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■? ARTICLE

The Future of Medicine: CAR-T Cell Therapy, CRISPR, And Gene Editing for Glioblastoma

Akhil Bollu

Independence High School, 23523 Bentley Grove Place, Ashburn, VA, 20148, USA; Akhilbollu09@gmail.com

ABSTRACT: Glioblastoma Multiforme is the most aggressive and fatal malignant brain cancer. Despite conventional treatment (surgery, radiation, and chemotherapy), the median survival rate remains less than 18 months. Conventional therapies have significant limitations due to GBM's inherent heterogeneity and its micro-environment's immunosuppression; therefore, new, more durable, targeted therapeutic approaches need to be developed to increase patient survival. Current evidence indicates that utilizing CRISPR-Cas9 gene editing in combination with chimeric antigen receptor (CAR)-T cell therapy will provide a new approach to overcoming several of the current barriers to effective GBM immunotherapy. CAR-T cells have been developed using various GBM-specific antigens (EGFRvIII, IL13Rα2, HER2, GD2, and EphA2). CAR-T cells directed at these antigens have demonstrated some level of clinical response as well as acceptable safety profiles, but generally lack long-term efficacy. Utilizing CRISPR-engineered CAR-T cells that incorporate deletion of inhibitory receptors (such as PD-1 and TIGIT) and integrate stable CAR constructs demonstrates increased persistence, immune activation, and tumor killing capabilities in all pre-clinical models evaluated. Therefore, integrating CRISPR-based genetic modification with CAR-T cell therapy has the potential to develop more personalized and durable immunotherapies for GBM, which could revolutionize the treatment of what is currently considered one of the most treatment-resistant malignancies.

KEYWORDS: Disease Treatment and Therapies, Glioblastoma, CAR-T Cells, CRISPR-Cas9, Immunotherapy.

■ Introduction

Glioblastoma multiforme (GBM) remains one of the most aggressive and treatment-resistant primary brain tumors, with median survival rarely exceeding 18 months despite maximal surgical resection, radiotherapy, and temozolomide chemotherapy.[1] Current therapeutic strategies are largely palliative, failing to eradicate infiltrative tumor cells or prevent recurrence due to GBM's high genetic heterogeneity, antigenic variability, and profoundly immunosuppressive microenvironment.[2] Immunotherapies such as checkpoint inhibitors and cancer vaccines have demonstrated limited efficacy in GBM, primarily because the blood–brain barrier, as mentioned in Table 2, restricts immune cell access, and the tumor's adaptive resistance mechanisms enable immune evasion and limit treatment options.[3,4] Consequently, there is an urgent need for therapeutic approaches that can specifically and effectively target tumor-associated antigens while overcoming immune suppression within the central nervous system.[5]

Immunotherapy has been proposed as a new generation of treatments for GBM that can overcome the limitations of traditional treatments. Immunotherapy involves using genetically engineered T lymphocytes that express chimeric antigen receptors (CAR).[6,7] CAR-T cells are engineered to specifically target and kill tumor cells that express particular antigens such as EGFRvIII, IL13Rα2, HER2, GD2, and EphA2.[8,9] Clinical trials have shown that CAR-T therapy can be safely administered to patients with GBM and, in some cases, cause measurable reductions in tumor size.[9,10] However, because of the heterogeneity of tumor antigens expressed by GBM cells, the potential for tumor cells to develop mechanisms to avoid destruction by the CAR-T cells, and the immunosuppressive environment of the tumor, CAR-T cell-mediated anti-tumor effects are usually transient and do not lead to durable therapeutic benefits.[1,3,11]

In some Preclinical studies, scientists have used the CRISPR/Cas9 gene-editing system to prolong the duration of CAR-T cell activity, more effectively recognize tumor antigens, be more resistant to immune suppression, and persist longer in the body, suggesting a possibility of longer-lasting and more effective anti-tumor effects with less toxicity.[12,13]

Considering the challenges of treating GBM with current therapies and the promise of using genetic engineering to create more effective and targeted treatments, the integration of the CRISPR system into CAR-T cell therapy is a timely and promising method of addressing many of the challenges associated with treating GBM. Therefore, the purpose of this study is to integrate the knowledge currently available regarding CAR-T and CRISPR-based therapies, assess the translational implications of these therapies, and explore the possible combination of these two therapies to provide new and innovative treatment options for glioblastoma, a cancer with one of the worst prognoses in human medicine. This literature review brings together many of the latest clinical trials of CAR-T cell therapy. Most of them include phase 1 trials that aim to develop long-lasting, individually designed treatments for Patients with Glioblastoma and are significant contributions to Translational Medicine and Cancer Immunotherapy.

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Methods

This study employed a narrative literature review design rather than an experimental or in-field study. The literature review method was chosen to synthesize information from previously published peer-reviewed research, clinical trial data, and institutional reports. No laboratory experiments, surveys, or human subjects were directly involved. The study did not require any ethical clearance, as it involved secondary data sources exclusively.

Relevant studies were identified and collected from credible online databases between 2010 and 2025. Databases searched included PubMed, Google Scholar, ClinicalTrials.gov, and the National Institutes of Health (NIH) repositories. The key search terms used were: "glioblastoma," "standard treatment," "CAR-T therapy," "CRISPR-Cas9," "GBM tumor antigens," and "immunotherapy in GBM." Studies were filtered to include those that provided data on treatment mechanisms, therapeutic efficacy, and translational relevance to clinical applications.

