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Review

Empowering brain tumor management: chimeric antigen receptor macrophage therapy

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Abstract

Brain tumors pose formidable challenges in oncology due to the intricate biology and the scarcity of effective treatment modalities. The emergence of immunotherapy has opened new avenues for innovative therapeutic strategies. Chimeric antigen receptor, originally investigated in T cell-based therapy, has now expanded to encompass macrophages, presenting a compelling avenue for augmenting anti-tumor immune surveillance. This emerging frontier holds promise for advancing the repertoire of therapeutic options against brain tumors, offering potential breakthroughs in combating the formidable malignancies of the central nervous system. Tumor-associated macrophages constitute a substantial portion, ranging from 30% to 50%, of the tumor tissue and exhibit tumor-promoting phenotypes within the immune-compromised microenvironment. Constructing CAR-macrophages can effectively repolarize M2-type macrophages towards an M1-type phenotype, thereby eliciting potent anti-tumor effects. CAR-macrophages can recruit T cells to the brain tumor site, thereby orchestrating a remodeling of the immune niche to effectively inhibit tumor growth. In this review, we explore the potential limitations as well as strategies for optimizing CAR-M therapy, offering insights into the future direction of this innovative therapeutic approach.

Keywords: Brain tumor, Immunotherapy, Chimeric antigen receptor, Macrophage

INTRODUCTION

Despite remarkable progress in the conventional treatment modalities for brain tumors (BTs), including surgical resection, radiotherapy, and systemic chemotherapy, patient outcomes remain unsatisfactory, primarily due to the intricate interplay within the BTs microenvironment [1, 2]. The microenvironment, characterized by a complex network of immune cells, stromal elements, and cytokines, exerts profound influence on tumor progression and therapeutic responses. Central to this are tumor-associated macrophages (TAMs), a prominent constituent of the immune infiltrate within BTs [3]. TAMs exhibit a remarkable plasticity and can adopt divergent functional states, often influenced by cues from the tumor microenvironment [4]. In the central nervous system, TAMs promote tumor growth and metastasis by fostering an immunosuppressive microenvironment characterized by the secretion of factors such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10), which inhibit anti-tumor immune responses and promote angiogenesis [5]. However, the balance between pro-tumorigenic and anti-tumorigenic functions of TAMs is delicately regulated by various factors, such as hypoxia, nutrient deprivation, and tumor-derived soluble factors [6].

Harnessing the immunomodulatory properties of TAMs represents a promising avenue for enhancing anti-tumor immune responses and improving patient outcomes [7]. However, challenges such as TAM heterogeneity, plasticity, and resistance mechanisms need to be overcome to realize the full therapeutic potential of targeting TAMs in BTs

therapy [8]. In this regard, engineered macrophages equipped with Chimeric Antigen Receptors (CARs) tailored for tumor specificity emerge as a compelling strategy. These engineered macrophages possess a multifaceted arsenal of capabilities crucial for combating tumor progression within the intricate microenvironment of the BTs [9]. By leveraging their inherent migratory ability and capacity for infiltrating deep into tumor sites, CAR-macrophages (CAR-Ms) can directly engage with tumor cells and mount potent immune responses [10, 11].

Facilitated by the innate ability to traverse the blood-brain barrier and penetrate deep within the tumor parenchyma, CAR-Ms navigate the complex landscape of the BTs microenvironment with precision [12, 13]. Once deployed within this hostile terrain, CAR-Ms unleash a formidable immune response characterized by targeted phagocytosis of tumor cells and efficient presentation of tumor antigens [14]. Concurrently, the presentation of tumor antigens by CAR-Ms facilitates the activation and proliferation of cytotoxic T cells, bolstering the adaptive immune response against the tumor [15]. This dual immunomodulatory action holds significant promise for overcoming the immunosuppressive microenvironment characteristic of BTs and fostering durable anti-tumor immunity [16].

Compared to Chimeric Antigen Receptor T cell (CAR-T) therapy, CAR-M therapy demonstrates distinct advantages in terms of therapeutic efficacy and suitability for targeting BTs. CAR-Ms exhibit superior infiltration and activation of both innate and adaptive immune responses within the BTs microenvironment [17]. This enhanced immunological engagement holds significant promise for overcoming the immunosuppressive barriers characteristic of BTs and bolstering anti-tumor immunity [18]. As such, the burgeoning field of CAR-M therapy in BTs treatment merits heightened attention and exploration. Its potential to revolutionize immunotherapy by leveraging the unique attributes of macrophages to orchestrate robust anti-tumor immune responses represents a transformative paradigm shift in the quest for more effective treatments against this devastating disease [19].

BIOLOGY OF BRAIN TUMOR AND MARCROPHAGES

Biology and Microenvironment of Brain Tumor

Primary BTs originate within the brain and are further categorized based on their cellular origin and molecular characteristics, such as gliomas and medulloblastomas. Secondary BTs originate from cancer cells that have disseminated from other regions of the body which are invariably malignant and are categorized according to the primary site of origin [20]. BTs exhibit diverse structural characteristics depending on their type and grade. High-grade tumors, such as glioblastoma multiforme (GBM), are characterized by rapid growth, necrosis, and extensive vascularization [21]. Low-grade tumors, such as grade I astrocytoma, tend to grow slowly and have well-defined borders [22]. The structural complexity of BTs poses challenges for surgical resection and treatment. The treatment of BTs is a multifaceted process that depends on the specific characteristics of the tumor, including type, size and location [23]. Surgical resection remains the primary treatment modality, with the objective of removing as much of the tumor as possible. Adjuvant therapies, including radiation and chemotherapy, are employed to eliminate residual tumor cells [24]. Even with all the continued advances in drug discovery and formulation that have substantially improved outcomes in systemic cancers, there has been disappointingly little impact on tumors of the brain [9]. The advent of targeted therapies and immunotherapies, such as CAR cell therapy, has demonstrated potential in treating specific tumor subtypes by targeting tumor-specific antigens. Nevertheless, challenges such as tumor heterogeneity and the intricate immune microenvironment continued research to optimize treatment outcomes [25].

The immune microenvironment of BTs is characterized by a complex interplay of immune cells, stromal cells, and tumor cells, contributing to an immunosuppressive milieu that supports tumor progression. Key players in this environment include TAMs, microglia, and regulatory T cells, which collectively promote immune evasion and tumor growth [26]. Although expected to induce apoptosis of tumor, cytotoxic T cells and natural killer (NK) cells are suppressed within TME either by direct contact with tumor or under the influence of inhibitory factors contributed by Treg cells and TAMs [27, 28]. TAMs also help in the promotion of tumors angiogenesis and proliferation. The structurally and functionally aberrant tumor vasculature contributes to the pro-tumorigenic and immunosuppressive TME by maintaining hypoxia, acidosis, and high interstitial pressure, while simultaneously generating a physical barrier to T cell infiltration [29]. Of note, hypoxia and hyperlactatemia induce metabolic reprogramming and epigenetic changes which are interconnected, and to a large extent, metabolic state dictated epigenetics in BTs. Furthermore, epigenetic alterations are

associated with all aspects of BTs, from BTs initiation to cancer progression and metastasis [30, 31].

The Role of Macrophages in Brain Tumor

Macrophages are innate immune cells that participate in immune defense, tissue homeostasis, and the regulation of diseases. TAMs are defined as macrophages that infiltrate tumor, which are an integral part of the immunity, exerting significant influence on the development and treatment of BTs [32]. TAMs play a dual role in tumor biology, either participating in tumor surveillance and eradication or facilitating tumor progression [33, 34]. TAMs polarize into the M1-like phenotype upon exposure to interleukin-12 (IL-12), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), granulocyte–macrophage colony-stimulating factor (GM-CSF), and bacterial lipopolysaccharide (LPS), characterized by CD68, CD80, CD86, major histocompatibility complex II (MHCII), and inducible nitric oxide synthase (iNOS) expression, exhibiting anti-tumor activity, reactive oxygen species (ROS) secreting, enhanced antigen presentation, proinflammatory cytokine production, and involvement in T helper (Th) type 1 responses [35, 36]. Exposure to IL-4, 5, 10, 13, colony-stimulating factor 1 (CSF1), transforming growth factor-beta (TGF-β), and prostaglandin E_2 (PGE_2), TAMs polarize into the M2-like phenotype, characterized by CD206, CD204, vascular endothelial growth factor (VEGF), CD163, and arginase-1 (Arg-1) expression, supporting protumor functions and contributes to BTs proliferation, growth, and invasive metastasis by promoting angiogenesis and stemness, creating an immunosuppressive environment that facilitates immune escape, thereby enhancing drug resistance and influencing neuronal activity [37-39].

