCL5/CCR5 can promote microglia M2 polarization and promote tumor cell proliferation and invasion; CXCL16/CXCR6 can promote tumor growth, recruit TAMs, and reduce T cell infiltration; CX3CL1/CX3CR1 can promote TAM infiltration and increase tumor angiogenesis.

2.3. Cytokines and chemokines play a critical role in inhibiting the immune response in glioblastoma

As a "cold tumor," glioblastoma exhibits significant alterations in the quality and quantity of these molecules within the TME, impacting tumor progression, recurrence, and metastasis. Immunosuppressive factors in glioblastoma include IL-8, IL-10, CCL2, EGF, VEGF, CXCL2, and CXCL12, among others, while immune-activating factors include IL-2 and IFN-γ [42]. These factors interact with their receptors to alter the immune microenvironment of glioblastoma, promoting tumor proliferation, growth, angiogenesis, and metastasis [84] (Fig. 3).

2.3.1. CXCL2/IL8/CXCR2 axis

IL-8, a significant inhibitory cytokine, is highly expressed in glioblastoma tissues. Research shows that tumor cells promote proliferation via autocrine secretion [85]. In addition, Liu et al. demonstrated that IL-8, by binding to CXCR2, recruits MDSCs in the TME and promotes angiogenesis. Inhibiting the IL-8/CXCR2 axis can enhance the effectiveness of PD-1 inhibitors in glioblastoma [86]. Hypoxic conditions in the TME induce IL-8 secretion, which recruits TAMs and consequently promotes tumor progression [87]. Regarding the CXCL2/IL8/CXCR2 signaling axis, CXCL2 is highly expressed in tumor tissues and facilitates tumor angiogenesis via its receptor CXCR2 [88]. This research indicates that the CXCL2/IL8/CXCR2 axis, which is highly expressed in tumors, is associated with poor prognosis and promotes tumor growth through several mechanisms, including TME inhibition [84].

2.3.2. CCL2/CCR2 axis

The CCL2/CCR2 axis plays a crucial role in driving glioblastoma progression. Deng et al. demonstrated that high CCL2 expression in tumor tissues correlates positively with the infiltration of $CD4^+$ T cells, macrophages, neutrophils, and myeloid dendritic cells in the TME [89]. Research indicates that tumor cells, microglia, and macrophages secrete CCL2, with CD163+ macrophages being the primary source of CCL2 in tumors [90]. The CCL2/CCR2 signaling axis recruits TAMs, including infiltrating macrophages and resident brain microglia, within the glioblastoma immune microenvironment, facilitating tumor progression [91–93]. Flores et al. have shown that the CCL2-CCR2 axis recruits MDSCs, and inhibiting CCR2 receptors can enhance $CD8^+$ T cell functionality in the TME and increase the sensitivity of PD-1 inhibitors in treating tumors [94].

2.3.3. CXCL12/CXCR4 axis

The CXCL12/CXCR4 signaling axis plays an important tumorigenic role in gliomas. Previous literature has reported that CXCL12 and its receptor CXCR4 are overexpressed in various types of gliomas [95], promoting tumor invasion and angiogenesis [96,97]. CXCR7 is a substitute receptor for CXCL12, which is also highly expressed in glioma cells, endothelial cells, and microglia, and mediates tumor angiogenesis [98]. In addition, the CXCL12/CXCR4 axis can also mediate radiotherapy and chemotherapy resistance in glioblastoma [99,100]. Alghamri et al. recently reported that using nanomaterials to encapsulate CXCR4 antagonists can inhibit tumor cell proliferation, induce tumor immunogenic death, and reduce infiltration of CXCR4+ monocellular myoid derived suppressor cells (M-MDSCs) into the TME [101]. What's more, Wei et al. also confirmed that blocking the CXCL12/CXCR4 signaling pathway enhances $CD8^+$ T cell infiltration and reduces the presence of tumor-associated myeloid cells (TAMCs) and Treg cells in the TME [102].

2.3.4. CCL5/CCR5 axis

CCR5 is overexpressed in glioblastoma and negatively correlated with patient prognosis [103] and its ligand CCL5 is generally believed to be secreted by monocytes/macrophages and T lymphocytes [104]. Previous studies have confirmed that The CCL5/CCR5 signaling axis promotes glioblastoma proliferation, migration, and invasion via autocrine and paracrine mechanisms, primarily by activating the PI3K/AKT pathway [103,105]. In addition, recent studies have indicated that CCL5, originating from peripheral blood cells, can enhance tumor cell resistance to temozolomide [106]. Inhibiting CCR5 can prevent the polarization of M2 microglia, subsequently reducing their migration [107].

2.3.5. CXCL16/CXCR6 axis

The cytokine CXCL16 is highly expressed in tumors, microglia, and endothelial cells, while its receptor CXCR6 is only expressed on a group of cells with stem-like proliferation capacity in glioblastoma [108] which suggests that CXCL16/CXCR6 can promote tumor proliferation. In addition, previous studies have reported that the CXCL16/CXCR6 axis facilitates glioma cell migration and invasion [109]. What's more, tumor-derived CXCL16 facilitates the transformation of glioma-associated microglia/macrophages (GAMs) into a pro-tumor phenotype, thereby enhancing glioblastoma growth, migration, and invasion [110]. Chia et al. demonstrated that the transmembrane cytokine CXCL16, expressed on the surface of tumor-associated myeloid cells (TAMCs), impairs T cell function in the tumor microenvironment (TME). Interestingly, the CXCL16-CXCR6 axis displays dual characteristics: it promotes T cell immune responses and T cell infiltration in the early stages, but inhibits T cell function during later stages [111].

2.3.6. CX3CL1/CX3CR1 axis

The role of the CX3CL1-CX3CR1 signaling axis is subject to debate. Several studies highlight CX3CL1 as one of the most abundantly expressed chemokines in the central nervous system, facilitating communication between neurons, glial cells, and microglia [112]. The ligand CX3CL1, expressed on tumor cells, is involved in cell adhesion, transendothelial migration, and mobilization [112]. CX3CR1 protein and mRNA are highly expressed in low-grade gliomas and glioblastomas [113,114], recruiting tumor-associated macrophages/microglia (TAMs) in the TME that upregulate the expression of MMP2, 9, and 14 [107]. Lee et al. reported that CX3CR1 promotes angiogenesis in low-grade gliomas by increasing CCL2 and MMP9 expression, contributing to the malignant transformation of these tumors [115]. On the other hand, it has been shown that IDH mutations have been shown to increase CX3CL1 expression in glioblastoma cells, subsequently recruiting NK cells within the TME [116]. Liu et al. observed a slight increase in tumor growth in CX3CR1−/− mice, although this did not significantly affect the cellular components of the TME [117]. Sciumè et al. also demonstrated that CX3CL1 may inhibit glioma invasion by promoting tumor cell aggregation [118].

