



Review

The Role of CRISPR and Its Therapeutic Applications in Glioblastoma

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Abstract

Glioblastoma multiforme (GBM) remains the most aggressive and treatment-refractory form of primary brain tumor in adults, characterized by rapid proliferation, intratumoral heterogeneity and resistance to current therapies. Despite therapeutic advancements in surgical resection, radiotherapy and chemotherapy, clinical outcomes remain poor, underscoring the need for innovative molecular strategies. This review examines the therapeutic potential of CRISPR/Cas9 genome-editing technologies in GBM, highlighting their ability to model, dissect and potentially correct the genetic alterations that drive GBM tumorigenesis. Key molecular targets, such as *EGFR*, *PTEN*, *TP53*, *NF1* and *PIK3CA*, are discussed within the context of GBM's mutational and signaling landscape. We further outline emerging CRISPR applications in preclinical models, the current status of CRISPR-based clinical trials and the major barriers hindering translation, including off-target effects, immunogenicity and the challenge of delivering gene-editing systems across the blood–brain barrier. Particular emphasis is placed on delivery technologies, viral and non-viral vectors, including lipid nanoparticles, polymeric systems, inorganic nanocarriers and DNA nanostructures, which are rapidly evolving to improve precision, safety and CNS penetrance. Collectively, this review highlights CRISPR/Cas9 as a powerful tool whose integration with molecular neuro-oncology and precision medicine may ultimately shift GBM treatment toward more personalized and durable therapeutic interventions.

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1. Introduction

Glioblastoma (GBM) is the most common and lethal form of primary malignant brain tumor in adults. According to the Fifth Edition of the World Health Organization (WHO) Classification of Tumors of the Central Nervous System (2021), GBM is defined as glioblastoma, *IDH*-wildtype (WHO grade 4) [1]. GBM has an annual incidence of approximately 3–5 cases per 100,000 individuals, with a higher prevalence rate occurring in males compared to females [2]. The median age at diagnosis is around 65 years, with an overall survival of 12–15 months despite aggressive treatment care plans, which include surgical resection, concurrent radiotherapy and temozolomide chemotherapy [2,3]. The 5-year survival rate is below 10% and tumor recurrence within the first year post-treatment is almost universal, highlighting the disease’s therapy-resistant nature. These poor clinical outcomes underscore the need for more effective therapeutic strategies [2,3].

In recent years, CRISPR/Cas9 genome-editing technology has emerged as a powerful tool for dissecting the molecular mechanisms driving GBM progression and resistance, while also representing a promising therapeutic approach for precise genetic intervention. This review explores CRISPR/Cas9 both as a powerful research tool for functional genomics and disease modeling, and as an emerging gene editing-based therapeutic strategy for GBM.

1.1. Pathophysiology

1.1.1. Molecular Alterations in GBM

According to the 5th Edition of the WHO Classification of Tumors of the Central Nervous System (2021), GBMs are now defined as GBM, *IDH*-wildtype (WHO grade 4), while tumors previously referred to as secondary glioblastomas are reclassified under astrocytoma, *IDH*-mutant (WHO grades 2, 3 or 4) [1,4–6] (Table 1).

Table 1. Comparison of glioblastoma, *IDH*-wildtype and astrocytoma, *IDH*-mutant according to WHO 2021 Classification [1,4,6–13].

Feature	GBM (Glioblastoma), <i>IDH</i> -Wildtype	Astrocytoma, <i>IDH</i> -Mutant
Key Mutations	<i>IDH</i> -wildtype, <i>TERT</i> , <i>EGFR</i> , chromosome +7/−10	<i>IDH1/2</i>
WHO Grade	4	2, 3, 4
Age Group	Older adults (sixth and seventh decades)	Younger adults (fourth and fifth decades)

Glioblastoma, *IDH*-wildtype, represents the most common and aggressive diffuse glioma in adults and is characterized by frequent amplification of the epidermal growth factor receptor (*EGFR*) [7], as well as loss of tumor suppressor *PTEN* [8] and telomerase reverse transcriptase (*TERT*) promoter mutations [9]. In contrast, astrocytoma, an *IDH*-mutant, develops through stepwise progression from lower-grade astrocytoma and is characterized by mutations in isocitrate dehydrogenase, *IDH1* or *IDH2*, early *TP53* mutations, and loss of *ATRX*. Both GBM and astrocytoma exhibit dysregulation of core oncogenic signaling cascades, including the PI3K/AKT and RAS/MAPK pathways, as well as alterations in cell-cycle control genes such as *CDKN2A/B* [10]. These genomic and

epigenetic alterations lead to uncontrolled proliferation, therapeutic resistance, and the aggressive clinical behavior characteristic of high-grade diffuse gliomas [1,11–13].

1.1.2. Intratumoral Heterogeneity in GBM

Intratumoral heterogeneity is a major cause of treatment failure in GBM. Intratumoral heterogeneity refers to having, within a single lesion, diverse tumor cell populations exhibiting distinct genetic and phenotypical profiles [12,14,15]. In GBM, large-scale transcriptomic analyses have shown that the canonical GBM expression subtypes, classical, mesenchymal and proneural, can be present simultaneously within the same tumor [15–17].

A critical component of this heterogeneity is the presence of glioma stem-like cells (GSCs), a subpopulation capable of self-renewal, possessing enhanced DNA repair mechanisms and the ability to enter a quiescent state. These properties enable GSCs to survive genotoxic stress induced by radiotherapy and temozolomide, subsequently driving tumor repopulation and recurrence [18–20].

This cellular and molecular diversity poses a major challenge for CRISPR/Cas9-based therapeutic strategies, as heterogeneous tumor subclones may harbor distinct genetic dependencies and variable accessibility to gene-editing systems. Incomplete or uneven genome editing across tumor populations may allow resistant cells, particularly GSCs, to persist and drive relapse, thereby limiting the durability of CRISPR-mediated interventions.

1.1.3. Tumor Microenvironment and Therapeutic Resistance

GBM is greatly immunosuppressive, which significantly contributes to therapeutic failure [21,22]. The GBM tumor microenvironment (TME) is enriched with tumor-associated macrophages (TAMs) originating from both resident microglia and infiltrating monocytes, which secrete cytokines, chemokines and growth factors that promote tumor invasion, angiogenesis and immunosuppression [23,24]. The TME also contains myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), both of which inhibit cytotoxic T cell activation and help in immune evasion [25]. At the molecular level, GBM employs multiple mechanisms to resist therapy, including enhanced DNA damage repair pathways, such as mismatch repair and base excision, along with phenotypical plasticity that enables tumor cells to transition between transcriptional subtypes. These adaptive processes allow GBM cells to survive radiotherapy and alkylating chemotherapy [4,11,26].

Immune recognition is further impaired by the tumor's low expression of major histocompatibility complex (MHC) molecules, which reduces antigen presentation and immune recognition [27]. Added to these challenges is the presence of the blood–brain barrier (BBB), which prevents effective drug delivery to the brain [28,29]. Collectively, these microenvironmental features limit the efficacy of current treatments.

1.2. Current Therapies

The current regimen in GBM consists of maximal safe surgical resection and systemic therapy alongside radiotherapy and adjuvant cycles [30,31]. Temozolomide (TMZ) is the current first-line chemotherapeutic agent, yet its efficacy depends on the methylation status of the O⁶-methylguanine-DNA methyltransferase (*MGMT*) promoter [19,32]. As a result, this regimen is limited to patients with the methylated *MGMT*, with little efficacy for those with unmethylated *MGMT*.

Multiple targeted therapies, targeting key signaling pathways such as *EGFR*, *PI3K/AKT/mTOR* and *VEGF* pathways has been attempted but have shown insignificant success in clinical trials due to the tumor's microenvironment, intralesional heterogeneity and adaptive resistance [33–35]. For example, monoclonal antibodies such as those

targeting *VEGF* have shown improvements in radiographic responses and a reduction in cerebral edema [36], yet they do not translate into prolonged overall survival [37–39].

Ongoing investigations include targeted PARP and *EGFRvIII* inhibitors, nanoparticle-based drug delivery systems and various immunotherapeutic approaches, but none have demonstrated effectiveness for integration into the therapeutic regimen of GBM [11,35]. Collectively, the limited efficacy of current interventions highlights the need for novel therapeutic strategies capable of directly targeting the genetic and molecular drivers of GBM. In this context, CRISPR-based gene-editing technologies represent a promising avenue for developing more precise and effective treatments.

2. CRISPR/Cas9 System

2.1. Discovery and Biological Role of CRISPR

The CRISPR system was first described in 1987 as clustered repetitive DNA sequences interspaced with unique segments in the genome of *Escherichia coli* [40]. These structures, later identified across diverse prokaryotes, were termed Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and their associated proteins became known as CRISPR-associated (Cas) proteins [41–46]. Subsequent studies revealed that CRISPR spacer sequences are derived from viral or plasmid DNA, functioning as an adaptive immune memory that enables sequence-specific recognition and cleavage of invading genetic material [42,47–50]. This mechanism forms the basis of the modern CRISPR/Cas9 gene-editing technology.

2.2. Classification

CRISPR systems are broadly categorized into two major classes based on the composition of their effector complexes, comprising six types and more than thirty subtypes [48–50] (Table 2). Class 1 systems (Types I, III and IV) employ multi-subunit protein complexes to mediate target recognition and cleavage, whereas Class 2 systems (Types II, V and VI) utilize a single, large effector protein, making them more amenable to biotechnological adaptation.

Among Class 2 systems, the Type II CRISPR/Cas9 system from *Streptococcus pyogenes* (SpCas9) rapidly became the most widely used platform for genome editing due to its simplicity and programmability [44,46]. Cas9 is guided by a dual-RNA structure comprising a CRISPR RNA (crRNA) and a trans-activating CRISPR RNA (tracrRNA). These two molecules can be fused into a single guide RNA (gRNA), enabling efficient and precise DNA targeting within eukaryotic cells [51,52]. The ability of Cas9 to function as a programmable nuclease, essentially acting as customizable “molecular scissors”, marked a transformative milestone in gene-editing technology [42–46].

Functionally, all CRISPR systems operate through three conserved phases of adaptive immunity: (1) adaptation (spacer acquisition)—integration of short fragments of invading viral or plasmid DNA into the CRISPR array; (2) expression (crRNA biogenesis)—transcription of the CRISPR locus into pre-crRNA, followed by processing into mature crRNA (with tracrRNA in Type II systems); and (3) interference—recognition and targeted cleavage of complementary foreign nucleic acids by Cas effector proteins [53].