Macrophages are critical effectors of antibody-based cancer therapy, and as antigen-presenting cells, activated macrophages can play a critical role in promoting an adaptive anti-tumor immune response. These considerations spurred previous attempts to transfer autologous macrophages to patients with tumors but failed to demonstrate notable anti-tumor efficacy [40, 41].

CAR-Macrophages: What They Are and How They Work

As an advancement, through receptor engineering, CAR-Ms possess extracellular antigen-binding domains, hinge regions, transmembrane domains and intracellular domains (Figure 1A) [42]. An antigen-binding domain is usually single-chain Fv (scFv) with a simple ectodomain and more exotic recognition components, yet recent innovations have expanded the constitution to include elements from other domains such as nanobodies, designed ankyrin repeat proteins (DARPins), ligands, or receptors [43]. The hinge region, an extracellular structure, links the antigen-binding domain (ABD) to the transmembrane domain, where variations in composition or length can influence CAR-antigen binding and signaling [44]. The transmembrane domain, characterized by a hydrophobic alpha helix that spans the cell membrane, anchors the CARs in the membrane, affecting its expression, stability, and function [45]. The intracellular domain is the functional end of the receptor. In CAR-Ms, the intracellular domains are typically derived from signaling molecules that can induce macrophage activation and polarization [17]. The most commonly used intracellular domain is CD3ζ chain, which is derived from the T-cell receptor complex and is responsible for initiating the primary activation signal [46]. However, to enhance the antitumor efficacy of CAR-Ms, additional costimulatory domains are often incorporated [47]. After receptor engineering, once the extracellular antigen-binding domain recognizes the antigen, intracellular signal transduction is initiated. CAR-Ms directly interact with the kinase Syk, which contains tSH2 domains, and utilize the intracellular CD3ζ domain, which contains immunoreceptor tyrosine-based activation motifs (ITAMs), to transmit phagocytic signals [48, 49]. In addition to CD3ζ, FcRγ effector function is antibody-dependent cellular phagocytosis (ADCP), while Megf10 can also induce the phagocytic function. All of these possess intracellular domains containing ITAMs [50]. After CAR-Ms identify and phagocytose tumor cells via CAR receptors, tumor engulfment is initiated in CAR-Ms. The subsequent degradation of tumor antigens leads to their presentation to $CD4^+$ T cells through MHC molecules, which activate the adaptive immune response to effectively eliminate tumor cells (Figure 2) [47].

DEVELOPMENT OF CAR-MARCROPHAGES FOR BRAIN TUMOR TREATMENT

Historical Context and Early Experiments with CAR-T cells

CAR technology was first described by Kuwana, Y. in 1987. This was followed by the discovery of the intracellular signaling domain of CD3ζ, which activates T-cell signaling [51]. The discovery enabled the development of first-generation CARs combining scFv domains, CD4 and other components in 1991 [52]. However, clinical trials using CAR-T therapy to eradicate HIV-infected cells and solid tumors were

unsuccessful in the mid-1990s [52, 53]. In 2003, a study showed that human CD19-directed CAR-T cells could kill leukemia cells in a mouse model, and CAR-T therapy has become a popular treatment for leukemia [54]. In 2010, the FDA approved the first clinical trials of CD19 CAR-T cells, and then two more products (Kymriah and Yescarta) were approved by the FDA in 2017 (Figure 3) [53]. To date, six CAR-T therapies have received FDA approval for hematological malignancies: Kymriah, Yescarta, Tecartus, Breyanzi, Abecma, and Carvykti [55].

Advantages and Limitations of CAR-Macrophages Compared to CAR-T Cells

CAR-M therapy and CAR-T therapy both utilize CARs to target and eliminate cancer cells, but their mechanisms of action differ significantly. CAR-T cells, derived from T lymphocytes, primarily exert their anti-tumor effects through cytotoxicity. Upon binding to the target antigen, CAR-T cells are activated through the CD3ζ signaling domain, releasing perforin and granzymes to induce apoptosis in target cells [56]. Despite the potential of T cells targeted to solid tumors, their efficacy is hampered by the TME, which impairs their infiltration and cytotoxic capabilities [57, 58]. In addition, T-cell therapy may induce severe side effects such as Graft-Versus-Host Disease (GVHD), Cytokine Release Syndrome (CRS), "on-target/off-tumor" toxicity, and immune effector cell-associated neurotoxicity syndrome (ICANS) [59]. Another challenge lies in the scarcity of tumor-specific antigens in solid tumors amenable to CARs, thereby limiting the effectiveness of CARs [60]. Furthermore, the high cost and time-consuming production of CAR-T therapy hinder widespread accessibility.

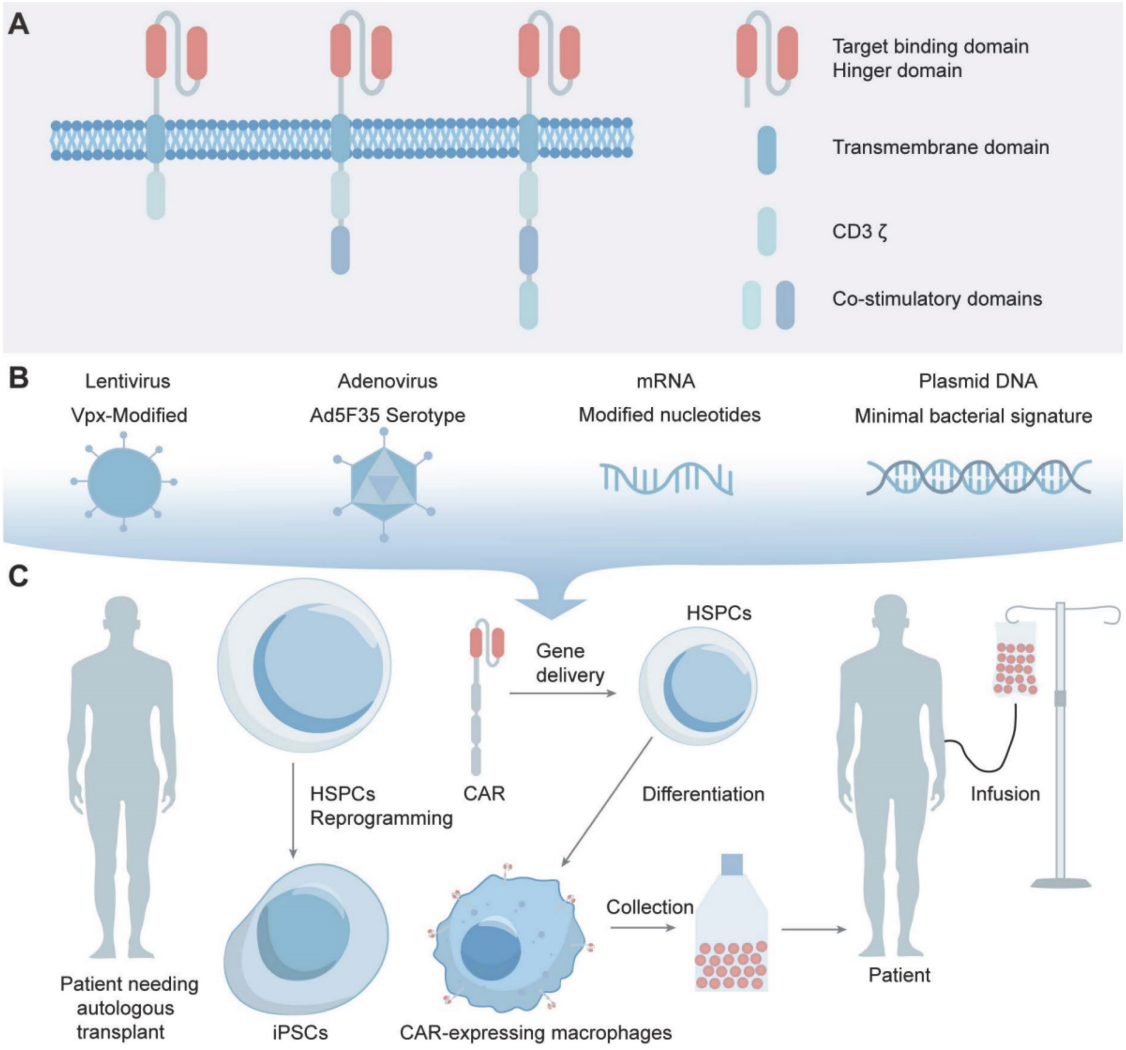

Figure 1. The structure of CARs and the construction of CAR-Macrophages. (**A**) CAR-macrophages possess extracellular antigen-binding domains, hinge regions, transmembrane domains and intracellular domains. (**B, C**) Advances in macrophage transduction technology have made it possible to transfer CARs into macrophages.