2.3.7. CXCL9/CXCL10/CXCL11/CXCL4/CXCR3 axis

The CXCR3 receptor, highly expressed in glioblastoma, serves as an independent prognostic factor for patients [119]. The main ligands of CXCR3 vary due to its different isoforms. For CXCR3-A, the primary ligands are CXCL9, CXCL10, and CXCL11; for CXCR3-B, they are mainly CXCL10 and CXCL4 [120,121]. CXCR3 is predominantly expressed on Th1 cells, $CD8^+$ T cells, and NK cells [104]. Zhao et al. confirmed that FGL2 recruits $CD69^+$ $CD8^+$ brain resident memory T cells via CXCL9/10 and CXCR3 chemokines [122]. Wang et al. reported that oncolytic adenovirus (oAds) expressing CXCL11 can recruit CAR-T cells, enhancing therapeutic efficacy. CXCL11 boosts the infiltration of $CD8^+$ T lymphocytes, NK cells, and M1-polarized macrophages, while reducing the presence of MDSCs, Tregs, and M2-polarized macrophages [123].

2.4. The immune cell composition in glioblastoma contributes to an immunosuppressive tumor microenvironment (TME)

Compared to intracranial metastases, glioblastoma—particularly IDH wild-type glioblastoma—exhibits a "cooler" immune microenvironment. Literature indicates that glioblastoma exhibits increased

numbers of tumor-associated macrophages (TAMs) and monocytes compared to intracranial metastases, including melanoma and non-small cell lung cancer. Glioblastoma shows significantly lower T cell levels compared to metastases (13 % ± 10 % vs 50 % ± 16 %). Additionally, plasma cells are moderately present in glioblastoma, while their expression is elevated in metastases. Specifically, microglia counts are significantly lower in metastatic tumors, while the presence of mononuclear-derived macrophages (MDM) is significantly higher compared to gliomas. The monocyte count remains essentially unchanged. Additionally, there is a notable increase in immature NK cells in glioblastoma compared to metastatic tumors. However, the trend for cytotoxic NK cells is the opposite [124].

Sun et al. confirmed the presence of numerous invasive immune cells, including T cells, mast cells, and macrophages, in brain metastatic lung adenocarcinoma, whereas astrocytes and microglia are significantly more prevalent in gliomas. Similarly, they observed a higher proportion of MDMs in metastatic tissue, whereas microglia were abundant in glioma tissue [125].

Klemm et al. demonstrated an increase in myeloid cells and a significant decrease in lymphocytes in gliomas compared to brain metastases. Specifically, microglia were most abundant in IDH mutant gliomas, followed by IDH wild-type gliomas, with reduced counts in metastatic tumors. The highest concentration of MDMs was observed in IDH wild-type gliomas. Additionally, $CD4^+$ and $CD8^+$ T lymphocytes were more prevalent in metastatic tumors, particularly melanoma, compared to gliomas. B lymphocytes were also more prevalent in metastatic tumors [126].

In summary, it is clear that TAM cells occupy a dominant position in glioma, with some studies suggesting they comprise up to 50 % of the total cellular composition [127]. However, T lymphocytes, especially for $CD4^+$ and $CD8^+$ T cells, were significantly reduced in gliomas. And Tregs comprise up to 30 % of infiltrating lymphocytes in GBM which suppress T-cell responses [128]. The number of cytotoxic NK cells is also reduced in gliomas. The complexity of this immune microenvironment arises from factors such as the characteristics of tumor cells, secretion of specific cytokines, and the presence of the blood-brain barrier.

2.5. Immune checkpoints suppresses the tumor immune microenvironment

2.5.1. PD-1/PD-L1 and CTLA-4

PD-1/PD-L1 checkpoints have been demonstrated to significantly inhibit $CD8^+$ T cells, thereby suppressing the TME [129]. The incidence of PD-L1 expression in GBM patients is frequent, similar to other malignancies that have been profiled for PD-L1 expression and higher expression of PD-L1 is correlated with worse outcome [130]. The expression of CTLA-4 is notably increased in GBM patients with IDH wild type and mesenchymal gliomas. Overexpression of CTLA-4 can induce the infiltration of immune cells (including $CD8^+$ T cells, Treg, macrophages, etc.), and the survival of glioma patients with low CTLA-4 expression is significantly prolonged [131].

2.5.2. TIM-3

TIM-3 is overexpressed in glioblastoma, and its expression is positively correlated with IDH wild-type and mesenchymal glioblastoma, and negatively correlated with patient prognosis. TIM-3 was closely related to T cell mediated immune response to tumor cell [132]. Recent studies have also shown that activation of TIM-3 checkpoint in glioblastoma can promote macrophage M2 polarization and promote tumor progression [133,134].

2.5.3. B7-H3

B7-H3 is overexpressed in glioblastoma, and B7-H3 is associated with mitotic cell cycle and cell proliferation, which promotes the proliferation of tumor cells. It also acts as an immune checkpoint to inhibit T cell activity, resulting in a suppressive TME. Higher B7-H3 expression indicates a worse prognosis for glioma patients [135].

2.5.4. LAG-3

A recent study has showed that LAG-3 is only rarely expressed on T lymphocyte in IDH-wildtype glioblastoma and LAG-3 expression was correlated with the presence of $CD3^+$, $CD8^+$, PD-1+ T lymphocytes, and PD-L1+ tumor cells [136]. Bookman et al. [137] also showed that anti-LAG-3 antibody alone or in combination with anti-PD-1 antibodies prolonged mouse survival. What's more, the literature also suggests that the human leukocyte antigen (HLA)-II is highly expressed in GBM and is associated with increased infiltration of LAG-3+ $CD4^+$ T cells. Furthermore, HLA-II high LAG-3 high was associated with worse patient survival. Combined anti-LAG-3 and anti-IL-10 treatment inhibited tumor growth in a mouse brain tumor model [138].

3. Clinical and basic research on CART therapy in glioblastoma

To date, no breakthroughs have been achieved in treating glioblastoma with immune checkpoint inhibitors or tumor vaccine therapy [42]. CART therapy has shown some progress in a limited number of cases. Therefore, we searched PubMed and ClinicalTrials.gov for ongoing CART clinical trials and compiled them into Table 1. We summarize the therapeutic progress and safety studies of each target as follows.