Table 2. Classification of CRISPR-Cas systems, key effector proteins and their main primary functions in nucleic acid targeting and microbial defense.

Class	Type	Key Cas Proteins	Main Function	Ref.
Class 1	Type I	Cas3	Degrades invading DNA through multi-protein cascade complex	[49]

	Type III	Cas10	Targets both DNA and RNA; co-transcriptional cleavage	[48,50]
	Type IV	Csf1 / Csf2	Defense against plasmids and mobile genetic elements	[49]
Class 2	Type II	Cas9	Cleaves double-stranded DNA guided by sgRNA (e.g., SpCas9)	[43,46]
	Type V	Cas12 (Cpf1)	Cleaves DNA with staggered cuts; used in genome editing	[54,55]
	Type VI	Cas13 (C2c2)	Targets and degrades RNA; enables RNA-based editing	[56,57]

2.3. Phase I: Spacer Acquisition and Immune Memory Formation

The first step of CRISPR-mediated adaptive immunity is spacer acquisition, during which the system captures and stores molecular “memories” of invading genetic elements. When a bacteriophage or plasmid infects a prokaryotic cell, short fragments of the foreign DNA, known as protospacers, are recognized and incorporated into the host CRISPR locus as new spacers within the CRISPR array [42,47,58]. This insertion is mediated by the conserved Cas1-Cas2 integrase complex. Cas1 functions primarily as a metal-dependent endonuclease that cleaves and processes foreign DNA, whereas Cas2 stabilizes the Cas1-DNA complex and facilitates accurate integration of the spacer into the CRISPR repeat array [58]. The addition of new spacers allows the establishment of memory from previous invasions, enabling the host to distinguish between self and non-self genes during subsequent infections [48]. Emerging findings suggest that Cas9 can guide the machinery, such as spacer acquisition, by interacting with its components and strengthening threat recognition along with downstream interference and defense functions [50,59].

2.4. Phase II: CRISPR RNA Processing and Cas9 Complex

After new spacers are integrated into the CRISPR array, the locus is transcribed into a long precursor RNA known as pre-crRNA, which contains alternating repeat and spacer sequences. In Type II CRISPR systems, including the CRISPR/Cas9 pathway, pre-crRNA processing requires a second small RNA molecule, the trans-activating CRISPR RNA (tracrRNA). The tracrRNA hybridizes with the repeat regions of pre-crRNA to form an RNA duplex that is recognized and cleaved by Ribonuclease III (RNase III), generating mature crRNA-tracrRNA units [51]. These units subsequently associate with the Cas9 protein to form an active ribonucleoprotein complex [43,52] (Figure 1).

Cas9 is composed of two major lobes: the α -helical recognition (REC) lobe, which stabilizes RNA-DNA interactions and the nuclease lobe, which contains two catalytic domains. The HNH nuclease domain cleaves the DNA strand complementary to the crRNA guide sequence, while the RuvC-like domain cleaves the non-complementary strand, producing a site-specific double-stranded DNA break. An arginine-rich bridge helix within the REC lobe plays a critical role in sensing guide RNA binding and activating the nuclease domains.

In engineered systems, crRNA and tracrRNA are typically fused into a single guide RNA (gRNA), simplifying Cas9 loading and enabling programmable, sequence-specific DNA targeting. Once assembled, the Cas9-gRNA complex scans the genome for sequences complementary to the crRNA spacer, initiating the next phase of CRISPR-mediated defense and forming the foundation of modern genome-editing applications [52,60,61].

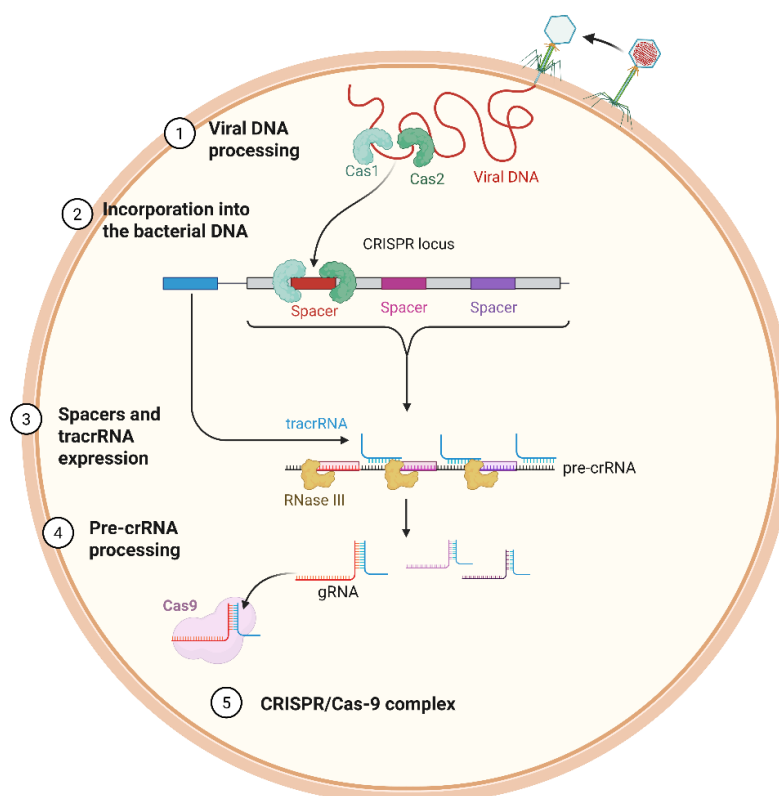


Figure 1. Overview of the CRISPR/Cas9 immune formation and processing of crRNA. This diagram illustrates the main stages of the CRISPR-Cas adaptive immune system in bacteria. (1) Upon viral infection, foreign DNA is recognized and processed by Cas1 and Cas2. (2) Short fragments, known as spacers, are incorporated into the bacterial CRISPR array, forming a genetic record of past infections. (3) The CRISPR locus is transcribed into precursor CRISPR RNA (pre-crRNA) alongside tracrRNA. (4) RNase III processes the pre-crRNA into mature guide RNA (gRNA). (5) Finally, the gRNA associates with Cas9 to form a ribonucleoprotein complex capable of recognizing and cleaving complementary viral DNA sequences during future infections. Created in BioRender. amer, S. (2026) <https://BioRender.com/3g0465y>.

2.5. Protospacer Adjacent Motif (PAM) and Target Recognition

Efficient and specific DNA targeting by Cas9 requires the presence of a Protospacer Adjacent Motif (PAM), a short nucleotide sequence located immediately downstream of the target site [52,60]. The PAM is a short nucleotide sequence required for Cas9 to recognize and bind to its DNA target. PAM recognition is an essential checkpoint that enables Cas9 to initiate DNA interrogation while preventing cleavage of the host's own CRISPR locus, as endogenous CRISPR arrays lack adjacent PAM sequences. This provides a fundamental mechanism for distinguishing self from non-self DNA, thereby preventing autoimmunity.

Structurally, PAM binding induces conformational changes within Cas9 that activate its nuclease domains and trigger local DNA unwinding. These changes allow the guide RNA to hybridize with the complementary DNA strand, forming an RNA-DNA heteroduplex that positions the HNH and RuvC nuclease domains for site-specific double-stranded cleavage. Different Cas proteins recognize distinct PAM sequences; for example, *Streptococcus pyogenes* Cas9 (SpCas9) requires the canonical 5'-NGG-3' motif to initiate target search and cleavage [59–61]. Therefore, the PAM-dependent mechanism ensures high specificity and prevents autoimmunity, forming the molecular basis for both microbial defense and programmable genome editing [43,46].

2.6. Adaptation of CRISPR/Cas9 for Genome Editing in Eukaryotes

Because eukaryotic cells lack endogenous CRISPR/Cas components, the *Streptococcus pyogenes* Cas9 system requires several modifications for efficient use in higher organisms. First, nuclear localization signals (NLSs) must be appended to Cas9 to ensure its import into the eukaryotic nucleus, where genomic DNA resides [43,44]. In engineered systems, the crRNA and tracrRNA are fused into a single-guide RNA (sgRNA), simplifying Cas9 loading and enabling programmable DNA targeting [62,63].

Once Cas9 induces a site-specific double-stranded DNA break, eukaryotic cells repair the lesion through one of two major pathways. Non-homologous end-joining (NHEJ) rapidly ligates DNA ends without the need for a template, but frequently introduces small insertions or deletions, making it useful for gene disruption. In contrast, homology-directed repair (HDR) uses an exogenous or endogenous DNA template to install precise genetic modifications, including point mutations or sequence insertions. To overcome limitations associated with double-strand breaks, next-generation CRISPR platforms such as base editors and prime editors have been developed. These systems enable precise nucleotide conversions or sequence rewrites without inducing DSBs, thereby reducing cytotoxicity and improving editing specificity [55,64,65]. Collectively, these innovations have expanded the utility of CRISPR/Cas9 in eukaryotic systems and are increasingly applied to model and correct disease-associated mutations, including those relevant to GBM.

2.7. Off-Target Effects and Optimization of sgRNA Design

Although CRISPR/Cas9 enables precise genome modification, off-target cleavage remains a major concern, particularly when the sgRNA partially complements unintended genomic loci. Cas9 can tolerate mismatches between the sgRNA and DNA, especially in distal regions away from the PAM, leading to cleavage at sequences that closely resemble the intended target [66]. Such off-target events may introduce undesired mutations, genomic instability or cellular toxicity, underscoring the need for improved specificity. In glioblastoma and central nervous system applications, these risks are amplified because neural cells have limited regenerative capacity and CRISPR-mediated genomic alterations are permanent. Unintended mutations in brain tissue may therefore result in long-term neurotoxicity, altered neural function or secondary oncogenic events, making off-target control particularly critical for GBM-directed therapies [67,68].