Primary sources for this review included landmark clinical and epidemiological studies, such as the Stupp regimen trial on chemoradiation in GBM and the CBTRUS report on glioblastoma incidence and survival data. Clinical trial data from ClinicalTrials.gov focusing on CAR-T therapies against EGFRvIII, IL13Rα2, HER2, GD2, and EphA2 antigens were also reviewed. Secondary sources included translational and molecular studies that explored the role of CRISPR-Cas9 in enhancing CAR-T cell safety, persistence, and specificity.

All selected studies were reviewed for methodological quality, relevance, and results. Data on therapeutic efficacy, antigen targets, clinical outcomes, and genetic editing strategies were categorized into thematic areas-namely: glioblastoma biology and standard treatments, CAR-T therapy design and results, CRISPR-based modifications, and clinical translation and challenges. Findings were compared and summarized to highlight the collective progress and limitations within the field.[8,14,15]

No physical tools, laboratory materials, or experimental equipment were used in this study. All information was obtained through online academic and clinical databases. Since this research relied solely on existing published literature and publicly available data, no ethical review or participant consent was required.

Prevalence and Epidemiological Profile of Glioblastoma:

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults. Globally, it affects about 3–5 people per 100,000 per year, making it a relatively rare cancer, but the most frequent among aggressive brain tumors. It represents about 15% of all brain tumors and nearly 50% of malignant gliomas. Men are slightly more likely to develop GBM than women. Most cases are diagnosed in people aged 45–70, though it can occur at younger ages as well.[14]

GBM is a WHO Grade IV glioma with necrosis, microvascular proliferation, and several genetic alterations, including TERT promoter mutation (a genetic alteration that increases telomerase expression, enabling tumor cells to maintain telomere length and achieve uncontrolled growth and survival),[16] EGFR amplification (an abnormal increase in the number of copies of the EGFR gene), which drives uncontrolled cell signaling, proliferation, and tumor growth),[17] chromosome 7 gain/10 loss (+7/–10), *and alterations in* critical pathways are RTK/RAS/PI3K, p53, and RB. GBM is strongly resistant to conventional therapy because of heterogeneity of the tumor and an immunosuppressive tumor microenvironment, hence the justification for targeted immunotherapy such as CAR-T.[17,18]

Genetic Mutations :

Many genetic mutations cause aggressive behavior in GBM.

TERT promoter mutations: Increasing the TERT activity due to mutations of the TERT promoter allows cancer cells to divide forever. Cancer cells become capable of dividing continually because the mutations increase telomerase activity of an enzyme called telomerase, which adds repetitive DNA sequences to the telomere's chromosome ends and keeps them from shortening. Telomerase prevents the normal process of aging of cells by preserving their telomere lengths, thereby allowing cancer cells to divide infinitely, beyond the bounds of typical cell division.[15]

EGFR mutations or amplifications: cause increased cell proliferation and survival, thus causing tumors to grow more quickly.

In many cases of GBM, the EGFR gene is amplified or mutated. For example, a particular type of EGFR mutation, EGFRvIII, causes a truncated receptor that signals continually without the need for binding with an EGF ligand, promoting uncontrolled cell proliferation, angiogenesis, and cell survival.[8]

Abnormalities of Chromosomes (+7/-10): Both gain of chromosome 7 and loss of chromosome 10 occur commonly in GBMs, and both are involved in malignancy.[19]

Loss of chromosome 10 and gain of chromosome 7 are common characteristics of GBM. Loss of chromosome 10 removes tumor-suppressing genes (e.g., PTEN) while gaining chromosome 7 adds oncogenes (e.g., EGFR), both of which result in the shift toward cancer.[23]

Disrupted signaling pathways: Many of the major signaling pathways involved in controlling cell growth and cell death, including the RTK/RAS/PI3K, p53, and RB pathways, are frequently altered in GBM, resulting in uncontrolled growth and failure to undergo programmed cell death.[20]

RTK/RAS/PI3K pathway: Activation of the RTK/RAS/PI3K pathway through mutations of EGFR or PDGFRA results in activation of pathways that promote cell survival and proliferation.[20,21]

p53 pathway: TP53 normally regulates cell death and DNA repair; when it is mutated, the ability of damaged cells to survive is enabled.[21]

RB pathway: The absence of Rb1 removes a critical regulatory point in the RB pathway that controls the cell cycle and allows for unregulated cell division.[19]

Other mutations: Other mutations, such as those in PTEN or IDH1/2, may influence prognosis and responsiveness to chemotherapy.

PTEN deletion: Inactivates a key inhibitor of the PI3K/AKT pathway, thus enhancing tumor growth and treatment resistance.

IDH1/2 mutations: IDH1/2 mutations are seen in approximately 50% of secondary GBMs, but less than 5% of primary GBMs. Secondary GBM patients who have these mutations may have a better prognosis and may respond to some types of chemotherapy.[22] All of these genetic alterations contribute to the uncontrolled growth of GBM and are the reason that targeted therapies such as CAR-T cells are of interest.

