Narrative Review Article

CRISPR-Cas13 Targeting of RNA in Glioblastoma Stem Cells: A Novel Approach to Disrupt Tumor Progression

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ABSTRACT

This review evaluates CRISPR-Cas13 as an RNA-targeting strategy to eradicate glioblastoma stem cells (GSCs) by knocking down transcripts that sustain self-renewal (e.g., SOX2, c-MYC, EGFRvIII, OLIG2). It focuses on guide design, delivery across the BBB to hypoxic/perivascular GSC niches, and preclinical endpoints that predict reduced recurrence. Glioblastoma Multiforme (GBM) is the most aggressive cancer of the brain, largely due to recurrence driven by Glioblastoma Stem Cells (GSCs). Treatment failure arises from interrelated factors such as therapy resistance, blood-brain barrier (BBB) impermeability, and immune evasion. CRISPR-Cas13 is a novel RNA-targeting system that directly silences key oncogenic transcripts of GSCs such as SOX2, c-MYC, and HIF-1α. Cas13 enables allele-specific targeting, and a reduced risk of genomic integration compared to other genetic tools including RNA-interference (RNAi), antisense oligonucleotides (ASOs), and Cas9. However, effective delivery remains challenging as viral vectors, non-viral vectors, and exosomes each offer distinct trade-offs. Combination strategies such as pairing Cas13 with immune checkpoint inhibitors (ICIs) may overcome immune suppression and barriers posed by oncogenic drivers. In the near-term, lipid nanoparticles (LNPs) with BBB-penetrating ligands are a promising approach for GBM treatment via GSC targeting, however as the engineering of exosomes develops, their natural origins, high biocompatibility and ease of integration into difficult tissues such as the brain becomes increasingly favorable. Ultimately, advances in Cas13 engineering and delivery could enable durable GSC targeting and transform the clinical outlook for GBM patients.

Keywords: Glioblastoma Multiforme (GBM); Glioblastoma Stem Cells (GSCs); CRISPR-Cas13; RNA-targeting therapies; Oncogenes; Lipid nanoparticles (LNPs); Exosomes; Immune checkpoint inhibitors (ICIs)

INTRODUCTION

Glioblastoma multiforme (GBM) is among the most aggressive and therapy-resistant tumors in oncology. It originates from the uncontrolled proliferation of glial cells or metastasis from other cancers. Typically, the tumor undergoes multi-stage therapy, often including surgical intervention, chemotherapy and radiation therapy. However, these therapies are often ineffective in the long term, resulting in one of the lowest five-year survival rates in oncology. This difficulty in managing malignancy can largely be attributed to the immense heterogeneity of tumor cells and the aggressive nature of glioblastoma stem cells (GSCs) (1).

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This subpopulation of tumoral cells drives the renewal and invasiveness of the tumor, posing an incredible challenge for doctors to eradicate. Interestingly, many therapies that work for other cancers however have been unsuccessful for GBM, particularly because of the GSCs behaviors and the tumoral microenvironment that they influence (2). As a result, the exploration of novel therapeutic approaches to disrupt GBM progression has become more and more urgent. When confronted with so many ineffective therapies, surgical outcomes and survival rates, novel methods have begun to be incorporated into these discussions. Particularly, a gene-editing technology called CRISPR has become increasingly relevant as a potential approach to cancer due to its ability to guide a protein effector to degrade particular sequences in the genome. Although the most widely utilised CRISPR system, CRISPR-Cas9, has been successful in the cleavage of targeted DNA nucleotides, recent research has expanded beyond DNA editing to RNA-targeting approaches. CRISPR-Cas13 has the ability to target and cleave RNA sequences, including the pre-mRNA that codes for many cancer-associated proteins (3). To expound, this means that gene expression can be silenced without permanent damage in the DNA of a cell, making the process easily reversible and therefore safer for such detrimental malignancies (4). This review evaluates CRISPR-Cas13 and potential combination therapies as an approach to disrupt GSCs and consequentially GBM progression.

OVERVIEW OF GLIOBLASTOMA MULTIFORME

Glioblastoma multiforme (GBM) is a WHO (World Health Organisation) grade IV glioma which remains a persistent challenge in neuro-oncology, with no definitive cure to date and a 5-year survival of only 4-5%. The tumor manifests in non-specific initial symptoms including headaches, nausea, blurred vision, among others, which are frequently masked by the brain's compensatory system and overlooked. This delays medical attention, impeding timely diagnosis and suspending interventions such as surgery, targeted therapy, chemotherapy or radiation, allowing the tumor to progress significantly (5).

GBM is among the most aggressive and treatment resistant tumors in oncology, with a ~90% recurrence rate attributed to several interrelated factors. These include its strong intratumoral heterogeneity—where subpopulations of cancer cells have varying responsiveness to treatment— its immunosuppressive microenvironment, and its location within the central nervous system, which complicates drug delivery due to the blood-brain barrier (6).

CURRENT GLIOBLASTOMA TREATMENT LIMITATIONS

Standard interventions for GBM, including surgical intervention, are often ineffective due to the infiltrative nature of the growth within the parenchyma of the brain. This frequently results in incomplete resection and recolonization. Recurrence occurs in the majority of patients within two years of surgical intervention as seen in Figure 1, demonstrating MRI scans post-resection and 18 months after surgical intervention (7).

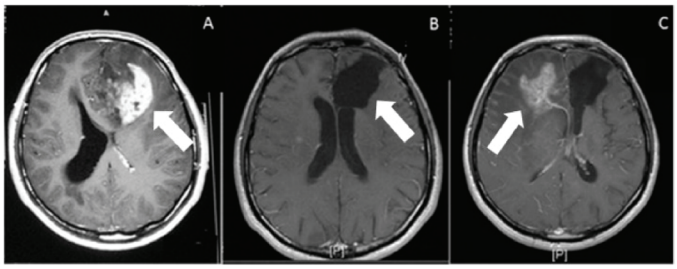

Figure 1. Axial MRI Scans of Glioblastoma Patient (A) Preoperative image shows irregular tumor enhancement in the left frontal lobe. (B) One-month postoperative image reveals the surgical cavity with no remaining tumoral residue. (C) After 18 months recurrence is observed in the contralateral hemisphere with peripheral edema (7).

Another frequent alternative in GBM treatment is the use of Temozolomide ($C_6H_6N_6O_2$)—an oral alkylating agent active against human cancers, known to interfere with the DNA of cancer cells and induce apoptosis as illustrated in Figure 2 (8).

