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Review Article

Dual Challenges and Innovative Strategies in Chimeric Antigen Receptor T-cell Therapy for Glioblastoma

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Abstract

Glioblastoma (GBM) is the most prevalent and aggressive form of primary brain malignancy in adults. Despite continuous advancements in standard treatment modalities, the prognosis for patients remains extremely poor, with a median survival of less than two years. In recent years, chimeric antigen receptor T-cell (CAR-T) therapy has achieved revolutionary success in hematologic malignancies, marking a significant breakthrough in the field of immunotherapy. However, the successful application of CAR-T therapy to GBM still faces dual challenges: antigen heterogeneity and the immunosuppressive tumor microenvironment. This review systematically summarizes these challenges encountered in CAR-T therapy for GBM and the innovative strategies currently under development to address these challenges, providing insights for the future clinical translation of CAR-T therapy in GBM.

Introduction

Glioblastoma (GBM) constitutes the most frequent malignant primary brain tumor in adults and accounts for nearly 15% of all intracranial neoplasms. Its incidence increases with age and is slightly higher in males than in females.[1] Despite continuous advancements in diagnostic and therapeutic techniques, the prognosis for GBM remains extremely poor, with a median survival of less than two years (approximately 15–16 months) and a five-year survival rate below 10%.[2] Furthermore, the recurrence rate is close to 100%, and median survival after recurrence is typically less than one year.[3] Approximately 90% of GBMs are immunogenic cell death (IDH)-wildtype, which are more aggressive and associated with worse outcomes, whereas patients with IDH-mutant GBM generally exhibit longer survival.[4–6] Despite the use of extensive surgical resection, radiotherapy combined with temozolomide (TMZ), and adjuvant TMZ treatment, the current standard therapy offers only temporary disease control, and multiple limitations render treatment outcomes unpredictable.

In recent years, breakthroughs in innovative immunotherapies have led to significant advances in cancer treatment. These strategies aim to modulate the immune system to target tumors, overcoming tumor-induced immune resistance and achieving durable clinical benefits.[7] Chimeric antigen receptor T-cell (CAR-T) therapy, as a major branch of immunotherapy, is based on genetically engineering a patient's own T cells to express a chimeric antigen receptor (CAR) that enables specific recognition and targeting of tumor cell surface antigens. This technology has produced revolutionary breakthroughs in hematologic malignancies, particularly in B-cell cancers such as acute lymphoblastic leukemia and lymphoma, where it has demonstrated remarkable efficacy, with a subset of patients achieving sustained remission or even being effectively cured.[8,9] CAR-T therapies directed against cluster of differentiation 19 (CD19) have received Food and Drug Administration ap-

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Table 1. Summary of clinical trials about chimeric antigen receptor T cell (CAR-T) cells in glioblastom

Target antigen	Trial	Phase	No. of Patients	Therapeutic effect	Adverse reactions	Reasons for failure
Epidermal growth factor receptor vII (EGFRvIII)	NCT02209376	I	10	Multiple cases: transient stable disease (SD); 1 case: significant long-term survival[15]	Symptoms like influenza, no severe cytokine release syndrome (CRS) or off-target toxicity[16]	Antigen loss, lack of pretreatment; insufficient amplification and persistence of CAR-T[17]
Interleukin13Rα2 (IL13Rα2)	NCT02208362	I	65	50% patients: SD or partial remission; overall survival (OS): 7.7 months overall[18]	Headaches, Fatigue, Myalgia, Encephalopathy, Ataxia, Mild cerebral edema[19]	Limited treatment scope; antigen heterogeneity and immune escape
IL-13Rα2	NCT00730613	I	3	1 case: partial remission[20]	Not mentioned	Expression differences of IL 13Rα2; microenvironment inhibition
EphA2	NCT03423992	I	3	1 case: SD; 2 cases: progressive disease (PD); OS:86–181 days[21]	Cytokine release syndrome accompanied by pulmonary edema[21]	The persistence of CAR-T is insufficient[21]
Human epidermal growth factor receptor 2 (HER2)	NCT01109095	I	17	1 case: partial remission; 7 cases SD. OS: 24.5 months[22]	Epileptic seizures, Headaches[22]	Antigen heterogeneity; insufficient amplification of CAR-T[22]
Disialoganglioside 2 (GD2)	NCT03170141	I	8	4 cases: PD; 3 cases: PD; OS: 10 months[23]	Epileptic seizures, Headaches[23]	Antigen loss, immunosuppressive microenvironment

proval, with early clinical trials reporting complete remission rates as high as 90%, highlighting their significant clinical potential.[10] Compared to hematologic malignancies, however, the application of CAR-T therapy in solid tumors faces unique challenges. The immunosuppressive tumor microenvironment (TME) can lead to inadequate T-cell infiltration and functional exhaustion. Additionally, tumor heterogeneity often results in antigen escape when targeting a single antigen, while the scarcity of tumor-specific antigens in solid tumors further limits therapeutic efficacy.[11–14]

Although recent innovations, such as combination therapies and engineered modifications, have partially improved T-cell function, and an increasing number of clinical trials have evaluated the efficacy of CAR-T therapy in GBM (Table 1),[15–23] CAR-T treatment for GBM still falls far short of the breakthroughs achieved in hematologic malignancies.[24,25]

This review aims to systematically examine the major obstacles limiting the efficacy of CAR-T cell therapy in GBM, with a particular focus on the tumor's unique microenvironmental features. We integrate the two core challenges, antigen heterogeneity and the immunosuppressive tumor barrier, within a triadic evaluative framework of "challenge identification", "mechanistic elucidation", and "strategy matching". Unlike previous reviews that primarily list isolated strategies, our manuscript emphasizes the alignment between innovative technological approaches and specific pathological mechanisms. We cover topics ranging from logic-gated and switchable modular CAR designs to the application of organoid and single-cell technologies in CAR-T development and evaluation. By bridging fundamental mechanistic insights with translational assessment, this review highlights the evolving therapeutic paradigm in GBM CAR-T therapy—from "single-target killing" toward "multidimensional modulation of the immune ecosystem". Our goal is to provide a comprehensive framework that offers both mechanistic depth and practical guidance to advance preclinical research and the clinical translation of CAR-T therapies in GBM.

