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Overcoming barriers and shaping the future: Challenges and innovations in nucleic acid therapies for Glioblastoma☆

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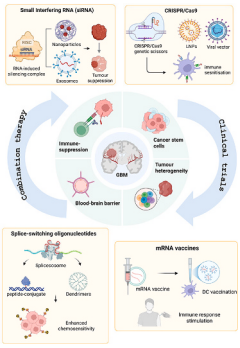
GRAPHICAL ABSTRACT



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ABSTRACT

Glioblastoma (GBM) is the most aggressive and treatment-resistant primary brain tumor in adults. Conventional therapies offer limited benefit due to the tumor's heterogeneity, invasive nature, and the presence of the blood–brain barrier, which restricts therapeutic access. Nucleic acid (NA)-based therapies, including small interfering RNA, microRNA, antisense oligonucleotides, splice-switching oligonucleotides, and CRISPR-based systems, have emerged as promising tools to modulate oncogenic pathways and overcome resistance mechanisms at the genetic level. However, effective delivery remains the primary challenge in translating these therapies into clinical success. This review examines the current landscape of NA-based strategies for GBM, with a focus on innovative delivery systems designed to navigate biological barriers and enhance therapeutic precision. We highlight clinical progress made with nanocarrier platforms such as liposomes, lipid nanoparticles, and exosome-based systems, and evaluate their safety, specificity, and delivery efficiency. Additionally, we discuss the most promising preclinical advances, including multifunctional, targeted, and stimuli-responsive carriers, that demonstrate strong potential for clinical translation. Our analysis underscores that the therapeutic efficacy of NA approaches in GBM is inseparable from the sophistication of their delivery platforms. Moving forward, the

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integration of rationally designed carriers with gene-targeted payloads holds the key to unlocking the full potential of precision medicine in GBM.

1. Introduction

1.1. Overview of Glioblastoma (GBM): a hard-to-treat cancer

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults, accounting for 54 % of all gliomas and 16 % of all primary brain tumors [2,3]. Although GBM can affect people of any age, it is most commonly diagnosed among older adults, with a median age at diagnosis of around 64 years. It affects men slightly more than women, and is more prevalent in the Caucasian population compared to other ethnic groups [4,5]. GBM is considered a fast-growing, highly invasive and resistant tumor, which makes it one of the most challenging cancers to treat [6].

GBMs usually develop in the cerebral hemispheres, typically in the frontal and temporal lobes, and originate from glial cells, though evidence now suggests they can arise from many cell types with a neural stem cell-like phenotype. Key characteristics of GBM include its ability to infiltrate the surrounding brain tissue, extensive angiogenesis, and areas of necrosis within the tumor [7]. These traits contribute to a poor prognosis, with a median survival of just 15 months, even with aggressive multimodal treatment [5].

1.2. Standard of care: Surgery, radiation, and chemotherapy

The first line of treatment in newly diagnosed GBM is maximal safe surgical resection. Surgical resection plays a key role in reducing tumor burden, mass effect relief, alleviation of neurological symptoms, and is a source of tissue which informs further treatment choices [8]. The aim of surgery is to remove the maximum amount of tumor tissue possible without compromising neurological function [9,10]. Repetitive surgical resection has been reported to be strongly correlated with improved progression-free survival in several studies [11].

Gross total resection (GTR), where all postoperative contrast-enhanced tumor visible on MRI has been removed, is the preferred intervention, with even small increases in extent of resection (EOR) translating to improved survival [12,13]. The development of sophisticated surgical techniques has allowed extensive resection to be performed while preserving neurological function. Such techniques include, intraoperative MRI for real-time navigation, fluorescence-guided surgery with 5-aminolevulinic acid (5-ALA) (which causes tumor cells to fluoresce pink under blue light), and awake craniotomy with cortical and subcortical mapping of tumors in eloquent sites [10,13–15]. In patients with deep-seated tumors, or patients where surgery is contraindicated, laser interstitial thermal therapy (LITT) has been developed and can be offered as a minimally invasive alternative [13,16–18].

Post surgery, patients are placed on the 'Stupp protocol', which consists of radiotherapy plus chemotherapy with temozolomide (TMZ) [19,20]. TMZ is a blood brain-barrier (BBB) penetrating alkylating agent which exerts its effects via methylation of guanine at the 06 position of DNA, causing cell cycle arrest and apoptosis [21,22]. Patients are treated with daily oral TMZ (75 mg/m^2) accompanied by radiotherapy e for a maximum of 49 days and followed up with 6 further cycles of adjuvant TMZ (150–200 mg/m^2/5 days every 28 days) [23].

1.3. Challenges in GBM treatment: surgical excision, microenvironment, chemoresistance, and drug delivery barriers

GBM treatment is faced by multiple challenges that contribute to its poor prognosis and therapeutic failure. Maximal safe resection is a cornerstone of GBM treatment, yet surgical excision is inherently limited by the infiltrative nature of GBM [24]. Even with modern advances residual tumor cells invariably remain [25], these infiltrating cancer cells can persist in the brain parenchyma and serve as the seedbed for tumor recurrence [26].

Chemoresistance is a major challenge in the treatment of GBM, significantly limiting the efficacy of current therapeutic approaches [27]. The primary chemotherapeutic agent used in GBM treatment, TMZ, faces multiple resistance mechanisms that severely impair its effectiveness. One of the best-characterized mechanisms is the expression of O6-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme that removes alkyl groups from the O6 position of guanine, thereby directly reversing the DNA damage caused by TMZ. Patients with an unmethylated MGMT promoter, which results in higher MGMT expression, have a significantly poorer response to TMZ treatment [28,29]. Apart from MGMT, GBM cells use multiple redundant strategies to circumvent chemotherapy. These include increased drug efflux via overexpression of ATP-binding cassette transporters [30], changes in apoptotic pathways [31], and activation of DNA damage response [31]. As such, even with the current standard of care (SOC) 5-year survival is only 5 % and even poorer in patients over 65 years (less than 2.1 %) [32].

The development of novel therapeutics is hindered by a number of barriers. The BBB represents the primary, and most obvious obstacle. Composed of endothelial cells, pericytes, and astrocytes, this highly regulated structure serves as a robust defence mechanism, restricting the entry of foreign substances to the brain [33,34]. Approximately 98 % of small molecules and nearly all large biological agents, including antibodies and growth factors, are unable to cross this barrier [34]. The BBB's protective function is further reinforced by the presence of drug-metabolizing enzymes and active efflux mechanisms, such as P-glycoprotein (P-gp) [35] and multidrug resistance-related proteins (MRPs) [36], which expel many therapeutic compounds from the brain parenchyma [22,33]. Moreover, the GBM microenvironment is characterized by hypoxic regions, which upregulate the expression of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), forming abnormal, leaky blood vessels [37]. This vascular dysfunction leads to increased interstitial fluid pressure, which impedes the delivery of therapeutic agents.

In addition to the BBB, the blood–cerebrospinal fluid barrier (BCSFB) represents another important regulatory interface that influences CNS exposure to therapeutics. The BCSFB is located primarily at the choroid plexus epithelium, where fenestrated capillaries supply CSF-producing epithelial cells joined by tight junctions. Unlike the BBB, which tightly restricts paracellular transport in brain capillaries, the BCSFB allows more selective trans-epithelial transport into the CSF [38]. For NA nanocarriers, this distinction is important because accumulation of nanoparticles in CSF or perivascular spaces does not necessarily indicate direct BBB penetration. Some delivery systems, including exosomes and LNPs may access the CNS preferentially via the BCSFB or meningeal lymphatic routes, particularly in regions where the choroid plexus environment remains relatively intact even when the BBB is focally disrupted in GBM [39].

Once a potential drug candidate has reached the tumor bed, there are additional obstacles due to the heterogeneity in GBM tumors, with different cell populations within the same tumor displaying variable sensitivities toward treatment [40]. In particularly, glioma stem-like cells (GSCs) are difficult to eliminate due to more efficient DNA repair mechanisms [41], higher expression levels of drug efflux pumps [42], and their ability to enter quiescence, thereby avoiding therapies that target rapidly dividing cells [43,44]. GSCs are believed to contribute significantly to the recurrence of tumors since they may survive initial

therapeutic interventions and then repopulate the tumor [45]. Not only do GBM exhibit heterogeneity on a cellular level, GBM is also highly diverse structurally. For example, while some tumor regions experience disruptions in the BBB, others, particularly at invasive margins where recurrence is most likely, maintain an intact barrier [34]. Therefore, reaching every cancerous cell in all regions of the tumor is particularly difficult.

In addition to intra-tumoral variation, patient-specific differences also influence therapeutic response and should guide the selection of NA-based strategies. Factors such as MGMT promoter methylation, IDH mutation status, GBM transcriptional subtype, and the degree of BBB integrity differ between patients and affect both the molecular susceptibility of tumor cells and the feasibility of systemic delivery [46]. Likewise, variation in the immune microenvironment influences the suitability of approaches such as mRNA vaccines or immunomodulatory antisense oligonucleotides. These patient-specific features highlight the need for personalized stratification when designing or selecting NA therapies for GBM [47].

Alternatives to traditional chemotherapy, such as immunotherapy, have shown limited success due to the profoundly immunosuppressive tumor microenvironment (TME). GBM is typically described as immunologically 'cold' TME, with low numbers of tumor-infiltrating lymphocytes (TILs) and other immune effector cells [48,49]. Notably, tumor-associated macrophages (TAMs), which can represent up to 50 % of the tumor mass, mostly acquire an immunosuppressive M2-like phenotype that favours tumor progression [50,51]. The microenvironment also exhibits elevated levels of immunosuppressive factors such as transforming growth factor beta (TGF-β) [52] and interleukin-10 (IL-10) [53], which hinders effective immune responses [54]. Furthermore, the interaction between tumor cells and surrounding astrocytes leads to the upregulation of immunosuppressive pathways, such as JAK/STAT and PD-L1, contributing to the persistence of the cold TME [55,56].

In addition to the BBB and the immunosuppressive tumor microenvironment, other biophysical features of GBM pose significant challenges to therapeutic delivery. The tumor extracellular matrix (ECM), composed of dense networks of hyaluronic acid, collagen, and tenascin-C, creates a physical barrier that restricts nanoparticle diffusion and hinders uniform drug distribution [57]. Furthermore, the abnormal and leaky vasculature characteristic of GBM leads to elevated interstitial fluid pressure (IFP), which counteracts convective transport and limits the penetration of therapeutics into the tumor core [58]. Together with the BBB and TME, these factors form an integrated set of biological barriers that significantly constrain the efficacy of NA–based therapies and highlight the need for rationally designed delivery systems capable of overcoming them.

2. Nucleic acid-based Cancer therapy for Glioblastoma

NA-based therapies have become a promising alternative with the potential to target GBM at the genetic and transcriptomic level. NA-based therapies encompass a broad spectrum of modalities, with distinct mechanisms of action and therapeutic potential. Gene therapy methods are based on modifying the genomic DNA to either introduce healthy genes to correct oncogenic mutations, or to inhibit oncogenic drivers. In contrast, on a transcriptomic level, RNA interference therapies utilize small RNA probes that selectively bind to complementary mRNA transcripts, leading to their degradation or translational repression. This ultimately results in the silencing of specific genes involved in tumor proliferation, invasion and resistance [59]. Other modalities include splice-switching oligonucleotides (SSOs) which bind to pre-mRNA to inhibit translation or modify splicing, influencing protein expression in tumor cells.