Temodar (the commercial name of Temozolomide), has extended the median survival of newly diagnosed adult GBM patients from approximately 12 to 14.6 months when administered alongside radiotherapy. This extension is attributed to specific subpopulations of tumor cells—A172, U87, U251 and U372—that demonstrate heightened sensitivity to Telozolomide's DNA-alkylating effect, leading to increased DNA damage and enhanced therapeutic results. Conversely, the tumor also inhabits Temozolomide resistant populations, including LN-18, T98G, U138, CCF-

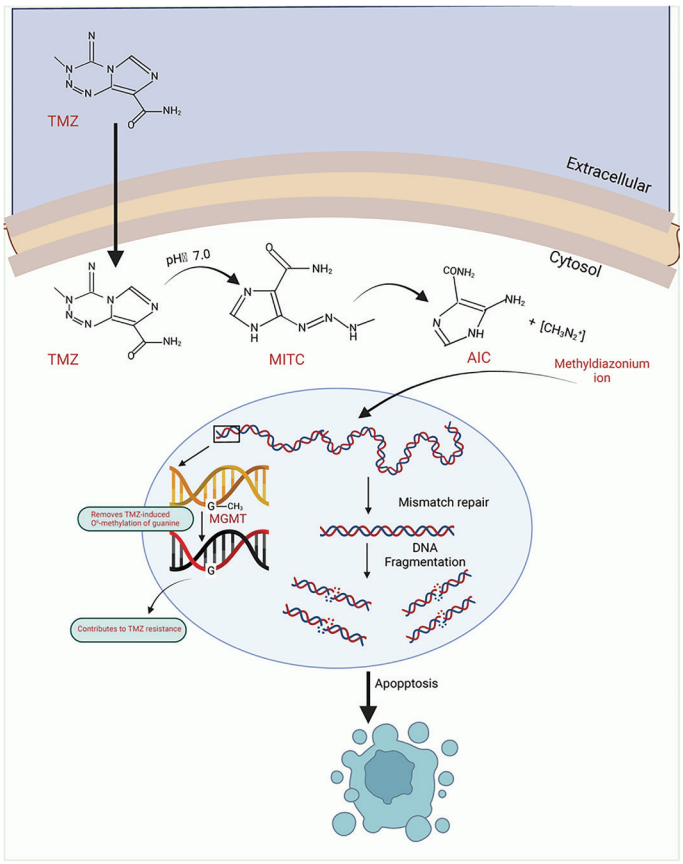

Figure 2. Temozolomide Mechanism of Action (8).

STTG1 and U343-MG, which overexpress O^6-methylguanine methyltransferase, interfering with Telozolomide efficacy and contributing to resistance in over 50% of treated patients (9).

An additional hallmark of cancer—particularly GBM—is tumor hypoxia, a condition characterised by reduced oxygen availability within the tumor's microenvironment. Hypoxia arises as cancer cells rapidly proliferate, spreading beyond functional vasculature and outpacing oxygen diffusion (10). This oxygen-deficient environment drives tumor progression, promotes metastasis and contributes significantly to the malignancy's treatment resistance, particularly in response to radiotherapy. To adapt, tumor cells preferentially utilize glycolysis as their primary metabolic pathway, even in normoxic conditions. This distinctive preference—known as the Warburg effect—leads to the accumulation of lactic acid, lowering the pH in the tumor microenvironment. The resulting acidosis compromises the function of immune cells and therapeutic perfusion, fostering its aggressive and invasive behaviour (11).

Moreover, a physiological limitation for the therapeutic delivery of GBM is the blood-brain barrier (BBB). The BBB is a protective, semi-permeable membrane that maintains homeostasis within the brain and prevents the passage of molecules larger than 400 daltons (12). This mechanism protects the brain's microenvironment, regulating the entrance of potentially harmful exogenous substances, but also hinders the delivery of therapeutic agents—including over 98% of small-molecule drugs, 100% of large-molecule drugs, and most RNA-based therapies—significantly inhibiting the effective treatment of intracranial malignancies (13).

RNA TARGETING THERAPIES

Ribonucleic Acid (RNA) therapies utilize oligonucleotides to disrupt RNA transcripts and investigate the function of particular genes. RNA plays a central role in protein synthesis and gene regulation, acting as a messenger transmitting genetic information to ribosomes (14). Over 80% of the genome is now known to be transcribed into RNA and therefore RNA-targeted techniques have been a prevalent tool in recent biomedical advances, with the two main approaches being RNA interference (RNAi) and antisense oligonucleotides (ASOs), whose mechanisms are demonstrated in Figures 3 and 4 (15).

However, a new tool with the potential to target and cleave RNA—CRISPR-Cas13— goes beyond the abilities of RNAi and ASOs with its capability to target both coding and non-coding RNAs, including nuclear transcripts, expanding its therapeutic scope. Moreover, CRISPR-Cas13 can enzymatically degrade RNA and provide sustained knockdown, whereas RNAi and ASOs typically yield transient effects and require repeated dosing, potentially seeming inefficient and financially inaccessible to many patients (16).

GLIOBLASTOMA STEM CELLS AND TUMOR RECURRENCE

The recurrence of Glioblastoma multiforme is also attributed to the presence of Glioblastoma Stem Cells (GSCs). GSCs are a tumoral cell subpopulation residing in protective environments such as hypoxic and perivascular zones with the ability to self-renew, differentiate into a heterogeneous population of cancer cells and initiate tumorigenesis. Their resistance to therapy can be attributed to distinctive biological mechanisms, including their quiescence, enhanced