This review is based on a comprehensive synthesis of current literature related to CAR-T cell therapy in GBM. Relevant studies were identified through searches of major scientific databases including PubMed, Web of Science, and Google Scholar. We focused primarily on peer-reviewed original research articles and high-quality review papers published in reputable journals over approximately the last 10 years to capture recent advances. Studies were selected based on their relevance to key topics such as antigenic heterogeneity, TME immunosuppression, clinical challenges, and emerging therapeutic strategies. Both preclinical and clinical studies providing mechanistic insights or reporting therapeutic outcomes were included to ensure a balanced and thorough overview. Studies lacking sufficient experimental detail or clinical relevance were generally excluded.

Dual core challenges in CAR-T therapy for GBM

Unlike hematologic cancers, solid tumors, especially GBM, exhibit significant antigenic diversity and a profoundly immunosuppressive TME, both of which hinder the effectiveness of CAR-T therapy.

Antigenic heterogeneity

The spatiotemporal heterogeneity of antigen expression in GBM significantly undermines the efficacy of CAR-T cell therapy. Core challenges include localized loss and dynamic evolution of tumor

cell surface antigens, as well as antigen escape by glioma stem cells (GSCs). For example, epidermal growth factor receptor variant III (EGFRvIII) is a deletion mutant of EGFR that is specifically expressed on tumor cells but absent in normal tissues, making it a tumor-specific antigen.[26–28] Early studies report that approximately 30–50% of GBM cases harbor EGFRvIII mutations,[29] making it one of the most frequently observed tumor-specific antigens in GBM. However, EGFRvIII is typically expressed only in a subset of tumor subclones, and its expression can be dynamically lost under therapeutic pressures such as radiotherapy.[30,31] The phenomenon of antigen loss is particularly prominent in CAR-T therapies targeting EGFRvIII, where residual antigen-negative subclones can undergo clonal expansion, resulting in immune escape.

GSCs, as key drivers of tumor recurrence, exhibit antigenic profiles that differ significantly from those of differentiated tumor cells. GSCs display distinct antigenic profiles compared to differentiated GBM cells, including higher expression of stemness markers such as CD133, aldehyde dehydrogenase 1, and transient receptor potential melastatin 7.[32] GSCs can be further divided into molecular subtypes. mesenchymal-type GSCs express serglycin (SRGN), supporting proliferation and stemness through the SRGN–NFκB pathway, while classical subtype/proneural subtype (CL/PN)-type GSCs express mesenchyme homeobox 2 (MEOX2) and depend on the MEOX2–NOTCH axis. Both SRGN and MEOX2 have been implicated in resistance to macrophage-mediated phagocytosis.[33] FOS like 1 is a key transcription factor in mesenchymal-type GBM. It promotes GSC stemness, self-renewal, and invasiveness, and enhances the expression of immune-evasive molecules such as CD44 and tenascin C through chromatin remodeling.[34] In addition, GSCs can suppress antigen presentation through epigenetic mechanisms (e.g., DNA methylation and histone modification), further reducing CAR-T cell efficacy.[35] Therapeutic stress, such as radiation, may also reshape GSC antigen profiles, leading to loss of targets like EGFRvIII and upregulation of stemness markers such as CD133.[36–38]

Immunosuppressive barriers of the TME

The immunosuppressive barriers in GBM are first manifested through unique physical obstacles. Both the blood–brain barrier (BBB) and the blood–tumor barrier (BTB) collectively limit the infiltration of immune cells and therapeutic agents. The BBB, composed of tightly connected endothelial cells and basement membrane structures, prevents CAR-T cells from entering the brain parenchyma. Studies show that only a small proportion of CAR-T cells can penetrate the BBB and reach the tumor core, thereby limiting therapeutic efficacy.[39,40] The BTB, a pathological barrier unique to GBM, is formed by tumor-associated endothelial cells, basement membrane components, pericytes, and other elements of the TME. While structurally similar to the BBB, the BTB possesses tumor-specific molecular characteristics that further reduce drug permeability.[41,42] Moreover, the BTB, together with immunosuppressive cells such as tumor-associated macrophages (TAMs), impairs CAR-T cell activity. For example, M2-polarized TAMs can suppress CAR-T cell function through the secretion of anti-inflammatory factors.[43,44] Although the BBB may be disrupted within the tumor core in GBM, the BTB at the invasive tumor margins often remains relatively intact, continuing to restrict the delivery of therapeutic agents.[42]

The complex immunosuppressive network within the GBM TME forms a biological barrier that inhibits CAR-T cell function through intercellular interactions and molecular signaling pathways. For example, glioblastoma-associated macrophages/microglia (GAMs) secrete immunosuppressive cytokines such as interleukin (IL)-10 and TGF-β, directly suppressing T-cell activation and proliferation.[44–46] Additionally, M2-polarized GAMs promote the accumulation of programmed cell death protein 1 (PD-L1^{+}) M2-GAMs via activation of the *ALOX5* gene and its metabolite 5-HETE, leading to high PD-L1 expression. This binds to programmed death-ligand 1 (PD-1) on T cells, inducing T-cell exhaustion.[47] Regulatory T cells (Tregs) are significantly expanded in GBM and further suppress antitumor immune responses through cytotoxic T-lymphocyte antigen 4 (CTLA-4) and adenosine signaling pathways.[48–52] Metabolic byproducts such as lactate and adenosine contribute to the immunosuppressive TME in GBM by modulating TAM polarization, Treg recruitment, and CAR-T cell dysfunction (Fig. 1).[53–58] As summarized in previous studies, these effects involve key mediators such as hypoxia-inducible factor 1-alpha, vascular endothelial growth factor (VEGF), CD39/CD73, and C-C chemokine receptor type 8.

Indirect obstacles to CAR-T therapy arising from dual challenges

The antigenic heterogeneity and immunosuppressive TME in GBM not only directly reduce the efficacy of CAR-T therapy but also indirectly create clinical challenges, such as increasing the risk of off-target toxicity and hindering the identification of reliable biomarkers to effectively monitor treatment responses.