3.1. EGFRvIII

EGFRvIII, resulting from the deletion of exons 2 to 7 in the EGFR gene, leads to the loss of 267 amino acids in the extracellular domain and activates the tyrosine kinase activity of the intracellular domain independent of ligand binding. Approximately 40 % of newly diagnosed glioblastoma patients exhibit EGFR amplification, and about 50 % of these have EGFRvIII mutations. Although prior literature has suggested that EGFRvIII can promote the proliferation, invasion, and angiogenesis of glioma cells in vitro [139], Felsberg et al. have confirmed that EGFRvIII has no effect on the prognosis of EGFR-amplified glioblastoma patients [140].

In 2017, the first CART therapy targeting EGFRvIII was intravenous administered to glioblastoma patients. O'Rourke et al. [141] collected 10 patients with recurrent glioblastoma and treated them with EGFRvIII-CART therapy. Among them, 7 patients underwent secondary surgery. One patient, followed for more than 18 months, maintained a stable condition. Observations revealed that T cells and EGFRvIII-CART cells, exhibiting proliferative and cytotoxic capabilities, were present in the tumor immune microenvironment two weeks after CART cell infusion, suggesting an improvement in the patient's TME due to CART therapy. Recently, results from another clinical trial were announced, where EGFRvIII-CART cells, combined with the PD-1 monoclonal antibody pembrolizumab, were intravenous administered to seven newly diagnosed glioblastoma patients. No dose-limiting toxicity was observed. However, the trial did not extend the patients' progression-free survival (PFS) (5.2 months; 90 % CI 2.9–6.0 months) or overall survival (OS) (11.8 months; 90 % CI 9.2–14.2 months). The presence of CART cells was also detected within the tumor. Analysis of the TME in three patients revealed no significant changes in the overall immune composition of the tumor post-treatment, although there was an increase in IFN-stimulated T cells [142].

Additionally, several ongoing clinical trials involving EGFRvIII-CART have been suspended (Table 1). According to available data, only a few patients have seen successful outcomes [143]. (including one recurrent glioblastoma patient who survived for 36 months), but due to the small sample size, we are unable to accurately analyze the efficacy of this target. However, the majority of patients have not experienced favorable outcomes.

Several experimental approaches have been tested in animal studies to enhance the efficacy of EGFRvIII-CART, achieving preliminary results. Considering that the expression of PD-1 after CAR-T treatment is clearly correlated with PFS in recurrent glioblastoma [144], some literature reported that PD-1 knockout [145] or aPD-1 monoclonal

Table 1
Clinical trials of CART in glioblastoma.

Trials	No.	Arms	Characteristic	Target	Phase	Outcome	Delivery routes	Toxicity	State
NCT04185038	90	Biological: SCRI-CARB7H3(s); B7H3-specific chimeric antigen receptor (CAR) T cel	Central Nervous System Tumor; Diffuse Intrinsic Pontine Glioma/ Diffuse Midline Glioma/Glioma	B7-H3	I	One patient had sustained clinical and radiographic improvement through 12 months on study	ICV	No dose-limiting toxicities	Recruiting
NCT04385173	12	Drug: B7-H3 CAR-T; Drug: Temozolomide	Recurrent/ Refractory Glioblastoma	B7-H3	I	NA	IT/ICV	NA	Recruiting
NCT04077866	40	Biological: B7-H3 CAR-T; Drug: Temozolomide	Recurrent/ Refractory Glioblastoma	B7-H3	I	NA	IT/ICV	NA	Recruiting
NCT05366179	36	Drug: CAR.B7-H3T cells infusion	Glioblastoma Multiforme	B7-H3	I	NA	IT	NA	Recruiting
NCT05835687	36	Drug: B7-H3-CAR T cells	Diffuse Midline Glioma, H3 K27M-Mutant/High Grade Glioma/ Glioblastoma	B7-H3	I	NA	locoregional (LR)	NA	Recruiting
NCT05474378	39	Drug: B7-H3 CART	Brain and Nervous System	B7-H3	I	NA	locoregional (LR)	NA	Recruiting
NCT05241392	30	Biological: B7-H3-targeting CAR-T cells	Glioblastoma	B7-H3	I	NA	locoregional (LR)	NA	Recruiting
NCT06221553	9	Biological: B7H3 specific CAR T cell with IL-7Ra signaling domain	DIPG Brain Tumor/ Diffuse Intrinsic Pontine Glioma	B7-H3 with IL-7Ra	I	NA	IT	NA	Not yet recruiting
NCT05768880	72	Biological: SC-CAR4BRAIN	Diffuse Intrinsic Pontine Glioma/ Diffuse Midline Glioma	B7-H3, EGFR806, HER2, and IL13-zetakine	I	NA	ICV	NA	Recruiting
NCT05660369	21	Drug: CARv3-TEAM-E T cells	Recurrent/ Recurrent Glioma	CARv3-TEAM-E	I	Radiographic tumor regression was dramatic and rapid, occurring within days after receipt of a single intraventricular infusion, but the responses were transient in two of the three participants.	IT/ICV	NO grade 3 or dose-limiting toxic effects	Recruiting
NCT04045847	31	Biological: CD147-CART	Recurrent Glioblastoma, CD147 Positive	CD147	I	NA	IT/ICV	NA	Unknown status
NCT05353530	18	Biological: Ex-Vivo expanded autologous IL-8 receptor (CXCR2) modified CD70 CAR (8 R-70CAR) T cells	Glioblastoma Multiforme	CD70	I	NA	IV	NA	Recruiting
NCT03941626	50	Biological: CAR-T/ TCR-T cells immunotherapy	Glioma	EGFRvIII	I	NA	IV	NA	Unknown status
NCT02209376	11	Biological: CART-EGFRvIII T cells	Patients With Residual or Reccurent EGFRvIII + Glioma	EGFRvIII	I	PFS:80day (28–159days, SD = 39)	IV	NA	Terminated
NCT03283631	2	Biological: EGFRvIII-CARs	Recurrent/ Recurrent Gliosarcoma	EGFRvIII	I	NA	ICV	NA	Terminated
NCT02844062	20	Biological: anti-EGFRvIII CAR T cells; Drug: cyclophosphamide; Drug: Fludarabine	Glioblastoma Multiforme	EGFRvIII	I	NA	IV	NA	Unknown status
NCT03726515	7	Biological: CART-EGFRvIII T cells; Biological: Pembrolizumab	Glioblastoma	EGFRvIII	I	median progression-free survival (5.2 months; 90 % confidence interval (CI),	IV	No dose-limiting toxicity	Completed