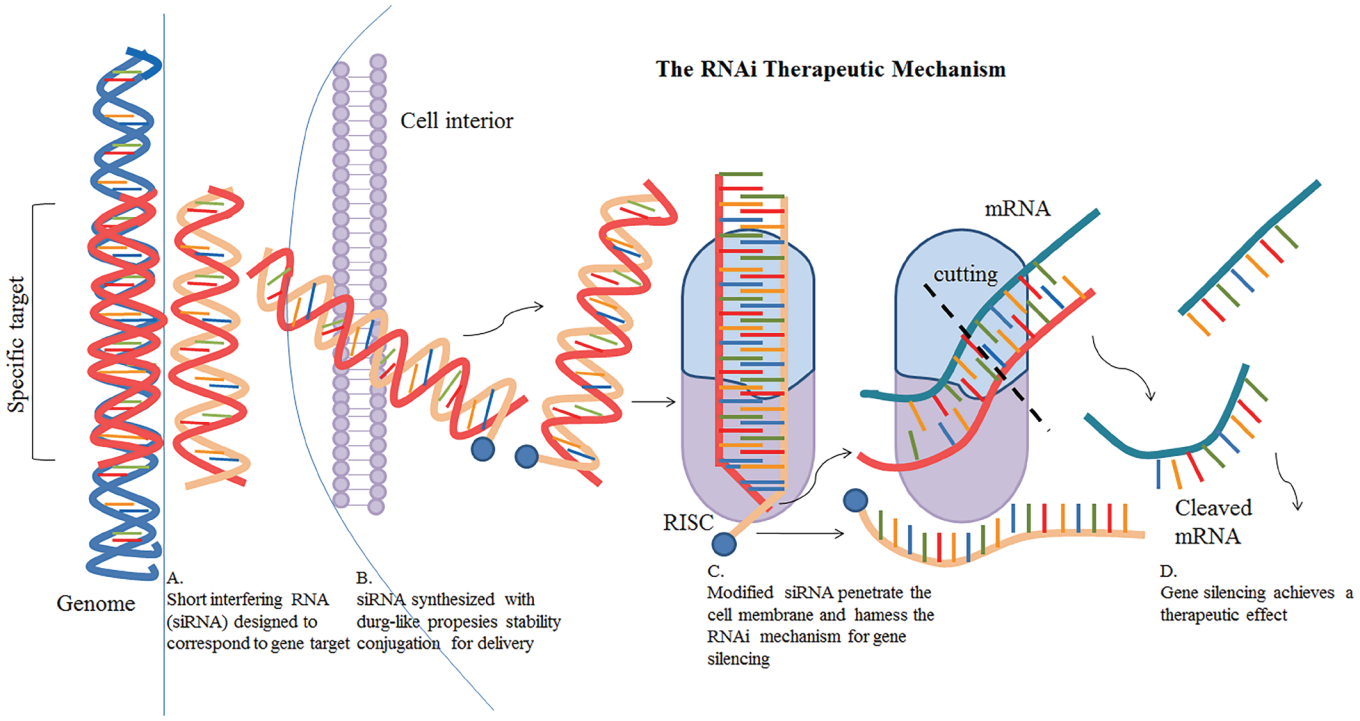

Figure 3. Mechanism of RNA Interference (RNAi) Therapeutics via siRNA-Mediated mRNA Cleavage (15).

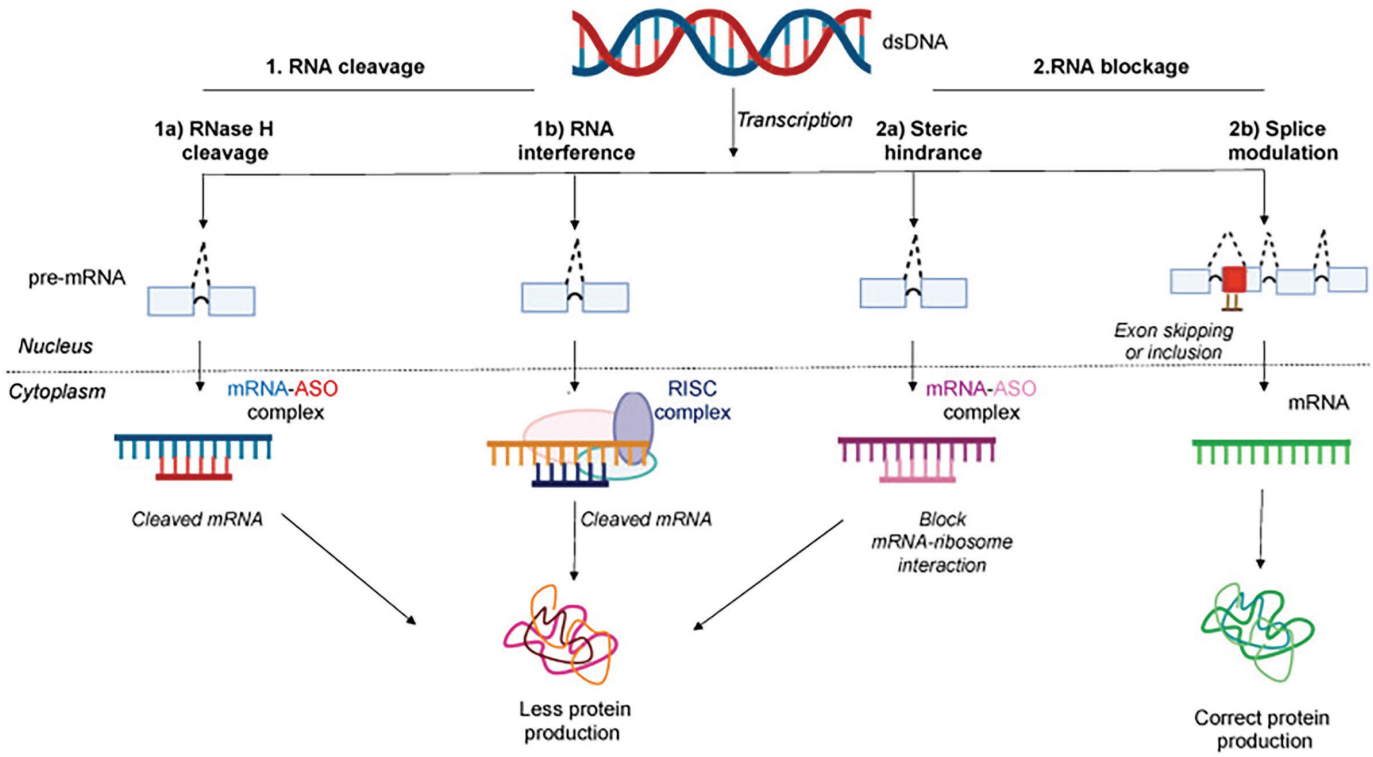

Figure 4. Mechanisms of Antisense Oligonucleotides (ASOs) Therapeutic Action (16).

oxidative capacity, localization in hypoxic regions, and extensive capacity to withstand DNA damage via activation of anti-apoptotic proteins. These characteristics combined with overexpression of survival pathways, such as c-MYC and SOX2, allow GSCs to evade conventional therapies and repopulate the tumor post-intervention. Their persistence poses a major obstacle to durable remission and underscores the necessity of GSC-specific targeting in GBM treatment (17).