Off-target toxicity of CAR-T therapy in GBM

GBM exhibits high antigenic heterogeneity, making single-target CAR-T cells prone to missing tumor subpopulations that do not express the targeted antigen. This promotes "antigen escape" and reduces therapeutic efficacy. To cover a broader range of tumor cells, researchers often select antigens that are widely expressed but not entirely specific, which may lead to off-target toxicity by damaging normal tissues expressing similar antigens. For instance, IL13Rα2, a potential GBM target, is expressed in more than 75% of cases but may also be present at low levels in normal brain tissues such as neurons and microglia, significantly increasing off-target risk.[59] Similarly, EGFRvIII-targeted CAR-T cell development requires systematic modeling to assess potential cross-reactivity with wild-type EGFR to ensure safety.[60] Insufficient tumor specificity of target antigens, combined with intratumoral heterogeneity, complicates clinical safety by causing unexpected damage to healthy cells during CAR-T therapy. To address this, multi-omics approaches such as single-cell sequencing and *ex vivo* cross-reactivity assays are essential for screening highly specific targets.[60,61]

Meanwhile, the TME can amplify off-target toxicity through multiple mechanisms. For example, hypoxia and metabolic stress within the TME impair CAR-T cell persistence, promoting the release of inflammatory cytokines such as IL-6 and interferon-γ (IFN-γ), which may trigger cytokine release syndrome (CRS). The high expression of immunosuppressive markers such as PD-1 and VEGF in the TME may necessitate combining CAR-T therapy with immune checkpoint inhibitors like anti-PD-1 antibodies, although nonspecific T-cell activation might broaden off-target effects.[60]

Lack of biomarkers for predicting CAR-T therapy response in GBM

The biological complexity of GBM severely limits the ability to predict CAR-T therapy efficacy. As the most aggressive malignant tumor of the central nervous system, GBM is characterized by a highly heterogeneous TME, making it difficult for single-target

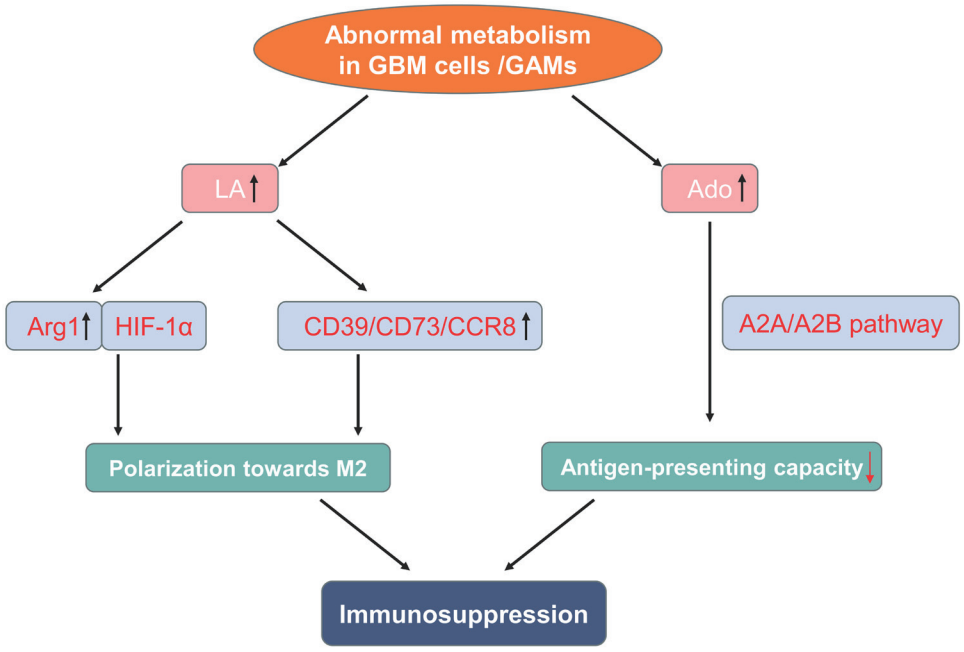

Fig. 1. Metabolic regulation of the immunosuppressive TME in GBM. ↑, accumulation of LA and Ado/upregulation expression of Arg1, CD39, CD73 and CCR8; ↓, diminished antigen-presenting capacity. A2A/A2B, adenosine A2A/A2B; Arg1, arginase 1; CCR8, C-C motif chemokine receptor 8; CD, cluster of differentiation; GAM, glioblastoma-associated macrophage; GBM, glioblastoma; HIF-1α, hypoxia-inducible factor 1-alpha; LA, lactate; TME, tumor microenvironment.

CAR-T therapies to address all tumor subpopulations and resulting in a lack of effective biomarkers reflecting therapeutic response.

Although targets such as IL-13Rα2 and EGFRvIII have been extensively studied, antigen expression heterogeneity and insufficient tumor specificity continue to hinder the identification of stable and reliable biomarkers. Moreover, real-time monitoring methods remain technically lacking.[62,63]

The presence of the BBB and BTB further limits the evaluation of systemically administered CAR-T cells.[64] While local injections can improve infiltration efficiency, they lack standardized assessment metrics.[65–67]

Even when appropriate biomarkers are identified, clinical translation faces significant multidimensional technical challenges. Patient stratification difficulties have prevented many clinical trials from achieving precise enrollment. While combination strategies (e.g., co-administration of PD-1 inhibitors) can reverse T-cell exhaustion, they may interfere with biomarker signals associated with monotherapy.[68,69] Furthermore, the diversity of resistance mechanisms makes it difficult for a single biomarker to comprehensively predict treatment failure.[70–72]

Innovative strategies

Antigenic diversity and the highly suppressive TME severely restrict the efficacy of CAR-T cell therapy in solid tumors, particularly GBM. To overcome these obstacles and enhance the antitumor activity of CAR-T cells, various innovative strategies have been explored. These include multi-target synergistic recognition technologies, logic-gated chimeric antigen receptors, switchable CAR systems, broad-spectrum antigen recognition using natural killer group 2, member D (NKG2D)-based CAR-T cells, combined targeting of tumor vasculature-associated antigens, integration of immune checkpoint inhibitors or cytokine engineering, optimization of delivery routes, and the combination of CAR-T therapy with radiotherapy or chemotherapy (Table 2).

Multi-target synergistic recognition technologies

Multi-target synergistic recognition technologies, through tandem or parallel CAR designs, have significantly improved the ability of CAR-T therapies to address GBM heterogeneity and antigen escape. Their core advantages lie in dual-target synergistic cytotoxicity and enhanced specificity through logic-gated designs.