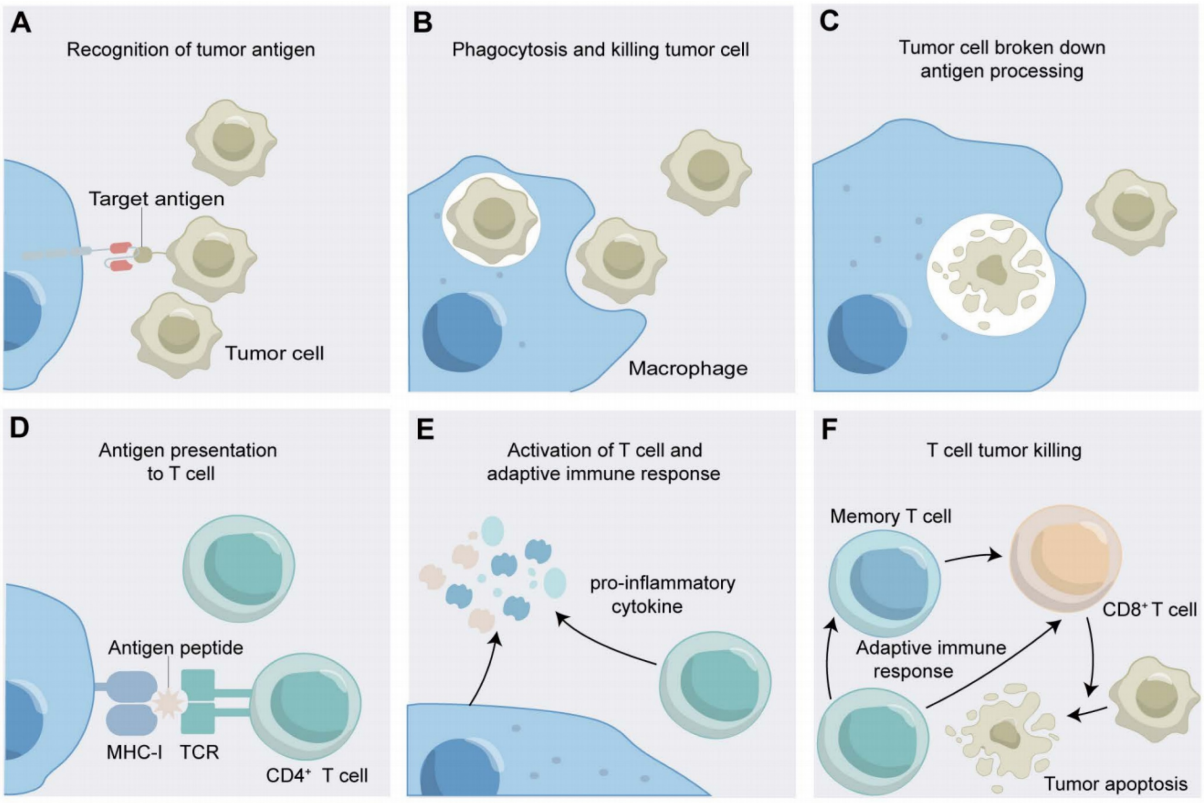

Figure 2. The Mechanism of Action of CAR- CAR-Macrophages. (**A**) CAR-macrophages recognize tumor antigens; (**B**) CAR-macrophages phagocytose tumor cells; (**C**) CAR-macrophages breakdown tumor antigens; (**D**) CAR-macrophages present tumor antigens to T cells; (**E**) CAR-macrophages activate adaptive immune response. (**F**) The adaptive immune response kills tumor cell.

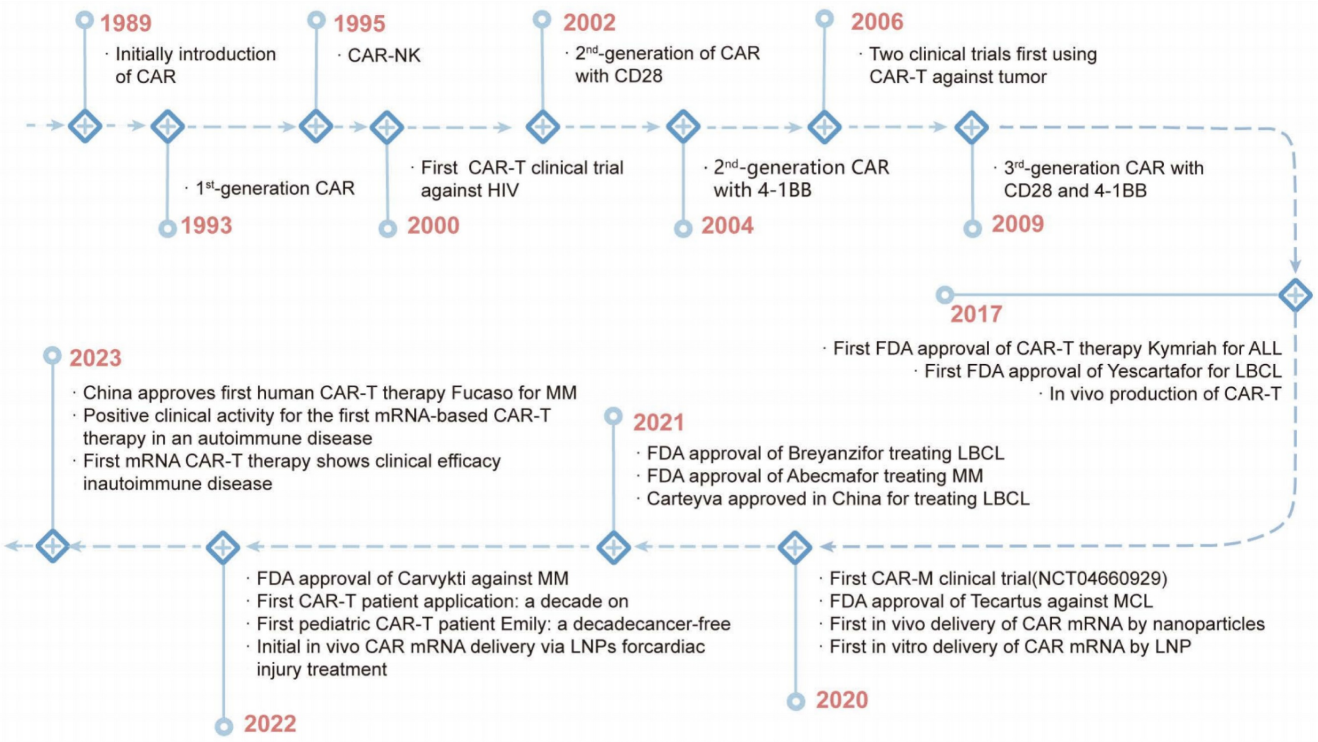

Figure 3. The History of CAR Cell Therapy Development.