Standard Treatments for GBM:

Glioblastoma (GBM) is highly aggressive and typically involves refractoriness to conventional treatment, requiring multimodal therapy:

Surgery: Cytoreduction by maximal safe resection removes much of the tumor and improves survival. Studies have shown that a maximal safe resection can significantly extend the median survival for certain patients. For example, some cohorts have shown an average survival of 37.3 months for patients who underwent aggressive resection, compared to 16.5 months for those with more modest resections.[23]

Radiation + Temozolomide (Stupp Regimen): Studies show that the combination of radiation and temozolomide will give you a much better chance of survival than either treatment alone. The results of a major study by EORTC (European Organization for Research and Treatment of Cancer) and NCIC (National Clinical Trials Group).[24] This showed that the median overall survival time with this combination was 14.6 months and 12.1 months when using radiation alone. Also, the researchers found that patients who received the combination therapy were twice as likely to be alive after two years (26.5%) as those receiving radiation alone (10.4%). Additionally, studies indicate that if your tumor has the MGMT promoter methylation biomarker, there may be a better response to temozolomide chemotherapy; therefore, identifying this biomarker could provide useful information regarding which chemotherapy to use.[25]

Tumor Treating Fields (TTFs): There is evidence indicating that the addition of TTFs to the Stupp regimen resulted in a statistically significant increase in survival time. The EF-14 study demonstrated that adding TTFs to the Stupp regimen increased the median overall survival time from 16.0 months to 20.9 months. Additionally, the study found that the addition of TTFs to the Stupp regimen resulted in significant improvements in long-term survival rates. Specifically, the two-year survival rate was increased from 30% to 43%, and the five-year survival rate was increased from 5% to 13%.[26]

Bevacizumab: A VEGF-targeting therapy that manages symptoms and edema. For newly diagnosed glioblastoma, two large Phase 3 trials showed that bevacizumab did not improve overall survival when added to standard therapy.[27,28] However, in patients with recurrent glioblastoma, it is a standard treatment that has demonstrated a significant effect on progression-free survival and response rates. For these patients, clinical data have shown a 6-month progression-free survival rate of approximately 42.6%.[22,28]

These therapies, in general, are capable of managing tumor growth and symptoms temporarily, though GBM is still largely an incurable disease overall; targeted treatments like CAR-T cell therapy are needed.

Table 1: Summary of Standard Treatments for GBM.

Treatment	Mechanism of Action	Survival / Response Benefit	Notes/Limitations
Surgery (maximal resection)	Physical removal of as much tumor mass as safely possible.	Median survival up to 37.3 mo vs. 16.5 mo.[23]	Improves outcomes but cannot remove all infiltrative cells; tumor recurrence is common.
Radiation + Temozolomide (Stupp)	RT damages DNA; Temozolomide alkylates DNA, triggering apoptosis in tumor cells.	Median OS 14.6 mo vs. 12.1 mo with RT alone; 2-yr OS 26.5%. [24]	Standard of care; effectiveness is higher with MGMT methylation; resistance often develops
Tumor Treating Fields (TTF)	Alternating electric fields disrupt mitotic spindle formation, blocking cell division.	Median OS 20.9 mo vs. 16.0 mo; 5-yr OS 13% vs. 5%.[26]	Non-invasive and improves long-term survival; requires continuous device use, which affects compliance.
Bevacizumab (recurrent GBM)	Monoclonal antibody against VEGF → reduces angiogenesis and tumor edema.	6-mo PFS 42.6%[29]	Effective for symptom relief and edema; no proven OS benefit in newly diagnosed GBM

The table illustrates that all of the established therapies will only marginally prolong life expectancy, but there are no established cures for Glioblastoma Multiforme (GBM). The table points out the shortcomings of traditional therapies and the need for the development of new therapeutic modalities that are both targeted and longer lasting than traditional therapies, i.e., immunotherapies.

Although the standard treatments - surgery, radiation therapy with temozolomide, Tumor Treating Fields, and bevacizumab for GBM- can extend survival and enhance quality of life, their advantages are still restricted. Surgery provides the greatest survival advantage when maximal safe resection is achievable. Even with the Stupp regimen, the median survival is only 16 months, and the five-year survival rate is around 6–7%.[14] This is also mentioned in Table 1, where it shows multiple differences in the survival median for each of the different kinds of treatments, and the highest amount of survival is 37.3 months with surgery, and the lowest is 6 months with Bevacizumab, a treatment used for recurrent GMB. Although add-on strategies like bevacizumab and TTFs offer slight increases in survival or symptom control, overall results show the urgent need for more potent and focused treatments like CAR-T cell therapy.

Key Challenges in GBM Treatment:

GBM has a number of inherent challenges that reduce the efficacy of available treatments. These difficulties are caused by the intricate biology of the tumor as well as how it interacts with the protective environment of the brain. Developing more successful treatment plans requires an understanding of these obstacles. The main difficulties in treating GBM are enumerated in Table 2, along with how they affect the results of therapy.

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Table 2: Key Challenges in the Treatment of GBM.

Challenge	Description	Example / Impact
Tumor heterogeneity	Cells within the same tumor can have different genetic and molecular profiles, which makes it hard for a single therapy to target all cancer cells effectively.[30]	EGFR amplification in one region, PTEN loss in another → therapy resistance.[31]
Immunosuppressive microenvironment	GBM can suppress immune activity in its surroundings, reducing the effectiveness of immunotherapies.[2]	PD-L1 expression inhibits T-cell activity, lowering immunotherapy efficacy.[5]
Blood–brain barrier (BBB)	This protective barrier prevents many drugs from reaching the tumor, limiting treatment options.[4]	Temozolomide crosses the BBB, but most monoclonal antibodies cannot.[32]
Infiltrative growth	GBM cells invade surrounding brain tissue, making complete surgical removal nearly impossible.[33]	Even after maximal surgery, recurrence occurs within ~2 cm of the margin.[33]

The table summarizes the major structural and biological barriers to treatment success in glioblastoma multiforme. Together, these interconnected barriers illustrate why current therapies are usually ineffective, as tumor heterogeneity promotes therapeutic resistance while the blood-brain barrier and immunosuppressive tumor microenvironment limit both drug delivery and immune-mediated tumor destruction. Therefore, these challenges reinforce the necessity for the development of novel methods to treat GBM, including CAR-T cell therapy and CRISPR-based approaches.