Recently, and following the success of the SARS-COV-19 mRNA vaccine, there has been an interest in using therapeutic vaccines for GBM. In this approach, NA which encodes GBM specific antigens or immune stimulatory sequences are injected either peripherally or locally, this activates the immune system to identify and eliminate tumor cells [60,61]. Recent reviews have discussed NA–based therapeutics for neurological and oncological applications. Luo et al. (2022) provided a comprehensive overview of gene therapy strategies across CNS diseases, focusing primarily on nanomedicine delivery and barriers to CNS targeting [62]. Similarly, Karlsson et al. (2021) offered an in-depth analysis of nanoparticle engineering principles for NA delivery in brain cancer [63]. Building on these works, the present review specifically focuses on GBM and uniquely integrates the molecular basis of gene silencing and editing (RNAi, ASOs, SSOs, CRISPR/Cas9, and mRNA therapies) with a critical evaluation of delivery systems and clinical translation barriers. By bridging molecular mechanisms, delivery design, and therapeutic outcomes, this review provides a disease-centred synthesis that highlights both current challenges and emerging innovations in the development of NA therapeutics for GBM.

As summarized in Table 1, several NA-based therapeutics have reached early-phase clinical evaluation in GBM, reflecting the growing translational momentum in this field. Among DNA-based strategies, SGT-53, a liposomal nanocomplex carrying wild-type p53 plasmid DNA, has demonstrated efficient tumor targeting through transferrin receptor-mediated uptake, BBB penetration, and encouraging safety in Phase Ib [64] and Phase II studies [65] (NCT00470613, NCT02340156). RNA-based approaches are also emerging: the miR-34a mimic MRX34 (NCT01829971) showed gene modulation and immune activation in advanced solid tumors, although systemic immune toxicity limited further development [66]. Diagnostic studies such as NCT01849952 explore circulating miR-10b as a prognostic biomarker in glioma, underscoring the multifaceted role of NA in both therapy and disease monitoring [67].

More recently, mRNA-based vaccines have entered clinical testing for GBM. A pilot study using an intravenous mRNA vaccine reported enhanced immune activation and increased T-cell responses post-surgery and chemoradiotherapy [68]. In contrast, a DC–based vaccine pulsed with GSC mRNA (NCT02010606) demonstrated improved progression-free and overall survival, with good safety and feasibility [69]. Despite these promising results, no NA-based therapy has yet achieved regulatory approval for GBM. Challenges such as immune activation, limited BBB penetration, and tumor heterogeneity continue to hinder clinical translation. Nevertheless, these early studies highlight the strong potential of rationally engineered delivery systems and immune-activating NA platforms to transform GBM therapy in the coming years.

Spherical nucleic acids (SNAs) represent a distinct class of nanostructures showing strong promise for GBM therapy. SNAs consist of a nanoparticle core densely functionalized with radially oriented siRNA oligonucleotides, resulting in a globular architecture with high nuclease resistance and efficient cellular entry. Unlike linear oligonucleotides, SNAs can cross the BBB without the need for additional targeting ligands, owing to their ability to engage scavenger receptors and initiate receptor-mediated endocytosis and transcytosis [70].

A first-in-human Phase 0 clinical trial (NCT03020017) evaluated siRNA specific for the GBM oncogene Bcl2Like12 (siBcl2L12)-SNAs (NU-0129) administered intravenously to patients with recurrent GBM. The treatment demonstrated favourable safety, and importantly, confirmed intratumoral accumulation of SNAs in resected tumor tissues. Gold enrichment was observed in tumor-associated endothelium, macrophages, and glioma cells, and SNA uptake correlated with reduced expression of the target oncogene Bcl2L12, indicating successful RNA interference in human GBM tissue [71].

To illustrate the broader landscape of NA-based strategies in GBM, Fig. 1A provides a summary of key therapeutic targets, and the corresponding modalities employed across published studies. This chart was generated following a targeted PubMed search of 567 studies published between 1997 and 2025. The analysis, based on study abstracts, reveals the diversity of NA therapeutics explored in GBM, with siRNA and miRNA being the most frequently studied modalities, particularly

Table 1
Clinical trials of NA therapies for GBM.

Trial Number	Trial Phase	Purpose	NA name	Route of administration/ delivery system	Objective	Testing System	Readout/outcome	Ref
NCT00470613	Phase Ib	Therapy	p53 plasmid DNA (SGT-53)	Intravenous, 3.6 mg DNA/infusion, twice weekly for 3 weeks/ liposomal nanocomplex	Efficacy and safety	Patients with advanced solid tumors	Tolerability, gene delivery, early antitumor efficacy	[64]
NCT02340156	Phase II	Therapy	p53 plasmid DNA (SGT-53)	Intravenous, 3.6 mg DNA/infusion, twice weekly + TMZ orally daily on days 9–13 of each cycle (liposomal nanocomplex)	Efficacy and safety in combination with TMZ	Recurrent or progressive GBM patients	PFS, OS, tumor apoptosis, BBB penetration	[65]
NCT01849952	Recruiting	Diagnosis	miR-10b	NA	Expression levels of miR-10b in glioma patients	Patient serum samples	Correlation with glioma subtype and prognosis	[67]
NCT01829971	Phase I	Therapy	MRX34 (miR-34a mimic)	Intravenous (daily for 5 days in 3-week cycles)/ Liposomes	Safety and immune response	Patient cohort with advanced solid tumors	miR-34a delivery, gene modulation, immune toxicity	[66]
Pilot study	Phase Ib	Therapy	mRNA vaccine	Intravenous	Enhance immune activation after SOC treatment	4 GBM patients post-surgery and chemoradiotherapy	Robust immune activation, increased T cell activity and immune protein expression	[61]
NCT02010606	Phase I	Therapy	GSC mRNA-pulsed DC vaccine	Intradermal injection of DCs	Trigger patient-specific immune response to tumor antigens	GBM patients post-TMZ, surgery, and radiotherapy	Improved PFS and OS, safety and feasibility demonstrated	[69]
NCT03020017	Phase 0	Therapy	SNAs encapsulating siRNA specific for the GBM oncogene Bcl2Like12	Intraveneous	Efficacy and safety	patients with recurrent GBM	Favourable safety, and confirmed intratumoral accumulation of SNAs in resected tumor tissues	[71]

against targets such as EGFR, STAT3, and miR-21. The figure demonstrated the growing emphasis on integrating targeted delivery with precise genetic modulation for improved therapeutic outcomes.

The following sections highlight key NA-based therapeutic strategies for GBM. These include approaches for gene silencing, and mRNA-based gene expression.

2.1. *Importance of novel delivery systems in addressing these challenges*

Therapeutic access to GBM is severely restricted by the failure of treatment agents to diffuse effectively across the BBB and into invasive tumor cells. These challenges require delivery systems that can circumvent these barriers and deliver therapeutics to their intended site [72–76]. Recent developments in delivery systems, especially in terms of NA-based therapies, have shown great potential in addressing these challenges [77–82].

Nanocarrier systems, such as liposomes [83–85], polymeric nanoparticles [86–88], and lipid nanoparticles (LNPs) [89,90], have emerged as pivotal platforms for the delivery of NA-based therapies, including small interfering RNA (siRNA) [91], microRNA (miRNA) [92], and messenger RNA (mRNA) [93]. These systems can be designed to encapsulate and protect labile NAs from enzymatic degradation, and surface functionalisation, e.g., with the addition of targeting ligands, enabling targeted delivery of these systems to GBM cells. For instance, LNPs loaded with siRNA targeting oncogenic pathways and conjugated with the Angiopep-2 (Ang) peptide have been shown to have higher delivery efficacy and therapeutic benefit in GBM preclinical models [94]. Moreover, pH-sensitive [95–97] and enzyme-responsive [98,99] nanocarriers enable targeted controlled release within the acidic and protease-rich TME, further enhancing specificity [82].

Although regions of the BBB in GBM are partially disrupted, particularly within the tumor core, this disruption is highly heterogeneous, and the invasive tumor margins often retain an intact and fully functional barrier [100]. Therefore, effective NA delivery requires strategies that actively engage BBB transport pathways rather than relying solely on passive diffusion. Targeted nanocarrier delivery across the BBB and into glioma cells relies heavily on receptor–ligand interactions that mediate active transcytosis. Among the most frequently exploited targets are the transferrin receptor (TfR) and the low-density lipoprotein receptor-related protein 1 (LRP1), both of which are overexpressed on BBB endothelial cells and glioma cells [101]. Ligands such as transferrin, lactoferrin, and Ang-2 bind to their respective receptors, triggering receptor-mediated endocytosis and transcytosis of functionalised nanoparticles into the brain parenchyma [102]. Similarly, integrin αvβ3, highly expressed in tumor neovasculature, enables RGD-peptide–modified nanocarriers to preferentially accumulate at the tumor site. The affinity and avidity of these ligand–receptor pairs determine not only binding strength but also the intracellular trafficking pathway, whether vesicular recycling, lysosomal degradation, or transcellular transport, thereby influencing the therapeutic efficiency and specificity of NA delivery systems [103].

Modulation of BBB is also gaining attention as a strategy to improve drug delivery and NAs. Targeted disruption of the BBB has been achieved with focused ultrasound (FUS) [104,105] and microbubbles [106–108]. Thermally induced alterations in the viscosity of extracellular fluids allow microbubbles to first transiently penetrate the BBB, after which the therapeutic agents (e.g., siRNA and gene-editing reagents) can be delivered to the tumor. Using this method, systemic toxicity can be reduced and the local concentration of the therapeutic payload in the brain can be maximized [109]. Other strategies bypass