KEY ONCOGENES AND RECEPTORS IN GSC MAINTENANCE

A key component of glioblastoma stem cell survival lies in the oncogenes and receptors which enable self-renewal and tumor propagation. Particularly, understanding their effects as outlined in Table 1 is crucial to mediating their summated function. One of the most commonly amplified oncogenes—found in approximately 60% of primary GBM and 10% of secondary GBM—is epidermal growth factor receptor (EGFR), especially EGFRvIII, a mutant isoform arising from an in-frame deletion that promotes constitutive oncogenic signaling by bypassing the need for ligand binding. The overexpression of EGFR contributes to a downstream cascade of PI3K/Akt and RAS/ERK1/2 as shown in Figure 5 by Xu *et al*., which significantly amplify hallmark features of malignancy—including enhanced cell survival, accelerated proliferation, and aggressive invasion (18).

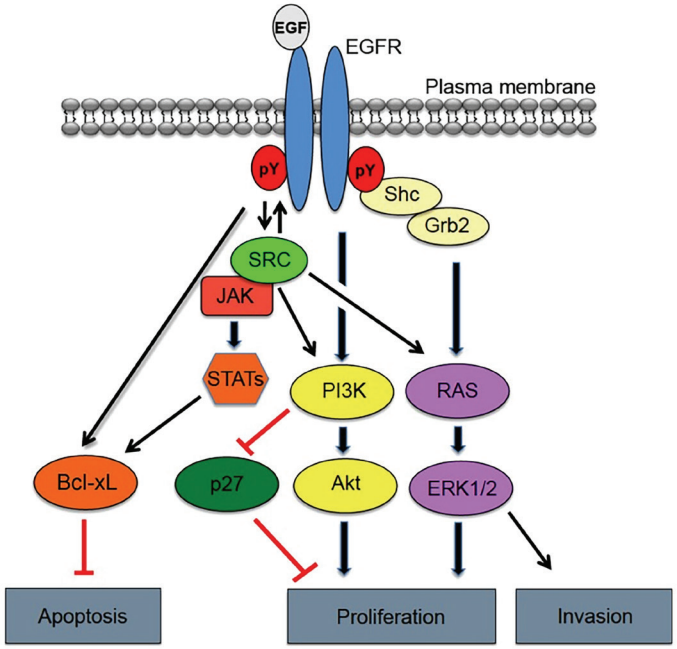

Figure 5. Mechanisms of EGFR Activation and Signaling in Cancers (18).

Table 1. Summary of the Functions, Roles and Rationales for Knockdown of Key Oncogenes/Factors that Sustain Glioblastoma Stem Cells

Oncogene/Factor	Function	Role in GSCs	Rationale for Cas13 Knockdown
EGFR/EGFRvIII	Tyrosine kinase activating PI3K/Akt and RAS/ERK1 & 2 pathways	Promotes downstream oncogenic signaling, increasing cell survival and invasion	Knockdown of EGFR/EGFRvIII will reduce the downstream signaling of detrimental pathways and tumor proliferation
SOX2	Regulates neural development and stem cell maintenance	Maintains stem-like phenotype, enhances therapy resistance and tumor plasticity	Knockdown will reduce self-renewing capacities and invasiveness of GSCs
c-MYC	Controls cell cycle and metabolism	Drives rapid proliferation and aerobic glycolysis, supporting tumoral growth	Knockdown limits proliferation and mitochondrial reprogramming, consequently impacting the tumor microenvironment.
HIF-1α	Hypoxia-inducible transcription factor	Promotes angiogenesis and survival in hypoxic microenvironments	Knockdown reduces tumor adaptation to hypoxia and angiogenesis
MGMT	DNA-repair enzyme	Repairs temozolomide-induced DNA damage leading to chemoresistance	Silencing restores sensitivity to Temozolomide and improves response

SOX2 is an additional significant gene, located on chromosome 3 position 3q26.3-q27, which encodes the SOX2 transcription factor and is key during the process of embryonic development—particularly for neural induction and differentiation (19). This protein is expressed in all gliomas, with the proportion of SOX-positive cells ranging from 6-80% and corresponding with the malignancy grade (20). SOX2 regulates stem-like properties of cancer stem cells; overexpression of the protein is associated with increased proliferation, invasiveness, therapy resistance and clonogenic capabilities of malignant cells. It also interacts with several downstream pathways including PI3K/AKT and Notch signaling—which further reinforce stemness and lineage plasticity (21). Collectively, these factors sustain a persistent, self-propagating tumor niche, contributing to the poor prognosis of GBM and other brain tumors with increased levels of SOX2.

The c-MYC protein is another oncogenic driver in GSCs. In non-pathological contexts, it is responsible for the regulation of cellular proliferation and tissue genesis through controlling the expression of cell cycle proteins. However, when overexpressed—which it is in most cancers and a contributor to the development of over 40% of tumors—the oncoprotein promotes proliferation, growth, and survival of glioma cancer stem cells (22). In an experiment by Wang *et al*., CD133+ glioma cells were infected with non-targeting control lentivirus or lentivirus expressing c-Myc shRNA and their effect on tumor propagation and neurologic signs was recorded as shown in Figure 6. The condition with c-MYC knockdown prevented tumor development, whereas the non-knockdown control did not.

These results show that the activation of c-MYC inevitably results in the formation of tumors. In addition, c-MYC has also been found to control metabolic changes within transformed cells. These changes are essential for the rapid rate of GSC proliferation as they support the increased need for nucleic acid, proteins and lipids needed for tumoral growth (23). Through the up-regulation of genes including GLUT1 and HK2, c-MYC drives the Warburg effect (aerobic glycolysis) and mitochondrial reprogramming in GSCs. This shift supports survival by rapidly generating ATP for quickly dividing tumors, even in hypoxic environments where tumors often reside. Furthermore, the resulting lactate accumulation allows GSCs to resist therapies including chemotherapy and radiotherapy by acidifying the tumor microenvironment, thus suppressing immune cell response and contributing to metastasis to neighboring regions.