New Treatment Options:

Immunotherapy, including CAR-T cell therapy:

In comparison to conventional treatments, immunotherapies for glioblastoma multiforme (GBM) show promise of better results. The principle of immunotherapy is to utilize a person's immune system to specifically identify and destroy the malignant cells that comprise a tumor. Immunotherapies currently under investigation for the treatment of GBM include dendritic cell (DC) vaccines, checkpoint inhibitors, and CAR-T cell therapy.

CAR-T-cell therapy represents a paradigmatic example of how an immunotherapeutic strategy can be used to design and engineer a patient's T-cells to specifically identify and destroy malignant cells. Modifying T-cells achieves this, so they will produce a chimeric antigen receptor (CAR), which allows them to recognize and kill tumor cells expressing a specific protein marker(s) on the surface of those cells. Researchers are now investigating whether or not this therapeutic approach can also be applied to the treatment of solid tumors, including GBM. CAR-T cells are genetically engineered T-lymphocytes designed to produce chimeric antigen receptors (CARs), which allow them to recognize and kill cancer cells displaying a specific protein(s) on their surface. In order to administer CAR-T cell therapy, the patient's T-cells are removed from the patient, then altered in a laboratory to recognize a particular antigen expressed on the surface of the tumor cells, and finally, the T-cells are returned to the patient to assist in destroying the cancer cells.[13]

CAR-T cell therapy has been studied in addition to dendritic cell vaccine-based treatments and checkpoint inhibitors, as other forms of immunotherapy, which could potentially provide patients with alternative treatment options.

Checkpoint inhibitors function by blocking proteins (such as CTLA-4 and PD-1/PD-L1) that inhibit the activation of T-cells, as illustrated in Figure 1. When administered, checkpoint inhibitors allow T-cells in the patient's body to travel to the tumor more easily and kill the tumor cells. Although checkpoint inhibitors have shown great promise in the treatment of some cancers, the low expression of tumor-associated antigens and high degree of immunosuppression found in the GBM tumor microenvironment have restricted the effectiveness of checkpoint inhibitors in treating GBM.[34,35]

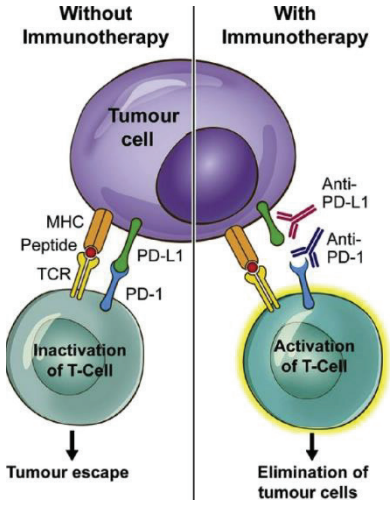

Figure 1: Visual representation of Checkpoint inhibitors. Genetically engineering T-cells with an antigen receptor is called CAR-T therapy; T-cells are taken from the blood of patients and put through a process of genetic modification in a laboratory using a viral vector to insert a gene that codes for a chimeric antigen receptor (CAR) on these T-cells. The chimeric antigen receptor allows the engineered T-cells to bind to a specific protein located on cancer cells. Once the CAR T-cells have been multiplied in the laboratory, they are injected back into the patient, where the CAR T-cells will continue to look for and kill cancer cells as they bind to their target antigen and induce apoptosis in the cancer cells.[33]

DC (dendritic cell) vaccines are to activate the body's immune system through exposure of dendritic cells to specific antigens present on cancerous cells (i.e., EGFRviii), as shown in Figure 2. As such, the hope is to create a targeted T-cell response to GBM without attacking healthy brain tissue. While the approach has proven successful for GBM patients on Provenge, the use of DC-based cancer vaccines is considered safe; they have failed to provide significant clinical benefits to patients, with only 5-15% of patients experiencing an objective immune response. It is possible that many of these failures in the efficacy of cancer vaccines resulted from the immunosuppressive mechanisms acting on the antitumor T cell responses; therefore, these factors represent a common rate-limiting step in most clinical trials and limit the overall clinical benefit of cancer vaccines.[36]

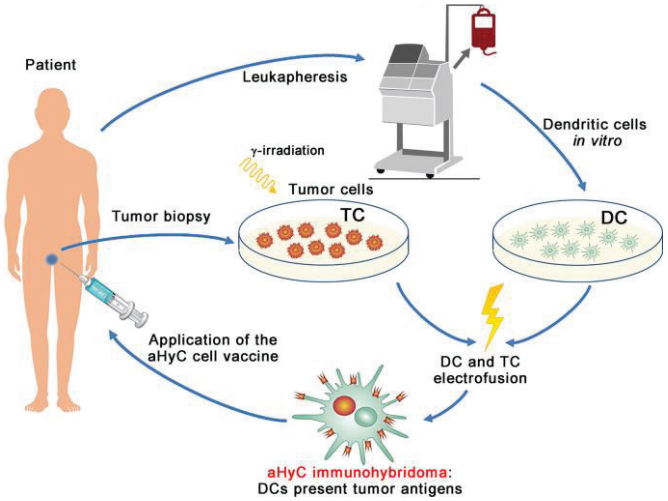

Figure 2: The hybrid personalized cancer vaccine involves preparing two different types of cells, namely tumor cells (from a biopsy) and dendritic cells (obtained through a simple blood draw), which are each treated separately and then combined through the use of electrical shock. The combination of the tumor's antigens with the dendritic cell's capacity for activating the immune system creates a "hybrid" vaccine. This vaccine is then returned to the patient as an injection, and causes the T-cells in the patient to recognize a wide variety of their own tumor markers, which results in a strong and targeted immune response against the patient's cancer. Visual representation of DC (dendritic cell) vaccines, adapted from Reference 37