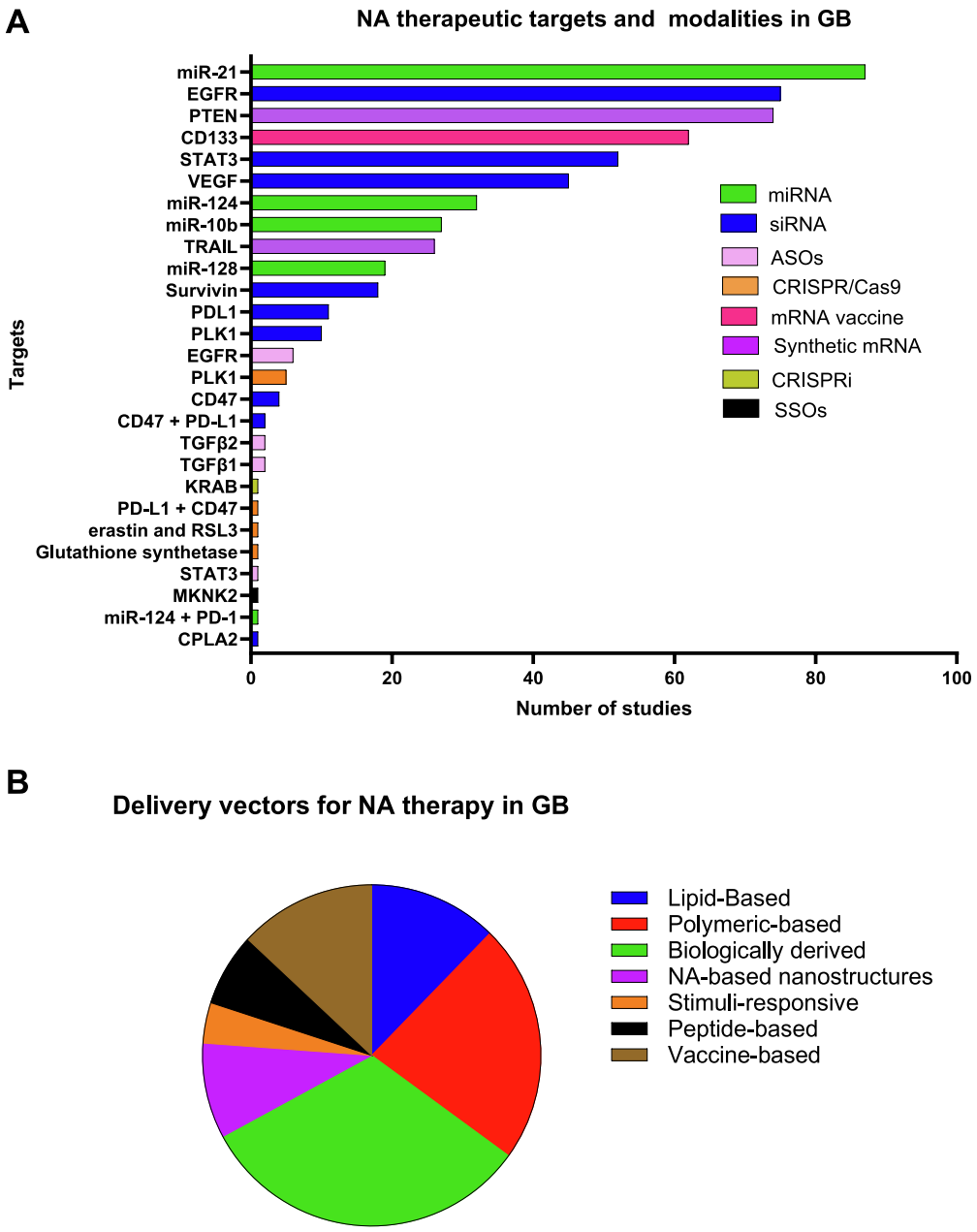


Fig. 1. Overview of NA therapeutic targets and delivery systems in GB. (A) The bar chart illustrates NA therapeutic targets and their corresponding modalities in GB. Each bar represents a specific target (e.g., EGFR, miR-21, CD47) and is color-coded by nucleic acid modality (e.g., siRNA, miRNA, ASOs, CRISPR/Cas9, mRNA vaccine). The data was compiled through a focused literature search on PubMed using the keywords "glioblastoma", the specific modality, and the target of interest. The search was based on the abstracts of 567 studies published between 1997 and 2025. **(B)** Proportional distribution of published studies using different delivery vectors for nucleic acid (NA) therapy in glioblastoma (GB) from 1995 to 2025. The chart illustrates the relative frequency of each delivery system type—ranging from lipid-based and polymeric carriers to biologically derived, DNA nanostructures, stimuli-responsive, peptide-based, and vaccine-based platforms—highlighting trends in research focus over time.

the BBB entirely and rely on localized delivery directly to the tumor. In these systems the vector serves to improve the pharmacokinetics of the NA or improve delivery on a cellular level. For example, researchers have developed a reactive oxygen species degradable injectable hydrogel loaded with a STING agonist (ADU-S100) and an AAV vector expressing soluble PD-1, enabling sustained local immunotherapy that, when combined with radiotherapy, enhanced T cell infiltration, restored effector function, and induced long-term immune memory to prevent GBM recurrence [110]. Another approach employed a thermosensitive PLGA-PEG-PLGA hydrogel loaded with a G5-BGG/shRNA complex targeting the CD47-SIRPα axis, effectively enhancing macrophage-mediated phagocytosis, downregulating immune escape mechanisms, and prolonging survival in a postoperative GBM model [111]. Convection-enhanced delivery is another key localized strategy, enabling direct infusion of therapeutic agents into the tumor or surrounding brain tissue via a pressure gradient. This technique improves distribution and is currently under clinical investigation for delivering chemotherapies, viral vectors, and immunomodulators in GBM patients [112].

Intranasal delivery has emerged as a non-invasive strategy to deliver NAs to intracranial tumors by bypassing the BBB entirely. This route exploits the olfactory and trigeminal neural pathways, enabling direct

transport from the nasal mucosa into the olfactory bulb and deeper brain parenchyma without systemic exposure [113]. Intranasal administration has been used to deliver siRNA [114] and miRNA [115] loaded nanoparticles in preclinical GBM models, showing efficient brain accumulation and gene-silencing effects. For example, chitosan- and PEG-based nanocarriers carrying siRNA have demonstrated enhanced distribution across both tumor cores and invasive margins following intranasal dosing, reflecting the advantage of neural pathway–based transport over vascular delivery [114].

Emerging delivery approaches are also being explored to enhance intracranial delivery of NAs. Microneedle platforms can provide minimally invasive, localized administration into the resection cavity or peritumoral tissue, offering sustained release while avoiding systemic exposure and invasive catheters [116]. Additionally, cationic vectors, including ionizable lipids and pH-responsive polymers, improve NA complexation, cellular uptake, and endosomal escape. Compared to earlier permanently cationic materials, these newer vectors are designed to reduce inflammatory toxicity, supporting safer and more efficient delivery in GBM [117].

Combined, advances in nanocarriers, BBB modulation, and localized delivery are demonstrating the feasibility of precise and effective NA based approaches for GBM therapy [82]. The following sections will review the NA targets, modalities and vectors currently under investigation for GBM.

Fig. 1B summarizes the types of delivery vectors employed across published studies from 1995 to 2025. This pie chart was generated through a targeted PubMed search of 520 studies focused on NA delivery in GBM. The analysis categorized the delivery systems reported, including lipid-based, polymeric, exosomes, DNA nanostructures, stimuli-responsive, peptide-based, and vaccine-based platforms. The distribution highlights the field's progressive shift toward more biocompatible and targeted delivery modalities that address key challenges such as BBB penetration and intratumoral heterogeneity in GBM.

A comparative overview of the key features of LNPs, exosomes, and polymeric carriers, including their BBB transport mechanisms, targeting strategies, safety considerations, and scalability, is provided in Table 2.

Table 2
Comparison of LNPs, exosomes, and polymeric nanocarriers for BBB traversal and NA delivery in GBM.

Feature	LNPs	Exosomes / EVs	Polymeric carriers
BBB penetration (in vivo evidence)	Can be improved with ligands (Ang-2, transferrin) and/or focused ultrasound; strongest data for intracranial/local delivery in GBM [118].	Naturally BBB-permissive in several models; tumor tropism can be enhanced by surface display (e.g. Ang motifs) [119,120].	Can reach the brain with Receptor-mediated transcytosis (RMT) ligands or local delivery [121].
Targeting options	Ligand modularity (Ang-2/LRP1; transferrin [122]; RGD/αvβ3 [123]; antibodies [124]); tunable protein corona via PEGylation [125].	Intrinsic cargo/ marker repertoire; can be engineered to display targeting peptides/ antibodies (e.g., Ang, RGD) [120]	Broad ligand chemistry (peptides, aptamers, antibodies, folate) [126].
Cargo compatibility	mRNA [127], si/miRNA [128], ASOs [129], CRISPR mRNA/ sgRNA [130].	si/miRNA [131,132], CRISPR-Cas9 [133], mRNA [134].	si/miRNA, plasmid DNA, mRNA, CRISPR plasmids [121,135].
Immunogenicity/ safety	Generally favourable with biodegradable ionizable lipids (pKa ~6.2–6.5) [89]; can lead to complement activation/ cytokines at high dose [136].	Low intrinsic immunogenicity, donor-source and batch heterogeneity must be controlled; minimal complement activation [137].	Composition-dependent; cationic polymers (e.g., PEI) can be cytotoxic/ inflammatory. Can be mitigated by biodegradable/ charge-shielded designs [86,125].
Manufacturing & scalability	Strong—robust, scalable (microfluidics), well-developed analytics; clear regulatory precedents [138].	Challenging, isolation, purity, yield, and identity; scale-up and release assays are still evolving [139].	Good for many systems (PLGA); reproducible, scalable; complex hybrids require stricter controls [140].

2.2. Gene silencing and suppression

Gene silencing/suppression has emerged as a potential therapeutic option for GBM treatment, as it can effectively modulate genetic regulators of tumor proliferation, invasion and resistance [59,141]. In contrast to traditional therapies with low specificity, gene silencing effectively targets selective oncogenes and other pathogenic pathways at the molecular level. The specificity of these techniques should reduce the off-target effects associated with traditional chemotherapy [142]. The main modalities for gene silencing and suppression in GBM include RNA interference (RNAi), SSOs, and CRISPR interference (CRISPRi), each with individual mechanisms and opportunities.

2.2.1. RNA Interference (RNAi)

RNAi involves using small RNA molecules to silence gene expression. This is usually achieved by degrading mRNA before its translation into proteins. This mechanism has been explored extensively in GBM [143].

2.2.1.1. Small Interfering RNA (siRNA). Small interfering RNA (siRNA) molecules have been used extensively in preclinical GBM models. siRNAs bind to complementary mRNA sequences, leading to transcript degradation and inhibition of protein expression. This process involves Dicer-mediated processing of siRNAs and their incorporation into the RNA-induced silencing complex (RISC), where Argonaute directs sequence-specific mRNA cleavage [144]. Typically, the use of siRNA in GBM has focused on the inhibition of aberrantly expressed genes involved in tumorigenesis, angiogenesis, and resistance. For instance, silencing of epidermal growth factor receptor (EGFR), a receptor highly expressed in most GBM tumors, via siRNA has been demonstrated to effectively inhibit the tumor growth and sensitise cancer cells to cytotoxic agents [145].

The therapeutic efficacy of siRNA largely depends on the selection of an appropriate delivery system, as non-modified siRNAs are inherently unstable and require carriers that ensure efficient cellular uptake, protection from degradation, and targeted delivery to tumor cells [146]. Early studies were performed using traditional lipidic or cationic polymeric transfection reagents [147]. For example, using Lipofectamine as the delivery system, plasmid-based siRNA constructs targeting EGFR were administered directly into tumors via intratumoral injection. This approach significantly reduced cell viability and tumor growth in U251 glioma cells, both in vitro and in vivo [148]. Likewise, InvitroRNA™ was used to effectively deliver VEGF siRNA and achieve silencing, inhibiting angiogenesis in GBM [149]. Cationic polymers have been widely explored as non-viral vectors due to their strong electrostatic interactions and ability to condense NA into nanoparticles for efficient cellular delivery [147]. Among the cationic polymers PEI is the most commonly used. PEI is complexed with NA and serves to enhance the cellular uptake and transfection efficiency of NAs [150]. A plasmid-encoded VEGF siRNA was delivered via polyethylenimine (PEI) and demonstrated significant reductions in tumor vascularisation in a xenograft mouse model [151].

In recent years, NA delivery has shifted from traditional carriers to more sophisticated and targeted systems. This shift has been particularly evident in GBM, where effective delivery remains a critical challenge. Various groups have developed aptamer targeted systems, based entirely on NA. Aptamers are NA with binding capabilities similar to monoclonal antibodies, however they are much smaller and may offer superior BBB penetrance [152]. For example, a DNA tetrahedron nanostructure [153] has been functionalized with the aptamer AS1411 to deliver survivin-targeted siRNA to GBM cells [154]. AS1411, a G-rich DNA aptamer, specifically binds to nucleolin, which is overexpressed on the surface of GBM cells and endothelial cells involved in tumor angiogenesis. This dual targeting enabled high-affinity binding and internalisation of the nanoconjugates, resulting in enhanced cellular uptake, improved targeting specificity, and increased apoptosis in U87 glioma cell lines [155]. Similarly, synthetic ligand-guided systems such as aptamer–siRNA chimeras have been developed to achieve precise molecular targeting without reliance on endogenous vesicle pathways. A notable example is the Gint4.T–STAT3 chimera, designed to selectively deliver siRNA to PDGFRβ-positive GBM cells. This platform demonstrated efficient internalisation and strong specificity, leading to reduced cell viability and migration in U87MG and T98G cells, and significantly suppressing tumor growth and angiogenesis in a subcutaneous xenograft model [156].