It is also important to address genes that promote tumor survival in adverse microenvironments, as well as resistance to therapeutic interventions and drug delivery. As previously discussed, tumors require a steady supply of oxygen and nutrients to proliferate, which is difficult to obtain in hypoxic environments such as the brain. Upon exposure to such environments, transcription factor hypoxia-inducible factor 1-alpha (HIF-1α) activates and binds to hypoxia-responsive elements (HREs), inducing the transcription of genes that promote tumor angiogenesis, invasion and survival

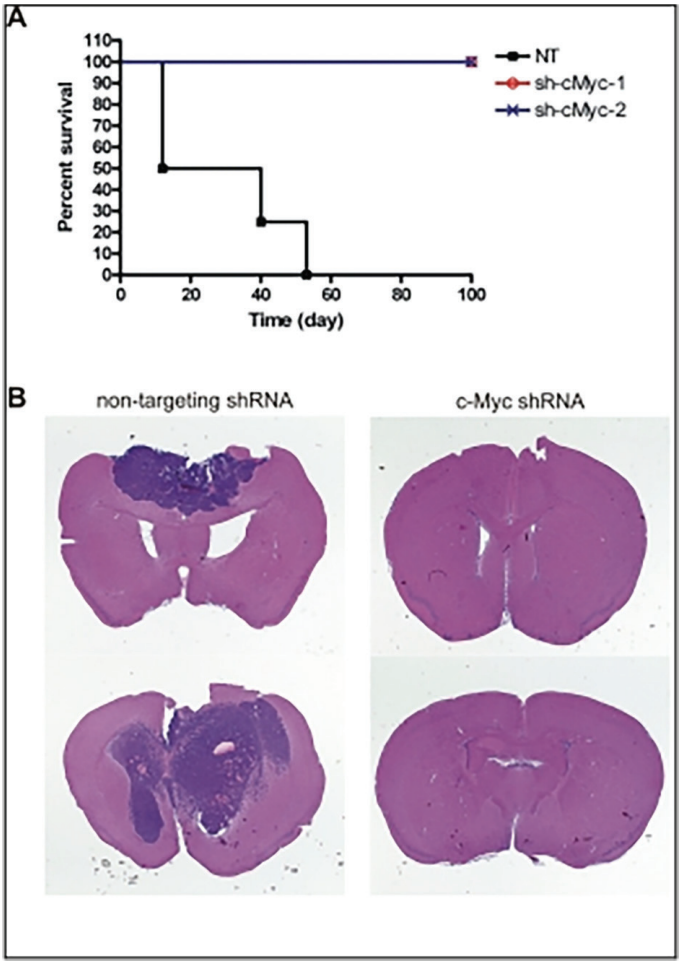

Figure 6. The effect of c-MYC expression vs c-MYC knockdown in nude mouse tumor formation by glioma cancer stem cells (17). (*A*) Kaplan-Meier survival curve of the different experimental conditions. (*B*) Hematoxylin and eosin-stained images of the intracranial tumors—the non-targeting shRNA revealed extensive tumoral tissue whereas the targeting shRNA showed no detectable malignant tissue.

(24). Angiogenesis is the formation of blood vessels that provide tumors with access to the circulatory system to thrive. HIF-1α activation is common in GBM and associated with therapy resistance and intense vascular hyperplasia that the tumor displays, ultimately leading the tumor to grow beyond a critical size and metastasize by facilitating the dissemination of cancer cells and GSCs through the bloodstream (25).

Another protein that promotes tumor survival is O^6-methylguanine-DNA methyltransferase (MGMT), encoded by MGMT mRNA. At low levels, the protein is unable to repair therapeutic TMZ-induced damage, rendering patients susceptible to the treatment. Conversely, elevated levels are clinically associated with resistance in some, but not all, GBM (26). Since TMZ exerts its cytotoxic effect by adding an O^6-methyl group to guanine in DNA, high MGMT expression rapidly repairs these O^6-MeG lesions by removing this methyl group from the O^6 position of guanine, preventing cytotoxic mismatch repair. Each repair process permanently inactivates MGMT, thereby diminishing the Temozolomide's therapeutic efficacy (27).

CRISPR-CAS13 AND RNA TARGETING

CRISPR-Cas13 is a rapidly emerging gene-editing tool first demonstrated as effective in human cells in 2017. Its system, as shown in Figure 7, is composed of a Cas13a enzyme most often extracted from *Leptotrichia wadei* and a CRISPR RNA (crRNA) of 22 to 28 nucleotides. The Cas13 enzyme is made up of a nuclease domain and a crRNA-recognition lobe which bind to the catalytic domains HEPN1 and HEPN2, enabling its enzymatic activity. The system also contains a protospacer flanking site (PFS), which prevents self-cleavage and ensures accurate binding to the target RNA sequence before crRNA-target duplex formation (28).

Once activated, the system can cleave target RNA, and potentially risk collateral cleavage, a phenomenon where non-targeted single-stranded RNAs surrounding the complex also get cut. This programmable cleavage has been applied to many strategies including RNA knockout, targeted cancer therapy, and delivery of CRISPR-based antimicrobials.

While Cas13 offers unique advantages in RNA targeting, Cas9 remains the most prominent CRISPR system. The most distinctive difference between the two is that Cas9 cleaves and edits DNA, whereas Cas13 applies the mechanism for RNA, enabling transient and reversible modification of gene expression without permanent genomic alterations. Regardless, Cas9's simpler design illustrated in Figure 8 makes it more accessible and affordable, increasing its market adoption and development. It can also modify large-scale genomes through the multiplex programming using multiple sequence-specific sgRNAs (29).

Despite these benefits, several limitations of Cas9 become evident when compared directly with Cas13. Unlike the Cas9 system, Cas13 can be designed to detect and cleave allele-specific RNA mutations within cells. This is partly due to its simpler modular construction, consisting of a single protein effector paired with its gRNA—which substantially increases its scalability and provides it with a higher sensitivity when

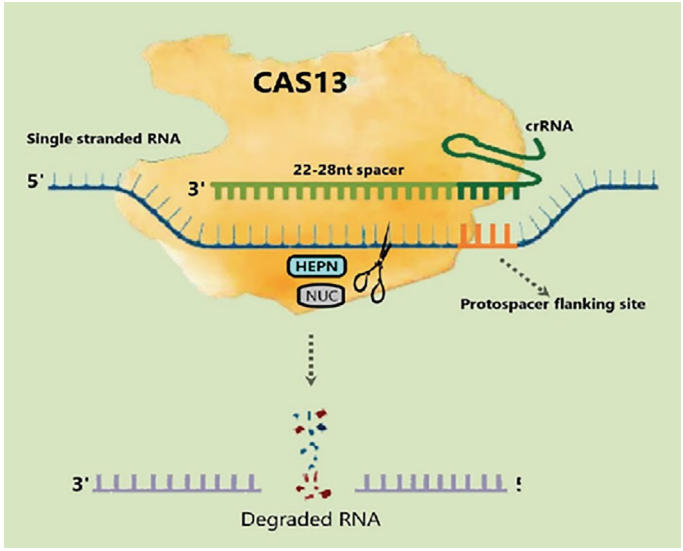

Figure 7. Mechanism of CRISPR-Cas13 System (28).