Tandem CARs achieve simultaneous recognition of two tumor-associated antigens within a single-chain structure. For example, a tandem CAR targeting CD44 and CD133 can effectively cover different tumor cell subpopulations within GBM, reducing treatment failure caused by the loss of a single antigen and lowering off-target toxicity. Preclinical studies have demonstrated that CD44/CD133 dual-target IL-7 receptor α-chain-armored CAR-T cells, co-expressing the IL-7 receptor α-chain, enhance T cell survival and proliferation, significantly inhibiting tumor growth and prolonging survival in xenograft models.[73] Similarly, EGFRvIII/IL-13Rα2 tandem CAR-T cells have shown synergistic cytotoxic effects in heterogeneous GBM models, outperforming single-target CAR-T therapies and overcoming the spatial heterogeneity of antigen expression.[74] Additionally, chlorotoxin-CARs, designed using a chlorotoxin peptide segment that specifically binds matrix metalloproteinase-2, further improve durability and antitumor efficacy when combined in tandem constructs with EGFRvIII or CD276 targets in xenograft models.[75]

Logic-gated CARs are advanced CAR-T technologies designed using synthetic biology principles. By introducing control mechanisms similar to computer "logic gates", they regulate T-cell activity through specific antigen input combinations, enabling CAR-T cells to recognize and attack target cells with greater precision

Table 2. Innovative strategies to overcome challenges in chimeric antigen receptor T cell (CAR-T) therapy for glioblastoma

Challenge	Innovative strategy	Experimental stage	Significance
Antigenic heterogeneity	Multi-target cooperative recognition technology	Phase I/II	Target multiple antigens to reduce immune escape
	logic-gated chimeric antigen receptor	Phase I	Precisely identify and attack cells; reduce the damage to normal tissues
	Switchable chimeric antigen receptor (CAR) systems	Phase I	Switching targeted antigens to reduce immune escape
	Natural killer group 2 member D (NKG2D) CAR-T	Phase I	Target multiple antigens to reduce immune escape; strengthen the immune killing system
	Combination of targeting tumor vascular related antigen	Preclinical trial	Non-specific recognition of antigens; bystander killing mechanism
Immunosuppressive tumor microenvironment	Combination of immune checkpoint inhibitors	Phase I	Reduce CAR-T cell depletion
	Cytokine engineering of CAR-T	Preclinical trial	Extend CAR-T cell viability and enhance its expansion capacity; Promote immune response
	Combination of temozolomide (TMZ) chemotherapy	Phase I	Increase tumor stress antigen; activate the immune response; remove immunosuppressive cells
	Combination of local radiotherapy	Phase I	Release damage-associated molecular patterns (DAMPs) to provide costimulatory signals for CAR-T; regulate cytokines and reduce CAR-T cell depletion; increase tumor antigen expression
Physical barriers	Change of the route of administration	Phase I	Avoid blood–brain barrie/blood–tumor barrier (BBB/BTB) barriers; reduce overall side effects

while minimizing off-target effects on normal tissues.[76]

The multi-antigen recognition strategies described above generally reflect the OR logic principle within logic-gated systems. Beyond OR gates, additional designs include AND, NOT, IF-THEN, and IF-BETTER logics (Fig. 2). The AND-gate CAR system separates activation and co-stimulatory signals into two distinct receptors, each recognizing a different antigen, and only activates the CAR-T cells when both (or more) antigens are simultaneously detected. In contrast, the NOT-gate CAR system inhibits activation if a specific antigen is recognized. Studies have shown that CAR-T cells combining an inhibitory domain and human epidermal growth factor receptor 2 (HER2) targeting successfully resisted lung cancer cells,[77] illustrating the effectiveness of combining AND and NOT logic principles, with potential applicability to solid tumors. In GBM models, IF-THEN logic has shown research significance. For instance, synNotch CAR systems recognizing EGFRvIII first induce downstream CAR expression (targeting antigens such as IL13Rα2 or CD276) in tumor cells expressing EphA2 or IL13Rα2, thereby reducing T-cell exhaustion and extending T-cell persistence.[78] As for IF-BETTER logic, the idea is that the presence of one antigen enhances CAR-T sensitivity toward another antigen, potentially offering unique advantages for tumors like GBM, where highly tumor-specific antigens are rare.

Beyond conventional logic-gated CARs, efforts have also focused on engineering intracellular signaling molecules to create reversible and dynamically regulated signaling networks. For example, the LINK CAR platform leverages the synergy between linker for activation of T cells and SH2 domain-containing leukocyte protein of 76 kDa to construct a fast-responding AND-gated system, enhancing antitumor activity while minimizing off-target risks.[79]

Collectively, these advances demonstrate that logic-gated CARs hold significant promise for overcoming challenges of antigen heterogeneity and off-target toxicity in GBM therapy. Future directions may focus on improving signal integration efficiency and reducing system complexity to facilitate their clinical translation into solid tumors.

Switchable CAR systems to address dynamic antigen changes

Dynamic changes in antigen expression represent a fundamental limitation to the sustained effectiveness of CAR-T cell therapy in GBM. Tumor cells can escape single-antigen targeting through immune editing or phenotypic plasticity. To address this challenge, researchers have proposed the concept of switchable CAR systems, which dynamically adjust antigen recognition modules or target combinations to enhance tumor coverage.

In traditional CAR designs, the antigen recognition domain and intracellular signaling domains are fused into a single unit, limiting each CAR-T cell to recognizing only a specific target. In contrast, switchable CAR systems use a modular design that separates the antigen recognition domain from the CAR's signaling domain, allowing flexible redirection without needing to re-engineer the CAR-T cells themselves (Fig. 3). This approach enables the development of universal CARs (UniCARs).[80] UniCAR-T cells achieve flexible targeting through soluble Targeting Modules that act as adaptors to direct T cell activity against different tumor antigens. In preclinical studies, UniCAR-T cells were effectively activated by biotinylated trastuzumab in HER2-positive solid tumor models, enabling penetration of the extracellular matrix and killing of hidden tumor cells in 3D spheroid models.[81] A similar strategy