To address this challenge, several high-fidelity Cas9 variants have been engineered, including eSpCas9, SpCas9-HF1 and HypaCas9, which reduce nonspecific DNA interactions and significantly diminish off-target cleavage without compromising editing efficiency [62,66]. Parallel to enzyme engineering, advances in sgRNA design algorithms, such as CRISPOR, Benchling and CHOPCHOP, enable computational prediction of optimal guide sequences based on thermodynamics, mismatch tolerance, chromatin accessibility and on/off-target scoring metrics [63]. Ongoing optimization efforts focus on integrating high-fidelity nucleases, improved sgRNA scaffolds, chemical sgRNA modifications and more sophisticated computational tools to enhance precision. Together, these developments increase the safety, reliability and therapeutic potential of CRISPR/Cas9, particularly in translational applications and emerging genome-editing strategies [69,70].

3. Potential CRISPR/Cas9 Targets in Glioblastoma

3.1. Tumorigenesis Drivers

3.1.1. Epidermal Growth Factor Receptors (EGFRs)

Aberrant activation of the epidermal growth factor receptor (EGFR) signaling axis is one of the most frequent oncogenic events in GBM. Approximately 40–60% of GBMs exhibit *EGFR* amplification and a substantial proportion express *EGFRvIII*, a constitutively

active mutant generated through deletion of exons 2–7. *EGFRvIII* lacks a ligand-binding domain, resulting in persistent downstream signaling that drives uncontrolled proliferation, enhanced invasion, metabolic reprogramming and therapeutic resistance [7,71].

CRISPR/Cas9 offers a powerful strategy to selectively target *EGFR*-driven tumorigenesis. Allele-specific sgRNAs can be designed to selectively disrupt *EGFRvIII* while sparing the wildtype *EGFR* allele, enabling precise elimination of mutant-expressing tumor cells. Such targeted editing significantly reduces GBM cell proliferation, colony formation and tumorigenicity [72,73]. Moreover, CRISPR-mediated knockout of *EGFR*, whether mutant or amplified, attenuates multiple oncogenic cascades, including PI3K/AKT/mTOR, MAPK/ERK and related survival pathways, thereby promoting apoptosis, suppressing angiogenic signaling and impairing overall tumor growth [7,68,74–76].

3.1.2. Phosphatidylinositol-4,5-Bisphosphate 3-Kinase (PI3K) Pathway: *PIK3CA*, Phosphatase and Tensin Homolog (*PTEN*)

The phosphatidylinositol-3-kinase (PI3K) pathway is one of the most frequently dysregulated signaling cascades in GBM and plays a central role in promoting cell survival, proliferation, invasion and therapeutic resistance [77]. *PIK3CA*, which encodes the p110 α catalytic subunit of class I PI3K, is responsible for converting phosphatidylinositol-4,5-bisphosphate (PIP2) into phosphatidylinositol-3,4,5-trisphosphate (PIP3). The accumulation of PIP3 activates downstream AKT/mTOR signaling, driving metabolic reprogramming and sustained tumor growth [78,79]. Mutations or amplification of *PIK3CA* result in constitutive activation of this pathway and are strongly associated with aggressive GBM phenotypes [77,80].

PTEN is a tumor suppressor lipid phosphatase that works by reverting PIP3 to PIP2 to antagonize the PI3K/AKT signaling pathway to control cell growth and survival [81]. *PTEN* loss, through mutation, deletion or loss of heterozygosity, is common in GBM and leads to persistent PI3K/AKT hyperactivation, enhanced invasion and increased resistance to both radiotherapy and chemotherapy [8,82]. The frequent co-occurrence of *PIK3CA* activation and *PTEN* inactivation produces a synergistic oncogenic effect, driving highly proliferative and treatment-refractory tumors [83].

CRISPR has been used to engineer GBM models combining *PIK3CA* activation with *PTEN* knockout to recapitulate the aggressive, therapy-resistant behavior observed in human tumors and are now being used to identify pathway vulnerabilities and evaluate targeted inhibitors [68,76]. Additionally, CRISPR-based strategies aimed at restoring *PTEN* function, either through direct gene correction or targeted editing of upstream regulatory elements, have shown promise in reducing tumor growth and re-sensitizing glioma cells to therapy [76,84].

3.1.3. RAS/MAPK Pathway: Neurofibromin 1 (*NF1*)

NF1 encodes neurofibromin, a GTPase-activating protein (GAP) that negatively regulates the RAS/MAPK signaling cascade by accelerating the conversion of active RAS-GTP to its inactive RAS-GDP form. Through this function, neurofibromin suppresses proliferative and pro-survival signaling in normal cells [85]. Loss of *NF1*, via mutation, deletion or loss of heterozygosity, is particularly enriched in the mesenchymal subtype of GBM and results in constitutive RAS/MAPK activation. This drives enhanced proliferation, increased invasiveness and broad resistance to conventional therapies [5,86].

CRISPR/Cas9 has played a key role in modeling *NF1* deficiency and elucidating its contribution to GBM biology. Somatic CRISPR-mediated deletion of *Nf1* in neural progenitors effectively reproduces the hyperactive RAS phenotype, validating *NF1* as a critical tumor suppressor. Moreover, in vivo CRISPR knockout models combining *Nf1*, *Pten* and *Trp53* loss robustly induce high-grade gliomagenesis in mice, establishing a causal

relationship between *NF1* inactivation and aggressive malignant transformation [5,13,87,88].

3.1.4. Stemness and Lineage Factors: *SOX2*, *FOXG1*

GBM is sustained by a subpopulation of glioma stem-like cells (GSCs) that exhibit self-renewal capacity, lineage plasticity and marked resistance to therapy [18]. Two transcription factors, *SOX2* and *FOXG1*, play central roles in maintaining these stem-like states and are frequently dysregulated in GBM [89].

SOX2 is a member of the SRY-box family of transcription factors and is a key regulator of pluripotency, neural stem cell maintenance and lineage specification. In the adult brain, *SOX2* supports neural stem cell renewal and preserves undifferentiated cell identity [90]. In GBM, *SOX2* is frequently upregulated, where it drives GSC survival, promotes therapeutic resistance and enhances tumor invasiveness. Elevated *SOX2* expression correlates with poor prognosis, aggressive tumor behavior and recurrence following therapy [91,92]. CRISPR/Cas9-mediated disruption of *SOX2* in GBM models has shown promising results, including decreased GSC proliferation, increased sensitivity to chemotherapy and radiotherapy, and reduced tumor growth and self-renewal in vitro and in vivo [93,94].

FOXG1, a member of the Forkhead box (FOX) family of transcription factors, is involved in neural development, forebrain formation and the regulation of progenitor proliferation [95]. Dysregulation of FOX family proteins broadly contributes to cancer initiation, progression and drug resistance [96]. In GBM, *FOXG1* is frequently overexpressed, where it acts to inhibit the differentiation of GSCs and maintain their stem-like properties. This promotes tumor propagation and contributes to treatment resistance [89]. CRISPR-based knockout of *FOXG1* has been shown to impair GSC self-renewal, drive differentiation toward non-tumorigenic cell fates, suppress proliferation in vitro and reduce tumorigenicity in orthotopic xenograft models [89].

3.1.5. DNA Repair and Resistance Genes: *MGMT*, *MSH6*, *PARP1*, *RAD51*

Resistance to temozolomide (TMZ), the frontline chemotherapeutic agent for GBM, is a major clinical challenge and is strongly influenced by alterations in DNA repair pathways. Among these, O6-methylguanine-DNA methyltransferase (*MGMT*) plays a central role. *MGMT* directly removes TMZ-induced O6-methylguanine adducts, thereby preventing mismatch formation and downstream apoptosis. High *MGMT* expression, typically driven by an unmethylated promoter, confers robust TMZ resistance [19,32,97,98]. CRISPR/Cas9-mediated *MGMT* knockout or promoter disruption has been shown to restore TMZ sensitivity in resistant GBM models, highlighting *MGMT* as an actionable target for overcoming chemoresistance [98,99].

Beyond *MGMT*, additional DNA repair genes contribute to resistance through complementary pathways. Loss-of-function alterations in *MSH6*, a key component of the mismatch repair (MMR) system, allow tumor cells to tolerate O6-methylguanine lesions, leading to hypermutation and acquired TMZ resistance. Similarly, *PARP1* and *RAD51*, essential mediators of base excision repair and homologous recombination, respectively, promote survival after DNA damage induced by TMZ or radiation [100,101]. CRISPR/Cas9 targeting of *MSH6*, *PARP1* or *RAD51* disrupts these repair mechanisms, increasing DNA double-strand breaks and promoting synthetic lethality when combined with alkylating chemotherapy or radiotherapy [76,102].

3.1.6. Tumor Suppressor Gene: *TP53*

TP53 is one of the most frequently mutated tumor suppressors in GBM and plays a central role in regulating cell cycle arrest, apoptosis, DNA repair and genomic stability. Loss-of-function mutations in *TP53* compromise these protective mechanisms, enabling

uncontrolled proliferation, impaired apoptotic responses and increased tolerance to genomic damage, hallmarks of GBM's aggressiveness [103,104]. CRISPR/Cas9-based strategies have explored multiple approaches to restore *TP53* function or compensate for its loss. Direct correction of *TP53* mutations or targeted reactivation of wildtype *TP53* alleles have been shown to re-sensitize GBM cells to apoptosis and suppress tumor growth. Alternatively, CRISPR-mediated activation of *TP53* downstream effectors, such as pro-apoptotic genes or cell cycle checkpoints, provides a complementary strategy to restore tumor-suppressive signaling in *TP53*-deficient contexts [74,76,105].

3.2. Genes Involved in GBM Invasion

GBM is characterized by marked local invasion rather than distant metastasis, driven by dysregulated signaling networks that remodel the cytoskeleton, alter cell–matrix interactions and promote extracellular matrix degradation. CRISPR-based functional genomic screens have helped identify several key regulators of GBM invasiveness, offering new therapeutic targets to limit tumor infiltration and recurrence.

3.2.1. MAP4K4

MAP4K4 is a serine/threonine kinase implicated in cytoskeletal remodeling, integrin signaling and cell motility [106]. In GBM, elevated *MAP4K4* activity promotes invasive behavior and facilitates the transition toward a mesenchymal phenotype [107]. CRISPR/Cas9-mediated knockout of *MAP4K4* significantly suppresses migration and infiltration in preclinical GBM models, underscoring its essential role in tumor invasion. As a potential target, *MAP4K4* and its downstream effectors may serve as promising targets for strategies aimed at restricting tumor spread following surgical resection [108].