Table 1. Advantages and Limitations of CAR-Ms Compared to CAR T-Cells

	CAR-T	CAR-M
Advantages	- Adequate number of circulating T cells	- Extensive infiltration in most solid tumors - Anti-tumor activity through phagocytosis, presentation of tumor antigen to Th1 cells and production of anti-inflammatory factors
Limitations	- Difficulty in infiltrating solid tumors - Immunosuppressive tumor microenvironment limits survival and persistence - Heterogeneity and loss of tumor antigens - CRS and OTOT toxicity	- Need to maintain M1 differentiation phenotype - CRS and OTOT toxicity

In contrast, CAR-Ms are engineered macrophages that express CARs similar to those in CAR-T cells [61]. However, the intracellular domains are often modified to include signaling motifs that promote macrophage activation and polarization. CAR-Ms, derived from macrophages, utilize phagocytosis to engulf and digest cancer cells [15]. Additionally, CAR-Ms secrete pro-inflammatory cytokines that modulate the tumor microenvironment and recruit adaptive immune cells, enhancing the overall anti-tumor response [25]. CAR-Ms have the potential to be an effective treatment for solid tumors due to their strong infiltration capacity and ability to act in both antigen-dependent and antigen-independent ways. The plasticity of macrophages allows for reversion to the M1-like phenotype characterized by antigen presentation to activate the adaptive immune response, further remodeling the matrix in the TME and recruiting more effector lymphocytes [48]. Furthermore, CAR-Ms are long-lived, have the ability to induce cytotoxic activity in the tumor niche and can be chemically modified to enhance their drug delivery [62-66], which means they can provide long-term therapeutic effects without the risk of toxicity associated with T-cell therapies, such as CRS and neurotoxicity [67].

Techniques for Developing CAR-Macrophages for Cancer Immunotherapy

CAR-Ms, while exhibiting potential in solid tumor therapeutics, encounter a multitude of formidable challenges [11]. Leveraging CAR-T therapy advancements, progress in optimizing CAR-Ms has been made, focusing on transfection efficiency and safety.

The main challenge of CAR-M therapy is the efficient transduction of the CAR gene or plasmid into macrophages. Macrophages possess a natural environment that is resistant to viral replication, as well as the ability to recognize and respond to foreign nucleic acids, exhibiting a high resistance to gene engineering [68]. Advances in macrophage transduction enable the transfer of CARs into macrophages, allowing for the engineering of cells to express and secrete therapeutic proteins targeting BTs. CD46, highly expressed in monocytes and macrophages, serves as the receptor for B-group adenoviruses such as Ad35, facilitating viral entry [69, 70]. To improve transduction efficiency, Vpx, a viral accessory protein that mediates SAMHD1 degradation, was incorporated into the lentivirus at the packaging stage to overcome SAMND1 inhibition and enabling viral transduction [71]. Ad5f35, a replication-incompetent chimeric adenoviral vector, maintains transduction of macrophages persists for at least one month *in vitro* and at least 62 days *in vivo*, indicating its potential as a vector for the long-term delivery of gene payloads to myeloid cells (Figure 1B) [48]. The vector not only efficiently delivers CAR genes to macrophages, but also activates the macrophage inflammasome, providing a proinflammatory stimulus that synergizes with CAR activity. Despite the advances, the clinical application of adenoviral vectors like Ad5f35 is limited due to concerns over oncogenic potential and immune reactions [72, 73].

Recent studies into non-viral methods have shown promise as a solution for gene therapy delivery challenges, merging the fields of nanotechnology, gene delivery, and synthetic biology to potentially transform cancer immunotherapy and immunomodulation strategies [74]. Recent studies highlight the potential of nanocomplexes to address CAR-M therapy challenges, such as reducing costs, simplifying manufacturing processes, and decreasing tumorigenic risks associated with viral vector transduction [75]. The nanocomplexes injected *in vivo* contain mannose-conjugated polyethyleneimine (MPEI), which significantly enhanced the macrophage targeting efficiency both *in vitro* and *in vivo* as mannose receptors are overexpressed in macrophages, and CAR-IFN-γ-encoding plasmid DNA with a nonviral piggyBac transposon system [76], which is a mobile genetic element that can be efficiently transposed between vectors and chromosomes via a "cut and paste" mechanism for the sustained expression of CAR transgenes [77]. The nanocomplexes induced a shift in macrophages from the M2-like to the M1-like phenotype and maintained the anti-tumor state post-phagocytosis of malignant cells *in vitro*. They also halted tumor growth in Neuro-2a tumor-bearing mice without causing systemic toxicity, through a reduction in Treg cells and an increase in activated CD8+ T cells, facilitated by CAR-dependent tumor cell phagocytosis (Figure 4A) [78]. Recent studies have utilized a CpG-free plasmid, which avoids Toll-like receptor 9 (TLR9)

recognition, inflammatory responses and transgene silencing, is effective for delivering a small plasmid (pCGfd-GFP) encoded by the vector. The method showed high transfection efficiency, sustained transgene expression and good cell viability in the transfections of Raw 264.7 and primary bone marrow-derived macrophages. Moreover, the method engineers the macrophages secreting anti-EGFR scFv-Fc, capable of effectively targeting and phagocytizing EGFR-expressing tumor cells through ADCP [65, 79].

Based on these innovative approaches of gene delivery and macrophage-targeted therapies, it is critical to further explore cutting-edge strategies that harness the potential of synthetic biology to augment macrophage functions within the complex TME. Researchers have demonstrated that a cavity-injectable nanoporter-hydrogel superstructure, which generates glioblastoma stem cells (GSCs)-specific CAR-Ms around the cavity, is effective in preventing BTs recurrence [80]. A nuclear localization sequence (NLS) peptide-synthesized nanomicelle was utilized for nuclear-targeted CAR plasmid (pCar) gene delivery and further coated with citraconic anhydride-modified dextran (CA-dextran) to achieve CD206-targeted pCAR-laden nanomicelles [80]. Brain ECM-derived laminin peptide and an immunostimulatory peptide were used as the precursors of the hydrogel. After intracavity delivery, the CAR gene-laden nanoporter introduced GSC-targeted CAR genes into macrophage nuclei, thus generating CAR-Ms in mouse models of BTs (Figure 4B) [80].

The widespread use of CRISPR/Cas9 in cell immunotherapy has yielded significant outcomes, particularly in CAR-Ms, where it effectively facilitates CAR gene knock-in and enhances macrophage proinflammatory effector functions to counter the TME [81]. CRISPR/Cas9 genome editing involves the following three steps: recognition, cleavage, and repair. An sgRNA is designed to recognize the target gene sequence through complementary base pairing. The Cas9 protein is then used to cleave DNA at the site, and the cell repair machinery repairs the break either through nonhomologous end joining or homology-directed repair [82]. The CRISPR/Cas9 complex can be delivered as plasmid DNA (pDNA), messenger RNA (mRNA), or ribonucleoprotein