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Table 1 (*continued*)

Trials	No.	Arms	Characteristic	Target	Phase	Outcome	Delivery routes	Toxicity	State
						2.9–6.0 months) and median overall survival (11.8 months; 90 % CI, 9.2–14.2 months).			
NCT06186401	2	Biological: E-SYNC T Cells	Recurrent Glioblastoma/ MGMT-Unmethylated Glioblastoma	EGFRvIII	I	NA	IV	NA	Not yet recruiting
NCT01454596	18	Biological: Epidermal growth factor receptor (EGFRv)III Chimeric antigen receptor (CAR) transduced PBL; Drug: Aldesleukin; Drug: Fludarabine; Drug: Cyclophosphamide	Malignant Glioma	EGFRvIII	I/II	pfs:1.1m–2.7m, ORR:0 %	IV	2 events $\geq$ Grade 3	Completed
NCT05802693	22	Drug: Targeted Epidermal Growth Factor Receptor Variant III(EGFRvIII) autochimeric antigen receptor T cell injection	Recurrent Glioblastoma	EGFRvIII	I	NA	locoregional (LR)	NA	Not yet recruiting
NCT05063682	10	Biological: EGFRvIII-specific hinge-optimized CD3 ζ-stimulatory/41BB-co-stimulatory Chimeric Antigen Receptor autologous T-lymphocytes	Malignant Glioma	EGFRvIII	I	NA	ICV	NA	Unknown status
NCT02664363	3	Biological: EGFRvIII CAR T cells	Malignant Glioma	EGFRvIII	I	1 case have Serious Adverse Events	IV	NO Dose-limiting Toxicity (DLT)	Terminated
NCT03423992	100	Biological: chimeric antigen receptor T cells	Malignant Glioma	EGFRVIII, IL13R2, Her-2, EphA2, CD133, GD2	I	one patient reported SD and two patients reported PD, with overall survival ranging from 86 to 181 days	IV	In two patients, there was grade 2 cytokine release syndrome accompanied by pulmonary edema, which resolved completely with dexamethasone medication.	Unknown status
NCT02575261	0	Biological: CAR-T cell immunotherapy	EphA2 Positive Malignant Glioma	EphA2	I	NA	IV	NA	Withdrawn
NCT03252171	0	Biological: CAR-T cell immunotherapy	GD2 Positive Glioma	GD2	I	NA	IV	NA	Withdrawn
NCT04406610	0	Biological: GD2 CAR-T immunotherapy	Glioma of Brain	GD2	I	NA	IV	NA	Withdrawn
NCT04099797	34	Genetic: C7R-GD2. CART cells	Diffuse Intrinsic Pontine Glioma| High Grade Glioma	GD2	I	NA	IV	NA	Recruiting
NCT04196413	54	Drug: GD2 CAR T cells; Drug: Fludarabine; Drug: Cyclophosphamide	Glioma of Spinal Cord|Glioma of Brainstem	GD2	I	Three of four patients exhibited clinical and radiographic improvement.	IV	Toxicity was largely related to the location of the tumor and was reversible with intensive supportive care. On-target, off-tumor toxicity was not observed.	Recruiting
NCT05544526	12	Biological: GD2 CAR T cells	Diffuse Midline Glioma, H3 K27M-Mutant	GD2	I	NA	IT	NA	Recruiting

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Table 1 (*continued*)

Trials	No.	Arms	Characteristic	Target	Phase	Outcome	Delivery routes	Toxicity	State
NCT05298995	54	Biological: GD2-CART01 (iC9-GD2-CAR T-cells)	Brain Tumor, Pediatric/ Medulloblastoma, Childhood/ Embryonal Tumor/ High Grade Glioma	GD2	I	NA	IV	NA	Recruiting
NCT01109095	16	Biological: HER.CAR CMV-specific CTLs	Glioblastoma Multiforme (GBM)	HER-2	I	NA	IV	NA	Completed
NCT03500991	10	Biological: HER2-specific chimeric antigen receptor (CAR) T cell	Glioma	HER-2	I	no dose-limiting toxicity	ICV	No dose-limiting toxicity	Active, not recruiting
NCT02713984	0	Biological: Anti-HER2 CAR-T	Glioma	HER-2	I/II	NA	IV	NA	Withdrawn
NCT03389230	29	Biological: HER2 (EQ)BBζ/CD19t + T cells/Other: Laboratory Biomarker Analysis/ Procedure: Leukapheresis	Glioblastoma/ Recurrent/ Refractory Glioma	HER-2	I	NA	IT/ICV	NA	Active, not recruiting
NCT05768880	72	Biological: SC-CAR4BRAIN	Diffuse Intrinsic Pontine Glioma/ Diffuse Midline Glioma/Recurrent CNS Tumor, Adult/ Recurrent, CNS Tumor, Childhood/ Refractory Primary Malignant Central Nervous System Neoplasm	HER-2	I	NA	IT	NA	Recruiting
NCT05752877	12	Biological: Targeted IL-13 Rα2 UCAR-T cell injection; Biological: Targeted B7-H3 UCAR-T cell injection	Advanced Glioma/ Complication of Chimeric Antigen Receptor (CAR-T) Cell Therapy	IL13Ralpha2/ B7-H3	I	NA	IT/ICV	NA	Recruiting
NCT02208362	82	Biological: IL13Ralpha2-specific Hinge-optimized 4-1BB-co-stimulatory CAR/Truncated CD19-expressing Autologous TN/MEM Cells	Recurrent Glioblastoma/ Recurrent Malignant Glioma/ Recurrent WHO Grade II Glioma/ Recurrent WHO Grade III Glioma	IL13Ralpha2	I	50 % (29/58) of patients, with two partial responses, one complete response and a second complete response, median overall survival for all patients was 7.7 months and for arm 5 was 10.2 months.	IT/ICV	No dose-limiting toxicities, Probable treatment-related grade 3+ toxicities were one grade 3 encephalopathy and one grade 3 ataxia.	Active, not recruiting
NCT04661384	30	Biological: IL13Ralpha2-specific Hinge-optimized 41BB-co-stimulatory CAR Truncated CD19-expressing Autologous T-Lymphocytes	Glioblastoma	IL13Ralpha2	I	NA	ICV	NA	Recruiting
NCT04003649	60	Biological: IL13Ralpha2-specific Hinge-optimized 4-1BB-co-stimulatory CAR/Truncated CD19-expressing Autologous TN/MEM Cells	Recurrent/ Refractory Glioblastoma	IL13Ralpha2	I	NA	IV	NA	Recruiting
NCT06186401	20	Biological: E-SYNC T Cells; Drug: Cyclophosphamide (non-investigational); Drug: Fludarabine (non-investigational);	Glioblastoma	IL13Ralpha2	I	NA	IV	NA	Not yet recruiting