An alternative approach has been to use biologically derived carriers such as exosomes which have endogenous tumor-homing and can cross the BBB [137]. Engineered exosomes functionalized with Ang have been employed to deliver siRNA targeting STAT3, a transcription factor frequently overexpressed in GBM. This approach reduced cell viability in U87MG cells and significantly inhibited tumor growth in an orthotopic xenograft model [157]. The exosome-based platform enhanced siRNA stability, cellular uptake, and tumor-specific accumulation, while reducing off-target effects [131,157,158]. In a related strategy, blood-derived exosomes were used to co-deliver siRNA against cytoplasmic phospholipase A2 (cPLA2) alongside chemotherapeutic agent metformin. This delivery system efficiently crossed the BBB disrupting mitochondrial metabolism in primary GBM cells and suppressing tumor growth in a patient-derived xenograft (PDX) model [158].

While the targeting of oncogenes within cancer cells has shown promise, NA has also been used to deliver immunotherapy. Specifically, siRNA has been used to silence immune checkpoints (ICPs) within the GBM TME. One of the most studied targets is programmed death-ligand 1 (PD-L1) which is a key ICP, overexpressed in GBM that enables tumor cells to evade T-cell-mediated immunity. Preclinical work has shown that knockdown of PD-L1 by siRNA in GBM cells reactivates T-cells, increasing immune recognition and tumor regression. For instance, a study developed CXCR4-targeted lipid-calcium-phosphate nanoparticles incorporating nitric oxide (NO) donors to deliver PD-L1 siRNA to GBM tumors [1]. This delivery system offers multiple synergistic advantages; CXCR4-targeting improves specificity by directing delivery to glioma cells and tumor-associated vasculature, where CXCR4 is overexpressed [159]; the lipid–calcium–phosphate core enables biocompatible, pH-responsive siRNA release [160], while NO donors transiently increase BBB permeability to enhance brain penetration [161]. The mechanism of action is illustrated in Fig. 2. Results showed enhanced BBB

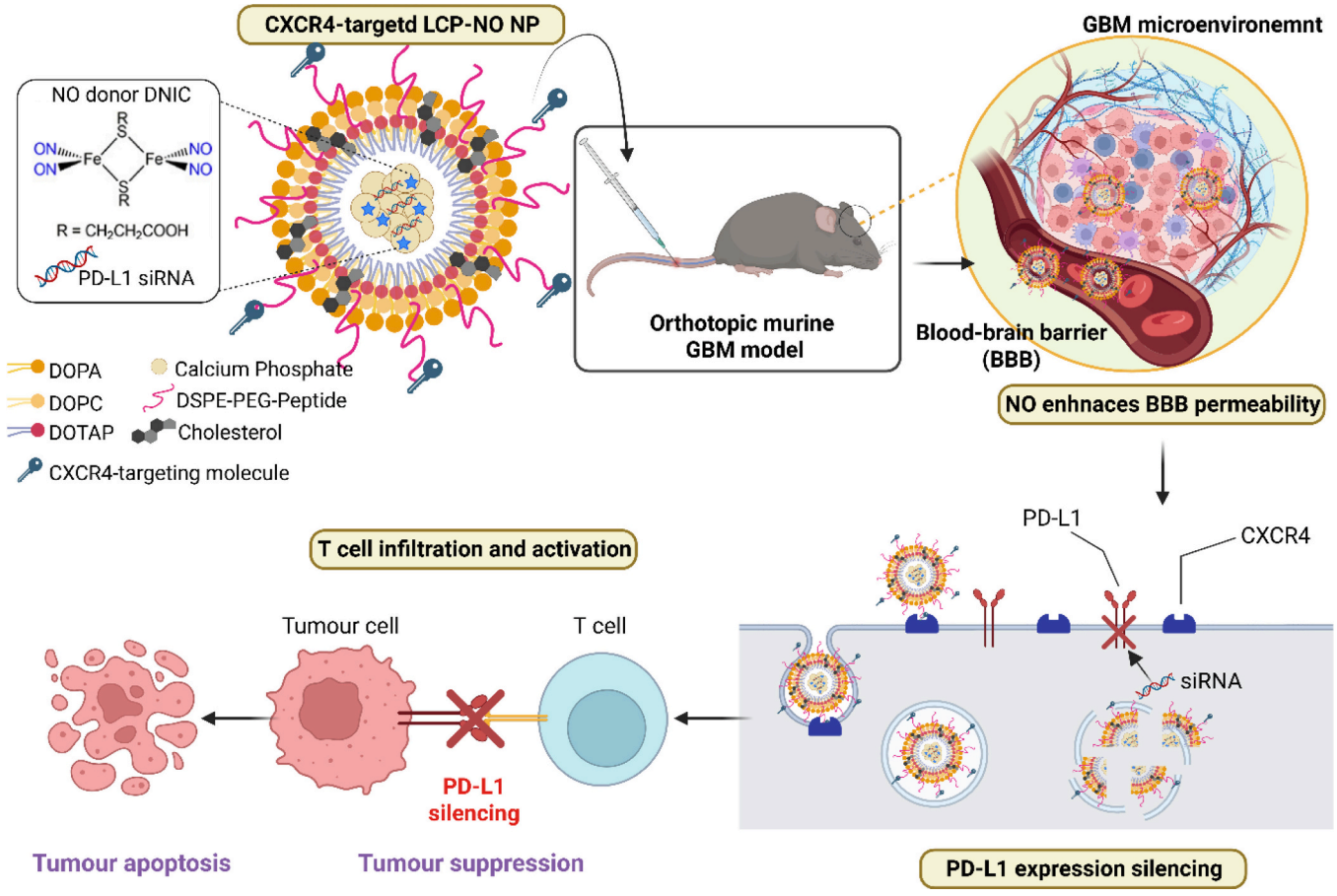


Fig. 2. CXCR4-targeted LCP-NO nanoparticles for siRNA delivery in GB immunotherapy.
Schematic representation of a CXCR4-targeted lipid-calcium-phosphate nanoparticle (LCP-NO NP) platform incorporating nitric oxide (NO) donors and PD-L1 siRNA for GB treatment. Systemic administration enables targeted accumulation at the BBB, where NO release transiently enhances BBB permeability. The nanoparticles then enter the TME and deliver PD-L1 siRNA to glioma cells, silencing PD-L1 expression and promoting T cell infiltration and activation. This immunomodulatory cascade leads to tumor suppression and apoptosis, highlighting the therapeutic potential of NO-enhanced, targeted siRNA nanodelivery in GB. Modified from [1].

permeability and targeted siRNA delivery leading to silenced PD-L1 expression, increased cytotoxic T-cell infiltration, and suppression of GBM progression [1].

In parallel to modulation of the PD-1 axis, focusing on mobilising T cell responses, several studies have assessed silencing of CD47 to engage macrophages. CD47 acts as a "don't eat me" signal that inhibits phagocytosis of cancer cells by binding to signal regulatory protein alpha (SIRPα) on macrophages [162]. CD47 knockdown has been reported to cause macrophage infiltration and tumor cell apoptosis, leading to potentiated anti-GBM activity and increased median survival [163]. Disrupting the CD47-SIRPα axis through siRNA knockdown, either alone or in combination with autophagy inhibition, enhanced macrophage-mediated phagocytosis, and significantly inhibited tumor growth in preclinical models [163]. Notably, this was achieved using a multi-component delivery platform comprising Ang-modified, Outer membrane vesicle (OMV)-coated ROS-responsive nanocarriers that co-delivered siCD47 and a DOX-aptamer complex (AO@PTP/47aD), ensuring efficient BBB penetration, targeted tumor accumulation, and potent immunogenic cell death [164]. The Ang enabled receptor-mediated BBB crossing [122], the OMV coating enhanced immune activation [165], and the ROS-responsive design allowed controlled drug release within the TME, together enabling synergistic chemo-immunotherapy with heightened specificity and efficacy [166]. Building on this strategy, targeting both the innate and adaptive immune evasion via the dual silencing of CD47 and PD-L1 has been trialled using cationic lipid nanoparticles (LNPs). The BAMPA-O16B/siRNA lipoplex efficiently delivered both siRNAs across the BBB, significantly reducing target gene expression in the tumor and enhancing the immune response within the TME. This enhanced performance was attributed to BAMPA-O16B's favourable endosomal escape properties and its optimal pKa (~6.5), which supports efficient ionization in the mildly acidic endosomal environment, thereby promoting membrane fusion and cytoplasmic siRNA release [89].

Together, these studies underscore the versatility of siRNA therapeutics in modulating both tumor-intrinsic pathways and the immune landscape of GBM. By integrating precise gene silencing with advanced delivery systems, siRNA-based strategies offer a robust framework for developing multifaceted treatments capable of addressing tumor heterogeneity and immune evasion [167].

2.2.1.2. MicroRNA (miRNA). MicroRNAs (miRNAs) are small, non-coding RNAs (~20–24 nucleotides) that play critical roles in post-transcriptional regulation of gene expression. In GBM, aberrant expression of specific miRNAs has been linked to key hallmarks of tumor progression, including proliferation, invasion, angiogenesis, and resistance to therapy [168]. Therapeutic strategies have focused on modulating miRNA activity by either restoring tumor-suppressive miRNAs using mimics (agomirs) or inhibiting oncogenic miRNAs with anti-miRs [169]. One of the most widely studied oncogenic miRNAs in GBM is miR-21, which is commonly overexpressed and contributes to tumor invasion, survival, and drug resistance [170–172]. Anti miR-21 has been delivered in a range of vectors, extensively using biodegradable polymerics. Noted examples include the use of engineered nanoparticles such as ApoE-coated poly(amine-*co*-ester) (PACE) polyplexes and poly (lactic acid)-hyperbranched polyglycerol (PLA-HPG) nanocarriers to deliver anti-miR-21 directly into brain tumors via convection-enhanced delivery [173]. These platforms protect NAs through electrostatic complexation, enhance uptake via ApoE-mediated targeting, and promote cytosolic release through endosomal escape mechanisms such as the proton sponge effect. Together, these features ensure effective gene silencing within the TME while minimising off-target effects [174]. A combined polymeric and lipid 'lipid–polymer hybrid nanoparticle' (LPHNP) system was developed for the co-delivery of pemetrexed and anti-miR-21 in GBM therapy. These LPHNPs, composed of Poly (lactic-*co*-glycolic acid) (PLGA), Poly-ε-caprolactone (PCL), and phosphatidylcholine, were designed for controlled release, high biocompatibility, and protection of nucleic acid cargo. Functionalisation with Pluronic F127 and Tween 80 further enhanced colloidal stability and cellular interaction, resulting in improved intracellular delivery, sustained release, and synergistic cytotoxicity in U87MG glioma cells. This system exemplifies the potential of multifunctional nanocarriers for combinatorial chemo–gene therapy [175]. While synthetic nanoparticles displayed encouraging preliminary results, exosomes naturally contain miRNA, and this property may be exploited for improved delivery [137]. In one study, genetically modified exosomes derived from HEK-293 T cells, were loaded with miR-21 sponge constructs or pri-miR-21. These exosomes efficiently modulated miR-21 targets and significantly reduced tumor burden in vitro and in orthotopic xenograft models [132].

In addition to miR-21, miR-10b, an oncogenic miRNA implicated in GBM progression and angiogenesis, has been targeted for treatment of GBM. Its inhibition has been shown to suppress tumor proliferation, invasion, and migration in both GBM tissues and stem-like cells [176]. To mediate silencing, PLGA-based nanoparticles have been formulated with antisense oligonucleotides targeting both miR-10b and miR-21. These nanocarriers provide enhanced cellular uptake, protection from enzymatic degradation, and efficient cytoplasmic release, thereby amplifying the therapeutic efficacy of miRNA-targeted interventions [177].