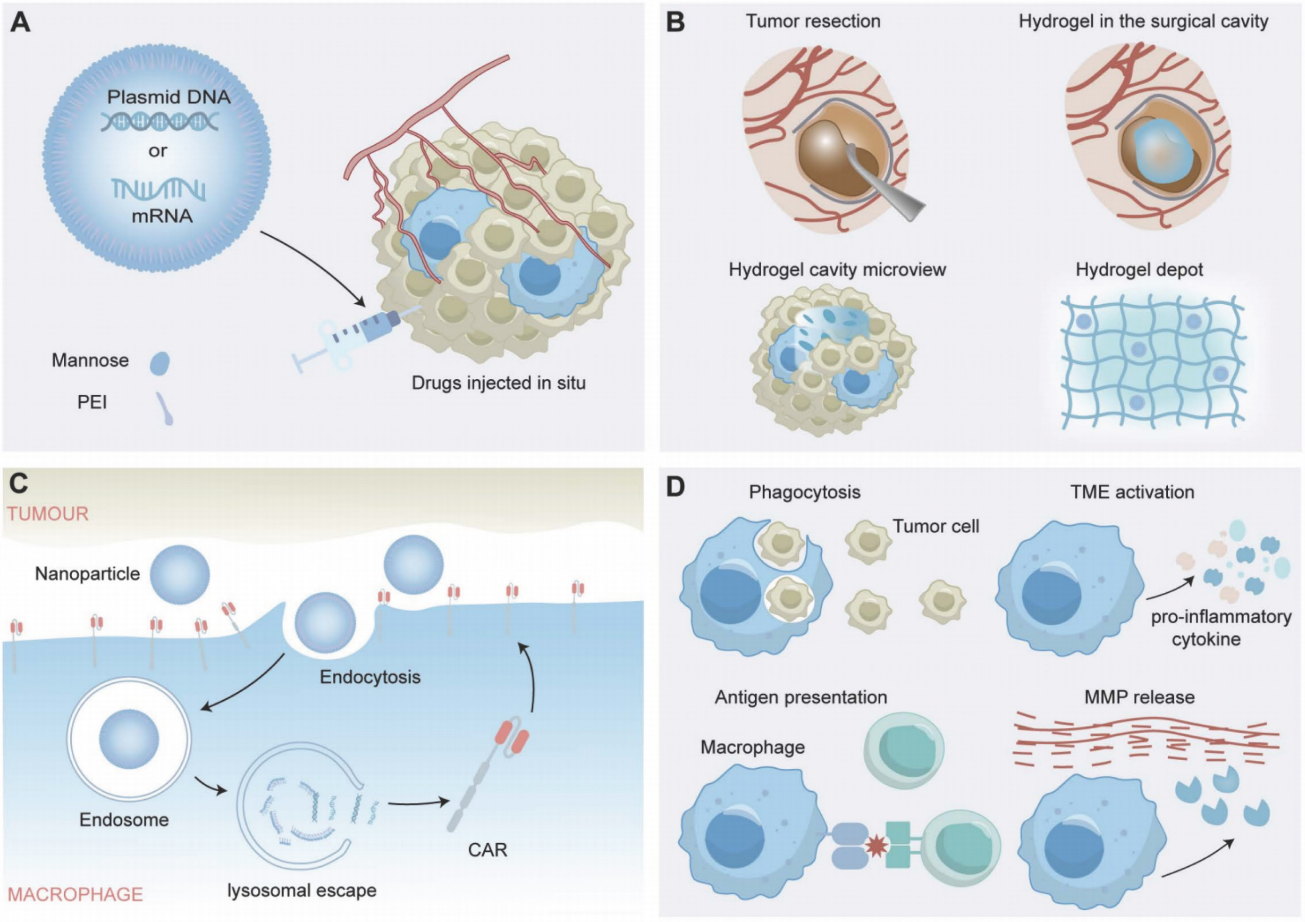

Figure 4. The Application of CAR-Macrophage Therapy in Postoperative Tumors. (**A, B, C**) The applications of nanotechnology, gene delivery, and synthetic biology in CAR-Macrophages. (**D**) The immune regulatory role of CAR-macrophages in the tumor microenvironment.

(RNP), with direct RNP delivery being advantageous due to its avoidance of many pitfalls associated with pDNA or mRNA delivery. RNP delivery facilitates the swiftest genome editing by eliminating the need for intracellular transcription and translation [80, 83-85]. In a study, CRISPR/Cas9-mediated depletion of SIRPα, a "don't eat me" signal of SIRPα/CD47, demonstrated synergistic efficacy in anti-tumor responses, whereas SIRPα knockout alone failed to enhance anti-tumor macrophage responses [86]. Additionally, researchers utilized CRISPR/Cas9 to integrate the anti-GD2 CAR into AAVS1 locus of hPSCs, resulting in CAR-Ms effectively targeting and eliminating GD2-expressing neuroblastoma and melanoma *in vitro* and *in vivo* [87]. Furthermore, CRISPR/Cas9 technology enhances CAR-Ms function by targeting regulators such as the aconitate decarboxylase 1 (ACOD1) and Kelch-like ECH-associated protein 1 (KEAP1), leading to improved persistence, polarization, ROS production, phagocytosis, and cytotoxicity *in vitro*, ultimately exhibiting high antitumor efficacy in mouse models of ovarian and pancreatic cancer. Moreover, synergistic effects were observed when combining this approach with immune checkpoint inhibitors [88]. Alternatively, catalytically dead Cas9 (dCas9) fused to transcriptional repression domains, [89] such as KRAB domains, chromatin remodeling factors, [90] and histone methylases, can be utilized to epigenetically silence targets known to precipitate M2-like phenotype polarization [91].

Selecting optimal cell sources is a pivotal consideration in cell therapy [92]. Peripheral blood mononuclear cells (PBMCs) offer easy access and are enriched with various immune cells for immunotherapy, although they exhibit limited genetic manipulation rates [17]. Alternatively, cell lines like THP-1, which possess macrophage-like characteristics induced by substances such as phorbol-12-myristate-13-acetate (PMA) and macrophage colony-stimulating factor (M-CSF), present advantages in terms of homogenous genetic background, ease of culture, rapid proliferation, and higher safety compared to PBMC-derived macrophages [93]. However, THP-1 lacks certain features such as LPS tolerance and specific cytokine secretion patterns, making them a viable but distinct option from PBMCs [94]. Induced pluripotent stem cells (iPSCs), reprogrammed from PBMCs, can differentiate into various somatic cell types, including immune cells. Rapid progress has been made in differentiating iPSCs into immune cells, including CAR-T and CAR-NK cells, demonstrating excellent cytotoxic effects against tumors. Researchers have developed iPSC-derived, CAR-expressing macrophages (CAR-iMac) for use in cancer immunotherapy [95, 96]. Based on the characteristics of iPSCs, researchers have obtained CAR-iMac with high yield and purity, exhibiting M1-associated gene expression and possessing phagocytic function. Cocultured with lymphoma cells expressing CD19 or ovarian cancer cells expressing the mesothelin, CAR-iMac were cytotoxic, employed ADCP, and polarized toward an M1-like phenotype (Figure 1C) [97].

The first-generation CAR-Ms were designed by incorporating the CD3ζ activating domain, similar to the first-generation CAR-T cells. This design aimed to leverage the phagocytic abilities of macrophages to target and eliminate tumor cells [9, 25]. It is not feasible to polarize macrophages towards a durable M1-like pro-inflammatory state in theory. Therefore, the design of a new macrophage-specific CAR to confer CAR-Ms with both phagocytosis abilities and polarization functions would contribute to fight against BTs [98]. Researchers engineered induced pluripotent stem cell-derived macrophages (iMACs) with toll-like receptor 4 intracellular toll/IL-1R (TIR) domain-containing CARs [99]. The design of a tandem CD3ζ-TIR dual signaling CAR endows iMACs with both target engulfment capacity and antigen-dependent M1 polarization and M2 resistance in a nuclear factor kappa B (NF-κB)-dependent manner, as well as the capacity to modulate the tumor micro-environment. The second-generation CAR-iMACs demonstrated superior antitumor functions, including orthogonal phagocytosis and polarization, resulting in a markedly enhanced antitumor effect over first-generation CAR-Ms [9, 99, 100].

Clinical Studies of CAR-M Therapy

The accumulating evidence attesting to the efficacy, security, and feasibility of CAR-M therapy is intensifying the enthusiasm for launching a clinical trial of this treatment. Despite the safety and efficacy of CAR-M therapy has been validated in animal experiments, clinical trials are crucial for assessing the safety and efficacy [101]. To date, a few clinical trials of CAR-M therapy are conducted and registered on clinicaltrials.gov. Currently, only three clinical trials associated with CAR-M therapy are in progress, and two clinical studies of CAR-monocyte therapy are also underway.

CT-0508 was developed by Carisma Therapeutics, the first CAR-M approved for clinical trials (NCT04660929). CT-0508 utilized Ad5f35-transduced PBMC-Macs to express HER2-targeted CARs and was administrated to 18 patients with relapsed or refractory tumors overexpressing HER2. Preliminary data reveals that CT-0508 is generally safe and well-tolerated with no dose-related toxicities. In

addition to monotherapy, the combined treatment of CT-0508 and Pembrolizumab has been examined for the possible supplementary impacts in the context of the clinical trial [102]. However, given the decision to prioritize CT-0525 as the product candidate in its HER2 program, Carisma has ceased further development of CT-0508 and new patients will no longer be enrolled in the Phase 1 clinical trial.

MAC-001, a CAR-M therapy developed by Macera Therapeutics, obtained approval to commence a phase 1 exploratory clinical trial targeting patients with advanced HER2-positive gastric cancer (NCT06224738). MAC-001 used an adenoviral vector system to genetically engineer autologous PBMC-Macs to express CAR molecules targeting HER2-positive tumors. Currently, the safety and feasibility of MAC-001 are being assessed in the ongoing clinical trial. Another clinical trial of CAR-M was an observational cohort study to determine the antitumor activity of CAR-Ms in breast cancer patients' derived organoids at different clinical stages. (NCT05007379).