3.2.2. GDF15

Growth differentiation factor 15 (*GDF15*) is highly expressed within the GBM tumor microenvironment, where it contributes to immune evasion by promoting immunosuppressive polarization of tumor-associated macrophages [109,110]. CRISPR/Cas9-mediated disruption of *GDF15*, delivered via nanoparticle-based systems, has been shown to reprogram macrophages toward pro-inflammatory phenotypes and significantly enhance cytotoxic T cell infiltration. Importantly, *GDF15* knockout synergizes with immune checkpoint inhibitors, improving antitumor immune responses and suppressing tumor growth more effectively than monotherapy [111]. These findings underscore CRISPR's potential not only to directly target tumor-intrinsic drivers but also to remodel the immunosuppressive microenvironment that enables GBM invasion, persistence and recurrence.

3.2.3. Mesenchymal /EMT Genes: STAT3, ZEB1, TWIST1

Transcription factors such as *STAT3*, *ZEB1* and *TWIST1* are major regulators of the epithelial-to-mesenchymal transition (EMT) [112] and play critical roles in driving the mesenchymal subtype of GBM. Their activation enhances cell motility, invasion, stem-like behavior and resistance to chemotherapy, thereby contributing to aggressive tumor progression [113]. CRISPR-based functional screens have identified these EMT regulators as essential drivers of GBM invasiveness [114,115]. Targeted CRISPR/Cas9 disruption, delivered using engineered exosome systems, effectively suppresses *STAT3*, *ZEB1* or *TWIST1* expression, leading to a reversal of mesenchymal phenotypes and restoration of more epithelial-like states [115]. These interventions also reduce TMZ resistance, highlighting the therapeutic potential of targeting EMT-associated transcription factors in GBM [116,117] (Table 3).

Table 3. Key oncogenic drivers, tumor suppressors and genes promoting invasiveness in GBM, their pathway involvement and CRISPR/Cas9 applications.

Gene	Primary Pathway(s)	Normal Function	Alterations in GBM	CRISPR/Cas9 Application	Ref
<i>EGFR / EGFRvIII</i>	PI3K/AKT/mTOR; MAPK/ERK	Promotes cell proliferation and survival signaling	EGFR amplification or EGFRvIII mutation → uncontrolled growth, angiogenesis	Allele-specific knockout of EGFRvIII reduces proliferation, tumorigenicity and suppresses downstream PI3K/MAPK signaling	[7,73]
<i>NF1</i>	RAS/MAPK	Negative regulator of RAS signaling (tumor suppressor)	Loss of NF1 → hyperactive RAS → proliferation, invasion, therapy resistance (mesenchymal subtype)	CRISPR-mediated NF1 KO models gliomagenesis and identifies synthetic vulnerabilities	[85,86,88]
<i>PIK3CA</i>	PI3K/AKT/mTOR	Generates PIP3 activating AKT & mTOR → cell growth & metabolism	Mutations/amplification → survival advantage & treatment resistance	CRISPR models combined PIK3CA activation + PTEN loss → identifies targets for PI3K inhibitors	[34,77,88]
<i>PTEN</i>	PI3K/AKT/mTOR	Converts PIP3 → PIP2 to suppress cell survival signaling	LOH, mutation or deletion → uncontrolled AKT activation → invasion & radio/chemoresistance	CRISPR correction or modeling PTEN loss → identifies therapeutic vulnerabilities	[81,84,88]
<i>MGMT</i>	DNA repair (O6-methylguanine reversal)	Removes alkyl groups from O6-methylguanine; prevents mutations	Overexpression → TMZ resistance	CRISPR knockout or promoter silencing restores TMZ sensitivity	[32,68,97,98]
DNA Repair Genes (<i>MSH6, PARP1, RAD51</i>)	DNA damage response	Maintains genomic integrity and DNA repair	Enhance repair → survival under chemo/radiation	CRISPR disruption induces synthetic lethality with TMZ/radiation	[101,102]
<i>TP53</i>	Cell cycle control, apoptosis	Tumor suppressor that mediates DNA repair and apoptosis	Mutation/inactivation → uncontrolled proliferation & apoptosis evasion	CRISPR restores WT-TP53 or activates downstream apoptosis signals	[103,104,118]
<i>FOXG1</i>	Transcriptional regulation; stemness	Regulates neuronal development and differentiation	Overexpression maintains GSC stemness and therapy resistance	CRISPR knockout induces differentiation and reduces tumorigenicity	[76,89,119]
<i>SOX2</i>	Stem cell pluripotency and self-renewal	Maintains neural stem cell identity	Upregulated → GSC maintenance, recurrence, poor prognosis	CRISPR silencing reduces proliferation & enhances treatment sensitivity	[89,92–94]
<i>MAP4K4</i>	Cytoskeletal remodeling & integrin signaling	Regulates cell motility and actin dynamics	Drives invasion, migration and mesenchymal transition → highly infiltrative GBM	CRISPR knockout reduces invasion and migration in preclinical GBM models; potential anti-recurrence target after surgery	[106–108]

<i>GDF15</i>	Tumor microenvironment remodeling & immune evasion	Regulates cell stress responses and immune signaling	Promotes macrophage polarization to immunosuppressive phenotypes → supports invasion and therapy resistance	Nanoparticle-delivered CRISPR knockout reprograms macrophages, improves T cell infiltration, enhances checkpoint inhibitor therapy	[109–111]
STAT3 / ZEB1 / TWIST1 (Mesenchymal TFs)	EMT-like transition, stemness & invasion pathways	Cell differentiation and stress response regulation	Promote epithelial-to-mesenchymal transition → increased invasion, stemness and TMZ resistance	CRISPR screening and exosome-delivery reverse mesenchymal traits and improve TMZ sensitivity	[113–117]

→ denotes “leads to”.

In comparison to current standard GBM therapies, conventional treatments such as temozolomide, radiotherapy and targeted kinase inhibitors primarily act at the protein or pathway level and are often limited by adaptive resistance mechanisms, pathway redundancy and intratumoral heterogeneity. In contrast, CRISPR/Cas9 enables direct and permanent modification of oncogenic drivers, tumor suppressors and resistance-associated genes at the genomic level, offering the potential for durable pathway suppression. However, unlike pharmacological therapies that can be discontinued or dose-adjusted, genome editing introduces irreversible genetic changes, raising important concerns regarding safety, off-target effects and long-term consequences in neural tissue. Thus, while CRISPR approaches provide unparalleled mechanistic precision and therapeutic promise, their clinical translation will require a careful balance between efficacy, specificity and biosafety.

4. Applications of CRISPR in GBM

4.1. Preclinical Applications in GBM

The application of the CRISPR/Cas9 (CRISPR/Cas) system in the context of GBM remains mostly at the preclinical stage but offers promising therapeutic avenues. Accumulating evidence demonstrates its substantial potential as both a research tool and a therapeutic strategy. CRISPR technologies have enabled precise examination and editing of genes driving GBM proliferation, invasion, metabolic reprogramming, therapy resistance and microenvironmental remodeling [75,76]. Similarly, CRISPR interference with stemness-associated transcription factors such as *SOX2*, *FOXP1* and *HER2/ERBB2* (and related RTKs) reduces GBM cell proliferation and tumorigenicity [72,76,88,89,94]. CRISPR deletion of *Tp53* in vivo models of gliomas accelerates high-grade glioma formation, confirming its central role in tumor initiation and progression [103,120]. Moreover, CRISPR/Cas9-mediated targeting of *EGFR* exon 17 can reduce *EGFR* and *EGFRvIII* expression, inhibiting glioma proliferation in vitro [73]. Beyond gene-level mechanisms, CRISPR has enabled genome-wide loss-of-function screening to uncover vulnerabilities essential for GBM cell survival [99]. Parallel advances in delivery platforms, such as nanoparticles and engineered vesicles capable of crossing the BBB, have further expanded the translational potential of CRISPR-based interventions, improving the feasibility of delivering genome-editing tools directly to intracranial tumors in preclinical models [121,122]. Collectively, these studies illustrate how CRISPR/Cas9 provides powerful opportunities to dissect GBM biology and lays the foundation for next-generation therapeutic strategies targeting the molecular architecture of GBM.

4.2. CRISPR-Based Clinical Trials in GBM

To date, no completed clinical trials have directly applied CRISPR/Cas9 genome editing to GBM cells in patients. The clinical use of CRISPR in GBM remains in its infancy, with current efforts primarily centered on CRISPR-engineered immune cells designed to enhance antitumor activity rather than direct editing of tumor cells.

One notable early phase trial involves CRISPR-modified CAR-T cells targeting *IL13Ra2*, a cell surface antigen overexpressed in GBM (NCT06815029). In this trial, T cells are engineered to disrupt *TGFB β 2*, a key mediator of immunosuppression in the GBM microenvironment, with the dual aim of enhancing CAR-T cell persistence and improving antitumor efficacy in patients with recurrent or progressive high-grade gliomas, including GBM.

Beyond GBM-specific applications, CRISPR-edited T cells have been evaluated across multiple clinical trials for hematologic malignancies and solid tumors, establishing a translational framework for genome-edited cell therapies [123,124].

Although direct CRISPR editing of GBM tumor cells has not yet reached clinical testing, early phase trials targeting relevant GBM antigens are underway. For example, *EGFRvIII*-directed CAR-T cells, although not CRISPR-edited in current trials, represent a therapeutic platform that could be enhanced through CRISPR technologies in future iterations [73]. Similarly, CRISPR-based checkpoint modulation (e.g., PD-1 silencing) in immune cells has entered clinical evaluation and may be adaptable to GBM-specific applications [125].

5. Delivery Methods for CRISPR

GBM research currently uses CRISPR/Cas9 mostly to understand molecular mechanisms, identify therapeutic targets and test gene-editing techniques in experimental models [126]. However, translation into clinical application remains at an early stage. Experiments from other cancer gene therapy trials offer a framework for future GBM interventions, but several barriers must still be overcome. These include efficient and safe delivery across the BBB, minimizing immunogenicity, avoiding off-target edits and generating robust evidence of safety and therapeutic benefit in humans [123,124,127]. Therefore, CRISPR/Cas9 holds significant promise for GBM, but its clinical deployment will require overcoming substantial challenges before routine clinical use becomes feasible.