Checkpoint inhibitor therapies and DC vaccine-based therapy often fail in glioma. This is because of the tumor's immunosuppressive microenvironment, as mentioned in Table 2, which limits the effectiveness of the immune activation, and glioma cells frequently lack strong neoantigen expression needed for robust T-cell responses, resulting in poor efficacy.[34] CAR-T remains the most heavily pursued immunotherapy in GBM as it can directly target tumor antigens and get around some of the limitations of other immune approaches.[9]

CAR-T Concept and Generation Using CRISPR Technology:

The CAR-T cell treatment process includes removing a patient's T-cells via leukapheresis and genetically modifying those cells so they can detect and kill cancer cells that contain the same proteins on their surfaces; proteins such as the EGFRvIII protein that some glioblastomas will express. Genetically modified T-cells are made by adding a Chimeric Antigen Receptor (CAR) to the T-cells' membrane. The main concept of CAR is to merge the native antigen recognition capacity of BCR (B cell receptor or antibody molecule) and the T cell activation capacity of TCR (T cell receptor). It thus bypasses the 'MHC-restriction' for recognition and killing of tumors by T cells. More in-depth into this idea, the CAR is made from an engineered receptor that contains both an antibody-derived recognition domain (the most common being a single-chain variable fragment, or scFv) and a T-cell signaling domain. CARs consist of three primary parts: an antigen-binding domain that sits outside the cell and binds to antigens on the surface of tumor cells; a transmembrane domain that holds the CAR in place; and an intracellular signaling region containing the CD3ζ chain and possibly one or two co-stimulatory molecules (CD28 or 4-1BB) to enhance T-cell activation, persistence, and ability to kill tumor cells.[10] After genetic modification, CAR-T cells are cultured *in vitro* and returned to the patient; usually after a course of lymphodepleting chemotherapy to allow the CAR-T cells to engraft and persist. Once returned to the patient, the CAR-T cells target and destroy tumor cells, produce cytokines, and attract other immune responses. Despite its potential, however, multiple barriers limit CAR-T cell treatment effectiveness in Glioblastoma Multiforme (GBM); among them are tumor heterogeneity resulting in antigen loss/escape, an immunosuppressive tumor microenvironment, limited T-cell penetration to tumors due to the presence of the blood-brain barrier, and the potential toxicity of CAR-T cell therapies such as cytokine release syndrome (CRS) and neurotoxicity, as summarized in Figure 3.[38]

Genome engineering advancements using CRISPR-Cas9 have improved CAR-T cell development and deployment to increase the efficacy and safety of CAR-T cells. By utilizing CRISPR to remove the inhibitory receptors PD-1 and/or TIGIT from CAR-T cells expressing an anti-EGFRvIII construct in glioblastoma models, researchers were able to demonstrate that CAR-T cells exhibited greater tumor killing capabilities and increased survival duration than did previous generations of CAR-T cells.[11] Additional enhancements include employing CRISPR to integrate the CAR transgene into "safe harbors" of the genome where it will be stably expressed, developing "universal" allogeneic CAR-T cells that lack TRAC and B2M genes to minimize the risk of graft-versus-host disease and immune rejection, and researching methods to generate CAR-T cells *in vivo* to decrease the need for large-scale manufacturing of CAR-T cells and associated costs. In total, the CRISPR-related advances above are expected to improve the persistence, uniformity, and overall effectiveness of CAR-T cells in treating glioblastoma multiforme.[40]

Recently, improvements in genome engineering technologies, specifically CRISPR-Cas9, have been utilized to enhance the design of CAR-T cells and improve their efficacy and safety. For example, CRISPR-Cas9 was used to ablate the inhibitory receptors PD-1 and/or TIGIT from CAR-T cells expressing an anti-EGFRvIII construct in preclinical glioblastoma models, which resulted in significantly enhanced tumor killing capability and prolonged survival upon intracerebral delivery. (34)[39] Additional modifications include knocking in CAR constructs to more controlled genomic locations and generating "universal" allogenic CAR-T cells through disruption of T-cell receptor (TRAC) and beta2-microglobulin (B2M) to diminish the risk of graft-versus-host and rejection. As such, the CRISPR-engineered CAR-T cell designs described herein should ultimately provide superior T cell persistence, uniform CAR expression, and may enable future *in vivo* generation of CAR-T cells without extensive ex vivo expansion, thus lowering the cost and time required for CAR-T cell manufacture.[40]

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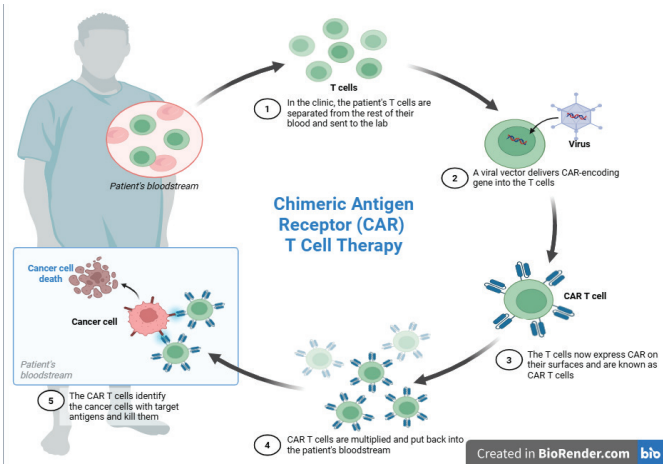