Monocytes are precursor cells of macrophages with *in vivo* persistence and are also capable of differentiating into pro-inflammatory CAR-Ms with multimodal anti-tumor mechanisms of action. As research into CAR-Ms progresses, it is becoming increasingly evident that monocytes have the potential to play a significant role in CAR-M therapy.

Following the cessation of CT-0508 development, Carisma Therapeutics redirected the efforts towards CT-0525, an *ex vivo* gene-modified autologous CAR-Monocyte cellular therapy intended to treat solid tumors that overexpress HER2 metastasis. The Phase 1 study for CT-0525 is designed to assess the safety, tolerability, and the manufacturing feasibility of CT-0525, expected to conclude in Match 2026 (NCT06254807).

MCY-M11 developed by MaxCyte, in which mRNA transduction is performed in PBMCs to express CAR-targeted mesothelin, is designed to treat recurrent/refractory ovarian cancer and peritoneal mesothelioma patients (NCT03608618). The production of MCY-M11 exploited MaxCyte's Flow Electroporation® technology bypassing viral components or cell expansion. Promising results from single-round administrations of MCY-M11 are motivating, however, the final results have not been published yet [103].

MT-101 is an mRNA-engineered CAR-M therapy developed by Myeloid Therapeutics to treat patients with relapsed or refractory, CD5-positive peripheral T-cell lymphoma (PTCL). The safety, tolerability, and efficacy of MT-101 are being assessed in the ongoing Phase 1/2 clinical trial, expected to conclude in October 2024 (NCT05138458).

In contrast to the majority of existing CAR-M therapies, which are derived from fully developed immune cells, CAR-iMAC is generated from induced pluripotent stem cells that have been modified with CAR molecules and subsequently transformed into specialized macrophages (iPSCs) [97]. SY001, an iPSC-derived CAR-Ms therapy developed by Cell Origin, is undergoing a single center, single-arm, dose-escalation, exploratory clinical trial to examine the safety, tolerability, pharmacokinetics and preliminary efficacy of SY001 from Cell Origin Biotechnology in patients with advanced solid tumors [104].

MECHANISMS OF ACTION OF CAR MARCROPHEGES IN BRAIN TUMOR TREATMENT

Target Antigens and Specificity of CAR-M Therapy

In BTs treatment, numerous tumor antigens have been identified and studied for the potential utilization in CAR-based immunotherapies. Here, we discuss the targets that have been explored in CAR-M therapy.

Erb-b2 Receptor Tyrosine Kinase 2 (HER2)

HER2 is a promising target for the treatment of BTs as the high abundance in BTs and low expression in brain tissues [105]. In a study of CAR-T cell therapy on 16 patients with progressive GBM (NCT01109095), the safety of HER2-targeted CAR cell therapy was instituted. Though the clinical trial demonstrated an acceptable safety profile, the therapeutic efficacy fell short of the desired level of efficacy, highlighting the need for further improvements in enhancing the functionality, persistence, and expansion capabilities.

Table 2. Clinical Studies of CAR Macrophages

Product	Target	Indication	Type of CAR-M	Phase	Country
CT-0508	Her2	Her2 overexpressing solid tumors	PBMC-Mac	1	USA
MAC-001	Her2	Her2-positive advanced gastric cancer	PBMC-Mac	Early 1	China
CT-0525	Her2	Her2 overexpressing solid tumors	PBMC	1	USA
MCY-M11	Mesothelin	Ovary cancer and peritoneal mesothelioma	PBMC	1	USA
MT-101	CD5	CD5+T-cell lymphomas	Myeloid cell	1/2	USA

To take advantage of macrophages, CAR-Ms have been developed to specifically target HER2 and have been tested in preclinical models. CAR-M therapy had efficient tumor cell killing capacity and improved survival rates [48]. To date, several CAR-Ms or CAR-monocytes targeting HER2 has been developed to treat HER2-positive solid tumors which are currently in the recruitment phase of clinical trials.

Epidermal Growth Factor Receptor (EGFR)

EGFR is one of the most commonly-mutated oncogenic sites in IDH-WT GBM. Approximately 50% of GBM multiforme samples exhibit mutations in their EGFR gene, with EGFRvIII (deletion of exons) being the most common mutation [106]. As a potential marker for therapeutic treatment, several EGFR-targeted therapies have been investigated, including small molecule inhibitors such as gefitinib and dacomitinib, as well as antibodies, vaccines, CAR-T, and other approaches limit the number of EGFRs [107-113]. However, these inhibitors have not been successful in patients suffering BTs with EGFR amplification (NCT01520870, NCT02447419), [107, 108] possibly due to the blood–brain barrier. Therefore, researchers have proposed another potential therapeutic strategy utilizing CAR-Ms, which have high infiltration in BTs, that focuses on targeting EGFRvIII to suppress the growth of GBM and improve treatment efficacy.

Interleukin-13 Receptor Alpha 2 (IL-13Rα2)

IL-13Rα2 is specifically overexpressed in the majority of BTs (>60%) with a significantly lower expression observed in brain tissue [114-116]. Clinically, IL-13Rα2 expression has been closely associated with the mesenchymal subtype of GBM, which has been identified as a prognostic indicator of lower patient survival rates [117]. The therapeutic efficacy of IL-13Rα2 as a therapeutic target for BTs has been demonstrated in clinical trials [118, 119]. A study by Brown and colleagues demonstrated the potential of multidose treatment with IL-13Rα2-CAR T cells, which induced complete tumor regression in 8 months in a patient with disseminated GBM (NCT02208362) [119]. These findings about IL-13Rα2 indicate that it could be a promising target of CAR-M therapy for BTs.

Mesothelin (MSLN)

MSLN is named for the expression in mesothelin cells, overexpressed in a variety of malignancies including lung adenocarcinomas and some other squamous carcinomas [120, 121]. CAR-T therapy targeting MSLN has been successively developed and applied showing great tumor elimination ability against cancers that highly express MSL [122, 123]. Phase 1 clinical trial is now underway to evaluate the safety and feasibility of CAR-M therapy for targeting MSLN (NCT03608618). MSLN also represents a promising potential target for the treatment of brain metastases.

Others

Human B7-H3, encoded by *CD276* is overexpressed in many types of cancers, including GBM, and has been associated with tumor aggressiveness and poor prognosis [124-127]. B7-H3 can act as an immune checkpoint that promotes tumor immune escape [128, 129]. B7-H3 is not only highly expressed in most types of solid tumors, but is also present in the vessels and fibroblasts within tumors, [130] implying that CAR-Ms directed against B7-H3 may be able to eliminate tumor cells through direct targeting, disrupt the stroma, and even inhibit angiogenesis. The findings suggest that B7-H3 could be a promising target of CAR-M therapy for BTs.

Glypican-1 (GPC-1) is notably overexpressed in GBM, contrasting its low expression in healthy tissues including liver, pancreas, cervix, esophagus, and brain [131-135]. GPC-1 is pivotal in regulating pathways related to tumor growth, angiogenesis, and invasion, highlighting its potential as a surface biomarker for developing targeted therapeutic and diagnostic agents [136].

Disialoganglioside 2 (GD2) has been identified as a marker of many malignancies and is specifically overexpressed in BTs, especially CSCs [137, 138]. Animal models and clinical trials have confirmed the potential of CAR T cell therapy targeting GD2 as safe treatments for GBM [137, 139, 140]. These findings indicate that GD2 is a potential therapeutic target for CAR-M therapy in BTs.

Prominin-1 (CD133) is a marker of CSCs in several human cancers, including GBM [141]. Disabling the CD133 subpopulation, regardless of the expression level, has been shown to inhibit tumor growth and provide a survival benefit for preclinical models [142]. Recently, investigators achieved successful *in situ* transfection of CARs targeting GBM CD133 into TAMs, leading to enhanced M1-like polarization and the inhibition of postoperative GBM recurrence. These findings suggest that CAR-Ms targeting for CD133 may hold promise as a potential treatment for BTs [80].

Although only a few of the potential targets for CAR-M therapy in BTs have been scientifically validated, their specific expression profile in BTs and application in CAR-T therapies demonstrates their potential to be exploited for CAR-M therapy in terms of BTs.