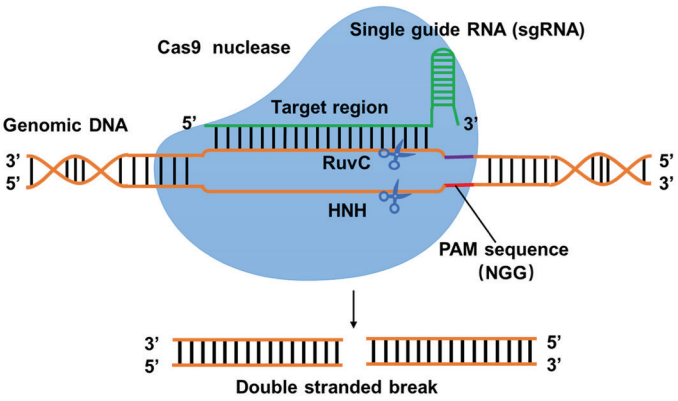

Figure 8. Mechanism of CRISPR-Cas9 System (30).

discriminating single nucleotide changes (30). Hence, the system appears more beneficial for diseases arising from minor mutations such as the toxic RNA repeats that cause Amyotrophic Lateral Sclerosis (ALS). This is also possible due to its faster downregulation of gene expression by directly degrading cytoplasmic mRNA transcripts, thereby inhibiting translation and harmful polypeptide production without permanent DNA modification. In addition, Cas13 systems pose a lower mutagenic risk, a vital component to consider when altering postmitotic cells such as the neurons involved in GBM as minimal damage could be fatal. However, Cas9 has a higher specificity and lower off-target effects in comparison to Cas13. This can be attributed to its relative novelty and ongoing development, as well as its HEPN activation, which can unintentionally degrade neighbouring molecules (31). The Cas therapies should be considered alongside other therapies, as contrasted in Table 2, to select the preferred approach for particular patients.

THE ROLE OF CRISPR-CAS13 IN SILENCING GSC ONCOGENES

To overcome the potential barriers in treatment previously discussed, multiple delivery methods have to be carefully compared as done in Table 3. One of the most established delivery approaches involves viral vectors, which fall into three major categories: adeno-associated virus (AAVs), adenoviral, and lentiviral. Vectors allow therapeutic genetic material like foreign DNA to enter living tissue via delivery with differing viral shells. When administered, AAV capsids bind to receptors on the cell's membrane, are internalized into vesicles, and transported to the nucleus, where the capsid degrades and releases the ssDNA within it—forming episomal DNA (non-integrated). This resulting transgene then produces mRNA which leaves the nucleus and gets translated into a functional protein. AAVs have been proven to have a long persistence (10+ years in organism) and a low immunogenicity in comparison to other viruses—meaning it has a low likelihood of triggering immune responses when administered in the body. However, many people might have had previous exposure to AAVs from viral infections, making them immune to treatment (32).

As opposed to AAVs' mechanisms, adenoviral vectors transport double-stranded DNA therapeutics. They do this as their cargo capacity is larger and can transport genetic material up to x8 the size of the AAV (~4.7kb for AAV → ~36kb for adenoviral). Additionally, adenoviral vectors trigger stronger immune responses despite being non-integrative; however, scientists have recently improved this characteristic, decreasing immunogenicity. Moreover, this subcategory can infect a larger range of cells, meaning its episomal expression

Table 2. Summary of the Advantages, Limitations and Risks Between Potentially Relevant Gene-Editing Technologies for Glioblastoma Stem Cells

Gene-Editing Technology	Advantages	Limitations	Risks
CRISPR-Cas9	Permanent DNA editing, high accuracy, scalable for multiplex genome modification	Can introduce double-strand breaks, irreversible, uneven alteration activity	Permanent collateral DNA cleavage potentially inducing oncogenesis, unintended immune responses
CRISPR-Cas13	Reversible RNA editing, higher detection sensitivity for single-nucleotide mutations, suitable for postmitotic cells	Potential for collateral cleavage, impermanent modifications (may require repeated dosing)	Unintended collateral degradation due to HEPN activation, delivery-related cytotoxicity
RNAi	Can simultaneously silence multiple genes, well supported by mammalian cells	Prone to off-targeting effects, unable to knockdown genes, might activate the immune response, delivery risks	Immune simulation due to dsRNA, potential amplification of intended effects
ASOs	Can modulate splicing and translation, chemically stable	Limited tissue penetration, requires multiple dosings, potential off-target hybridization	Hepatotoxicity, nephrotoxicity, immune stimulation related to reduction in platelet numbers.

Table 3. Summary of the Features, Advantages and Limitations/Risks of Relevant Delivery Mechanisms for CRISPR-Cas13 Therapeutics

Delivery Mechanism	Features	Advantages	Limitations/Risks
Adeno-Associated Virus (AAV)	Delivers single-stranded DNA that forms episomal DNA in the infected nucleus	Long persistence and low immunogenicity compared with other viruses	Small genetic cargo, many patients have pre-existing immunity reducing treatment efficacy
Adenoviral Vectors	Transport double-stranded DNA	Strong, short-term expression and large genetic cargo	Could trigger an immune response and transient expression due to mitotic dilution
Lintiviral Vectors	Carries single-stranded RNA which permanently integrates into the host genome	Permanent expression of the integrated RNA sequence, infect dividing and non-dividing cells	Risks of insertional mutagenesis and irreversible genomic alteration
Lipid Nanoparticles (LNPs)	Composed of nucleic acids and a variety of lipids (PEG, cholesterol, phospholipids, etc.)	High biocompability, can be engineered with ligands to cross the blood-brain barrier, efficient RNA encapsulation	Limited targeting specificity, could be cleared rapidly depending on circulation
Polymeric Nanoparticles (PNPs)	Built from synthetic or natural polymers such as dextran	Highly customizable in size, release kinetics and more	Possible toxicity risk, circulatory instability, poor oral bioavailability
Exosomes	Naturally occurring extracellular vesicles released via exocytosis	Low immunogenicity and toxicity, capable of crossing the blood-brain barrier and bypassing lysosomal degradation	Difficult to standardize preparation, risk of contamination and potency variability across batches

is briefer from mitotic dilution, but more intense due to its size (33).