Delivering the CRISPR/Cas9 system effectively into cells is one of the biggest challenges in applying genome editing for therapeutic purposes. Different strategies have been developed to introduce Cas9 and guide RNA into the desired cells, including physical, viral and non-viral methods. Each approach has its own strengths and drawbacks depending on the target tissue, delivery efficiency and safety [128–130]. In the case of GBM, delivery remains particularly challenging due to the presence of the BBB.

5.1. Physical Delivery Methods

Physical delivery approaches rely on externally applied mechanical or electrical forces to facilitate the entry of CRISPR/Cas9 components into cells. These methods are primarily suited for in vitro and ex vivo applications, where direct manipulation of cells is feasible. Although their clinical relevance in GBM is limited, mainly due to the inaccessibility of tumor cells deep within the brain and the constraints imposed by the BBB, they remain essential tools for optimizing delivery efficiency, validating gene-editing constructs and establishing baseline editing performance prior to in vivo experimentation.

5.1.1. Microinjection

Microinjection is a highly precise physical delivery technique in which CRISPR/Cas9 components, such as plasmid DNA, mRNA or ribonucleoprotein (RNP) complexes, are directly introduced into the cytoplasm or nucleus of individual cells using a micromanipulator under high-resolution microscopy. This method bypasses extracellular and membrane barriers, enabling efficient delivery of cargos with diverse molecular sizes and allowing tight control over dosage and composition [131] (Figure 2).

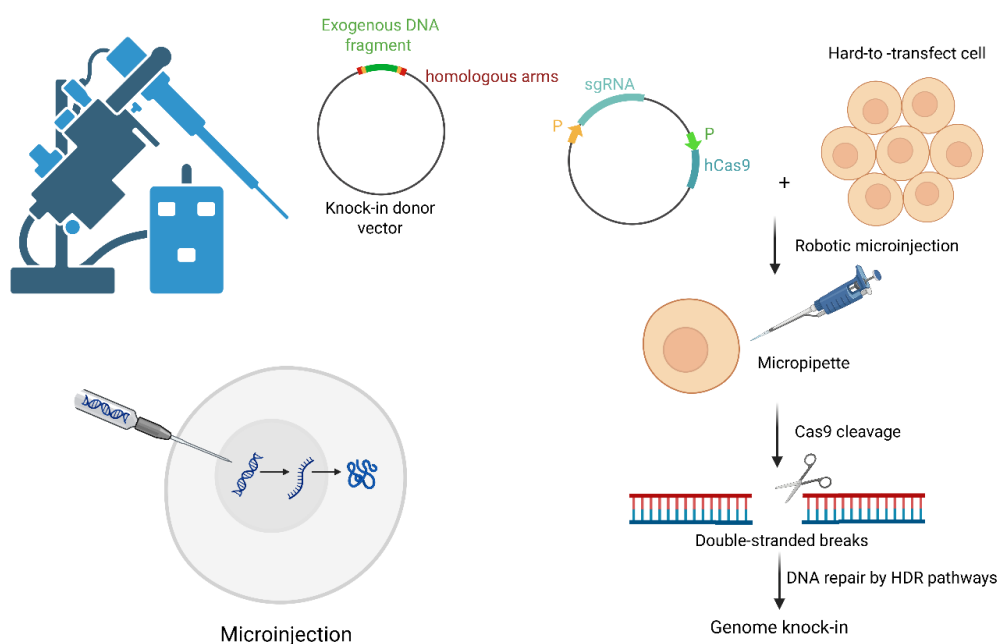


Figure 2. Schematic representation of CRISPR/Cas9-mediated genome knock-in via microinjection. This diagram illustrates the microinjection process used to deliver CRISPR/Cas9 components into hard-to-transfect cells or embryos. A knock-in donor vector containing the exogenous DNA fragment and homologous arms is co-delivered with the Cas9/sgRNA construct using a micropipette under robotic or manual control. The injected Cas9 induces site-specific double-stranded DNA breaks, which are subsequently repaired through homology-directed repair (HDR) pathways using the donor template, resulting in targeted genome knock-in. The use of automated or robotic microinjection systems enhances precision, throughput and reproducibility of the gene-editing process. Although microinjection is not applicable for in vivo GBM therapy due to the tumor's diffuse infiltration into brain tissue and the inaccessibility of individual tumor cells, it remains a valuable in vitro tool [132–135]. Created in BioRender. Badawy, M. T. M. (2026) <https://BioRender.com/ypfszqlin>.

Because it enables direct introduction of CRISPR components, microinjection offers excellent precision, minimal off-target distribution and high editing accuracy. It is frequently used in early embryos and cultured cells to generate knockout or knock-in models and to validate gene-editing constructs [136]. Automation technologies, including piezo-assisted and robot-guided microinjection systems, have substantially improved throughput and survival rates in experimental settings, making the technique more scalable compared to traditional manual injection workflows [132–134].

However, microinjection remains impractical for clinical applications in GBM. The method is limited to handling small numbers of cells and requires direct visual access to the target, making it unsuitable for the heterogeneous and deeply invasive tumor architecture of GBM. Thus, while microinjection provides valuable mechanistic insights and serves as an important in vitro optimization tool, its translational relevance is low

compared with viral or nanoparticle-based delivery systems that can reach tumor cells within the brain.

5.1.2. Electroporation

Electroporation is a widely used physical method for introducing CRISPR/Cas9 components into cells by applying short, high-voltage electrical pulses that transiently disrupt the plasma membrane. This rapid and reversible permeabilization allows nucleic acids, proteins and ribonucleoprotein (RNP) complexes to enter the cytoplasm efficiently, making electroporation a powerful tool for in vitro gene editing. The technique is compatible with cargos ranging from plasmids to Cas9 RNPs and nanoparticles, offering considerable flexibility and high editing efficiency in many cell types [137].

Electroporation parameters, such as voltage, pulse duration and pulse number, must be precisely optimized for each cell type to balance delivery efficiency with cell viability. Excessive electrical stress can induce cytotoxicity, making the method unsuitable for fragile or highly stress-sensitive cells. Advances in electroporation protocols and device engineering have improved consistency and enabled high-throughput editing in cultured cells [138].

Recent developments include the use of electroporation to load CRISPR/Cas9 components into extracellular vesicles, such as red blood cell-derived EVs, enabling efficient encapsulation of Cas9 mRNA and gRNA for targeted delivery in experimental cancer models [139]. Although promising, these applications remain at an early preclinical stage.

Despite its utility, electroporation has limited translational relevance for GBM. The technique requires direct access to cells in suspension or culture, which is incompatible with the invasive, heterogeneous architecture of GBM tumors in the brain. Moreover, electroporation cannot circumvent the BBB or achieve widespread distribution across tumor regions in vivo. As such, electroporation serves primarily as a valuable in vitro optimization and screening tool, but alternative delivery platforms, such as viral vectors, nanoparticles or BBB-penetrant exosomes, are required for therapeutic gene editing in GBM [138].

5.1.3. Hydrodynamic Injection

Hydrodynamic tail vein injection (HTVI) involves the rapid, high-pressure infusion of a CRISPR/Cas9-containing plasmid dissolved in physiological saline. The sudden increase in venous pressure transiently expands liver sinusoids, enabling efficient gene transfer into hepatocytes [140–142] (Figure 3). This technique has been widely used in vivo to model liver diseases by delivering CRISPR/Cas9 directly into mouse or rat livers, for example, to generate oncogenic fusions, induce liver tumors or edit metabolic genes such as *Pten* [141,142]. However, despite its value in hepatic gene-editing research, HTVI is not suitable for GBM applications. The method relies on hemodynamic forces unique to the liver and demonstrates extremely low transfection efficiency in non-hepatic tissues, including the brain. Moreover, the rapid high-volume injection can cause significant cardiac and vascular stress, making it unsafe and impractical for clinical translation. Because of these limitations, poor targeting of brain tissue, inability to cross the BBB, low efficiency outside the liver and substantial systemic risks, HTVI has no feasible application for CRISPR/Cas9 delivery in GBM therapy. As such, research on GBM gene editing has shifted toward more brain-compatible strategies, including nanoparticles, viral vectors, intratumoral delivery and convection-enhanced delivery systems.

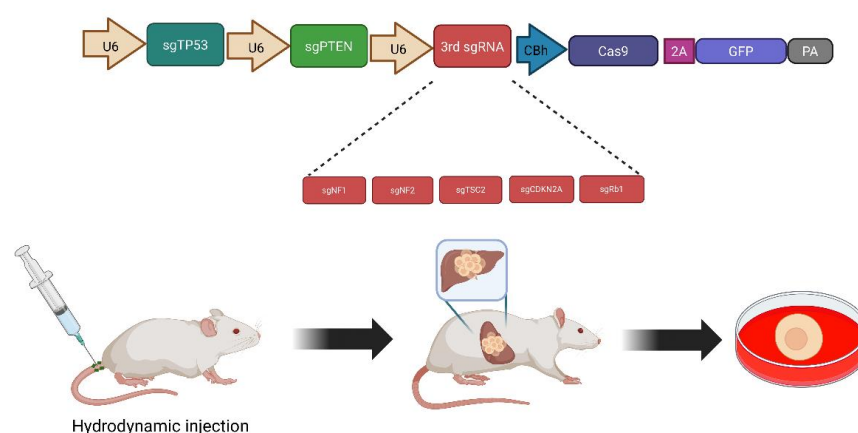


Figure 3. Schematic representation of CRISPR/Cas9 delivery via hydrodynamic tail vein injection (HTVI). The CRISPR/Cas9 vector containing multiple sgRNAs (e.g., targeting *TP53*, *PTEN* and additional genes) is rapidly injected into the mouse tail vein in a large volume of physiological saline. The high-pressure injection causes transient liver expansion, facilitating efficient plasmid uptake by hepatocytes. This enables in vivo genome editing and tumor induction in the liver. Following injection, liver tumors can be isolated and cultured as organoids for further experimental analysis. HTVI offers a convenient and efficient approach for liver-specific genome editing and disease modeling, although it remains limited by low transfection efficiency in non-hepatic tissues and potential cardiac stress in animal subjects. Although HTVI is not applicable to GBM, because the method is inherently liver-restricted and ineffective in brain tissue due to the BBB, it remains an important reference delivery system in the CRISPR literature [140–142]. Created in BioRender. Badawy, M. T. M. (2026) <https://BioRender.com/x7ucjd6>.