Beyond targeting oncogenic miRNAs, restoring tumor-suppressive miRNAs, such as miR-124, has demonstrated significant therapeutic promise. One advanced strategy employed engineered umbilical mesenchymal stem cells (UMSCs) equipped with a PiggyBac transposon system to co-deliver miR-124 and PD-1 plasmid construction. Exosomes derived from these stem-cells were administered via intra-carotid artery injection, enabled selective tumor homing, immune modulation, and sustained release of bioactive cargo directly within GBM tissue [178]. Co-delivery of anti-miR-21 and miR-124 was achieved via Ang-functionalized, ROS-responsive nanomedicines (Ang-NM@miRNA). This offered a non-invasive, systemic delivery across the BBB and facilitated spatiotemporally controlled release in the TME, leading to potent inhibition of GBM progression and modulation of oncogenic signalling pathways [179]. Other tumor-suppressive miRNA frequently downregulated in GBM include miR-128 which has been shown to promote apoptosis, inhibit tumor growth, and enhance sensitivity to TMZ [180]. Polyhydroxybutyrate (PHB)-*co*-PEI nanoparticles have been developed to encapsulate plasmids expressing miR-128, offering stable, serum-resistant, and efficient intracellular delivery while minimising cytotoxicity [181].

Collectively, these innovative delivery strategies, summarized in Table 3, ranging from polymeric nanoparticles to stem cell-derived exosomes, demonstrate the growing potential of miRNA-based therapeutics in GBM, offering multifaceted platforms to overcome the barriers of the BBB, enhance targeting specificity, and modulate key oncogenic pathways for improved treatment outcomes.

2.2.2. Splice-switching oligonucleotides (SSOs)

SSOs are synthetic antisense molecules that modulate pre-mRNA splicing by binding to specific splice sites or regulatory elements, redirecting the splicing machinery to produce therapeutically beneficial transcript variants [182]. A pivotal study in GBM demonstrated that SSOs can modulate MKNK2 splicing, skewing the balance of prooncogenic Mnk2b toward tumor suppressive Mnk2a. This modulated p38-MAPK pathway inhibited proliferation and enhanced chemosensitivity in GBM cells, along with suppressed tumor growth in vivo [183]. These therapeutic effects were facilitated by the use of TMC-SA nanocomplexes, a biocompatible, trimethylated chitosan-based delivery system functionalized with stearic acid, that ensured serum-stable complexation, efficient cellular uptake, and endosomal escape, thereby facilitating precise intracellular delivery of SSOs for effective splice correction [184]. In parallel, Peptide nucleic acid (PNA)-based

Table 3
NA-based therapies used in GBM treatment.

Modality	Target	Delivery system	Delivery system composition	In vitro model	In vivo model	Route of administration	Outcome	Ref
Small Interfering RNA (siRNA)	EGFR	Transfection agent	Lipofectamine	U251	Subcutaneous tumor model	Intratumoral	growth inhibition effect on U251 glioma cells in vitro and in vivo	[148]
	VEGF	Polymer-based	Polyethylenimine (PEI)	U87 human GBM cells	Xenograft SCID mouse model	Intratumoral	Reduced tumor vascularisation, complete tumor inhibition with IL-4	[150]
	Survivin	Nanostructure	DNA tetrahedron nanostructures with aptamer AS1411	U87	N/A	N/A	Increased apoptosis, reduced tumor cell survival sensitising GBM cells to radiotherapy and overcoming radioresistance.	[151]
				LN229	N/A	N/A		
	STAT3	Exosome	Exosomes functionalized with Angiopep-2	U87MG	Orthotopic xenograft model	Intravenous	Reduced tumor growth, improved immune recognition	[156,157]
		Aptamer	aptamer-siRNA chimera (Gint4. T-STAT3)	U87MG and T98G	Subcutaneous NOD/SCID nude mice xenograft mouse	Intra-peritoneal	Reduced cell viability and migration	
	cPLA	Exosome	Engineered blood-derived exosomes	Primary GBM cells	Patient-derived xenograft (PDX) model	Intravenous	Impaired mitochondrial metabolism, reduced tumor growth	[158]
	PD-L1	LNPs	CXCR4-targeted lipid-calcium-phosphate nanoparticles	Murine GBM ALTS1C1 cell line and Human brain capillary endothelial cells	Xenograft C57BL/6JNarl mouse model	Intravenous	Increased cytotoxic T-cell infiltration, reduced tumor progression	[1]
	CD47	Nanocarriers	Outer membrane vesicle (OMV)-coated ROS-responsive nanocarriers	GBM cell lines	Xenograft model	N/A	Reduced CD47 expression, enhanced immune response	[164]
MicroRNA (miRNA)	miR-21	Nanoparticles	ApoE-coated PACE polyplexes or PLA-HPG-based nanocarriers	U87	intracranial mouse model	intracranial (convection-enhanced)	Robust PTEN upregulation and cell apoptosis	[173]
	miR-21	Nanoparticles	lipid-polymer hybrid nanoparticle	U87MG	N/A	N/A	Improved accumulation of LPHNPs in the nucleus of U87MG cells	[175]
	miR-128	Nanoparticles	Polyhydroxy butyrate (PHB)-*co*-PEI-based nanoparticles	U87	N/A	N/A	Enhanced therapeutic efficacy and minimized cytotoxicity	[181]
Splice-switching oligonucleotidesSplice-switching oligonucleotides	MKNK2	Nanocomplex	TMC-SA nanocomplexes	U87MG, HuH7 and MDA–MB–231	U87MG intracranial model	Intratumoral	Inhibited proliferation and enhanced chemosensitivity in GBM cells	[183]
		Peptide conjugate	PNA–peptide bioconjugates	U87MG	N/A	N/A	Cancer cell death	[185]
Antisense oligonucleotides	EGFR	Dendrimers	Folate-conjugated PAMAM dendrimers	The rat C6 cerebral glioma cell line	Rat glioma model	Intratumoral	Decreased tumor growth, and prolonged survival	[189]
	TGFβ1/TGFβ2	modified antisense oligonucleotide	phosphorothioate-locked nucleic acid (LNA)-modified antisense oligonucleotide gapmers	Human glioma lines LN-308 and LN-229 Mouse glioma line SMA-560	Crl: CD1 Foxn1 nude xenograft model	Subcutaneous	Diminished expression of TGFβ, decreased tumor growth, and increased survival.	[188]
	STAT3	Neural stem cells (NSC)	CpG-conjugated STAT3 antisense oligonucleotides loaded NSCs and packaged into secreted extracellular vesicles	Modified U-251 MG Patient-derived GSCs	N/A	N/A	Reduced DRR/FAM107A expression, marking the first instance of utilising an antibody-antisense strategy against cancer stem cells	[119]
CRISPR/Cas9	Glutathione synthetase	Evs	Dual-modified EVs functionalized with Ang and trans-activator of transcription (TAT) peptides	LN229	Orthotopic mouse model	Intratumoral	Sensitising glioma cells to ferroptosis following radiotherapy	[199]

(*continued on next page*)

Table 3 (*continued*)

Modality	Target	Delivery system	Delivery system composition	In vitro model	In vivo model	Route of administration	Outcome	Ref
	PLK1	Nanocapsule	Acid-responsive nanocapsule system	U87MG	U87MG-Luc orthotopic tumor-bearing nude mice	Intravenously	Extended median survival rate	[206]
	PD-L1/CD47	Nanoparticles	MC3-LNPs	GL261 and NPE—IE GBM stem cells	Orthotopic tumor models	Intracranial	Reduced tumor growth and improved cancer cell senzitisation to the immune system	[200]
Synthetic mRNA	PTEN	Nanoparticles	ApoE-decorated biomimetic nanoparticles	U87MG	Intracranial mouse model	intraveneously	Induced apoptosis in glioma cells	[215]
	PTEN + TRAIL	Non-viral transfection reagent	TransIT transfection kit	DBTRG	xenograft mouse model	intratumoral	Tumor growth inhibition	[216]
mRNA vaccine	CD133	DCs	DCs vaccination	N/A	Humanized mouse model	N/A	Immune response stimulation	[219]

SSOs were conjugated to cell-penetrating peptides like CLIP6 or nuclear localisation sequences (NLS), forming chemically defined PNA, peptide bioconjugates that enabled stable intracellular delivery and nuclear localisation in GBM cells without the need for traditional transfection agents. This carrier-free, covalent conjugation strategy facilitates receptor-independent uptake and sustained splice correction, marking a versatile and potent alternative for intracellular SSO delivery [185].

Together, these studies highlight that the success of SSO therapies hinges not only on sequence design but also on the development of sophisticated and targeted delivery systems as summarized in Table 3. These results underline the therapeutic potential of SSOs in the correction of aberrant splicing patterns and offer a new and targeted approach in the treatment of GBM.

2.2.3. Antisense oligonucleotides (ASOs)

ASOs have been investigated as therapeutics for GBM therapy to downregulate gene expression via recognition of specific mRNA sequences and subsequent inhibition of translation, primarily through RNase H-mediated degradation or steric blockade of translation [186]. A number of studies, summarized in Table 3, utilized ASOs to a specific set of genes that have been associated with GBM progression [187]. As ASO are short single stranded RNA molecules it is relatively simple to synthesise them entirely chemically, incorporating a number of stabilising modifications, thus negating the need for a particulate carrier. For example, systemically administered locked nucleic acid (LNA) gapmers ISTH1047 and ISTH0047, targeting TGFβ1 and TGFβ2, respectively, achieved sustained, isoform-specific silencing in both xenograft and syngeneic intracranial GBM models. Notably, these ASOs crossed the BBB after subcutaneous injection, as confirmed by digoxigenin-labelled ISTH1047, demonstrating that advanced chemical stabilisation can eliminate the need for invasive or nanoparticle-assisted delivery. This strategy simplifies the therapeutic regimen and facilitates repeat dosing with minimal toxicity, representing a favourable approach for clinical translation [188].

In contrast, other studies have pursued the development of dedicated delivery vehicles to improve ASO bioavailability, targeting specificity, and intracellular delivery. One group of studies has focused on polymeric nanocarrier-based delivery systems to enhance ASO bioavailability and targeting. For instance, phosphorothioate-modified ASOs targeting EGFR were delivered using folate-conjugated poly(amidoamine) (FA-PAMAM) dendrimers, a highly branched, nanoscale polymer designed to exploit folate receptor-mediated endocytosis. This system significantly improved ASO stability, cellular uptake, and nuclear delivery in vitro, leading to reduced EGFR expression, slower tumor growth, and extended survival in an orthotopic rat glioma model. These results underscore the value of rational carrier design in amplifying the therapeutic impact of ASOs in GBM [189].

A more complex approach has utilized cell-based delivery platforms to address the challenges of tumor specificity and immune modulation. In a novel study, neural stem cells (NSCs) were used as biocarriers to deliver CpG-conjugated STAT3 antisense oligonucleotides (CpG-STAT3ASO) to the GBM microenvironment. NSCs were first loaded with CpG-STAT3ASO ex vivo; the therapeutic cargo was then packaged into secreted extracellular vesicles (EVs), which were systemically or intracranially administered. This dual-layered delivery system, leveraging NSC tumor-homing properties and EV-mediated intracellular transport, achieved precise delivery to dendritic cells and macrophages within the TME. Beyond enhancing ASO bioavailability and uptake, this strategy also reprogrammed the glioma immune landscape, promoting antitumor immune activation [119].

Together, these studies underscore the importance of both molecular design and delivery strategy in the success of ASO-based therapies for GBM. By combining chemical modification with tailored delivery platforms, ranging from dendrimers to stem cell-derived vesicles, researchers are increasingly able to overcome the challenges of CNS drug delivery and unlock the full potential of gene-silencing approaches in

GBM treatment.