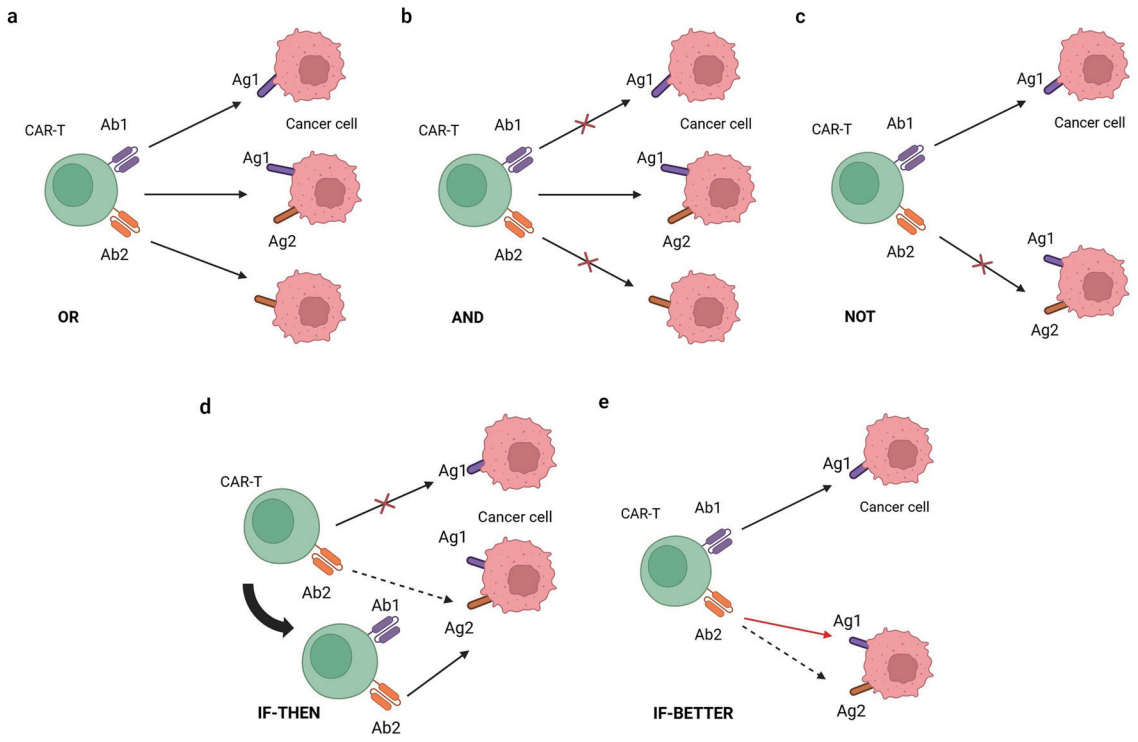

Fig. 2. Schematic diagram of logic-gated CARs. (a) OR Logic: CAR-T cells engineered with tandem antibodies Ab1 and Ab2 can recognize tumor cells expressing either Ag1, Ag2, or both Ag1 and Ag2. (b) AND Logic: CAR-T cells are only activated by tumor cells co-expressing both Ag1 and Ag2; cells expressing only Ag1 or Ag2 individually do not activate CAR-T cells. (c) NOT Logic: CAR-T cells are not activated when they encounter inhibitory antigens, thereby protecting normal cells that express such inhibitory antigens. (d) IF-THEN Logic: When the antibody Ab2 on the CAR-T surface recognizes Ag2 on tumor cells, it induces the transient expression of Ab1, enabling CAR-T cells to subsequently recognize and kill tumor cells expressing Ag1. (e) IF-BETTER Logic: CAR-T cells can recognize and kill tumor cells expressing Ag1 via Ab1; however, if tumor cells also express Ag2, the sensitivity of CAR-T cells toward Ag1-positive tumor cells is further enhanced. The figure was created using BioRender. Ab1/2; antibody 1/2; Ag1, antigen 1/2; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell.

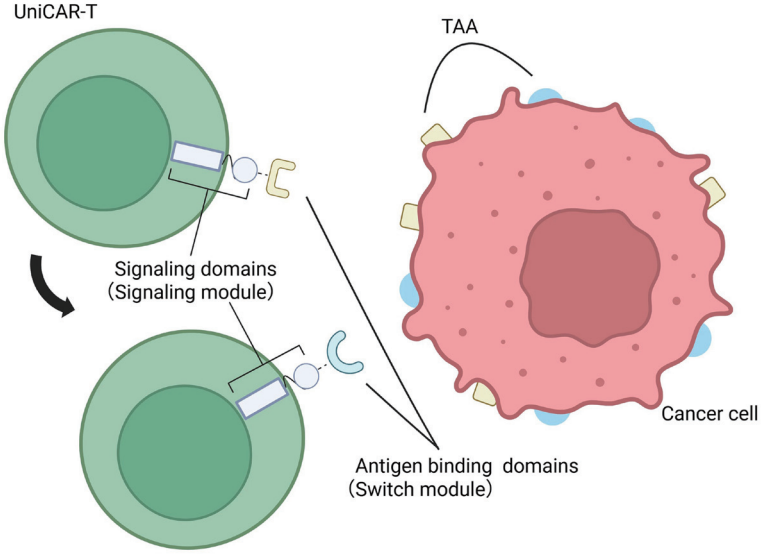

Fig. 3. Schematic diagram of UniCAR-T structure and mechanism. The UniCAR system divides the antigen recognition domain into two components: the Switch module and the Signaling module. The Switch module specifically recognizes tumor cell surface antigens and can rapidly switch between different antigen-binding domains. The Signaling module contains a domain for recognizing the Switch module, a hinge region, a transmembrane domain, and an intracellular signaling domain, which collectively provide activation signals to T cells. The figure was created using BioRender. TAA, tumor-associated antigen; UniCAR, universal chimeric antigen receptor; UniCAR-T, universal chimeric antigen receptor T-cell.

may be applied to target overexpressed antigens such as EGFR in GBM.[82] Regarding safety, CD123-targeted UniCAR-T cells have already demonstrated controllability in a phase I clinical trial (NCT04230265), laying an important safety foundation for potential application in GBM.[83,84]

By shifting from "static targeting" to "dynamic adaptation" through modular design, UniCAR technology offers a novel approach to overcoming GBM's heterogeneity, aggressiveness, and immunosuppressive microenvironment. Its synergistic potential with existing therapies and its favorable safety profile make it a highly promising strategy in the GBM treatment field, although further research is needed to optimize target selection, fine-tune regulatory mechanisms, and develop effective combination therapies.

Broad-spectrum antigen recognition by NKG2D CAR-T cells

As a key activating receptor within the innate immune system, NKG2D is highly conserved and recognizes numerous ligands upregulated under cellular stress, including MHC class I polypeptide-related sequence A (MICA), MHC class I chain-related protein B (MICB), and UL16-binding protein.[85,86] NKG2D-based CAR-T cells, by engaging multiple tumor-associated antigens simultaneously, help mitigate antigen escape and hold considerable therapeutic potential for solid tumors like GBM.