5.2. Viral Vector Systems

Viral vectors remain the most widely used platforms for delivering CRISPR/Cas9 into mammalian tissues because of their high transduction efficiency and ability to infect non-dividing cells such as neurons and glioma stem cells [143]. For GBM, viral delivery must overcome additional challenges, including efficient penetration of the tumor core, infiltration of diffuse tumor margins and delivery across or around the BBB. Among existing systems, lentivirus (LV), adeno-associated virus (AAV) and adenovirus (AV) are the dominant vectors evaluated for CRISPR-based applications in the CNS [144].

5.2.1. Lentiviral Vectors

Lentiviral vectors can accommodate relatively large genetic payloads (~7 kb), enabling the delivery of SpCas9 (~4.2 kb) plus sgRNA(s) in a single construct [145]. This makes LVs attractive for CRISPR applications targeting large GBM-associated genes such as *NF1*, *PTEN* and *TP53*. Third-generation “self-inactivating” (SIN) LV vectors reduce risks of insertional mutagenesis and improve biosafety [146]. However, LV tropism remains a challenge for CNS applications [147]. In GBM models, LV-CRISPR systems have been successfully used to generate functional knockouts in *SOX2* [94] and *PD-1* [148], supporting tumor modeling and the identification of synthetic lethal interactions. Despite their efficiency, LV vectors integrate into the host genome, raising concerns for permanent Cas9 expression, potential genotoxicity and immune activation [145]. For this reason, non-integrating LV vectors (NILVs) are being increasingly explored, although they currently show lower efficiency and remain technically more challenging to produce.

5.2.2. Adeno-Associated Virus

AAV is considered the most promising viral platform for in vivo CRISPR delivery in CNS disorders, owing to its natural neurotropism, low immunogenicity and ability to transduce neurons and glial cells [149]. Its serotype specificity (e.g., AAV2, AAV9, and AAV-PHP.B) allows selective targeting of brain tissue [150,151], making AAV particularly relevant for GBM applications. However, AAV's packaging limit (~4.7 kb) poses difficulty for delivering SpCas9 [152]. To overcome this, researchers frequently use SaCas9 (~3.2 kb), Split-Cas9 systems and self-deleting AAV-CRISPR constructs that limit prolonged Cas9 expression. They have also developed a creative integrated self-deleting AAV-CRISPR-Cas9 system. This system includes sgRNA and the *Staphylococcus aureus* Cas9 nuclease (SaCas9). In this system, the Cas9 protein cleaves the AAV vector in addition to facilitating the cleavage of the targeted *PCSK9* gene. This clever method significantly reduces off-target effects, avoids excessive self-expression and improves safety [153]. AAV is already widely used as a delivery system in many different fields and clinical trials will soon replace in vitro trials [149,152].

For GBM specifically, AAV has been used to deliver CRISPR targeting *EGFRvIII* and *PDGFRA*, demonstrating effective gene disruption and reduced tumor growth in preclinical models [154,155]. A central limitation is the immune response to both AAV capsid proteins and Cas9, which can impair delivery, clear edited cells and induce neuroinflammation [156,157]. Emerging solutions include alternative AAV serotypes, Cas9 orthologs with lower immunogenicity and sensitive immune-monitoring platforms [158]. This would improve the safety and effectiveness of the AAV-CRISPR system when used for human gene editing by enabling researchers to better understand and manage possible immune reactions.

5.2.3. Adenoviral Vectors (AVs)

Adenoviral vectors (non-enveloped, dsDNA viruses) offer high payload capacity (up to 8 kb in standard AVs and ~35 kb in helper-dependent AVs), enabling co-delivery of Cas9, multiple sgRNAs and reporter genes in a single vector. Importantly, they do not integrate into the genome, reducing the risk of insertional mutagenesis [159,160]. Third-generation AVs such as HCAdV and HDAdV show markedly reduced viral DNA content and improved safety profiles. Engineering self-cleaving adenoviral CRISPR/Cas9 constructs has further minimized long-term Cas9 expression and off-target risks [159].

For GBM, AVs have been evaluated for editing *EGFR* and *EGFRvIII* [161], modifying immune pathways (e.g., *PD-1* [148]) and delivering CRISPR to resected cavity margins due to their strong transduction capacity. Nevertheless, AVs remain highly immunogenic, which is a major concern for CNS applications, where inflammation can worsen edema or neurological deficits [159].

5.3. Non-Viral Vector Delivery

Non-viral delivery systems are gaining momentum as safer and more adaptable alternatives to viral vectors for CRISPR/Cas9 transport, particularly relevant in GBM, where immunogenicity, insertional mutagenesis and persistent Cas9 expression pose serious risks. Non-viral carriers offer several advantages, such as high cargo capacity, reduced immune activation, transient Cas9 activity and tunable physicochemical properties [162], all of which are critical for genome editing in the CNS. Below, we summarize the major non-viral platforms with emphasis on GBM-specific applications and translational potential.

5.3.1. Lipid Nanoparticles

Lipid nanoparticles (LNPs) have emerged as one of the most promising non-viral delivery platforms for CRISPR/Cas9 genome editing, owing to their biocompatibility, formulation flexibility, high nucleic acid loading capacity and ability to promote endosomal escape. Typically composed of ionizable lipids, phospholipids, cholesterol and PEG-lipids, LNPs can be further optimized through the incorporation of permanently cationic or disulfide-cleavable lipids, which enhance intracellular release and improve the stability of the CRISPR cargo [163]. These physicochemical properties make LNPs particularly attractive for gene-editing applications within the central nervous system, where delivery is hindered by the BBB and the highly infiltrative nature of GBM.

Recent studies have demonstrated that LNP-mediated CRISPR delivery is increasingly feasible in GBM models. Rouatbi et al. reported efficient co-delivery of Cas9 mRNA and sgRNA in mesenchymal glioma stem cells (GSCs), achieving up to 80% gene knockout in orthotopic tumors, underscoring the ability of LNPs to target aggressive GSC populations [164]. Similarly, Zhang et al. developed biodegradable LNPs encapsulating Cas9 mRNA and sgRNA targeting *miR-10b* in orthotopic GBM models; intracerebroventricular administration resulted in successful editing within both the tumor core and invasive margins, suppression of tumor progression, enhanced CD8⁺ T cell infiltration and improved survival in orthotopic GBM models [165]. These findings highlight the potential of LNPs as clinically translatable vectors capable of targeting heterogeneous and deeply infiltrative tumor compartments.

Several considerations remain central to advancing LNP-based CRISPR therapy for GBM. First, BBB and blood–tumor barrier (BTB) penetrance remains variable; although BTB permeability is increased in GBM, it is spatially heterogeneous, necessitating surface functionalization strategies such as transferrin or angiopep-2 ligands to facilitate receptor-mediated transport. Second, the marked intra- and inter-tumoral heterogeneity of GBM requires broad nanoparticle distribution to reach subclonal populations, including therapy-resistant GSCs scattered throughout the brain parenchyma. Third, transient expression achieved by delivering Cas9 mRNA or RNP complexes via LNPs may reduce long-term off-target editing, a significant advantage in the sensitive neural environment, where persistent nuclease expression carries higher toxicity risks. Nonetheless, payload immunogenicity, potential neuroinflammation and off-target editing in neuronal and glial cells must be rigorously evaluated in preclinical studies. Finally, the translational potential of LNP-based CRISPR therapy will depend on the ability to manufacture reproducible, scalable nanoparticle formulations that maintain consistent size, encapsulation efficiency and stability under clinical-grade production [163]. When combined with standard therapies, such as temozolomide, radiotherapy or immune checkpoint blockade, LNP-delivered CRISPR editing, particularly of resistance-associated targets such as MGMT or *miR-10b*, may ultimately represent a powerful adjunctive strategy for improving therapeutic outcomes in GBM.

5.3.2. Polymer Nanoparticles

Polymeric nanoparticles represent one of the most versatile non-viral platforms for delivering CRISPR/Cas9 components, owing to their tunable physicochemical properties, biocompatibility and ability to encapsulate diverse cargos such as CRISPR/Cas9 ribonucleoproteins (RNPs), plasmids or mRNA. These carriers are typically constructed from natural or synthetic polymers that form stable complexes with nucleic acids through electrostatic interactions. The positively charged surfaces of cationic polymers facilitate binding to the negatively charged CRISPR cargos and enhance uptake through receptor-mediated endocytosis [166]. Within the context of GBM, the ability to modulate parameters such as polymer charge density, hydrophobicity and molecular weight is particularly

advantageous, as efficient delivery in GBM requires enhanced stability, improved endosomal escape and minimal toxicity in the highly sensitive brain microenvironment.

Several polymer-based systems have demonstrated promising results in GBM models [167]. Ban et al. developed a supramolecular substrate-mediated delivery (SNMD) platform employing supramolecular nanoparticles (SMNPs) assembled on adamantane-modified silicon nanowire substrates [168]. This system enabled highly efficient delivery of Cas9 RNPs to U87 GBM cells. Importantly, near-infrared stimulation triggered the release of the CRISPR cargo via reactive oxygen species, thereby enhancing photodynamic therapy sensitivity [168]. These findings highlight the capacity of polymeric carriers to be engineered for stimuli-responsive gene editing, a desirable feature in tumors such as GBM where spatiotemporal control is needed.

Although not GBM-specific, Chou et al. demonstrated the adaptability of SMNP vectors for local delivery of CRISPR/Cas9 plasmids in ocular tissues, supporting the flexibility of polymeric nanoparticles across biological barriers [169]. Extending this concept, Wang et al. engineered PEG-based hybrid nanoparticles that substantially improved Cas9 and sgRNA delivery compared with traditional polycationic systems [170]. Their work underscores the importance of PEGylation in enhancing nanoparticle stability and reducing cytotoxicity, two parameters critical for safe intracranial application.

Dendritic polymers have also attracted significant interest due to their hyperbranched architecture and high density of functional groups. Polyamidoamine dendrimers, in particular, provide defined branching patterns suitable for CRISPR/Cas9 complexation through cation- π interactions or nitrogen-boronate coordination [171]. More recently, Zhang et al. introduced a zwitterionic branched copolymer (ZEBRA) comprising agarose, low-molecular-weight polyethylenimine and hyaluronic acid. ZEBRA achieved high transfection efficiency and selective targeting of GBM cells through hyaluronic acid-mediated interaction with the *CD44* receptor, which is frequently overexpressed in GBM stem-like populations [172].