2.2.4. CRISPR-based therapies

CRISPR-based gene editing technologies have revolutionized functional genomics, enabling precise and efficient manipulation of genetic material in a wide range of cell types [190]. Traditional CRISPR/Cas9 gene-editing technology operates through a single guide RNA (sgRNA) that directs the Cas9 endonuclease to a specific DNA sequence, where it induces double-strand breaks, prompting the cell's repair machinery to disrupt or correct the target gene [191,192]. In GBM, CRISPR/Cas9 systems are increasingly utilized to dissect the roles of oncogenes [133], tumor suppressors [193], and DNA repair pathways [194], offering insights into the molecular underpinnings of tumor progression and resistance mechanisms [190]. More recently, CRISPR interference (CRISPRi) has emerged as a powerful RNA-guided gene repression system based on a catalytically inactive Cas9 (dCas9) fused to transcriptional repressors. Unlike CRISPR nucleases, CRISPRi enables sequence-specific transcriptional silencing without inducing double-strand breaks, allowing for reversible and tunable gene knockdown [195,196]. CRISPRi has been successfully deployed in high-throughput screens to dissect essential genes and long non-coding RNAs (lncRNAs) involved in GBM proliferation, therapy resistance, and stem cell maintenance, highlighting its potential in elucidating non-mutational vulnerabilities and informing precision therapies [196].

Beyond target discovery, CRISPR is being actively explored as a therapeutic modality, with strategies aimed at disrupting oncogenic signalling [197], reprogramming the TME [198], and sensitising GBM cells to standard therapies [199].

The first successful demonstration of CRISPR-Cas9 gene editing in GBM using LNPs was reported by Rosenblum et al. In this study, LNPs incorporating a novel ionizable amino lipid were engineered to co-deliver Cas9 mRNA and sgRNAs targeting PLK1 via intracranial injection into orthotopic GBM tumors. This approach achieved up to ~70 % gene editing in vivo, leading to tumor cell apoptosis, 50 % reduction in tumor growth, and a 30 % improvement in survival. This work established a precedent for the use of nonviral, systemically safe LNPs in the treatment of brain tumors and demonstrated the therapeutic potential of genome editing in GBM [130].

One well-established approach involves the use of LNPs formulated with ionizable lipids. In this study, LNPs composed of the ionizable lipids Dlin-MC3-DMA were employed to co-deliver Cas9 mRNA and sgRNAs targeting PD-L1 and CD47 directly into the TME via intracranial injection. These LNPs were optimized for NA encapsulation, stability, and cellular uptake, and demonstrated effective in vivo gene editing with significant immunomodulatory and antitumor effects as shown in Fig. 3 [200]. MC3 is a pH-sensitive lipid with a tertiary amine headgroup that becomes protonated in acidic endosomal environments, facilitating endosomal membrane destabilisation and efficient cytosolic release of NAs (Fig. 3). At physiological pH (~7.4), MC3 remains relatively neutral, minimising toxicity and enhancing systemic tolerability [201,202]. A similar MC3-based LNPs system was also used to deliver Cas9 mRNA and sgRNAs targeting GFP or PLK1 into GBM and other cancer models, showing efficient in vivo gene editing following intracerebral injection with selective tumor uptake and low toxicity [130].

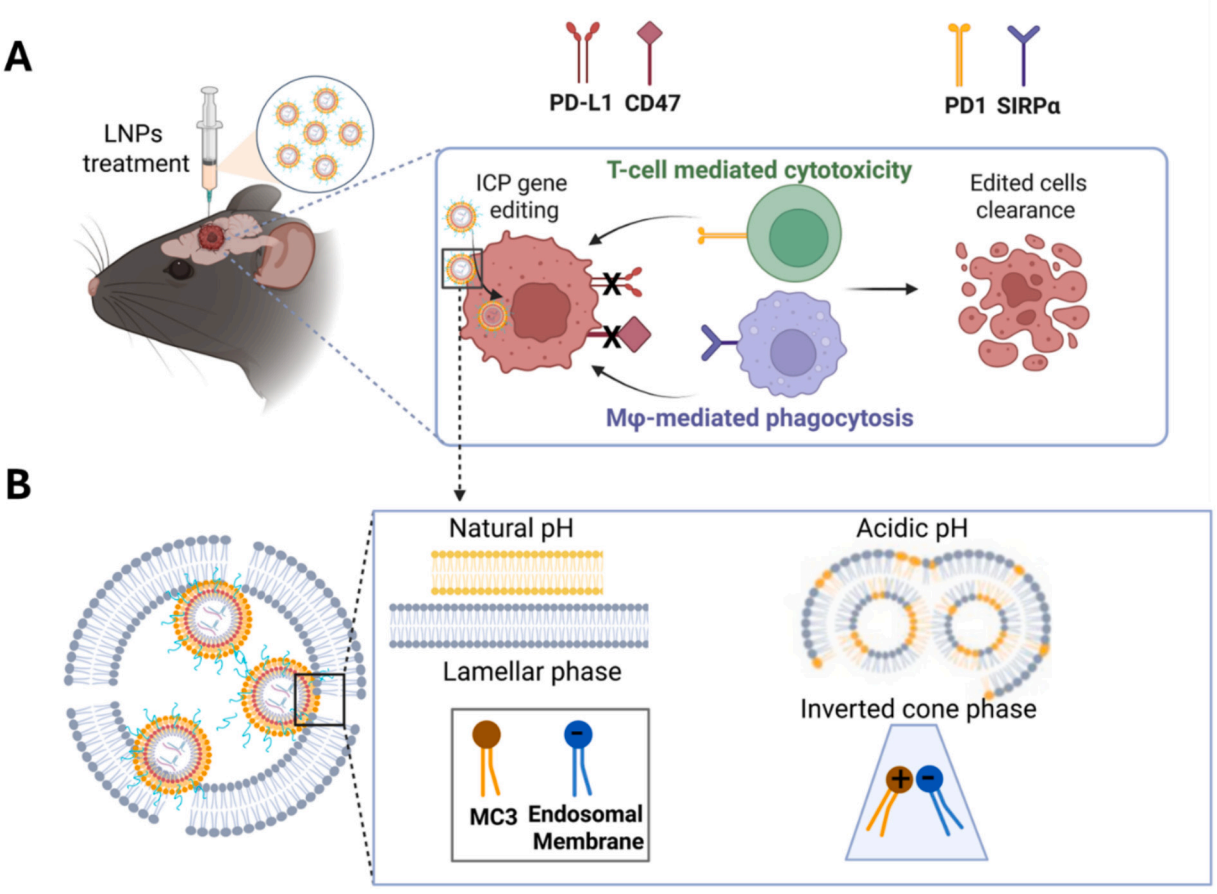


Fig. 3. MC3-based LNP delivery system for CRISPR-mediated ICP editing in GBM. (A) Intracranial injection of LNPs co-encapsulating Cas9 mRNA and sgRNAs targeting PD-L1 and CD47 enables gene editing within GBM cells. This results in reduced expression of immune checkpoint proteins, enhancing T cell-mediated cytotoxicity and macrophage (Mϕ)-mediated phagocytosis, ultimately leading to tumor cell clearance. (B) The ionizable lipid MC3 remains neutral at physiological pH (lamellar phase) for systemic stability, but becomes protonated in acidic endosomes, adopting an inverted cone shape that disrupts membranes and promotes endosomal escape for cytoplasmic delivery of CRISPR components. (B) modified from [1]. Drawn using Biorender.

Alternative nanoparticle platforms have been developed to transport Cas9 and sgRNA encoded in plasmids. These platforms included nanoliposomes [203], LPHNPs [135], and liposome-based hydrogel systems [204,205]. Interestingly, liposome-based hydrogel systems were also used to deliver Cas9/sgRNA as ribonucleoprotein complexes (RNPs) [204,205].

Besides the use of nanoparticles, other delivery platforms have emerged for the safe and efficient delivery of CRISPR components, including EVs and stimuli-responsive nanocarriers, to overcome biological barriers like the BBB and improve targeting precision. Another example involved the use of dual-modified EVs functionalized with Ang and trans-activator of transcription (TAT) peptides to deliver RNPs targeting glutathione synthetase (GSS), a suppressor of radiotherapy-induced ferroptosis. CRISPR-mediated knockout of GSS disrupted glutathione synthesis, inactivated GPX4, and promoted iron accumulation, sensitising glioma cells to ferroptosis. The Ang/TAT-modified EVs enabled efficient BBB crossing and tumor accumulation, achieving up to 67.2 % GSS editing in vivo with minimal off-target activity [199].

Further advancing the field, acid-responsive nanocapsule systems (ANCSS), composed of polymer shells surrounding RNPs targeting PLK1, a key regulator of glioma cell proliferation, and functionalized with Angiopep-2 to enhance BBB targeting. These nanocapsules provided protection from RNase degradation, demonstrated efficient systemic delivery and BBB penetration, and facilitated endosomal escape for high-efficiency gene editing within the TME. As a result, ANCSS significantly improved therapeutic efficacy while limiting systemic toxicity and off-target effects [206].

Together, these diverse delivery platforms mentioned in Table 3 highlight a fundamental principle in CRISPR-based GBM therapy: the efficacy of gene editing is intrinsically tied to the delivery system. As such, rationally engineered nanocarriers—capable of traversing biological barriers, ensuring intracellular delivery, and releasing therapeutic payloads with spatial and temporal precision—will be instrumental in translating CRISPR therapeutics from bench to bedside. Continued innovation in this space will likely define the next generation of personalized and minimally invasive gene therapies for GBM.

2.3. Gene expression: mRNA-based therapies

Messenger RNA (mRNA)-based therapies have emerged as a novel approach for treating GBM, offering unique advantages in addressing the complex challenges posed by this aggressive brain cancer [207]. These therapies encompass synthetic therapeutic mRNA delivery and mRNA vaccines, each with distinct mechanisms of action and potential benefits.

A key translational challenge for RNA therapeutics is their potential to trigger innate immune responses. Unmodified RNA molecules can be recognised by endosomal Toll-like receptors (TLR3, TLR7, and TLR8) and cytosolic sensors such as retinoic acid-inducible gene I (RIG-I) and Melanoma differentiation–associated protein 5 (MDA5), leading to activation of interferon signalling pathways and proinflammatory cytokine release [208]. Likewise, certain cationic or ionizable lipids used in LNPs may activate the complement cascade or induce cytokine secretion through membrane perturbation. These immunostimulatory effects can limit therapeutic efficacy and safety, particularly in the CNS, where inflammation may exacerbate neurotoxicity [209].

To mitigate these responses, recent formulations employ chemically modified nucleosides such as pseudouridine (ψU) and N1-methylpseudouridine ($m^1\Psi$), which reduce TLR recognition and enhance mRNA stability and translational efficiency [210]. In parallel, biodegradable ionizable lipids have been developed with optimized pKa values (~6.2–6.5) to balance endosomal escape and minimize off-target immune stimulation. These strategies have collectively improved the tolerability and translational feasibility of mRNA and lipid-based delivery systems for GBM therapy [211].

2.3.1. Synthetic mRNA therapies

Synthetic mRNA therapeutics function by delivering in vitro-transcribed mRNA encoding therapeutic proteins, such as tumor suppressors, cytokines, or genome-editing tools, into GBM cells, where they are translated to exert transient, controlled biological effects without altering the host genome [212]. Clinically, mRNA-based therapies delivered via LNPs have shown promise in other cancer types; for instance, Moderna's ongoing trials (NCT03739931, NCT02872025) with mRNA-2752 demonstrated tolerability, immune activation, and signs of tumor regression in solid tumors [213,214]. Building on this clinical momentum, preclinical studies in GBM have explored mRNA delivery to modulate the TME and introduce genome-editing components with encouraging therapeutic effects.