Figure 3: CAR-T Cell Therapy - Production and clinical use. This graph shows the full cycle of a CAR-T cell from its production to its implementation back into the body. Tumors can shut down T-cells by binding their PD-L1 to the T-cell's PD-1 receptor and essentially shut down the T-cells' ability to find and kill the tumors. Checkpoint Inhibitors are a class of immunotherapies that target PD-1 or PD-L1 and stop the interaction with the "off" switch for the T-cell, so they do not go into a dormant state and can continue to identify, target, and destroy cancer cells that may have otherwise gone undetected by the body's immune system. This graph was built on BioRender.

CAR-T Cells in Glioblastoma: Mechanism and Clinical Insights:

CAR-T cell therapy relies on identifying tumor-specific antigens that can distinguish malignant cells from normal brain tissue. These antigens serve as molecular "flags" on the tumor surface, guiding CAR-T cells to recognize and destroy cancer cells. In glioblastoma (GBM), several key antigens have been investigated as therapeutic targets due to their overexpression, tumor specificity, and association with aggressive disease biology (Table 3).[10,41]

Table 3: Antigen Targets in GBM.

Antigen	Description and Clinical Insight	Current Clinical Status
EGFRvIII	A mutant form of the epidermal growth factor receptor is present in ~25–30% of GBMs. It promotes uncontrolled cell proliferation and enhances tumor survival, making it an attractive but heterogeneous target.	Early-phase clinical trials have shown safety but limited efficacy due to antigen loss and tumor heterogeneity.
IL13Rα2	A receptor subunit is overexpressed in recurrent GBM but not in normal brain tissue. It is associated with tumor aggressiveness and poor outcomes, providing tumor specificity for therapies.	CAR-T trials targeting IL13Rα2 have shown dramatic individual responses, including complete remission in one case, though durability remains a challenge.
HER2	A member of the epidermal growth factor receptor family found in ~40% of GBMs. It sustains tumor growth and cell survival and is also targeted in breast cancer, providing translational therapeutic insights.	HER2 CAR-Ts are in early clinical testing; responses are modest but demonstrate feasibility and safety.
GD2	A disialoganglioside is highly expressed in pediatric brain tumors such as diffuse midline gliomas and DIPG. It plays a role in tumor cell adhesion and signaling.	CAR-Ts targeting GD2 have shown promising activity in pediatric gliomas, with ongoing studies exploring applications in GBM.
EphA2	A receptor tyrosine kinase involved in cell migration and invasion. Its overexpression in GBM correlates with tumor invasiveness and poor prognosis.	Preclinical studies support EphA2 as a target; CAR-T trials are underway but still at an early stage.

The table illustrates the wide variety of tumor-associated antigens that have been identified for use in CAR-T cell therapy for patients with glioblastoma multiforme. This also demonstrates how the diversity of the tumor antigens among individual tumors results in variable responses to CAR-T cell therapy, as no single antigen is used across all the different glioblastoma cells. Antigen heterogeneity helps explain why there is a consistent barrier in achieving a long-term solution, as demonstrated in the early stages of the clinical trials. Due to the antigen heterogeneity, future CAR-T cell strategies will likely require a multi-antigen targeting approach to improve the long-term efficacy and minimize antigen escape.

Mechanism of Action: CAR-T cells target tumor antigens via CARs, which engage cytotoxic activity such as cytokine release and direct tumor killing. GBM is tough, though, as summarized in Table 4.

Table 4: Challenges for CAR-T Therapy in GBM.

Challenge	Description / Impact	Example / Notes
Tumor antigen heterogeneity	GBM cells express different antigens; some may not carry the target CAR-T antigen, leading to incomplete killing.	EGFRvIII-targeted CAR-T may not affect cells lacking EGFRvIII.[11]
Immunosuppressive tumor microenvironment	GBM secretes TGF-β, recruits Tregs, and expresses checkpoint proteins, reducing CAR-T cell activation and persistence.	Limits the durability and efficacy of therapy.
Restricted T cell trafficking / BBB	The blood-brain barrier restricts CAR-T cells from efficiently reaching tumor sites when administered systemically.	Strategies like intraventricular or intracranial delivery are being explored.
Risk of cerebral edema and neurotoxicity	Immune activation in the brain can cause swelling or neurotoxic effects, posing safety concerns.	Dose titration and careful monitoring are required in clinical trials.

The table describes the major constraints imposed by using CAR-T Cells to treat solid tumors such as glioblastoma multiforme. It stresses that if we wish to improve the persistence, safety, and efficacy of CAR-T Cell therapy, these constraints must be overcome.

Clinical Trials:

The Major Car-T cell clinical trials discussed in this section will be summarized in Table 5.