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Table 1 (*continued*)

Trials	No.	Arms	Characteristic	Target	Phase	Outcome	Delivery routes	Toxicity	State
		Procedure: Leukapheresis; Procedure: Surgical resection							
NCT04510051	18	Drug: Cyclophosphamide; Drug: Fludarabine; Biological: IL13Ralpha2-specific Hinge-optimized 41BB-co-stimulatory CAR Truncated CD19-expressing Autologous T-Lymphocytes	Malignant Brain Neoplasm/ Recurrent Malignant Brain Neoplasm/ Refractory Malignant Brain Neoplasm	IL13Ralpha2	I	Additionally, of the five patients evaluable for disease response, three experienced transient radiographic and/or clinical benefit not meeting protocol criteria for response.	ICV	No dose limiting toxicities (DLTs)	Recruiting
NCT05540873	18	Drug: YYB-103	Recurrent Malignant Glioma	IL13Ralpha2	I	NA	IV	NA	Recruiting
NCT04214392	36	Biological: Chlorotoxin (EQ)-CD28$^-$CD3zeta-CD19t-expressing CAR T-lymphocytes (via ICT delivery)	Recurrent/ Recurrent Malignant Glioma	MMP2	I	NA	ICV	NA	Recruiting
NCT02617134	20	Biological: anti-MUC1 CAR-T cells	Malignant Glioma of Brain	MUC1	I	NA	IV	NA	Unknown status
NCT05131763	3	Biological: NKG2D-based CAR T-cells	Glioblastoma	NKG2D	I	NA	IV	NA	Unknown status
NCT04717999	20	Biological: NKG2D CAR-T	Recurrent Glioblastoma	NKG2D	Not Applicable	NA	IT/ICV	NA	Unknown status
NCT04270461	0	Biological: NKG2D-based CAR T-cells	Glioblastoma	NKG2D	I	NA	IV	NA	Withdrawn

IV:intravenous. IT:intratumoral.ICV: intracerebroventricular.LR:locoregional.

antibody [146] in animal experiments can enhance the efficacy of EGFRvIII-CART, but this approach has failed in clinical trials [142]. In addition, Dong et al. [147] reported that, compared to sole EGFRvIII-CART cell therapy, the addition of an anti-mouse VEGF antibody (B20) enhances CAR T cell infiltration and distribution in GBM TME, suppresses tumor growth, and extends survival in GBM mouse models. Swan et al. [148] demonstrated that CART cells modified to express IL7 not only increased their abundance but also improved overall survival rates in tumor-bearing mice. Agliardi et al. [149] also reported that IL-12 enhances the cytotoxicity of CART cells and remodels the TME, resulting in increased $CD4^+$ T-cell infiltration, reduced Treg cell numbers, and extended survival in mice. But these methods have not yet been used in the clinic.

Several ongoing clinical trials are investigating the combination of radioimmunotherapy and CART therapy for recurrent intracranial neuroblastoma, ependymoma, and diffuse intrinsic pontine glioma (DIPG). Recently, clinical trials involving B7-H3 CART for treating recurrent glioblastoma have been initiated, as summarized in Table 1. However, most of these trials have yet to yield results. A report noted that a patient with recurrent glioblastoma, exhibiting overexpression of B7-H3, experienced rapid tumor regression lasting 50 days following intracerebral administration of B7-H3 CART via the ommaya device. Regrettably, the tumor recurred after six infusion cycles, leading to the patient's withdrawal from the study during the seventh cycle [157]. Nonetheless, B7-H3 CART treatment effectively inhibited tumor growth in mouse models bearing tumors [152,158].

3.2. B7-H3

B7-H3 (CD276), part of the B7 family, has been identified as a novel immunotherapy target for refractory blood cancers and solid tumors [150,151]. B7-H3 is overexpressed in gliomas, with expression levels rising as tumor grade increases [152,153]. Overexpression of B7-H3 promotes tumor cell proliferation and invasion [154]. Furthermore, B7-H3 contributes to a suppressive immune microenvironment and promotes tumor angiogenesis [153,155]. In a clinical trial, 38 patients with high-risk or recurrent primary or metastatic CNS malignancies, including neuroblastoma, retinoblastoma, medulloblastoma, and rhabdomyosarcoma—all exhibiting high B7-H3 expression—received compartmental radioimmunotherapy (cRIT) using radioactive anti-B7H3 murine monoclonal antibody ^{131}I-omburtamab by intraventricular administration. The results indicated that the combination therapy was safe and significantly prolonged survival in neuroblastoma patients, with a median progression-free survival (PFS) of 7.5 years [156].

3.3. IL13Rα2

IL13Rα2 is overexpressed in glioblastoma [159], with database analyses revealing that 40 % of glioblastoma patients exhibit mRNA overexpression of this receptor [160]. IL13Rα2 overexpression is associated with poor patient prognosis and contributes to tumor resistance to temozolomide [161]. IL13Rα2 can promote tumor growth and metastasis through the PI3K/Akt/mTOR pathway [162].

Previous clinical trials have shown that IL13Rα2-CART treatment is safe for 3 patients with recurrent glioblastoma, achieving locally controlled responses after intracranial administration. Antitumor effects were observed in 2 patients [163]. Brown et al. also reported a clinical trial of IL13Rα2-CART therapy for inoperable recurrent glioblastoma. Six patients received intracranial administration of modified glucocorticoid-resistant CART cells, and four patients showed signs of transient tumor shrinkage and/or tumor necrosis [164].

A recent clinical study indicated that IL13Rα2 CART therapy has yielded some significant results. This clinical trial involved 65 patients

with recurrent high-grade glioma, treated via various routes of locoregional T cell administration. The trial reported probable treatment-related grade 3+ toxicities, including one instance each of grade 3 encephalopathy and ataxia. 50 % of patients (29 out of 58) achieved stable or improved conditions. The median overall survival for all patients was 7.7 months, while for the arm 5 group (Tn/mem manufacturing and local/intraventricular administration), it was 10.2 months. The increase in inflammatory cytokines (including IFNγ, CXCL9, and CXCL10) in the central nervous system is related to the administration and biological activity of CAR-T cells [165]. In a case of multifocal glioblastoma, both intracranial and spinal lesions regressed following dual administration, with the regression maintained for 7.5 months and a survival period extending beyond 11 months [162].