Preclinical studies have demonstrated that NKG2D CAR-T cells can eliminate over 90% of tumor cells in glioblastoma organoid (GBO) models derived from patients with other solid tumors,[87] with no significant toxicity observed toward normal neural stem cells.[88] Further research has revealed that NKG2D ligands such as MICA/B and UL16-binding proteins are expressed in the majority of GBM cases, and NKG2D CAR-T cells have recently been shown to effectively treat GBM.[89] Although the expression level of NKG2D ligands in GBM is not as high as in some other cancers, and in certain cases GBM tumor cells may even downregulate these antigens to evade immune surveillance,[90] studies have shown that small molecules such as valproic acid can upregulate NKG2D ligand expression, thereby enhancing NK cell-mediated cytotoxicity against tumor cells.[91] Beyond their broad-spectrum antigen recognition capabilities, NKG2D CAR-T cells, derived from innate immune receptors, can also synergistically activate immune responses by mimicking NK cell cytotoxic mechanisms. This includes not only direct activation of T cell-mediated cytotoxicity but also recruitment of NK cells to reshape the tumor immune microenvironment and enhance antitumor immune responses.[92,93]

Synergistic effects of targeting tumor vasculature-associated antigens

The aberrant vascular network in GBM serves not only as a nutrient supply for tumor growth but also as a critical component of its immunosuppressive microenvironment. Designing CAR-T cells to target tumor vasculature-associated antigens enhances antitumor efficacy by exploiting the broader, nonspecific distribution of these antigens. In this regard, several research teams have explored fibroblast activation protein (FAP) as a promising target. FAP-targeted CAR-T therapy enhances treatment efficacy through the following dual mechanisms:

1. Anti-angiogenesis and direct cytotoxicity: FAP is specifically overexpressed in perivascular cells and cancer-associated fibroblasts within GBM. FAP-specific CAR-T cells can disrupt tumor vasculature, inhibit neovascularization, and directly kill FAP-positive tumor cells.[94,95]
2. Endogenous bystander effect: Novel FAP-targeted CAR-T cells can activate innate immune mechanisms within the TME, such as macrophage phagocytosis and cytokine release, indirectly eliminating neighboring antigen-negative tumor cells. This broadens the antitumor response beyond FAP-positive cells.[96]

Combination with immune checkpoint inhibitors

The highly immunosuppressive TME in GBM, characterized by elevated PD-L1 expression, induces CAR-T cell exhaustion through the PD-1/PD-L1 signaling pathway.[97,98] To address this limitation, combining CAR-T cell therapy with immune checkpoint inhibitors has been explored. Preclinical evidence suggests that inhibiting the PD-1/PD-L1 axis can restore CAR-T cell function and improve antitumor efficacy. For example, in PD-L1–high GBM models, EGFRvIII-targeted CAR-T cells with PD-1 gene knockout demonstrated enhanced cytokine secretion (IFN-γ, IL-2), increased cytolytic activity, and significantly prolonged survival in tumor-bearing mice.[68,99] Similarly, combining disialoganglioside 2 (GD2)-targeted CAR-T cells with nivolumab (an anti-PD-1 monoclonal antibody) improved CAR-T persistence, tumor infiltration, and tumor control in animal models.[100] In addition, the combination of IL-13Rα2-targeted CAR-T cells with anti-CTLA-4 minibodies further supports the synergistic potential of multi-target immunotherapy.[101,102]

Despite promising preclinical data, early-phase clinical trials (e.g., NCT03726515) reported that EGFRvIII-targeted CAR-T cells combined with pembrolizumab were safe and biologically active but showed limited overall survival benefit due to lack of efficacy.[103] Notably, PD-1 expression levels in infused CAR-T products were significantly correlated with peripheral CAR-T cell expansion and progression-free survival, suggesting that PD-1 could serve as a predictive biomarker for treatment response.[104] To optimize combination strategies, researchers are focusing on genetically engineering CAR-T cells. Approaches such as clustered regularly interspaced short palindromic repeats/Cas9-mediated PD-1 knockout or overexpression of costimulatory molecules (e.g., 4-1BB) have been shown to improve CAR-T resistance to PD-L1–mediated suppression.[62,105] For instance, CAR-T cells expressing checkpoint reversal receptors targeting PD-1 have demonstrated enhanced tumor clearance in GBM models.[105]

While the combination of immune checkpoint inhibitors with CAR-T therapy offers strong theoretical advantages for GBM treatment, further studies are needed to elucidate precise mechanisms and optimize therapeutic protocols for clinical translation.

Cytokine engineering enhances CAR-T cell therapy

While certain cytokines in the TME contribute to immunosuppression and hinder CAR-T cell efficacy, others—such as IL-7, IL-15, and IL-21—support T-cell activity by stimulating the JAK-STAT signaling pathway, thereby promoting CAR-T cell survival and proliferation.[106–108] This has led to the emergence of cytokine-engineered CAR-T cell strategies. These approaches involve incorporating cytokines or their receptors into CAR-T cells to significantly enhance their proliferation, persistence, and antitumor activity while simultaneously remodeling the immune microenvironment to support synergistic immune responses.

In cytokine-engineered CAR-T strategies, IL-7 is of particular interest for its role in maintaining T-cell homeostasis and promoting survival. In an EGFRvIII-heterogeneous GBM model, IL-7–modified CAR-T cells showed enhanced expansion and increased mouse survival from 9% to 67%, largely due to IL-7–mediated self-renewal and reduced exhaustion.[109] Moreover, CAR-T cells co-engineered with IL-7 and Flt3L further amplified these effects by increasing conventional dendritic cells and $CD103^{+}XCR1^{+}$

migratory dendritic cells in the TME, thereby enhancing antigen cross-presentation and endogenous immune activation to eliminate antigen-negative tumor cells.[109]

Beyond IL-7 and Flt3L, additional studies have developed CAR-T cells that co-express cytokines upon activation. For instance, GD2-targeted IL-18–secreting CAR-T cells release IL-18 in a CAR-dependent manner.[110] Others have engineered autocrine cytokine-receptor circuits, such as IL-7/C-C motif chemokine ligand 21 (CCL21) combinations, to further improve CAR-T efficacy.[111]

However, challenges remain regarding safety, tumor heterogeneity, and clinical translation. Proliferative cytokines like IL-6 may increase the risk of CRS, highlighting the need for optimized regulatory systems.[112] Tumor heterogeneity necessitates the use of multi-targeted CAR-T cells or combination therapies with immune checkpoint inhibitors.

Cytokine engineering offers a paradigm shift for CAR-T therapy—from "single-target cytotoxicity" to "systemic immunomodulation". By synergistically enhancing CAR-T function and reprogramming the immune microenvironment, this approach holds promise for overcoming the limitations of solid tumor treatment. Future research should explore combinations of cytokines such as IL-15 and IL-21, dynamic regulatory strategies, and integration with localized delivery platforms or biomaterials to further enhance therapeutic outcomes.