Immunomodulatory Effects of CAR-Macrophages in the Brain Tumor Microenvironment

CAR-Ms are genetically engineered immune cells that express CARs on their surface, which allows them to specifically recognize and target tumor cells. In the context of the tumor microenvironment, CAR-Ms can exert various immunomodulatory effects, including the following (Figure 4D):

Phagocytosis of Tumor Cells: CAR-Ms can recognize and bind to specific tumor-associated antigens on tumor cells, leading to their engulfment and destruction. Such phagocytic activity helps to clear tumor cells and reduce tumor burden [48, 78, 80, 143].

Cytokine Production: Upon activation, CAR-Ms can produce and secrete a range of cytokines that modulate the immune response. For example, proinflammatory cytokines such as interleukin-12 (IL-12) and IFN-γ can promote antitumor immunity, which can be suppressed by anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta (TGF-β) [48, 78, 80, 143].

Activation of Anti-tumor Immunity: CAR-Ms have been shown to play a key role in the immune response to BTs, not only by actively seeking out and destroying tumor cells through direct phagocytosis but also by recruiting and activating other immune cells to the tumor site. These immune cells, including T cells, NK cells and dendritic cells, assist in improving overall tumor clearance. Additionally, CAR-Ms can induce adaptive antitumor immunity by processing and presenting tumor antigens to T cells, leading to the activation of tumor-specific cytotoxic T lymphocytes (CTLs), which improves tumor clearance [78, 143].

Reprogramming of the TME: BTs are known for highly immunosuppressive microenvironment, which can hinder the effectiveness of immune-based therapies. CAR-Ms can help counteract such immunosuppression by producing factors that reprogram the microenvironment, converting it into a more immunostimulatory state, including reducing the amount of immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs) and Tregs, as well as downregulating the expression of immune checkpoint molecules such as PD-L1 [48, 78, 80].

With the immunomodulatory effect of CAR-Ms in the tumor microenvironment, CAR-Ms have potential to overcome the immunosuppressive barriers associated with these types of cancer and enhance the natural defense mechanisms of human body against tumor cells. However, further research and clinical trials are needed to optimize the design and delivery of CAR-Ms for BTs treatment as well as to fully understand their safety and efficacy profiles.

STRATEGIES FOR OPTIMIZING CAR-M THERAPHY IN BRAIN TUMOR TREATMENT

Target Selection

Identifying and validating the specific targets expressed in tumor cells will decrease off-target effects, increase specificity, leading to effective target selection for BTs treatment [144]. Bioinformatics tools are capable of analyzing large datasets in order to identify potential tumor-specific antigens and to predict their immunogenicity. Single-cell RNA sequencing offers insights into the tumor microenvironment, elucidating heterogeneity and identifying distinctive cell populations that can be targeted. The multi-target strategy has the potential to enhance therapeutic efficacy by addressing the challenges of tumor heterogeneity and antigenic escape [145, 146]. A combination therapy targeting IL13Rα2 and HER2 by bispecific CAR-T cells co-expressing IL13Rα2 and HER2 CAR molecules has been shown to have significant potential for the elimination of tumor cells [147]. By combining these technologies, off-target effects can be minimized and the efficacy of CAR-M therapies can be improved.

CAR Design

Optimization of CAR design will enhance binding affinity, specificity, and signaling capacity [148]. Costimulatory domains may also be incorporated to improve the activation and effector function of CAR-Ms [149, 150]. The design of first-generation CAR-Ms draws upon the established framework of CAR-T cells, which nevertheless requires further refinement and innovation to facilitate enhanced CAR-M therapy. The therapeutic efficacy of CAR-M can be markedly enhanced by incorporating the intracellular signaling structural domains of TLR4 or IFN-γ into the CAR framework [151]. The discovery of new CAR domains or signal pathway is necessary for the development of novel CAR-M therapy. Artificial Intelligence (AI) algorithms, which can integrate multi-omics data to predict the most effective constructs and optimize CAR design, may advance CAR-M therapy.

Combination Therapy

The optimal design of CAR-M therapy is intended to facilitate macrophages to phagocytose tumor cells in the maximum extent, with the objective of eradicating the tumor. Nevertheless, the capacity of macrophages to phagocytize is markedly constrained

by the phagocytosis checkpoint and immune checkpoint [152, 153]. Consequently, the combined use of immune checkpoint inhibitors and phagocytic checkpoint inhibitors greatly enhanced *in vivo* macrophage phagocytosis and suppressed tumor development [154, 155]. In addition, macrophage has the potential to promote T cell activation and potential advantages in infiltrating to BTs, while T cells has limited infiltrating ability into the dense extracellular matrix of tumor [156]. It was demonstrated that CAR-M and CAR-T cells exhibited a more powerful antitumor reaction compared to each treatment individually. The inflammatory factors secreted by CAR-T cells augment the cytotoxicity of CAR-Ms by inducing M1 polarization and increase the expression of costimulatory ligand, that may promote the fitness and activation of CAR-T cells in turn [61]. Through these approaches, the effectiveness of CAR-M therapy can be improved, ultimately leading to improved patient outcomes.

Personalized Therapies and Monitoring

CAR-M therapy can be adapted to the specific characteristics of an individual tumor by developing CARs that target antigens expressed on the tumor cells. Patient-specific CAR-M therapy can be designed based on the distinctive tumor molecular subtypes of each patient. Methods for the non-invasive monitoring of CAR-M therapy, such as imaging technique, can be used to assess treatment efficacy, detect relapse, and guide potential modifications to the therapy [157, 158]. Imaging techniques can be used to track the distribution and activity of CAR-Ms *in vivo* [159]. Regular monitoring of biomarkers can help assess the effectiveness of CAR-M therapy and detect any adverse effects early on [160]. Monitoring the immune response can provide insights into the therapy impact on the immune system and help in managing potential side effects [161]. Additionally, the role of AI and Machine Learning (ML) in predicting effectiveness and response could offer a novel perspective on advancing personalized medicine in BTs treatment.

These strategies have the potential to significantly improve patient outcomes, but further research is needed to ensure the safe and effective implementation in clinical practice. The development of new strategies to enhance the safety and efficacy of CAR-M therapy for BTs treatment is an important step towards improving patient outcomes. However, it is important to note that many of these strategies are still at the preclinical or early clinical stages of development. It is required to ensure in more research that these strategies can be used safely and effectively in clinical practice.

THE CHALLENGES FACING CAR-M THERAPHY

The Challenges of Administration and Biodistribution

Despite the considerable potential of CAR-M as a powerful cancer immunotherapy, numerous challenges must be addressed to achieve the desired outcomes. The most significant limitation is the number of cells that can be obtained [9, 25]. CAR-Ms exhibit minimal proliferation after injection *in vivo*, which may impact the efficacy of the treatment [162]. Currently, the predominant approach for CAR cell therapy is peripheral intravenous infusion [163]. When CAR-Ms are administered intravenously at a restricted dose, the number of infiltrated macrophages in BTs is not the full extent of the injected [164]. In contrast to CAR-T cells, CAR-Ms, despite the capacity for substantial immersion to the tumor microenvironment, potentially due to larger size or *in vivo* migratory characteristics, tend to accumulate in the lung, liver, and kidney, which may influence the effectiveness of treatment [48, 78]. It was observed that the administration of CAR-Ms via peritoneal injection resulted in an enrichment of CAR-Ms in the intra-abdominal tumor tissue, implying that intertumoral or subarachnoid administration may be a better administration way [163].

The Challenges of Clinical Translation

The production of CAR-M involves complex processes, including the isolation, genetic modification, and expansion of macrophages. The high cost and complexity of manufacturing remain the primary challenges to the clinical application of current CAR-based immunotherapies [165]. Efforts to control costs are focused on the refinement of manufacturing techniques to improve the efficiency of the cell expansion, which could lead to a significant reduction in costs. It has been demonstrated that iPSC-derived macrophages facilitate the scalable production of therapeutic cells, which may result in a significant reduction in costs. Nevertheless, the technical challenges associated with the induction of functional and competent CAR-Ms from iPSCs remain significant [166]. The *in vivo* editing technology can circumvent the extraction and expansion production cycle of CAR-Ms, representing a promising avenue of research and a crucial area of development for CAR-M therapy in the future [167]. Several companies, including Carisma Therapeutics, are engaged in the development of CAR-M *in vivo* editing, but none have yet reached the clinical trial stage. Further research is required to enhance and

standardize the manufacturing protocol for the production of clinical-grade products [168].