The last major viral vectors are lentiviral vectors. Distinctively from AAVs and adenoviral vectors, lentiviral vectors carry ~13.5kb of single-stranded RNA as their genetic payload. It also integrates the therapeutic into the host genome—causing permanent expression in both dividing and non-dividing cells. Due to this, lentiviral vectors have a broad applicability, capable of infecting any cell without dilution of its effects as well as low immunogenicity (34). In summary, AAVs are typically utilized for in-vivo treatments on non-dividing cells, adenoviral vectors are commonly utilized in vaccine deliveries, and lentiviral vectors are most efficient for ex vivo gene therapy, such as engineering T-cells (35).

In addition to viral delivery, non-viral strategies like lipid nanoparticles, polymeric NPs, and exosomes are also promising. Non-viral systems are composed of synthetic organic/inorganic substances capable of transporting drugs to cells, tissues and even entire organs. They also exhibit low toxicity, mutagenesis risk and a larger production feasibility as they are immunogenically safe and very simple to design. However, they also demonstrate efficiency in mostly somatic cells (not germline), lower transduction potentials, and decreased target specificity. Recently, lipid nanoparticle vehicles have been gaining popularity, especially in RNA therapeutics used in clinical applications such as virus vaccines, cancer immunotherapy and gene editing (36). Lipid nanoparticles demonstrate high biocompatibility and improved biodistribution of the RNA within by promoting cellular uptake and cytosolic entry. They typically consist of five main components: nucleic acids, PEGylated lipids, cholesterol, phospholipids, and ionizable lipids. These are essential to the function of RNA carrying nanoparticles as their positive charge at a low pH allows for efficient encapsulation of initially negative RNA as demonstrated in Figure 9.

Another non-viral delivery mechanism is polymeric nanoparticles. Their structural nature allows them to be

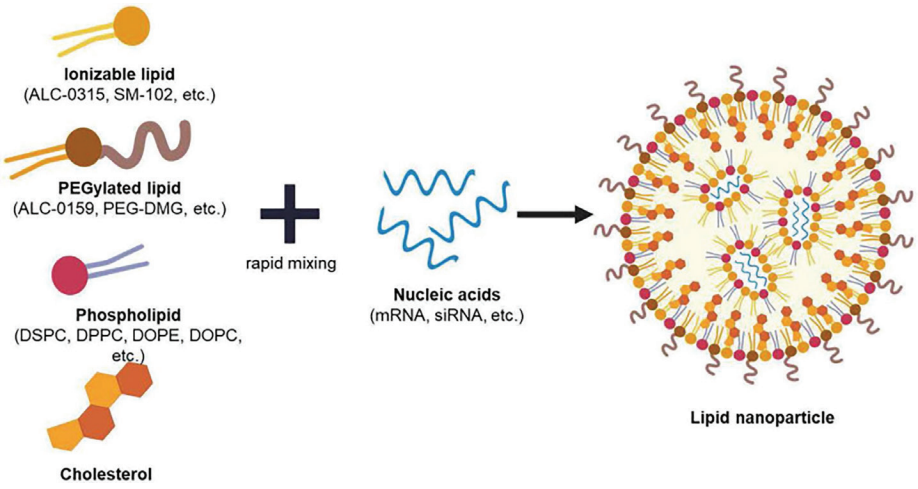

Figure 9. The Composition of Lipid Nanoparticles (36).

extremely customizable, especially in properties such as hydrophobicity, molecular weight, biodegradability, and release kinetics. However, they also face challenges regarding poor oral bioavailability, circulatory instability, and toxicity depending on the polymers used (37). This is being investigated and improved with techniques such as particle replication in no wetting templates (PRINT), which are promising for reducing unintended consequences of polymeric NP administration. Poly-lactic acid, polyglycolic acid and polylactic-co-glycolic acid are among the most frequent synthetic materials used to compose polymeric NPs, as well as naturally-derived polymers such as dextran, gelatin, and collagen. Lastly, exosomes are a novel but scarce delivery mechanism. They occur naturally in the body as a result of cell exocytosis (extracellular vesicles), thus, they exhibit low immunogenicity and toxicity when administered. This also allows them to bypass biological barriers (etc. the blood-brain barrier) as well as endosomal pathways and lysosomal degradation, directly entering the cytoplasm. However, their inconsistent preparation including isolation, characterization, and purification, poses a challenge in administrations as they can lead to the introduction of bacteria, viruses or other contaminants as well as fluctuating content and potency (38).

Cas13 can be programmed to degrade mRNA transcripts of key oncogenes, including EGFR (7p11.2), SOX2 (3q26.33), MYC (8q24.21), HIF-1α (14q23.2), and MGMT (10q26.3) (39). In addition, a beneficial therapy that could be employed alongside CRISPR-Cas13 is immune checkpoint inhibitors (ICIs). These immunotherapy drugs function by blocking checkpoint proteins from binding with their ligands (preventing the "off" signal from transmission), thus allowing T cells to kill cancer cells. Specifically, ICIs target checkpoints such as cytotoxic T-lymphocyte associated protein 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed death ligand-1 (PD-L1) (40). Although only ~20% of patients respond to ICIs, combining them with Cas13-mediated silencing of GSC oncogenes and immune-suppressive transcripts could improve responsiveness by reducing tumor immune evasion (41).

CURRENT LIMITATIONS AND ETHICAL CONCERNS OF THE CRISPR SYSTEMS

CRISPR systems present numerous technical limitations and profound ethical considerations. CRISPR technologies lack complete precision, with off-target or inaccurate cleaving potentially causing unforeseen nucleotide edits. However, these risks may diminish as technology advances.

Moreover, editing human germ lines raises concerns that altered genes could be passed to future generations, producing unpredictable effects. Such genetic uncertainty complicates application as the intervention would affect not only the treated individual, but also their descendants (42). Few diseases result from a single gene alone; environmental and epigenetic factors also shape biological phenotypes. This would decrease the efficacy of CRISPR technologies as multiple administrations with various gene cleavages would have to be condoned to potentially target a single

disease. Although the system is significantly improving approaches to diseases such as cancers, it is difficult to apply to human genomes from prior testing of simple organisms such as *Drosophila melanogaster* (fruit fly) or *Danio rerio* (zebra fish).