Immunomodulatory role of TMZ chemotherapy combined with CAR-T cell therapy

Despite aggressive treatment involving surgical resection followed by radiotherapy and TMZ chemotherapy, patients with GBM face a median overall survival of just 12 to 15 months and a five-year survival rate under 10%.[2,113,114] TMZ exerts its cytotoxic effect by inducing DNA damage via alkylation, yet its effectiveness is constrained by poor BBB permeability, tumor heterogeneity, and an immunosuppressive TME.[115,116] However, combining TMZ with CAR-T cell therapy has demonstrated unique synergistic mechanisms. Firstly, TMZ activates the DNA damage response pathway, leading to upregulation of stress-induced antigens on tumor cells, which enhances CAR-T cell recognition and cytotoxicity.[117] In parallel, TMZ-induced tumor cell apoptosis results in antigen release, thereby activating endogenous antitumor immune responses.[118,119] Secondly, TMZ's lymphodepleting effect eliminates immunosuppressive cells such as Tregs, creating a favorable niche for CAR-T cell expansion.[120,121] To address the cytotoxic effects of TMZ on normal T cells, genetic engineering strategies have been explored to improve CAR-T cell viability. For instance, co-expression of miR-17-92 suppresses pro-apoptotic proteins, preserving T-cell activity and IFN-γ production.[122] Moreover, *MGMT* gene modification renders γδ CAR-T cells resistant to TMZ-induced DNA damage.[117]

The integration of TMZ and CAR-T cell therapy represents an innovative strategy for GBM, as TMZ increases tumor-antigen exposure, remodels the immune microenvironment, and improves T-cell survival. The clinical translation of this combination approach will benefit from multicenter trials and the integration of personalized treatment strategies.[2,115]

Combination of local radiotherapy and CAR-T cell therapy

As a commonly used therapeutic approach for GBM, radiotherapy not only exerts direct cytotoxic effects on tumor cells but also modulates the TME through immunoregulatory mechanisms. These include the induction of immunogenic cell death (ICD), stimulation of innate immune responses, and enhancement of T-cell infiltration, thus offering a solid basis for combination with CAR-T cell therapy.[123,124]

Radiotherapy can enhance CAR-T efficacy through multiple mechanisms. Firstly, First, it induces tumor cell stress leading to ICD. Dying tumor cells release various damage-associated molecular patterns (DAMPs) that activate DAMP signaling, triggering phagocytosis and the production of inflammatory cytokines. These processes promote antigen presentation by dendritic cells, thereby enhancing the killing capacity of CAR-T cells and reversing their exhaustion.[125] Secondly, irradiated tumor cells release abundant radiotherapy-induced microparticles. Upon uptake by non-irradiated cells, these radiotherapy-induced microparticles significantly upregulate surface MHC-I expression, enhancing T-cell recognition of tumor-associated antigens.[126] Thirdly, radiotherapy has dose-dependent effects on immune checkpoint molecule expression (e.g., PD-L1, CTLA-4). Systematic investigation of this dose-dependent regulation could help design optimized radiotherapy protocols that synergistically enhance CAR-T-mediated tumor eradication.[127] Furthermore, emerging studies have revealed that radiotherapy can induce systemic abscopal effects in cancer treatment—beyond eliminating localized lesions, it can cause regression of distant tumors. While the precise mechanisms remain incompletely elucidated, this phenomenon likely involves radiotherapy-induced ICD and the subsequent release of DAMPs.[128] However, such abscopal responses have been observed in only a minority of clinical cases.

Compared to traditional chemotherapy, localized radiotherapy offers superior spatial precision, significantly reducing post-treatment systemic immunosuppression. This preserves CAR-T cell functionality and minimizes interference with concurrent immunotherapeutic regimens. Several ongoing clinical trials are investigating this combinatorial strategy. For instance, emerging studies reveal that FLASH radiotherapy (ultra–high dose rate radiotherapy) enhances GD2 CAR-T cell efficacy against medulloblastoma through dual metabolic-immune reprogramming. It remodels lipid metabolism in TAMs, driving their polarization toward the pro-inflammatory M1 phenotype while suppressing the immunosuppressive M2 phenotype.[129] Future experimental investigations of this combination strategy must address several challenges. First, optimal radiation dose and timing need to be determined: low-dose irradiation synergizes well with CAR-T therapy, whereas high doses may induce fibrosis and hinder CAR-T infiltration.[123] Second, radiotherapy can impose selective pressure on tumors, potentially leading to the expansion of antigen-negative clones. To address this, multi-targeted CAR-T cells or combined tumor vaccines may be necessary to overcome antigen heterogeneity.[130,131]

In conclusion, combining local radiotherapy with CAR-T cell therapy presents a promising strategy for treating GBM. Further optimization and approaches to address the immunosuppressive TME are crucial for successful clinical translation.

Delivery routes of CAR-T cell therapy for gliomas

The therapeutic efficacy of CAR-T cell therapy in gliomas is highly dependent on optimizing delivery routes. Traditional systemic intravenous administration, though convenient, is significantly limited by the BBB, which hampers CAR-T cell infiltration into the tumor and results in poor delivery efficiency.[132,133] Moreover, systemic administration may induce off-target toxicity and CRS.[132,134]

Local and regional delivery strategies, which involve direct administration of CAR-T cells into the tumor site or cerebrospinal

fluid (CSF), effectively circumvent the BBB. Intratumoral injection, often performed using stereotactic techniques, delivers CAR-T cells targeting antigens such as HER2, EGFR, or B7-H3 directly into the tumor cavity. This approach has demonstrated safety in clinical trials for pediatric gliomas,[135] although repeated dosing is often required, and there is a risk of infection. Intraventricular or intrathecal administration is suitable for tumors near the ventricles or meninges. When combined with epigenetic modulators, it can enhance efficacy against metastatic medulloblastoma.[136] However, the persistence of CAR-T cells in the CSF and the risk of neurotoxicity remain concerns.

With advances in nanomedicine and biomaterial sciences, emerging strategies have been developed to enhance CAR-T function via nanocarriers and engineered delivery platforms. For example, macrophage-based CAR-MΦ therapies leverage the natural tumor-penetrating properties of macrophages. ErbB2-targeting CAR-MΦs have shown durable antitumor effects in glioma models, with localized administration reducing systemic side effects.[137] Similarly, biomimetic nanoparticle-based CAR-neutrophil systems, designed to mimic neutrophil responses to TME-derived inflammatory signals, have enabled targeted CAR-T delivery and synergized with chemotherapeutic agents to elicit enhanced antitumor responses.[138]

Prospective technological advances related to CAR-T therapy

With the rapid advancement of multiple scientific disciplines, cutting-edge technologies from various fields are being actively integrated into CAR-T research. This section briefly introduces the utilization of single-cell sequencing techniques and organoid systems within CAR-T treatment strategies for GBM.