GD2 CAR T Cells in Diffuse Intrinsic Pontine Gliomas (DIPG) & Spinal Diffuse Midline Glioma (DMG):

This is a Phase I study testing CAR-T cells targeting GD2 antigen in eleven kids and young adults with a tough type of brain tumor called diffuse midline glioma (including DIPG). They gave the treatment IV first, and if it worked, some patients got more doses directly into the brain. Some patients had brain swelling and inflammation, but the doctors were able to manage it with ICU care. Even with the risks, 4 patients had major tumor shrinkage (one over 30 months), and 9 patients had symptom improvement, so this therapy works very well but needs a phase II.[42]

EphA2-CAR T Cells in Recurrent Glioblastoma:

Three patients with recurrent glioblastoma were treated with a form of CAR-T cells that were designed to target a protein called EphA2. Each patient received a single dose of these specially made T-cells via IV injection, and each received chemotherapy before the CAR-T cells to condition their bodies. Two of the patients developed cytokine release syndrome (CRS), which caused them to experience breathing problems due to inflammation in the lungs. However, the CRS was con-

trolled with steroid medication. The CAR-T cells were present in the body for several weeks, but the tumors continued to grow in all three cases. In one case, the patient's tumor was stable for a short time; however, the median overall survival among the three cases ranged between 3 and 6 months. The study demonstrated that the treatment is possible and has a level of safety, but it does not currently work very well.[43]

IL-13Rα2-Targeted CAR-T Cells in Recurrent Glioblastoma:

Doctors conducted a clinical trial involving a total of 65 individuals with recurrent high-grade gliomas. Those in the clinical trial were treated with CAR-T cell therapy that targeted the IL-13Rα2 antigen. In order to treat the patients, the CAR-T cells were administered directly into the tumor, into the spinal fluid surrounding the brain, or both. Overall, the treatment was safe; however, the two most serious adverse reactions experienced during the trial were grade 3 encephalopathy and ataxia. Fifty percent of the patients (29 of 58) were stable or better after receiving the treatment. Within the group of patients that were better off after receiving the treatment, there were two patients who experienced a partial response and another two patients who experienced a complete response (one of the two complete responses was from a patient who received an additional CAR-T after the trial protocol). In total, the median for overall survival among the patients that received the CAR-T cells was 7.7 months; however, the median for overall survival among those patients that received the personalized dual delivery treatment was 10.2 months. The administration of the CAR-T cells resulted in an increase in the levels of pro-inflammatory cytokines within the central nervous system, including IFNγ, CXCL9, and CXCL10. This increase in cytokines indicates that the CAR-T cells were active. Additionally, patients who had tumors that contained more CD3+ T cells than other patients tended to live longer.[44]

HER2-CAR T Cells in Progressive Glioblastoma:

A Phase I clinical trial examined HER2-specific CAR-modified virus-specific T cells (VSTs) in 17 patients with progressive HER2-positive glioblastoma. The VSTs used in this clinical trial were produced from each patient and genetically modified to produce a second-generation HER2-CAR containing a CD28.ζ signaling domain. The patients in the clinical trial received the HER2-CAR VSTs without first undergoing lymphodepletion. The treatment was tolerable in that none of the patients experienced a dose-limiting toxicity. The one patient that achieved a partial response lasted for more than 9 months, while seven of the sixteen patients that were evaluated were able to maintain stable disease for periods of time ranging from 8 to 29 months. Three of the patients in the clinical trial were able to remain free of further disease for periods of time up to 29 months, and the median time until death from the date of infusion was 11.1 months, and the median time until death from the date of diagnosis was 24.5 months. The findings from this clinical trial indicate that HER2-CAR VST therapy is both safe and capable of producing clinical benefits for some patients with glioblastoma, the degree of benefit was relatively small in many cases, indicating a need for additional strategies to improve the effectiveness of HER2-CAR VST therapy against glioblastoma.[9]

EGFRvIII-CAR T Cells in Recurrent Glioblastoma:

Similar to the above-mentioned clinical trials, a Phase I clinical trial also examined a third-generation EGFRvIII-targeted CAR T product in 18 patients with recurrent glioblastoma. Following a course of lymphodepletive chemotherapy, the patients in the clinical trial received CAR T cells at various doses ranging from approximately 6.3×10^7 to 2.6×10^8 cells along with IL-2 support. The treatment was generally safe; however, serious adverse reactions were noted, including severe hypoxia and treatment- related death in one of the patients who received the higher doses of CAR T cells. There were limited efficacy outcomes noted among the patients in the clinical trial, as evidenced by the fact that the median time until disease progression was only 1.3 months (IQR = 1.1 – 1.9 months) and the median time until death from the date of infusion was 6.9 months. While no objective tumor responses were noted in the majority of the patients in the clinical trial, a couple of long-term outliers survived for more than 1 year, with one patient remaining alive at 59 months. These findings highlight both the biological potential of EGFRvIII-directed CAR T cells, as well as the significant challenges presented by the heterogeneity of glioblastoma, antigen escape, and the immunosuppressive nature of the tumor microenvironment.[11]

Role of CRISPR in CAR-T:

CRISPR enhances CAR-T therapy through the removal of inhibitory receptors, upregulation of CAR, and enabling future *in vivo* production of CAR-T.[12,38] Such advances can potentially overcome GBM's immunosuppressive tumor microenvironment and increase the specificity and durability of CAR-T cells.

Table 5: Clinical Trial Highlights.