Beyond these biological risks, it is also important to evaluate the risks of CRISPR systems in comparison to other mutagenic therapies. For cancers, common therapies including radiation and chemotherapy lead to double strand breaks of DNA and intercalations. Thus, repeated exposure to such therapies poses a comparable risk to CRISPR technologies. Both can introduce genetic mutations and unintended genomic alterations that can be passed to future generations.

In addition to scientific risks, CRISPR also raises broader social concerns. Even if only used for medical purposes, the technology could reintroduce eugenics, the belief that humans can be "improved" through selective breeding of populations and forced sterilization of those deemed "unfit." (43). Moreover, CRISPR technologies could also widen the socioeconomic gap, where the costliness of the treatment would exacerbate disparities in access, allowing only the most fortunate individuals to receive the therapy. This is unethical, as much of CRISPR's development is funded by public taxation, yet many who contributed may ultimately be denied its benefits (44).

POTENTIAL USE IN THE FUTURE

While CRISPR and drug delivery tools are still being developed, many have promising approaches, whether in the near-term or in the future. A nearer-term solution is lipid nanoparticles (LNPs) with BBB-penetrating ligands such as transferrin which can cross the blood-brain barrier, a major obstacle in GBM therapy delivery. Additionally, LNPs have been clinically validated through COVID-19 mRNA vaccines to be efficient in the delivery of mRNA and easily engineered to be adaptable to specific tumor microenvironments such as hyperacidity. Their nanoscale size facilitates uptake by GSCs, improving tumor-specific delivery of the Cas-LNP system. Furthermore, their non-integrative mechanism promotes efficient metabolism and clearance, reducing mutagenesis and long-term toxicity risks (45). In combination with Cas13 technologies, such LNPs could effectively target the GSCs in tumors and overcome numerous tumoral obstacles of GBMs such as hyperacidity, hypoxia, and inefficient delivery.

To evaluate the efficacy of Cas13-based therapies in targeting GSCs, clear experimental endpoints must be established in both in-vitro and in-vivo models. In-vitro models such as cell cultures and organoids should be validated across numerous parameters before translation into in-vivo models or clinical settings. Firstly, it is necessary to check transduction efficacy, quantifying the percentage of GSCs exhibiting Cas13-mediated knockdown. This is also pivotal at the mRNA/protein level, as tracking oncogene expression via methods like qPCR or western blotting after alterations determines whether the therapy will be efficient for the suppression of GSC proliferation. Secondly, off-target cytotoxicity and specificity of the system must be assessed, ensuring minimal toxicity to non-target cells such as healthy astrocytes, a particularly critical factor in the brain. It is also important to quantify changes in expression of differentiation markers (SOX2, Nestin, CD133) to determine persistence of GSC stemness. Finally, immunogenicity markers such as IL-6, TNF-α should be measured as indicators of immune activation and potential adverse effects. Together, these endpoints establish the molecular efficacy, specificity and safety of Cas13-based therapies before advancing to in-vivo models.

In-vivo models such as mouse or patient-derived xenografts necessitate additional endpoint assessments. Firstly, BBB penetration represents a fundamental parameter to quantify: if the drug cannot cross the barrier, its delivery system would require reformulation. Moreover, its biodistribution must be analyzed, determining the distribution of nanoparticles within tissues (if they remain targeted in the brain or get cleared by the liver/spleen system). Furthermore, in-vivo models enable MRI imaging to assess tumor volume reduction after administration as well as survival analysis using Kaplan-Meier curves. Finally, immune response should also be evaluated in-vivo through detection of inflammatory cytokines, T-cell infiltration and antibody development (46). Together, these endpoints provide critical translation data on therapeutic efficacy and survival.

The selection of patients is also critical to ensure the system's effectiveness. From a molecular standpoint, the most suitable patients for this system would have high expressions of previously discussed oncogenes to ensure sufficient transcript silencing to disrupt GSC maintenance and renewal. This would include patients who overexpress key oncogenes such as SOX2, c-MYC, EGFR, HIF-1α, and MGMT as silencing them would impede traits including therapy resistance, stemness

and angiogenesis, addressing the root drivers of GBM recurrence.

As for limitations of this paper, it is based on current preclinical evidence, hence limited by the absence of clinical and experimental evidence. Several assumptions underlie the framework of the paper including the assumption of Cas13 specificity to cleave particular oncogenic transcripts, and the feasibility of the suggested delivery systems to reliably reach their target. The suggested approach to not only use CRISPR-Cas13 but to combine it with other therapies has not yet been supported by sufficient data and thus remains speculative. Moreover, GBM heterogeneity further complicates the application of this framework to patients as the variation of oncogenic expression varies largely, also reducing generalizability. Finally, long-term safety considerations including potential immune responses and the effect of collateral RNA cleavage remain unpredictable and highlight the need for further exploration in-vivo models.

CONCLUSION

This review highlights Cas13's potential to overcome GBM recurrence by silencing key oncogenes within GSCs. Successful translation will depend on optimizing delivery combinations that enable the system to cross the BBB, minimize off-target cleavage, and ensure in-vivo and clinical safety. Advances in both CRISPR-Cas13 technologies as well as LNP engineering are very promising, both individually and combined with immunotherapies such as ICIs. Surpassing testing of the system with LNP transport in model organisms such as *Drosophila melanogaster*—which possess similar neuroanatomies to humans—is essential to assess its suitability the technology for GSC intervention and arranging alterations for the maximization of therapeutic efficacy (47). This treatment could be designed to target the mRNA transcripts of various oncogenes supporting GSCs, particularly the prevalent ones discussed earlier in the paper and monitor their transcript concentrations alongside MRI imaging as markers of therapeutic success. If this treatment reduces tumor initiation or recurrence, the therapy would be considered a substantial improvement to GBM. Potentially, the oncogene EGFRvIII could also be targeted alongside CAR-T therapy, a form of immunotherapy which modifies a patient's own T-cells to attack cancer cells (48). This could further eradicate the oncogene's downstream cascade of harmful proteins and improve immune recognition of the tumor. With further development, Cas13-based therapies could transform the treatment of GBM by directly targeting its most resilient and problematic subpopulation, with the potential to significantly improve patient survival and quality of life.

CONFLICTS OF INTEREST

The author declares that there are no conflicts of interest related to this work.

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