Single-cell sequencing to guide personalized multi-antigen CAR-T design

The introduction of single-cell RNA sequencing technologies has provided a novel perspective for dissecting the molecular complexity of GBM. Multi-target CAR-T design based on single-cell data is a key strategy to overcome tumor heterogeneity. Single-cell sequencing enables systematic profiling of cancer cell subpopulations within the TME, allowing identification of combinatorial antigen signatures specific to different cell subsets. For instance, single-cell RNA sequencing has revealed distinct antigen expression patterns between GSCs and differentiated tumor cells, with antigens such as GD2, IL13Rα2, and EphA3 being highly expressed in the stem-like populations.[73,139,140] Using transcriptomic screening, researchers have identified combinations like CD44 and CD133 that can cover heterogeneous GBM populations, with their synergistic anti-tumor effects validated in organoid models.[73,141] Furthermore, integrating single-cell epigenomic data allows prediction of pathways associated with tumor cell plasticity, providing insights for combination therapies aimed at halting resistance evolution.[142,143]

Single-cell RNA sequencing can also reveal the dynamic trajectories of tumor cell subpopulations, such as the enrichment of specific GSC-like subsets in recurrent GBM and their interactions with the immune microenvironment.[142,143] Additionally, single-cell multi-omics analyses can identify CAR-T exhaustion markers like CD38 and durable T-cell subpopulations, offering molecular targets to optimize CAR-T function.[144,145]

In summary, single-cell sequencing sheds light on the evolution of GBM heterogeneity at the molecular level and offers a data-driven framework for the precise design of multi-antigen CAR-T strategies. By integrating target selection, microenvironmental modulation, and delivery optimization, personalized multi-target CAR-T approaches may overcome the limitations of current GBM therapies. Their clinical translation will require further interdisciplinary innovation and the development of adaptive therapeutic systems.

Organoid models to optimize preclinical evaluation systems

The inter- and intra-tumoral heterogeneity of GBM poses significant challenges to traditional *in vitro* models. Patient-derived GBOs, which can be rapidly generated within two to four weeks and cryopreserved, successfully retain tumor heterogeneity and molecular characteristics, making them an ideal platform for CAR-T research.[146,147] GBOs not only mimic the immunosuppressive features of the TME but also allow real-time evaluation of CAR-T cell infiltration, cytotoxicity, and cytokine release profiles. For example, GBOs preserve GSC subpopulations and vascular structures, enabling assessment of CAR-T–mediated cytotoxicity toward stem-like and perivascular cells.[148] Moreover, the similarity in cytokine release patterns between GBOs and patient CSF further validates their clinical relevance.[73,149]

In CAR-T studies targeting GBM, research teams have used dual-target strategies (e.g., CD44/CD133 tandem CAR-Ts) and broad-spectrum antigen recognition approaches (e.g., 806 CAR-Ts targeting EGFRvIII) to overcome antigen heterogeneity and immune evasion.[73,150] Combination therapies, such as CAR-Ts with the IAP antagonist birinapant, enhance efficacy by inducing bystander killing and activating the NF-κB pathway.[151] Locally delivered engineered CAR-Ts, such as those secreting IL-7/CCL19 or Tanζ-T28-Δ7R CAR-Ts, have also been validated in GBOs and orthotopic xenograft models.[27,73] These advances underscore the pivotal role of GBOs in dynamic efficacy evaluation.

To enhance the predictive value of GBO-based models, it is essential to integrate multi-omics data and functional parameters such as CAR-T persistence and exhaustion markers into efficacy prediction frameworks.[152] Innovations such as the development of GBM–blood vessel assembloids and microfluidic chip systems have enabled simulation of BBB penetration and CAR-T migration/killing dynamics under perfusion conditions.[27,148] Standardized biobanking of GBOs representing various molecular subtypes and treatment histories is another critical step in model optimization.[146,147] However, challenges remain in model standardization, dynamic modeling of resistance evolution, and the full representation of immune microenvironment complexity.[73,146–148,151]

In summary, GBO-based preclinical systems offer a highly relevant platform by recapitulating tumor heterogeneity and the GBM microenvironment. Future efforts should focus on integrating engineered CAR designs, multi-omics analysis, and dynamic modeling to accelerate the development of personalized immunotherapeutic strategies against GBM.

Limitations

This review has several limitations. First, many of the studies referenced are based on preclinical animal models or early-phase clinical trials, and their reproducibility and applicability in broader patient populations remain to be fully validated. Second, while we focused on summarizing innovative strategies and their therapeutic potential, practical issues such as safety, cost-effectiveness, and clinical feasibility were not discussed in depth. Future research should integrate multi-center clinical trials with multi-omics analyses to further elucidate GBM immune evasion mechanisms and facilitate the efficient clinical translation of CAR-T therapy.

Conclusions

CAR-T cell therapy for GBM faces two fundamental challenges: antigenic heterogeneity and an immunosuppressive TME. Recent research has progressed from single-target strategies to multi-dimensional and combinatorial approaches. Innovative designs, such as multi-target CAR-Ts, dynamic antigen-adaptive systems, and TME-remodeling modifications, have significantly enhanced therapeutic efficacy. For example, logic-gated CAR systems enable precise recognition of tumor regions co-expressing dual antigens; NKG2D-based CAR-T cells provide broad-spectrum targeting to reduce immune escape; and IL-7/Flt3L engineering improves CAR-T persistence while stimulating endogenous immune responses. In addition, combination therapies involving radiotherapy, TMZ chemotherapy, or immune checkpoint inhibitors have shown synergistic effects. Novel delivery methods, including local administration and nanocarrier technologies, also hold promise for overcoming the BBB.

CAR-T therapy for GBM must evolve beyond a tumor-centric "killing" paradigm toward a model of systemic immune regulation. The integration of interdisciplinary innovation with robust clinical trial design will be key to overcoming existing bottlenecks and advancing the next generation of CAR-T therapies for GBM.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

Conceptualization (QLJ, YLH, LJZ), writing - original draft preparation (LJZ, ZQZ, JLH), figure preparation (JLH, ZHZ), writing - review & editing (HXZ, YHJ, XZ), writing - revision (YMY, ZHZ), and funding acquisition (NNL, WX). All authors have given informed consent for the publication of this article.

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