Besides, recent researches suggested that altering the structure of IL13Rα2 CART cells, such as combining the CD28 transmembrane domain [166], humanized scFv domain [167] or 4-1BB domain [168], can improve the efficacy of CART therapy for glioblastoma. Additionally, clinical trials involving other third-generation CART cells are currently in the patient recruitment stage (seen in Table 1).

3.4. HER2

HER2, a member of the epidermal growth factor receptor family, is overexpressed in many types of tumors, including 80 % of glioblastomas [169]. Ahmed et al. demonstrated that HER2-CART cells exhibit potent anti-tumor activity against autologous HER2-positive tumors, including stem cells, leading to the sustained regression of GBM xenografts in severe combined immune deficiency mice [170]. In another study, CART cells targeting HER2 and IL13Rα2 showed reduced antigen escape, enhanced anti-tumor efficacy, and improved survival in animal models [171]. HER2-CART cells exhibit potent, antigen-specific cytotoxicity and cytokine secretion to patient-derived diffuse intrinsic pontine glioma (DIPG) cells. In addition, HER2 CAR-T cells showed significant regression in intracranial DIPG xenograft tumors [172].

A clinical study involving 17 recurrent glioblastoma patients reported good tolerance to intravenous HER2-CART cell therapy, with no dose-limiting toxic effects observed. Among the 16 evaluable patients, one experienced partial remission for over 9 months, seven had stable disease ranging from 8 weeks to 29 months, and eight showed disease progression. Three patients with stable disease remained progression-free for 24–29 months during follow-up. Across the cohort, the median overall survival post-first T-cell infusion was 11.1 months (95 % CI 4.1–27.2 months), and post-diagnosis, it was 24.5 months (95 % CI 17.2–34.6 months) [173]. In another clinical trial, HER2 CART cells were administered to children and young adults with recurrent or refractory CNS tumors, including diffuse midline glioma. Following local treatment via CNS catheter (into either the tumor cavity or the ventricular system), three patients exhibited no dose-limiting toxicity and showed clinical and laboratory evidence of localized central nervous system immune activation. However, the results of this trial have not yet been published [174]. There is also a clinical study using HER2 CAR-NK cells to treat patients with HER2-positive recurrent glioblastoma, which achieved certain clinical effects [175]. Additionally, several clinical trials are ongoing, and results have yet to be published (Table 1).

3.5. GD2

Disialoganglioside (GD2), a subtype of gangliosides, is highly expressed in nervous system tumors, including neuroblastoma, retinoblastoma, and glioma [176]. In 2015, the FDA approved the first anti-GD2 antibody for neuroblastoma treatment [177]. Most GD2-CART treatments target H3K27M-mutated diffuse midline gliomas (DMGs), including diffuse intrinsic pontine glioma (DIPG)—a highly malignant intracranial glioma predominantly affecting children and young adults. Mount et al. [178] reported that GD2-CART cells effectively killed patient-derived H3K27m-mutant glioma cells both in vitro and in vivo. However, it is noteworthy that peritumoral neuroinflammation during the acute phase of antitumor activity led to hydrocephalus, which proved lethal in some of the animals.

A study of GD2-CART treatment in patients with DMGs confirmed its safety. Following intravenous and intracranial administration of GD2-CART cells, three out of four patients showed clinical and radiographic improvements. No on-target, off-tumor toxicity was observed [179]. A recent clinical trial involved eight patients with newly diagnosed, high-GD2-expressing glioblastoma, treated either with post-operative intravenous administration alone or in combination with intracavitary administration of GD2-CART therapy. Of the eight evaluable patients, four experienced a partial response between 3 and 24 months, three showed disease progression between 6 and 23 months, and one remained stable at four months post-infusion. The median overall survival across the cohort was 10 months post-infusion. Both single and combined infusions of GD2-CART cells targeting GBM were safe, well-tolerated, and free of severe adverse events. However, the treatment did not extend overall survival [180]. In summary, clinical GD2-CART therapy has been extensive and has made some progress against neuroblastoma, but less breakthrough has been made against high-grade gliomas and most clinical trials are ongoing (Table 1).

3.6. Other targets

In recent years, numerous new targets have been explored for treating glioblastoma, with some progress noted in both clinical trials and basic research. Choi et al. [181] reported on a clinical trial utilizing CARv3-**TEAM-E** T cells to treat recurrent glioblastoma. CARv3-TEAM-E T cells, engineered to target both the tumor-specific antigen EGFRvIII and the wild-type EGFR protein, function through the secretion of a T-cell-engaging antibody molecule (TEAM). This therapy resulted in no grade 3 adverse events or dose-limiting toxic effects. Although tumor regression was dramatic and rapid, as shown radiographically, the responses were transient in two of the three participants. Weiss et al. [182] reported the construction of **NKG2D**-CART cells, which have been used to treat various solid tumors [183], and demonstrated that these cells exhibit synergistic efficacy with radiotherapy in treating glioblastoma and prolonging survival in mice. Jin et al. [184] discovered that while **CD70** was not detected in normal brain tissues, it was overexpressed in IDH wild-type primary low-grade gliomas, glioblastomas, and recurrent tumors. Overexpression of CD70 was also associated with poor survival. They developed CD70-CART cells and demonstrated that these cells can kill glioblastoma cells in vitro and in vivo, and also extend the survival of mice. Lin et al. [185] published results from a clinical trial using **EphA2**-CART for treating recurrent glioblastoma, where three patients received an intravenous infusion of CART cells. Two patients experienced grade 2 cytokine release syndrome, accompanied by pulmonary edema, which fully resolved following dexamethasone treatment. Among these three patients, one had stable disease (SD) and two had progressive disease (PD), with overall survival times ranging from 86 to 181 days. Yang et al. [186] confirmed that **CD147**, also known as the extracellular matrix metalloproteinase inducer, is overexpressed in glioblastoma. This overexpression significantly correlates with poor patient prognosis and serves as an independent prognostic marker for overall survival in GBM patients. Clinical trials targeting these markers have been conducted, but the results have not yet been published.