Collectively, these studies illustrate the broad versatility of polymeric nanoparticles as customizable carriers for CRISPR/Cas9 delivery in GBM. Their modular design allows the incorporation of tumor-targeting ligands, imaging probes and controlled-release mechanisms, making them highly adaptable to the heterogeneous and infiltrative nature of GBM. Nonetheless, several challenges remain, including potential polymer-induced toxicity, variability in *in vivo* editing efficiency and difficulties in scaling production for clinical translation. Continued optimization of polymer chemistry and architecture is therefore necessary to enhance endosomal escape, improve tumor penetration and ensure biocompatibility for future therapeutic applications in GBM.

5.3.3. Inorganic Carriers

In recent years, inorganic nanomaterials, such as AuNPs, MSNs, graphene derivatives and magnetic nanoparticles, have gained increasing attention as delivery platforms for CRISPR/Cas9 therapeutics due to their distinctive physicochemical features, including tunable size, high surface area, chemical stability and facile surface modification. Such features allow the ideal delivery of gene-editing machinery to tumor cells, including those within the CNS [173]. The ability to functionalize the surface of these nanocarriers is key to their conjugation with targeting ligands or polymers that may enable their transport across the BBB and selective accumulation in GBM tissue.

Gold-Based Nanocarriers

Gold-based nanostructures, including AuNPs, gold nanorods (AuNRs) and gold nanoclusters (AuNCs), are characterized by unique optical and photothermal properties, which can be exploited for both therapeutic and diagnostic purposes [174]. When used as

CRISPR/Cas9 delivery vectors, AuNPs can be functionalized with polymers, peptides or lipids to enhance cellular uptake and reduce degradation [128,175]. Gold nanorod–polyethylenimine (AuNR-PEI) composites were bioengineered for CRISPR/Cas9 delivery [176], while a multifunctional lipid–AuNP hybrid was developed to deliver Cas9/sgRNA complexes targeting the *PLK1* gene [177]. High transfection efficiency was achieved through caveolae-mediated endocytosis and efficient endosomal escape due to the proton sponge effect of PEI [178,179]. Similarly, the incorporation of TAT peptides allows nuclear localization, while photothermal activation under near-infrared irradiation promotes plasmid release and enhances gene knockdown efficiency [180,181].

AuNCs, with an ultrasmall size of less than 2 nm, exhibit superior biodistribution, renal clearance and biocompatibility when compared with larger AuNPs [182]. A hypoxia-activated CRISPR-Cas9 nanosystem (APACPs) was developed using AuNRs coupled to azobenzene linkers. In this work, near-infrared irradiation and hypoxic conditions closely resembled the GBM microenvironment; cleavage of azobenzene caused CRISPR release for heat shock protein 90 alpha (*HSP90α*) gene knockout, reducing thermotolerance in tumor cells and enhancing photothermal therapy outcomes [183]. Other studies further demonstrated self-assembled SpCas9-AuNC complexes that could deliver Cas9 effectively into nuclei through pH-dependent disassembly, enabling intracellular release and precise gene editing with minimal cytotoxicity [184]. Given their ability to respond to hypoxia, penetrate tumoral regions and combine photothermal activation with gene editing, gold-based nanocarriers represent a particularly promising non-viral platform for CRISPR/Cas9 delivery in GBM, where deep infiltrative growth and an immunosuppressive, hypoxic microenvironment limit the efficacy of conventional vectors.

Magnetic Nanoparticles

SPIONs (superparamagnetic iron oxide nanoparticles) have also emerged as multifunctional nanocarriers suitable for targeted CRISPR delivery and MRI-based monitoring. These nanoparticles can be externally guided using magnetic fields, offering site-specific targeting in the brain, an essential advantage for GBM therapy [185,186]. PEI-SPIONs (polyethylenimine-coated SPIONs) demonstrated lower cytotoxicity while delivering CRISPR/Cas9 plasmids, achieving efficient gene editing via both non-homologous end-joining (NHEJ) and homology-directed repair (HDR) pathways. This approach shows promise for localized editing of oncogenes or tumor suppressor genes in GBM while minimizing off-target effects in healthy tissue [187].

Mesoporous Silica Nanoparticles

Mesoporous silica nanoparticles (MSNs) are advantageous for CRISPR/Cas9 delivery owing to their tunable pore sizes, large surface area and modifiable surface chemistry. These features enable co-loading of CRISPR components and therapeutic drugs for synergistic effects [188]. A multifunctional MSN system that co-delivers CRISPR/Cas9 plasmids with the anti-inflammatory agent VX-765 to edit *GSDMD* and reduce pyroptosis-related inflammation was developed [189]. In GBM models, MSNs can be functionalized with nuclear localization signals (NLSs) or tumor-targeting ligands to enhance BBB penetration and selective uptake. Furthermore, surface modifications that improve protein–silica interactions significantly enhance Cas9 loading efficiency and editing performance [140,188,190]. These attributes make MSNs promising candidates for combinatorial gene and drug therapy in GBM.

Graphene-Based Nanocarriers

Graphene oxide (GO) and its derivatives have recently been explored as nanoplat-forms for CRISPR/Cas9 delivery due to their high mechanical strength, large π - π

conjugated surface and efficient cargo loading capabilities. Functionalization of GO with polyethyleneimine or hyaluronic acid enables complexation with negatively charged nucleic acids and selective binding to *CD44* receptors, which are overexpressed on GBM cells. GO-based carriers have demonstrated enhanced Cas9/sgRNA transfection efficiency with minimal cytotoxicity in glioma cell lines, highlighting their potential for targeted genome editing and photothermal combination therapy [191].

5.3.4. DNA Nanocarriers

Recently, DNA nanostructures, including DNA nanoflowers, DNA nanospheres and DNA nanotubes, have attracted great interest due to their versatility in biomedical applications. These structures can be engineered into various forms such as DNA probes, nanochannels and templates, which are capable of undergoing conformational changes in response to specific stimuli. This adaptability makes them promising tools for drug delivery, molecular assembly and gene regulation. DNA nanocarriers offer several advantages, including high drug-loading capacity, excellent biocompatibility and facile functionalization, making them suitable for targeted drug delivery and biological imaging [192]. Ding et al. developed a non-cationic DNA-crosslinked nanogel-based CRISPR/Cas9 delivery platform in which DNA was grafted with polycaprolactone brushes and crosslinked through nucleic acid hybridization to encapsulate the Cas9/sgRNA complex. This nanogel protected the CRISPR/Cas9 system from degradation while preserving efficient target gene editing and held potential for cytoplasmic protein delivery and site-specific genome editing [193].

Drawing inspiration from microRNA regulation, Shi et al. constructed a miRNA-responsive CRISPR/Cas9 delivery system using DNA nanoflowers prepared by rolling-circle replication. *MUC1* aptamers were included to potentiate lysosomal escape, while *miR-21* binding sequences allowed for intracellular release of Cas9/sgRNA, leading to markedly improved gene-editing efficiency compared with non-responsive controls [194]. Li et al. presented a proton stimulus-activated DNA-based nanosystem for the co-delivery of Cas9/sgRNA and DNase. The system, compacted into nanoparticles by Mn^{2+} ions, released the cargo in the acidic lysosomal environment, enabling efficient gene regulation and therapeutic effects in breast cancer models [179]. Sun et al. introduced DNA nanoclews synthesized via rolling-circle amplification, which formed yarn-like DNA structures capable of loading Cas9 RNP complexes through base-pairing with partially complementary sgRNA sequences [195].

Collectively, these studies demonstrate the significant potential of DNA nanostructures as CRISPR/Cas9 delivery vehicles, emphasizing their programmable architecture, cellular responsiveness and multifunctional capacity as a promising platform for precise and efficient genome editing. Although DNA-based nanocarriers have not yet been extensively evaluated in GBM models, their programmable architecture, biocompatibility and ability to co-deliver multiple molecular cargos highlight their potential as a future platform for CRISPR/Cas9-mediated genome editing in GBM.

Table 4 summarizes popular CRISPR/Cas9 delivery methods along with their benefits and drawbacks.

Table 4. Summary of delivery strategies for CRISPR/Cas-based genome editing in glioblastoma (GBM).

Types of Delivery	Delivery Strategies	Delivery Formats	Advantages	Limitations	Ref.
Physical delivery methods	Microinjection	Plasmid DNA, mRNA	Highly precise; dosage controllable; direct delivery;	Technical limitations; in vitro only; time-consuming;	[132–134]

		or RNP complexes	minimal off-target effects	low throughput	
Viral vector delivery methods	Electroporation	DNA; mRNA; Protein	Simple to use; no restrictions on cargo size; high transfection efficiency; reproducible	Affects cell viability; optimization required per cell type	[137]
	HTVI	DNA; protein	Simple to use; low price; allows systemic delivery	High tissue stress; low specificity	[142]
	AAV	DNA	Low immunogenicity; long-term expression; broad tissue tropism	Limited packaging size (~4.7 kb); potential immune response; persistent expression may cause toxicity	[149,152–154,158]
	AV	DNA	High transduction efficiency; large cargo capacity (~8 kb)	High immunogenicity; transient expression; challenging to produce on a large scale	[159]
Non-viral vector delivery methods	LV	DNA	High capacity for packaging; persistent expression	Insertional mutagenesis risk; prolonged Cas9 activity increases off-target effects	[145]
	LNPs	mRNA; RNP	Biocompatible; scalable; low immunogenicity; low cost; can cross BBB with modification	Specific cell tropism; transient expression	[163]
	Polymeric nanoparticles	mRNA, protein	Easy to operate; tunable release; biodegradable	Variable efficiency; cytotoxicity	[166]
	Inorganic nanocarriers (e.g., gold, silica, graphene)	Protein, RNP	Superior chemical stability; easy surface modification; potential for BBB penetration via magnetism or ultrasound	Limited biocompatibility; potential toxicity; cell types determine variable efficiency	[121,191,196]
	DNA nanostructure (DNA origami)	Protein, RNP	Good histocompatibility; excellent biocompatibility; nuclear targeting possible	Complex synthesis; requires modification of template DNA	[192]

This table categorizes delivery methods into physical, viral and non-viral systems, outlining their respective formats, advantages and limitations. Physical approaches provide direct delivery and experimental control but are limited to in vitro or preclinical use. Viral vectors offer high transduction efficiency and long-term expression, though they present immunogenicity and integration concerns. Non-viral systems represent emerging, biocompatible alternatives capable of crossing the BBB and enabling transient Cas9 activity.