Trial Name / Identifier	Number of Participants and Patients Population	Treatment Protocol / Delivery Method	Primary Outcomes / Results	Key Notes / Side Effects
GD2 CAR T Cells in DIPG & DMG[42] Phase I Trial	11 patients, who included children and young adults with midline glioma	IV infusion first; some received repeat intracerebral doses	4 had major tumor shrinkage (one >30 months); 9 had symptom improvement	Brain swelling, inflammation; higher IV doses caused more toxicity, but were manageable in the ICU
EphA2-CAR T Cells in Recurrent GBM[42] Phase I Trial	3 adult patients with recurrent GBM post-standard therapy	Single IV infusion after chemotherapy conditioning	Limited efficacy: tumors mostly progressed; OS 3–6 months; brief stable disease in 1 patient	Cytokine release syndrome in 2 patients (managed with steroids); lung shortness of breath
IL-13Rα2-CAR T Cells in Recurrent GBM[44] Phase I Trial	65 patients with recurrent high-grade glioma	Intratumoral, intracerebrospinal, or dual delivery	29/58 patients had stable or improved disease; 2 partial + 2 complete responses; OS median 7.7 months (10.2 in dual-delivery group)	Grade 3 encephalopathy & ataxia in 2 cases; CAR-T activity correlated with cytokine elevation & CD3 T-cell presence
HER2-CAR T Cells in Progressive GBM[9] Phase I Trial	17 patients with HER2-positive progressive GBM	Autologous virus-specific T cells engineered with HER2-CAR; IV infusion without lymphodepletion	1 partial response >9 months; 7 stable disease (8–29 months); median OS 11.1 months from infusion, 24.5 from diagnosis	Well tolerated; no dose-limiting toxicities; modest responses suggest need for improved strategies

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EGFRvIII (3rd gen)-CAR T Cells in Recurrent GBM[11] Phase I Trial	18 patients with recurrent EGFRvIII+ GBM	IV infusion after lymphodepletion + IL-2; doses from 6.3×10^7 to 2.6×10^8	Median PFS: 1.3 months; median OS: 6.9 months; no objective responses; 2 patients >1 year survival, 1 at 59 months	Generally safe, but serious toxicities include severe hypoxia & 1 treatment-related death at higher doses

The table outlines the main conclusions drawn from several large-scale studies of CAR-T Cell therapy for glioblastoma multiforme. Although the treatments were found to be relatively safe and biologically effective, they provided little or no significant clinical benefit. Hence, the studies emphasize the necessity for further modification of CAR-T Cell therapy through either the use of combination therapies or genetic manipulation like CRISPR to enhance their therapeutic potential.

■ Discussion

Despite advances in surgical and systemic treatments (radiation and chemotherapy with temozolomide), patients with glioblastoma (GBM) continue to experience short-term survival gains from standard care, and recurrence is almost universal. The failure of current treatments has led to investigations into targeted therapies, specifically CAR-T cell therapy as a form of precision immunotherapy.

While clinical trials using CAR-T therapy for GBM have demonstrated safety and biological activity, it has not achieved long-term therapeutic benefit due to: 1) the heterogeneous nature of tumors; 2) the inability of CAR-T cells to traffic effectively through the blood-brain-barrier; and 3) the suppressive effects of the tumor's immune environment on the function of CAR-T cells. Despite this, many studies have used antigen-specific targeting (EGFRvIII, IL13Rα2, HER2, EphA2, GD2), resulting in only transient anti-tumor responses and localized tumor regression or symptom relief. Antigen escape and heterogeneous expression remain significant barriers to successful CAR-T therapy.

CRISPR-Cas9 gene editing technology has the potential to overcome the limitations associated with CAR-T therapy. In preclinical trials, CRISPR has been able to increase CAR-T persistence, eliminate inhibitory checkpoint molecules (PD-1), and enable more consistent CAR expression.[9,38] New methods of delivering CRISPR constructs across the blood-brain barrier using nanocarriers and exosomes have also become available. Although many applications of CRISPR technology are currently experimental, it is expected to improve both the efficacy and safety of CAR-T therapies for GBM.

The intersection of CAR-T cell therapy and CRISPR-based engineering presents a new and exciting area of research. Future clinical evaluations will need to evaluate combinations of CAR-T therapies (i.e., multi-antigen targeting and/or checkpoint inhibition) to overcome the complex immunological environment of GBM.

The following are several limitations of this review. First, few CAR-T clinical trials have been published in GBM with heterogeneous small sample sizes, study design, antigen target, and route of administration, making comparison challenging. Second, the majority of CRISPR studies are preclinical and thus results will not likely be extrapolated directly to patients due to heterogeneity in tumor biology and the blood–brain barrier. Third, publication bias would also be a risk because positive findings are more likely to be published than null or negative findings. Finally, while every effort was made to obtain all relevant studies using systematic searching, there is the potential that some new studies or non-English studies were not captured.

■ Conclusion

Glioblastomas (GBMs) are cancers that have an extremely poor prognosis and cannot currently be cured by any established standard treatments. CAR-T cells have shown safety and early signs of efficacy in treating glioblastoma; however, CAR-T has not yet demonstrated its ability to produce long-lasting or durable responses against this cancer type. The gene editing capability of CRISPR could potentially enhance the ability of CAR-T cells to target glioblastoma tumors by addressing the major hurdles associated with tumor heterogeneity, immune suppressive mechanisms, and persistence of CAR-T cells. As additional clinical trials are completed, the application of CRISPR-based modifications to CAR-T cells may usher in a new era of glioblastoma treatment. It will be necessary to employ multi-modality approaches using genetic engineering, immunomodulation, and targeted therapy to provide significant improvement in the overall survival rates of patients diagnosed with glioblastomas.

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Author

Akhil Bollu is a junior at Independence High School with a strong interest in biology and the medical field. He enjoys basketball, spending time with family, and go-karting with them, and is committed to volunteering at hospitals and serving his community.