In summary, most innovative CART therapies are currently in phase 1 clinical trials or are still recruiting participants. Some results indicate that CART therapy is safe, whether administered intravenously or intracranially. Grade 3 adverse reactions occurred in a few cases but were all reversible following treatment. Regarding therapy effectiveness, most CART treatments have not significantly improved patient survival on a broad scale. However, the small sample sizes and varied administration methods preclude a precise evaluation of CART treatment effects. Nevertheless, it is encouraging that some patients still benefit from CART treatment.

4. The challenges and future of CART therapy

4.1. Blood-brain barrier impediments

Comprised of vascular endothelial cells, pericytes, basement membranes, and astrocytes, the blood-brain barrier effectively prevents most foreign substances, including various immune cells, from entering brain tissue. Over 98 % of molecules are unable to penetrate the central nervous system. Drugs that conform to the Lipinski's rules—molecular weight under 500 Da, no more than five hydrogen bond donors, fewer than ten hydrogen bond acceptors, and a log P not exceeding 5—are exceptions that can cross the blood-brain barrier [187]. The brain parenchyma lacks traditional lymphatic vessels, and the blood-brain barrier stringently regulates the entry of immune cells into the central nervous system. This limits the presence of leukocytes in the brain, preventing inflammation and maintaining internal stability [188]. The occurrence of brain tumors, whether gliomas or metastatic, disrupts the integrity of the blood-brain barrier, evident in the incomplete coverage by pericytes, dysfunctional astrocytes, and ruptured basement membranes [189,190], allowing some leukocytes to penetrate the blood-brain barrier. This also explains why there are fewer leukocytes in the immune microenvironment of low-grade gliomas, as low-grade gliomas have less damage to the blood-brain barrier. However, despite the varying degrees of blood-brain barrier disruption in GBM, achieving uniform and effective drug concentrations within the tumor tissue remains challenging, and T cells continue to face barriers to intracranial entry [191].

Recent clinical trials have demonstrated that intratumoral, intraventricular, and intrathecal injections of CART cells increase their intracranial content, potentially enhancing their antitumor effects. Brown et al. found that a combination of intravenous and intraventricular administration of IL13Rα2-CART yielded superior antitumor effects, indicating that intraventricular infusion is more effective than intravenous infusion alone [165]. Phase I clinical trials have confirmed the safety and effectiveness of this approach. However, local CART therapy is strongly discouraged for patients who have not undergone surgical treatment, as it may increase intracranial pressure and pose a risk to patient safety. Similarly, GD2-CART cell therapy for DMGs has shown that CART cell-induced brain stem inflammation can cause obstructive hydrocephalus, increased intracranial pressure, and hazardous tissue displacement [179]. In addition, local CART therapy can cause seizures, sterile inflammation, fever, and more serious complications such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) [192].

4.2. Heterogeneity and antigen loss in glioblastoma

Glioblastoma exhibits significant heterogeneity in both its tumor cells and the tumor immune microenvironment [193]. Moreover, changes in the surface antigens of tumor cells are observed in glioma patients, both within the same patient pre- and post-treatment. Traditionally, glioblastoma cells are classified into four types: neural, pro-neural (PN), classical (CL), and mesenchymal (MES), each expressing distinct molecular characteristics. The classical type exhibits 100 % amplification of chromosome 7 coupled with deletion of chromosome 10, genetically manifesting as amplification of the EGFR gene and deletion of the PTEN gene. In addition, the deletion rate of CDKN2A/CDKN2B gene is the highest in the classical type, and RB1 gene deletion is the lowest. In the mesenchymal type, deletions of the NF1 gene are more common than in other types, along with frequent deletions of the PTEN, CDKN2, and RB1 genes. The mesenchymal type has the poorest prognosis and is resistant to treatments. The pro-neural subtype of glioblastoma is primarily characterized by PDGFRA alterations and IDH1 point mutations. Neural subtypes typically express neuronal markers, including NEFL, GABRA1, SYT1, and SLC12A5. Heterogeneity among subtypes is evident, with only about 38 % exhibiting NF1 gene deletion and 95 % showing EGFR amplification, though only 29 % display high-level amplification [194]. Recent single-cell sequencing has confirmed that glioblastoma typically comprises these subtypes, rather than homogeneous populations [195]. Yu et al. utilized multi-site biopsy samples for single-cell sequencing and discovered significant heterogeneity in the initiation, progression, and interaction with the TME of glioblastoma [196]. Recently, Mathur et al. [197] conducted an analysis of intratumoral heterogeneity in glioblastoma using single-cell sequencing and 3D technology, finding that transcriptome and chromatin heterogeneity correlate with diverse cells of origin. Additionally, it has been observed that under certain conditions, PN can transform into MES. For instance, Xiong et al. recently demonstrated that macrophages with high GPNBB expression can induce PN to MES transformation in glioblastoma, as evidenced by single-cell sequencing [198]. Furthermore, as previously discussed, glioblastoma's low tumor mutational load (TML) and limited neoantigen presence diminish the cytotoxic effectiveness of T cells.

The phenomenon of antigen loss, observed in previous clinical trials, was evident in seven patients who received EGFRvIII-CART therapy followed by secondary surgery; five of these patients exhibited significantly reduced antigen expression, which impaired the cytotoxic activity of the CART cells [141]. Similarly, in a clinical trial of IL13Rα2-CART therapy, one of three patients demonstrated a decrease in overall IL13Rα2 expression within the tumor following T cell therapy [163]. More recently, Liu et al. observed a loss of GD2 antigen during GD2-CART therapy for glioblastoma [180].

Multi-target CAR-T therapy aims to prevent tumor recurrence due to target antigen loss and tumor heterogeneity. This therapeutic approach has been clinically tested in various hematologic and solid malignancies, demonstrating promising efficacy [199]. For glioma, multi-target CART therapy has been employed and tested in clinical trials. For instance, ongoing clinical trials such as those combining B7H3 CART with IL-7RαCART, and IL-13 Rα2/B7H3 CART, are implementing double-target treatments for glioblastoma [Table 1]. However, the efficacy of this approach remains to be validated by clinical trial outcomes.

4.3. Suppression of CART therapy by the tumor microenvironment

Previously, we discussed the factors contributing to the suppressive tumor immune microenvironment in glioblastoma. These include the overexpression of various inhibitory factors, a tumor-associated macrophages (TAMs)-dominated immune environment, and a scarcity of T lymphocytes, predominantly Treg cells. When CART cells infiltrate the tumor, they encounter inhibitory cells and chemokines or cytokines that can induce T cell dysfunction. However, research is still limited regarding which specific mechanism predominantly influences this effect.