Among these, restoring the function of tumor suppressors through synthetic mRNA delivery, such as PTEN or p53, has shown notable potential in counteracting oncogenic signalling and enhancing treatment responses in GBM. One preclinical study aimed to enhance the therapeutic efficacy of synthetic mRNA by using ApoE-decorated biomimetic nanoparticles (ABNPs) as a non-viral delivery platform. These ABNPs were specifically engineered to facilitate efficient mRNA encapsulation, protect against degradation, and promote selective accumulation within GBM tumors by traversing the BBB via ApoE-mediated targeting. This delivery strategy significantly improved cellular uptake, transfection efficiency, tumor penetration, and apoptosis induction, while minimising systemic toxicity, highlighting its promise for safe and precise gene therapy in brain tumors [215].

Studies have also demonstrated that multiple mRNA can be co delivered achieving synergistic effects. TransIT formulations of mRNAs encoding PTEN and TRAIL, a tumor necrosis factor-related apoptosis-inducing ligand, injected intracranially demonstrated efficient transfection, enhanced mRNA stability, and robust intracellular uptake directly within the tumor. This localized, non-viral delivery method reduced systemic exposure and immune activation while promoting targeted gene expression and potent antitumor activity, thereby maximising therapeutic efficacy with a favourable safety profile [216].

2.3.2. mRNA vaccine strategies

mRNA vaccines are a novel class of immunotherapeutics that deliver in vitro-transcribed mRNA encoding tumor or viral antigens to immune cells, enabling the host to generate a targeted and adaptive immune response without introducing live pathogens or altering the genome. In GBM, mRNA vaccines aim to stimulate anti-tumor immunity by directing the immune system against tumor-specific antigens, either shared or patient-specific [217].

One prominent strategy targets tumor-associated or viral antigens. An example that was conducted in humanized GBM mouse model involves the use of DCs transfected with mRNA encoding the cytomegalovirus (CMV) pp65 protein, a viral antigen uniquely expressed in GBM but absent from normal brain tissue. This ex vivo delivery system uses patient-derived DCs as cellular carriers to present the antigen and stimulate a targeted immune response upon intradermal injection [218]. The use of DCs ensures effective antigen presentation, strong T cell activation, and durable immune memory, even when administered alongside standard therapies such as TMZ. Similarly, another approach utilized mRNA-loaded DCs transfected with modified CD133 mRNA, a glioma stem cell marker, engineered to direct the antigen to both MHC class I and II compartments for optimal presentation. When administered intradermally in humanized NOG mice bearing intracranial GBM xenografts, these vaccines triggered potent $CD4^+$ and $CD8^+$ T cell responses and extended survival [219].

In contrast to 'common tumour antigens' shared between GBMs, personalized neoantigen vaccines, selectively target patient-specific mutations identified through comprehensive genomic profiling [220]. These approaches aim to circumvent tumor heterogeneity and minimize opportunities for immune escape. Personalized neoantigen vaccines usually comprise mRNA encoding neoantigens which are either

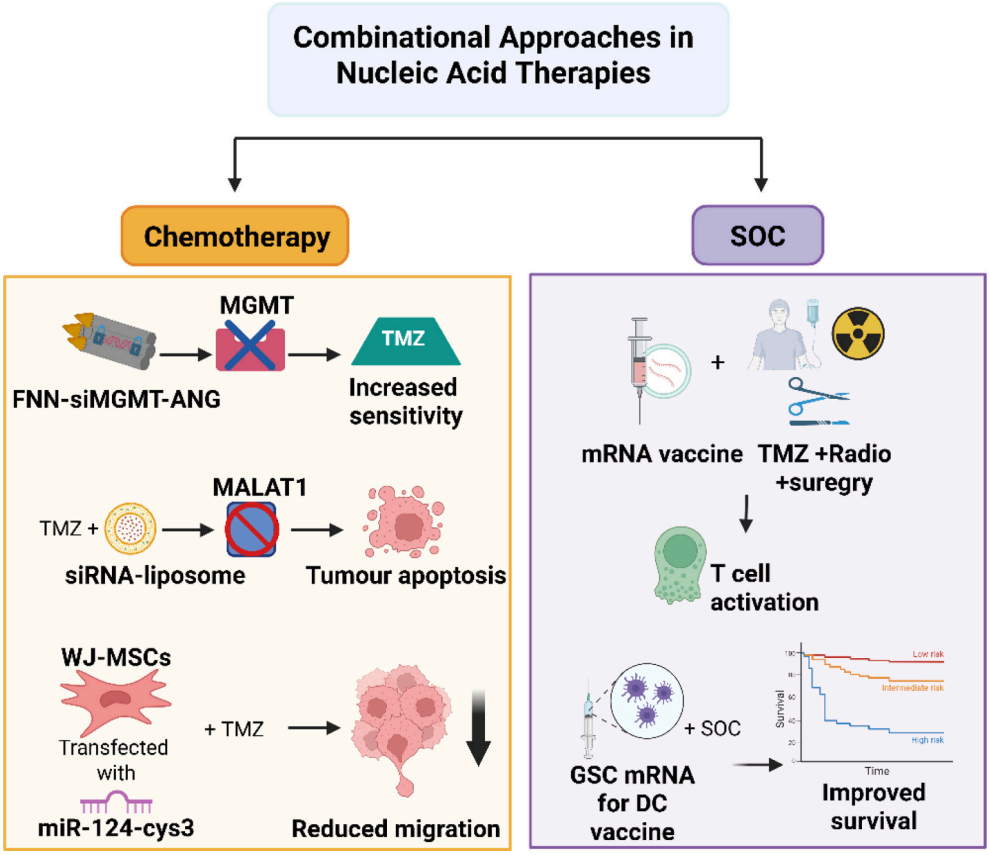
administered peripherally in a suitable vector or used to ex vivo load DCs, as described above. For example, GSC-targeted vaccines involve electroporating autologous DCs with amplified mRNA derived from patient-derived GSC cultures. Repeated intradermal administration of these DCs elicits a broad and patient-specific immune response by capturing a wide range of tumor-associated antigens reflective of each individual's tumor biology [221].

Together, these approaches highlight the therapeutic potential of mRNA vaccines when paired with rationally designed delivery systems, paving the way for more effective and durable immune-mediated tumor control.

2.4. Combinatorial approaches in nucleic acid therapies

Combinatorial approaches to NA therapy have been designed as a promising approach to enhance the treatment of GBM. By integrating NA-based interventions with other therapeutic modalities, researchers aim to overcome the limitations of single-agent treatments and address the complex challenges posed by GBM's heterogeneity and aggressive nature. These combinatorial strategies take advantage of the unique properties of DNA and RNA to block specific molecular events in conjunction with conventional therapies or other novel therapies [63,222]. The integration of NA therapies with chemotherapy, targeted drugs, immunotherapy, and advanced delivery systems offers the potential to improve treatment outcomes and quality of life for GBM patients [223,224]. These combinatorial strategies are conceptually illustrated in Scheme 1, which summarizes the diverse NA-based therapeutic combinations employed in GBM, the delivery systems facilitating their transport across biological barriers, and the synergistic outcomes achieved through co-administration with chemotherapy, immunotherapy, or standard-of-care treatments.

Several delivery systems have been developed to enhance NA therapy efficacy in GBM, as summarized in Table 4. A well-characterized combination strategy involves integrating NA therapy with TMZ chemotherapy to overcome chemoresistance in GBM. An example includes the use of a DNA-based dual-locking nanocarrier (FNN-siMGMT-ANG), which co-delivers siRNA targeting MGMT, a DNA repair enzyme implicated in TMZ resistance. This multifunctional delivery system consists of a DNA origami nanostructure loaded with siMGMT and surface-modified with the Angiopep-2 peptide to facilitate BBB penetration via LRP1-mediated transcytosis. The FNN construct ensures high structural stability, siRNA protection, and pH-responsive release within the TME. When administered in combination with TMZ, FNN-siMGMT-ANG enhanced chemosensitivity, suppressed tumor growth, and prolonged survival in orthotopic GBM models. This strategy exemplifies how rationally engineered, brain-targeted nanocarriers can be leveraged to precisely modulate gene expression and potentiate existing therapies in a synergistic, minimally invasive manner [225]. Another compelling approach used a transferrin receptor–targeted scFv-conjugated liposomal nanocarrier (scL) to deliver metastasis-associated lung adenocarcinoma transcript 1 (MALAT1)-specific siRNA (scL-siMAL) to chemoresistant GBM cells. This targeted, non-viral system enabled efficient delivery across the BBB, endosomal escape, and cytoplasmic release of siRNA. By silencing the long non-coding RNA MALAT1, implicated in stemness, migration, and therapy resistance, scL-siMAL sensitized TMZ-resistant GBM cells (e.g., T98G and U87R) to



Scheme 1. Schematic overview of combinatorial NA therapy strategies in GB. This illustration categorises current NA-based combinatorial approaches into three main therapeutic modalities: chemotherapy, SOC, and cytokine-based immunotherapy. DNA origami-based nanocarriers like FNN-siMGMT-ANG facilitate BBB penetration and deliver siMGMT to silence MGMT, thereby enhancing TMZ sensitivity. Similarly, liposome-encapsulated siRNAs targeting MALAT1 promote apoptosis and improve TMZ efficacy, while WJ-MSC engineered to express miR-124 leverage tumor-homing capacity to reduce GB cell migration and further potentiate TMZ response. In SOC-integrated regimens, mRNA and DC vaccines derived from GSC antigens augment T cell-mediated immunity when administered alongside surgery, radiotherapy, and TMZ. Drawn using Biorender.

Table 4
Combinatorial modalities in GBM therapy.

Target	Combination modality	Delivery system	Testing system	Outcome	Ref
MGMT	siRNA + TMZ	Framework NA-Based Nanoparticles (FNN)	in vitro (T98G, U87, U251, U178, A172, LN229, U118, NHA and bEnd.3 cell lines) & in vivo (TBD0220 GBM mouse model)	Increased TMZ sensitivity and improved survival in GBM models	[225]
MALAT1-specific siRNA	siRNA + TMZ	Liposomes	GBM cells (e.g., T98G and U87R) & in vivo orthotopic models	Reduced tumor sphere formation, and induced apoptosis	[226]
BRCA1, POLD1	shRNA/CRISPR + TMZ	Lipofectamine 2000	in vitro (GSCs)	BRCA1 knockdown sensitized p53 wild-type GSCs to TMZ	[227]
miR-21	miRNA inhibitor + TMZ	Liposomes	in vitro (U87MG cell line)	Suppressed anti-apoptotic signalling and increased TMZ efficacy in U87 cells	[228]
miR-124	miRNA mimic + TMZ	Wharton's jelly-MSCs (WJ-MSCs) mediated delivery	in vitro (U87 cells)	Reduced migration and increased sensitivity to TMZ in U87 cells	[229]
Autologous GSC mRNA	mRNA + SOC	DC vaccine	Clinical trial	Improved PFS and OS, safe and feasible	[231]

chemotherapy, reduced tumor sphere formation, and induced apoptosis in both in vitro and orthotopic models. This underscores how antibody-guided liposomal nanocomplexes can offer tumor-selective, systemically administered RNA interference therapy with translational potential [226]. Similarly, another study employed a pooled shRNA screening approach targeting 350 DNA repair genes in GSCs to identify synergistic interactions with TMZ. Following initial screening, candidate targets such as BRCA1 and POLD1 were silenced using siRNAs and CRISPR/Cas9 systems. The delivery of these gene-silencing agents was achieved via Lipofectamine 2000, a lipid-based non-viral vector that facilitates endosomal escape and cytoplasmic delivery of NAs. Lipofectamine-mediated transfection enabled efficient intracellular uptake and gene silencing in GSCs, resulting in increased DNA damage, apoptosis, and sensitisation to TMZ. This underscores the utility of lipid-based delivery systems for rapid, scalable functional screening and therapeutic gene modulation in resistant GBM cell populations [227]. These reports illustrate the potential of NA therapeutics in combination with TMZ to overcome TMZ resistance and to improve survival in GBM patients. However, there remain some limitations in the delivery of NA therapeutics to the brain tumor cells effectively.