Furthermore, CAR-M therapy must undergo rigorous preclinical and clinical trials to obtain regulatory approval. This includes demonstrating safety, efficacy, and quality control in accordance with guidelines set by regulatory agencies. The regulatory path for CAR-T cells has set a precedent that emphasizes stringent evaluation of safety and efficacy. However, the patient safety and therapeutic outcomes may be different implications cause of multifunctional role in immune modulation [169]. The long-term effects of CAR-Ms are particularly controversial, given the potential to profoundly alter the dynamics of the immune system [170]. The tailored regulatory approaches are necessary, considering the unique biological behaviors of macrophages and their interaction with the TME. The challenge for regulators is to develop guidelines that adequately address these concerns [171].

POTENTIAL FOCUS OF FUTURE DEVELOPMENTS IN CAR-M THERAPHY

Despite recent advancements, CAR-M therapy remains in infancy and requires further development to become the next generation of immunotherapy in clinical. *In situ* gene editing for CAR-Ms, mRNA-based CAR gene engineering and the combination with other immunotherapies will be the emerging strategy for the next generation of CAR-Ms, which have already been discussed extensively. Here, we examine two additional potential focus of CAR-M therapy in BTs treatment.

Microglia in CAR-M Therapy

Microglia, arising from progenitor cells in the embryonic yolk sac, are resident phagocytes in the brain parenchyma with highly efficient phagocytic abilities. Microglia are involved in maintaining brain homeostasis, responding to injury, and modulating immune responses within the brain [172]. In the context of BTs, microglia are often the dominant macrophage population and can significantly influence tumor progression and the immune landscape. The tumor environment confers microglia with an immunosuppressive phenotype with weaker phagocytic capacity, antigen presentation and T cell-stimulatory function, which supports the tumor growth [173]. As with CAR-M therapy, CAR editing may potentially be able to reverse the tumor-promoting phenotype of microglia and exert an anti-tumor effect on BTs. Especially, multiple ways have been demonstrated to differentiate iPSCs into microglia [174]. As the most abundant immune cell with intrinsic properties and functional population in the brain, the potential of microglia for use in CAR-M therapy in the treatment of BTs is considerable.

The Role of Imaging in CAR-M Therapy

Leveraging imaging modalities within CAR-M therapy holds immense promise in enhancing our comprehension of therapy efficacy and limitations. As discussed above, CAR-M therapy has achieved remarkable success in the treatment of BTs, but its application still faces challenges. Leveraging imaging modalities within CAR-Ms therapy holds immense promise in enhancing our comprehension of therapy efficacy and limitations [157]. Moreover, imaging facilitates the assessment of therapeutic responses and the identification of potential obstacles impeding CAR-M efficacy, such as tumor heterogeneity, immune evasion mechanisms, and off-target effects [158]. By elucidating the interplay between CAR-Ms and the complex tumor milieu, imaging empowers clinicians and researchers to tailor interventions, mitigate risks, and maximize therapeutic outcomes. Incorporating advanced imaging technologies into CAR-M therapy not only enhances the ability to monitor treatment efficacy but also fosters a deeper understanding of the underlying biological mechanisms governing CAR-M function.

Cellular and Molecular Imaging *in vitro*

In vitro cellular and molecular imaging plays a pivotal role in elucidating the intricate interactions between Chimeric Antigen Receptor (CAR) cells and tumor cells, offering invaluable insights for the development of future immunotherapies. Utilizing fluorescent proteins and their derivative biosensors has emerged as a powerful approach in this pursuit.

Fluorescent proteins, along with live-cell staining dyes and fluorescent-conjugated antibody labeling, enable the visualization of both tumor cells and CAR-Ms in real-time, facilitating the assessment of CAR therapy's antitumor efficacy [175]. By tracking the expression and spatial distribution of specific signaling molecules at the subcellular level, fluorescent proteins encoded by genes provide a means to decipher the determinants and regulatory factors governing CAR cell function [176].

These sophisticated imaging tools have proven instrumental in unraveling the molecular mechanisms underlying CAR cell activation upon encountering tumor cells. By visualizing dynamic cellular processes and signaling events, researchers could gain critical insights into the intricacies of CAR macrophage-tumor cell interactions, thereby informing the refinement of CAR therapy strategies.

Imaging *in vivo*

The efficacy of the CAR-M therapy relies on the successful trafficking of CAR-Ms to the designated targets within BTs. Leveraging imaging-based strategies enables real-time tracking and visualization of CAR-Ms, offering invaluable insights into treatment outcomes. Assessment of *in vivo* process through imaging holds the potential to predict the success or failure of the treatment [159]. Utilizing Bioluminescence Imaging (BLI) as a foundation, tumor imaging can be augmented with various imaging modalities including Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), or Photoacoustic Imaging. This amalgamation of techniques allows for precise tracking of CAR-M distribution, providing a comprehensive view of cell localization, functionality, and interactions within the BTs microenvironment. The integration of these diverse imaging methodologies allows researchers to delve into the intricate dynamics of immune responses, thereby refining therapeutic strategies for enhanced cancer treatment outcomes [158, 177]. Furthermore, imaging serves a crucial role in diagnosing and monitoring toxicities or adverse effects associated with CAR-M therapy. In particular, imaging is instrumental in assessing the clinical implications of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) toxicity subsequent to CAR therapy, which PET/CT playing a central role [178]. Imaging-based approaches offer invaluable insights into the dynamics of CAR-M therapy, enabling real-time assessment of treatment efficacy, localization of CAR-Ms, and detection of potential adverse effects. By providing a deeper understanding of treatment mechanisms and outcomes, imaging contributes significantly to the refinement and optimization of CAR-M therapy for enhanced cancer treatment.

OUTLOOK

The prospect for CAR-M therapy in the treatment of BTs is promising yet multifaceted. While preclinical studies have unveiled the potential of CAR-Ms in targeting tumor cells and eliciting robust anti-tumor immune responses, several challenges lie ahead on the path to clinical translation. Addressing the complexity of the tumor microenvironment, optimizing CAR-M design and engineering, navigating regulatory pathways, and conducting rigorous clinical trials are pivotal steps in advancing CAR-M therapy towards clinical application. Collaborative interdisciplinary approaches, leveraging expertise from diverse fields, will be instrumental in overcoming these challenges and refining therapeutic strategies.

As research endeavors persist in delving deeper into the complexities of CAR-M therapy, it becomes increasingly evident that interdisciplinary collaborations and rigorous clinical validation are indispensable. These collaborative efforts, drawing upon the collective expertise of researchers across various disciplines, are essential for unlocking the full potential of CAR-M therapy as a transformative modality in the field of neuro-oncology.

By fostering synergistic partnerships between immunologists, oncologists, bioengineers, and regulatory experts, interdisciplinary collaborations enable the convergence of diverse perspectives and methodologies. This interdisciplinary approach facilitates a comprehensive understanding of CAR-M therapy, from its molecular mechanisms to its clinical application, thereby accelerating its translation into effective treatments for BTs.

Furthermore, clinical validation plays a pivotal role in bridging the gap between preclinical promise and clinical reality. Rigorous clinical trials, guided by robust scientific evidence and conducted in collaboration with healthcare professionals and regulatory authorities, are essential for assessing the safety, efficacy, and long-term outcomes of CAR-M therapy in patients with BTs.

In conclusion, although formidable challenges persist, the collective efforts of researchers, clinicians, and stakeholders in the field of CAR-M therapy offer a beacon of hope for revolutionizing the treatment landscape of BTs. With continued dedication to research, innovation, and clinical validation, CAR-M therapy holds the promise of ushering in a new era of precision medicine, providing renewed hope and improved outcomes for patients confronting this devastating disease.

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Author contributions

F.F., Y.Z. and S.N. conceived the subject. Q.Q. and Y.Z. devised proof outline. F.F. and J.S. drafted the original draft. Q.Q., Y.Z. and S.N. reviewed the manuscript. F.F. and Y.Z. revised and edited the manuscript. J.S. revised and edited the figures.

Competing Interests

The authors have declared that no competing interest exists.

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