Larson et al. demonstrated that deletion of IFNγR1 in glioblastoma cells diminished CAR-T cell activity, IFNγR signaling in glioblastoma was required for sufficient adhesion of CAR T cells to mediate productive cytotoxicity [200]. To mitigate the adverse effects of the tumor microenvironment on T cells in glioblastoma, cytokine administration has been used to enhance CART cell activity. Zhu et al. [201] confirmed that stimulating IFN-γ release with oncolytic herpes simplex virus-1 (oHSV-1) enhances the anti-tumor effect of CD70-CART on intracranial tumors in mice. Additionally, incorporating cytokines or chemokine receptor structures into CART cells (4th generation CART) can enhance their anti-tumor efficacy. One of the most commonly targeted cytokines is IL-15. Zannikou et al. [202] demonstrated that IL15-modified IL13Rα2-CART cells target GBM tumor cells and myeloid-derived suppressor cells (MDSCs), exerting potent anti-tumor effects. In addition, literature suggests that IL15-modified GD-2 [203] and IL13Rα2-CART [204] can improve T cell proliferation, persistence, and cytokine production, resulting in better anti-tumor effects.

What's more, receptor-modified CAR-T cells can improve the

inhibitory TME to increase their invasion in solid tumors. Liu et al. [205] confirmed that CXCR2-modified CAR-T cells can significantly accelerate in vivo trafficking and tumor-specific accumulation due to high expressions of CXCR2 ligands in hepatocellular cancer. Sun et al. [206] found that CXCR4-modified CAR-T cells targeting CLDN18.2 could traffic more into tumor sites and suppress MDSC migration in pancreatic cancer. Trinh et al. [207] also reported that CX3CR1-overexpressed CAR-T cells targeting NKG2D increased their infiltration in hepatocellular cancer. The application of this option in glioblastoma requires more basic and clinical experimental support.

In addition, the hypoxic microenvironment in glioblastoma can lead to the failure of immunotherapy. Recent studies [208] have confirmed that elevated PHGDH expression in glioblastoma endothelial cells promotes tumor hypoxia and angiogenesis via metabolic pathways, contributing to tumor resistance to CAR-T therapy. Previous studies have demonstrated that anti-angiogenic therapy can enhance the efficacy of CART treatment [209].

Of course, CART therapy faces several challenges, including maintaining T cell activity, ensuring infiltration in solid tumors, ensuring safety post-CAR modification, identifying new targets, managing tumor resistance and recurrence, and reducing the costs associated with CART therapy [210]. All these issues require further research (Fig. 4).

5. Conclusion

Glioblastoma is a highly malignant intracranial tumor and the current treatment progress is very slow. Previously, targeted therapy and immune checkpoint blockade have shown no significant benefit, which is closely related to the complex and suppressive immune microenvironment of glioblastoma. Fortunately, recent CART therapy has shown a reliable safety in Phase I clinical trials and some cases have achieved survival benefits, which has become a hope for future treatment of glioblastoma. Recent research indicates that CAR-T therapy has the potential to improve the TME. Alizadeh et al. [211] illustrated that IL13Rα2-CART cells could activate patient-derived endogenous T cells and monocytes/macrophages via IFNγ signaling, inducing tumor-specific T-cell responses in GBM patients. CAR-T cell therapy could potentially modulate the TME, creating an environment that allows for the initiation of endogenous antitumor immunity. However, similar to other immunotherapies, CART therapy also faces with the tumor immunosuppressive microenvironment, the strong heterogeneity of tumor cells, and other problems. We still have a long way to go.

Availability of data and material

The data and materials used in the study are available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Jie Lu: Writing – original draft, Software, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Wen Huo:** Writing – review & editing, Software. **Yingze Ma:** Writing – review & editing. **Xin Wang:** Visualization, Validation, Supervision, Funding acquisition. **Jinming Yu:** Writing – review & editing, Visualization, Validation, Supervision, Project administration.

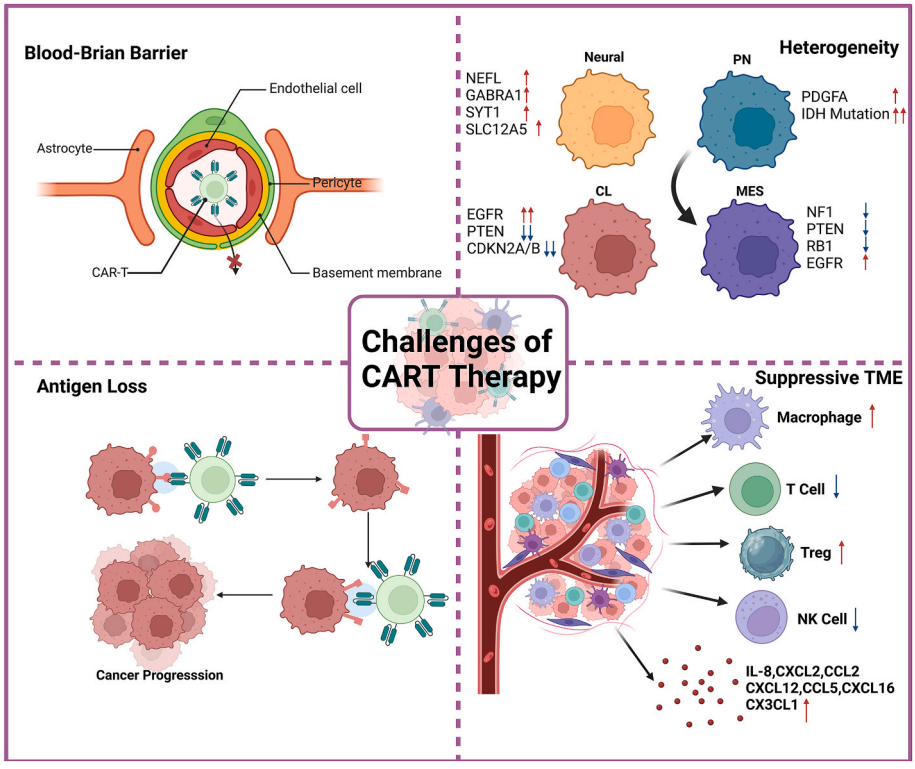

Fig. 4. Challenges of CART therapy in glioblastoma. Including blood-brain barrier mpediments, tumor heterogeneity, antigenic loss during therapy, and a suppressive TME.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

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