In parallel, other NA combinations with chemotherapy are also being explored. For instance, a miR-21 inhibitor was delivered via liposomes, which serve as biocompatible and biodegradable carriers capable of encapsulating and protecting RNA molecules from enzymatic degradation. In U87MG GBM cells, this delivery approach allowed the miRNA inhibitor to suppress anti-apoptotic signalling pathways more effectively, thereby sensitising the cells to TMZ and enhancing its cytotoxic effects [228]. Liposomes are particularly attractive for miRNA delivery due to their ability to fuse with cell membranes and facilitate cytosolic release of their cargo, making them an efficient tool for intracellular delivery in vitro and potentially in vivo [91].

Another promising strategy involves the use of Wharton's jelly-derived mesenchymal stem cells (WJ-MSCs) as delivery vehicles for a miR-124 mimic. MSCs possess intrinsic tumor-homing capabilities, allowing them to migrate toward and integrate within the GBM microenvironment. This feature makes them suitable for targeted delivery of therapeutic NAs. In this study, WJ-MSCs were engineered to deliver miR-124 to U87 GBM cells, resulting in reduced cell migration and enhanced sensitivity to TMZ [229]. The cell-based delivery method as illustrated in Table 4 helps bypass some of the limitations associated with synthetic carriers, such as poor penetration into the brain and off-target effects and offers a biologically responsive platform for miRNA delivery in GBM [230].

The use of NA vaccine in combination with SOC treatment for GBM showed promising results in several studies. These techniques are specifically designed to improve the existing immune response against GBM and to escape the complex immunosuppressive TME found in GBM. An mRNA vaccine created by the University of Florida led by Elias Sayour, M.D., pH.D. et al has demonstrated encouraging results in pilot clinical studies. In a pilot study of 4 GBM patients, the vaccine was given via intravenous administration up to four doses over the course of 6 weeks after standard surgery, chemotherapy and radiation. Treatment stimulated fast and robust immune responses, including increased expression of immune-related proteins and T cell activity against tumors in blood samples of patients [61]. Another approach involves using DC vaccines pulsed with tumor-specific antigens. A clinical trial used autologous GSC mRNA to create a DC vaccine, which was administered alongside standard TMZ treatment after surgical and radiotherapy. This strategy aims to stimulate each patient's T cells toward their unique array of antigens, addressing the heterogeneity of GBM tumors. The trial has been completed and demonstrated safety, feasibility, and potential clinical benefit, with improved progression-free (PFS) and overall survival (OS) observed in vaccinated patients compared to controls [44,231]. Nevertheless, researchers acknowledge the critical demand for continued optimisation of delivery strategies and for further research to fully exploit the therapeutic promise of these combinatorial approaches [69].

Despite these breakthroughs, clinical translation remains challenging due to the difficulty of delivering NA-based therapies across the BBB and the heterogeneity and immunosuppressive nature of GBM. Advanced delivery systems like LNPs and focused ultrasound are being explored to overcome these barriers, with ongoing clinical trials reporting encouraging early results [232].

3. Discussions and conclusions

Despite the significant progress in NA-based therapies for GBM, their clinical success remains tightly constrained by the effectiveness of delivery systems [233]. While numerous therapeutic strategies have demonstrated preclinical efficacy, only a limited number have successfully advanced into clinical trials [234]. These include ligand-targeted liposomal nanocomplexes such as SGT-53 delivering wt p53 [64], miRNA mimics such as MRX34 [66], and DC vaccines encoding tumor-specific antigens [231]. In all these cases, the delivery platform played a critical role in ensuring the stability of the therapeutic payload, its biodistribution, and safety [82]. SGT-53, for example, leveraged transferrin receptor-targeting liposomes to cross the BBB and achieve selective tumor uptake, offering early signs of clinical benefit [65]. Similarly, DC-based mRNA vaccines demonstrated immunogenicity and feasibility when combined with standard-of-care treatment [231]. However, MRX34 also underscored the risks of systemic delivery, with severe immune-related adverse events prompting trial termination [66]. These outcomes stress that successful delivery, not just molecular targeting, is essential for translation. However, even well-characterized delivery platforms such as LNPs, while highly effective for hepatic or systemic delivery, face notable limitations in the context of GBM. Their low intrinsic brain tropism, potential for off-target accumulation, and

limited ability to penetrate the BBB reduce their efficacy in targeting intracranial tumors. This challenge is compounded by the immune-privileged and heterogeneous nature of the brain microenvironment, which demands not only robust delivery but precise spatial control [235].

What distinguishes clinically tested delivery systems is their design adaptability to physiological constraints. They share a set of key attributes: the ability to cross the BBB, low immunogenicity, and compatibility with systemic or minimally invasive administration [236]. These platforms often exploit targeting ligands (e.g., transferrin [141], Angiopep-2 [122]) or endogenous carriers (e.g., DCs [231], exosomes [119,131,158]) to enhance tumor specificity and cellular uptake. Furthermore, their modular structures allow for surface modifications that can improve pharmacokinetics or endosomal escape. Such delivery vehicles not only enable effective NA transport but also influence the therapeutic index by mitigating off-target effects and toxicity [237].

In the preclinical space, an emerging class of delivery systems appears poized to enter clinical evaluation. These include Ang-modified exosomes [157], ionizable lipid-based LNPs [200], OMV-coated ROS-responsive nanocarriers [164], and DNA origami frameworks [225]. Their shared innovation lies in multifunctionality, integrating targeting ligands [122,157,225], stimuli-responsive elements [97,98,166,238], and immune-modulating components into a single platform [225]. Notably, several of these platforms achieve BBB penetration, tumor-selective accumulation, and controlled cytosolic release, all while minimising systemic exposure [82]. Many also employ biodegradable or endogenous materials, which enhances their translational feasibility [231]. Importantly, some are already being tested in orthotopic GBM models and show compatibility with standard-of-care agents like TMZ, which may facilitate combinatorial clinical strategies [90,96,188,200,215].

The success of any delivery system in GBM ultimately depends on more than its ability to carry genetic cargo. It must demonstrate scalability, biocompatibility, batch reproducibility, and safety under repeated administration. Current trends favour non-viral vectors due to their lower immunogenicity, although viral and cell-based systems continue to offer unique advantages for specific applications [239]. Furthermore, delivery platforms that can accommodate multiplexed therapies, such as co-delivery of siRNA and immune modulators, offer added value in tackling GBM's complexity [91].

Despite the encouraging outcomes observed in early-phase clinical trials, several biological and translational barriers continue to limit the successful clinical implementation of nucleic acid (NA) therapeutics in glioblastoma. A primary obstacle remains the BBB, which restricts systemic delivery of large or negatively charged nucleic acid molecules. Even when partially disrupted within tumor cores, the BBB often remains intact at the infiltrative margins, precisely where residual glioma cells persist, thereby preventing uniform drug exposure across the tumor mass [240]. The intratumoral heterogeneity of GBM represents another major challenge. Distinct cellular subpopulations within the same tumor exhibit highly variable genetic, epigenetic, and metabolic profiles, leading to differential sensitivity to gene modulation [241]. For instance, GSCs display enhanced DNA repair capacity, efficient efflux pump activity, and a quiescent state that makes them inherently resistant to cytotoxic or gene-targeting therapies. This heterogeneity complicates both the prediction of therapeutic efficacy and the identification of universal molecular targets [242].

Furthermore, inflammatory and immunogenic responses remain a critical safety concern. Unmodified or partially purified RNA can activate innate immune sensors such as TLR3/7/8 and cytosolic sensors like RIG-I and MDA5, triggering cytokine release and systemic inflammation [243]. Similarly, certain lipid-based carriers may induce complement activation or hepatotoxicity, as reflected in recent clinical holds by the FDA, for example, the temporary pause of Intellia's Phase 3 CRISPR trial due to elevated liver enzyme levels. These events highlight the delicate balance between therapeutic potency and immune tolerability in the development of NA-based drugs [244]. Finally, the intrinsic instability of NAs in biological fluids, driven by nuclease degradation and poor cellular uptake, significantly compromises their bioavailability and therapeutic half-life. While chemical modifications (e.g., 2'-*O*-methylation, phosphorothioate backbones) and protective nanocarrier systems mitigate degradation, ensuring consistent delivery and sustained gene modulation in the complex GBM microenvironment remains a formidable task [245].

Future work must incorporate predictive safety assessments earlier in the development pipeline and adopt more physiologically relevant models to assess delivery efficiency [222,246]. It is equally important to consider patient-specific factors, such as BBB integrity and tumor heterogeneity, when designing or selecting delivery strategies [233].

Looking forward, the most viable path to clinical translation involves the integration of sophisticated, adaptable delivery platforms with rationally designed NA payloads. This includes combining gene silencing with immune modulation or deploying mRNA and CRISPR components via responsive carriers that react to the TME. Advances in biomaterials, nanotechnology, and synthetic biology will be instrumental in shaping the next generation of delivery systems that can meet these complex demands [247]. One of the most exciting frontiers lies in integrating RNA therapeutics with next-generation ICP inhibitors. Beyond the classical PD-1/PD-L1 and CTLA-4 axes, new targets such as lymphocyte activation gene-3 (LAG-3) [248], T cell immunoreceptor with Ig and ITIM domains (TIGIT) [249], T cell immunoglobulin and mucin-domain containing-3 (TIM-3) [250], and V-domain Ig suppressor of T cell activation (VISTA) [251] are emerging as promising modulators of T cell exhaustion and tumor immune evasion. Rationally combining these ICP inhibitors with RNA-based therapeutics or vaccines could help reprogram the immunologically "cold" GBM microenvironment into a responsive, inflamed phenotype.

Recent findings have also demonstrated that clinically available mRNA vaccines, originally designed for infectious diseases, can exert strong immune-sensitising effects against tumors. Notably, SARS-CoV-2 mRNA vaccines were shown to increase type I interferon production, activate antigen-presenting cells, and prime cytotoxic T lymphocytes capable of targeting tumor-associated antigens. When administered concomitantly with ICP inhibitors, these vaccines enhanced PD-L1 expression and improved overall survival even in patients with immunologically cold tumors. This observation underscores the broad immunomodulatory potential of mRNA vaccine platforms, which could be leveraged to augment immunotherapy responsiveness in GBM [252].

Furthermore, advances in synthetic biology and exosome engineering are redefining delivery paradigms for NA therapeutics in the CNS. A recent study in Huntington's disease demonstrated the successful use of a hepatocyte-based genetic circuit to generate rabies glycoprotein-tagged exosomes carrying siRNA against mutant huntingtin (mHTT). These exosomes traversed the systemic circulation and selectively delivered their cargo to neurons in the cortex and striatum, resulting in reduced mHTT aggregation and improved behavioral outcomes. Although demonstrated in a neurodegenerative setting, this self-assembling, neuron-targeted exosomal delivery system provides a compelling blueprint for overcoming the BBB and achieving efficient, targeted gene silencing in GBM [253].

In conclusion, while NA therapies hold transformative potential for GBM treatment, their success is significantly linked to delivery. The carriers that escort these potent molecules across physiological barriers and into the TME are not passive tools, they are critical enablers of therapeutic success. To realise the promise of precision neuro-oncology, future efforts must prioritise delivery innovation as the cornerstone of translational progress in NA therapeutics for glioblastoma.

Declaration of generative AI and AI-assisted technologies in the writing process

Statement: During the preparation of this work the author(s) used

Grammarly and ChatGPT in order to improve the readability and language of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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