6. Challenges and Limitations

6.1. Technical and Biological Challenges

The therapeutic application of CRISPR/Cas9 in GBM is still limited by several issues, despite notable advancements. Effective intracellular delivery and BBB penetration are still significantly hampered by the large molecular size of Cas9 and the limited loading capacity of current viral and non-viral vectors. Even with advanced nanoparticle formulations, there are still issues related to target specificity, stability and biodistribution problems [197]. Off-target editing, in which CRISPR/Cas9-induced cleavage takes place at unexpected genomic loci, is a second significant limitation [198]. Although strategies such as high-fidelity Cas9 variants, dual-guide RNA systems and siRNA-assisted silencing improve precision, complete elimination of off-target activity remains challenging [199]. Furthermore, the immunogenic nature of Cas9, derived from bacterial species, raises concerns about inflammatory or autoimmune responses, particularly upon repeated administration [200]. These limitations highlight the need for single-dose therapeutic models, immune-evasive Cas variants and next-generation genome editors such as Cas9 nickase systems or prime/base editors.

6.2. Experimental and Translational Constraints

The limited experimental diversity of current CRISPR-based GBM studies is another drawback. Most genome-wide CRISPR/Cas9 screens rely on a narrow panel of GBM cell lines or glioma stem cell (GSC) models, which do not adequately reflect the extensive inter- and intra-tumoral heterogeneity seen in patient-derived tumors. As a result, findings from these studies might not be broadly applicable or may have limited translational value. Another major limitation is the scarcity of robust *in vivo* validation. A substantial proportion of studies remain restricted to *in vitro* systems, resulting in limited understanding of long-term editing effects, delivery efficiency, immune interactions and tumor-microenvironment dynamics in physiological conditions [201]. This gap slows progress toward clinically relevant insights. To bridge these limitations, future work must emphasize diversified, patient-derived GBM models, comprehensive *in vivo* mechanistic studies and rigorous preclinical validation. Such efforts are essential for accurately determining therapeutic efficacy, biosafety, delivery performance and the durability of CRISPR-mediated modifications.

6.3. Ethical and Regulatory Concerns

Beyond technical barriers, the clinical translation of CRISPR/Cas9 in GBM raises important ethical and regulatory concerns. Permanent genome modification in neural tissue carries potential risks of long-term neurotoxicity, unintended cognitive or neurological effects and irreversible off-target alterations, which may only manifest years after treatment. Furthermore, ethical issues surrounding informed consent, risk–benefit balance in terminal malignancies and equitable access to advanced gene-editing therapies must be carefully addressed. Robust regulatory frameworks and long-term monitoring will therefore be essential to ensure responsible clinical implementation of CRISPR-based therapies in neuro-oncology.

7. Future Directions and Considerations

Despite the significant therapeutic promise of CRISPR/Cas9 in GBM, substantial biological, technical, ethical and regulatory challenges remain. Many GBM-associated genes exhibit context-dependent or dual functions, acting as oncogenes or tumor suppressors depending on the cellular microenvironment, which complicates target selection and necessitates deeper mechanistic insight into gene–pathway interactions [11]. Addressing

these gaps requires moving beyond discovery-level studies toward rigorous in vivo validation to assess long-term efficacy, genomic stability and off-target consequences in physiologically relevant models. Future progress will also depend on the development of next-generation, compact and high-precision editing platforms, including base editors, prime editors and alternative Cas systems, alongside advanced delivery strategies capable of crossing the BBB with minimal toxicity [202,203]. Given the pronounced intra- and inter-tumoral heterogeneity of GBM, personalized and multi-targeted CRISPR approaches, supported by integrated multi-omics, single-cell and spatial profiling, and AI-driven computational modeling, may be necessary to achieve durable therapeutic responses. However, the immunogenicity of bacterial Cas proteins [200], potential neurotoxicity [204] and ethical concerns [205] related to genomic safety and equitable patient access underscore the need for robust regulatory frameworks and patient-centered governance to ensure the responsible clinical translation of CRISPR-based precision therapies in neuro-oncology.

8. Conclusions

CRISPR/Cas9 genome editing presents a transformative but still evolving opportunity for GBM therapy. Its capacity to precisely modify oncogenic drivers, restore tumor-suppressor function, and reprogram resistance pathways positions CRISPR as a powerful platform for developing patient-specific therapeutic strategies. However, several barriers continue to impede clinical translation, including off-target genetic alterations, the immunogenicity of Cas nucleases, delivery limitations imposed by the BBB and incomplete evaluation in vivo.

In the near term, the most realistic applications of CRISPR/Cas9 in GBM are likely to remain within functional genomics and disease modeling.

To advance CRISPR/Cas9 toward clinical readiness, future research must prioritize the development of safer and more efficient delivery systems, robust preclinical validation in physiologically relevant models and computational tools that refine target selection and editing precision.

Overall, the integration of CRISPR/Cas9 with molecular oncology, multi-omics profiling and precision medicine technologies, as well as its combination with established therapies, has the potential to shift GBM management from largely palliative care toward individualized, mechanism-based interventions. Continued interdisciplinary efforts will determine whether CRISPR/Cas9 can ultimately reshape the therapeutic landscape of GBM.

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Abbreviations

The following abbreviations are used in this manuscript:

2-HG	D-2-hydroxyglutarate
α -KG	Alpha-ketoglutarate
AAV	Adeno-associated virus
AKT	Protein kinase B
ATRX	ATRX chromatin remodeler
AuNCs	Gold nanoclusters
AuNPs	Gold nanoparticles
AuNRs	Gold nanorods
AV	Adenoviral vectors
BBB	Blood–brain barrier
BTB	Blood–tumor barrier
CAR-T	Chimeric antigen receptor T cells
Cas	CRISPR-associated
Cas9	CRISPR-associated protein 9
CD44	Cluster of differentiation 44
CD8 ⁺	Cluster of differentiation 8 positive T cells
CDKN2A/B	Cyclin-dependent kinase inhibitor 2A/2B locus
CHOPCHOP	CRISPR guide RNA design tool
CNS	Central nervous system
crRNA	CRISPR RNA
CRISPOR	CRISPR guide RNA design and off-target prediction tool
CRISPR	Clustered regularly interspaced short palindromic repeats
DSB	Double-strand break
EGFR	Epidermal growth factor receptor
EGFRvIII	Epidermal growth factor receptor variant III
EMT	Epithelial-to-mesenchymal transition
eSpCas9	Enhanced-specificity <i>Streptococcus pyogenes</i> Cas9
FOXP1	Forkhead box G1
GBM	Glioblastoma multiforme
G-CIMP	Glioma CpG island methylator phenotype
GDF15	Growth differentiation factor 15
GO	Graphene oxide
gRNA	Guide RNA
GSCs	Glioma stem-like cells
GSDMD	Gasdermin D
HCAAdV	Helper-dependent adenoviral vector
HDR	Homology-directed repair
HDAAdV	High-capacity adenoviral vector
HNH	Histidine–asparagine–histidine nuclease domain
HSP90 α	Heat shock protein 90 alpha
HTVI	Hydrodynamic tail vein injection
HypaCas9	Hyper-accurate Cas9 variant
IDH1	Isocitrate dehydrogenase 1
IL13R α 2	Interleukin-13 receptor alpha 2
KO	Knockout
LOH	Loss of heterozygosity
LNPs	Lipid nanoparticles
LV	Lentiviral vector
MAP4K4	Mitogen-activated protein kinase 4
MAPK	Mitogen-activated protein kinase
MDSCs	Myeloid-derived suppressor cells
MHC	Major histocompatibility complex
miR	MicroRNA

miR-10b	MicroRNA-10b
miR-21	MicroRNA-21
MGMT	O6-methylguanine-DNA methyltransferase
Mn ²⁺	Manganese (II) ions
MRI	Magnetic resonance imaging
MSH6	MutS homolog 6
MSNs	Mesoporous silica nanoparticles
NHEJ	Non-homologous end-joining
NF1	Neurofibromin 1
NILVs	Non-integrating lentiviral vectors
NIR	Near-infrared
NLS	Nuclear localization signal
PAM	Protospacer adjacent motif
PARP1	Poly(ADP-ribose) polymerase 1
PCSK9	Proprotein convertase subtilisin/kexin type 9
PD-1	Programmed cell death protein 1
PDGFRA	Platelet-derived growth factor receptor alpha
PEG	Polyethylene glycol
PEI	Polyethylenimine
PI3K	Phosphatidylinositol 3-kinase
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PIP2	Phosphatidylinositol-4,5-bisphosphate
PIP3	Phosphatidylinositol-3,4,5-trisphosphate
PTEN	Phosphatase and tensin homolog
RAD51	RAD51 recombinase
RAS	Rat sarcoma GTPase family
REC	Recognition lobe
RNase III	Ribonuclease III
RNP	Ribonucleoprotein
RTKs	Receptor tyrosine kinases
RuvC	RuvC-like nuclease domain
SaCas9	Staphylococcus aureus Cas9
sgRNA	Single-guide RNA
SIN	Self-inactivating
SMNPs	Supramolecular nanoparticles
SNMD	Supramolecular substrate-mediated delivery
SOX2	SRY-box transcription factor 2
SpCas9	Streptococcus pyogenes Cas9
SpCas9-HF1	High-fidelity Streptococcus pyogenes Cas9 variant 1
SPIONs	Superparamagnetic iron oxide nanoparticles
STAT3	Signal transducer and activator of transcription 3
TAMs	Tumor-associated macrophages
TERT	Telomerase reverse transcriptase
TGFβR2	Transforming growth factor beta receptor 2
TME	Tumor microenvironment
TMZ	Temozolomide
TP53	Tumor protein p53
tracrRNA	Trans-activating CRISPR RNA
Tregs	Regulatory T cells
TWIST1	Twist family bHLH transcription factor 1
U87	U87 glioblastoma cell line
VEGF	Vascular endothelial growth factor
VX-765	Caspase-1 inhibitor VX-765
WHO	World Health Organization
WT	Wildtype
ZEB1	Zinc finger E-box binding homeobox 1
ZEBRA	Zwitterionic branched copolymer delivery system

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