

Strategies to enhance the safety and efficacy of non-viral gene delivery to the central nervous system

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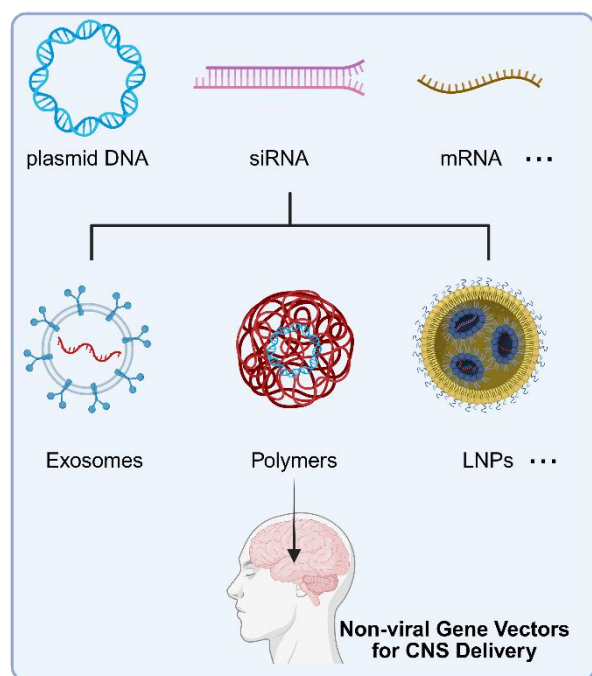
Nano Res., **Just Accepted Manuscript** • <https://doi.org/10.26599/NR.2026.94908987>

<https://www.sciopen.com/journal/1998-0124> on Jul. 1, 2026

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This review highlights recent advances in non-viral central nervous system gene delivery and emphasizes the need to co-optimize safety and efficacy for clinically translatable brain gene therapy.

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Received: 14 April 2026; **Revised:** 28 June 2026; **Accepted:** 1 July 2026

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Cite this article: *Nano Research*, 2026, 19, 94908987 <https://doi.org/10.26599/NR.2026.94908987>

ABSTRACT: Non-viral gene delivery offers a promising route for treating central nervous system (CNS) disorders, but its translation remains limited by the difficulty of achieving both safety and efficiency in the brain. This review summarizes recent advances in non-viral CNS gene delivery from the perspectives of safety and efficacy, highlighting progress in biodegradable and biomimetic materials, cargo engineering, blood-brain barrier (BBB)-crossing strategies, administration route optimization, and cell-type-specific targeting. We further discuss representative clinical studies. Overall, this review emphasizes that the future of non-viral CNS gene therapy will depend on the rational co-optimization of safety and efficacy.

KEYWORDS: central nervous system; non-viral gene delivery; blood–brain barrier; lipid nanoparticles; polymeric carriers

1. Introduction

Central nervous system (CNS) disorders represent an increasingly urgent public health challenge worldwide[1]. In addition to neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS), CNS diseases also include primary and metastatic brain tumors, which are often associated with high morbidity and mortality[2-4]. These disorders are broadly characterized by the devastating consequence of progressive neural dysfunction, ultimately leading to severe cognitive, motor, sensory, and autonomic impairment. The complexity of CNS pathology, together with the limited regenerative capacity of neural tissue, makes the development of effective therapeutic strategies particularly challenging. In this context, gene therapy has emerged as a therapeutic paradigm with transformative potential[5]. Unlike conventional pharmacological interventions that mainly act at the downstream protein level, gene therapy enables intervention at the genetic or transcriptional level through gene replacement, gene silencing, transcript modulation, or precise genome editing. By delivering therapeutic nucleic acids, including plasmid DNA (pDNA), messenger RNA (mRNA), antisense oligonucleotides (ASOs), small interfering RNA (siRNA), and CRISPR-based genome-editing systems, gene therapy offers a promising strategy for the precise and effective treatment of complex neurological disorders and brain tumors[6-8].

The therapeutic potential of gene therapy depends critically on delivery vectors that can safely and efficiently transport fragile nucleic acids to target cells. Although viral vectors, especially adeno-associated virus (AAV) and lentivirus (LV), have dominated CNS gene delivery because of their natural tropism and high transduction efficiency, their intrinsic limitations have become increasingly evident, including restricted payload capacity, manufacturing complexity, high

production cost, immunogenicity, and the limited feasibility of repeat dosing[9, 10]. These constraints have driven growing interest in non-viral delivery systems as a highly versatile and clinically attractive alternative. In general, non-viral gene vectors can be classified into three major categories. The first comprises lipid-based vectors, including lipid nanoparticles (LNP) and liposomes, which are particularly attractive because of their high nucleic acid encapsulation efficiency and translational success in mRNA vaccine[11, 12]. The second includes polymer-based systems, such as polyethylenimine (PEI) and poly (β -amino esters) (PBAEs), which offer strong nucleic acid condensation capability and highly tunable physicochemical properties[13-15]. The third consists of extracellular vesicles (EVs) and biomimetic hybrid vectors, which exploit endogenous intercellular communication pathways and therefore exhibit superior biocompatibility, low immunogenicity, and an intrinsic ability to cross biological barriers[16, 17]. Owing to their structural flexibility, high nucleic acid loading efficiency, and capacity to accommodate cargos of diverse sizes and formats, non-viral vectors have become an important platform for next-generation CNS gene delivery.

Despite these compelling advantages, the clinical translation of non-viral gene delivery for CNS disorders remains challenging, with the blood-brain barrier (BBB) continuing to represent a major unresolved obstacle. Physiologically, the BBB is formed by brain microvascular endothelial cells connected by tight junctions and supported by pericytes, astrocytic end-feet, and the basement membrane, which collectively restrict paracellular permeability, limit nonspecific transcytosis, and severely limit the brain entry of most therapeutic agents. Under pathological conditions, including neurodegenerative diseases, neuroinflammation,

ischemic stroke, and brain tumors, BBB integrity and homeostatic transport functions are often compromised, accompanied by tight-junction disruption, endothelial activation, abnormal vascular permeability, altered expression of influx and efflux transporters, and inflammatory-cell infiltration. Beyond the BBB, another central challenge is that safety and efficacy are often difficult to optimize simultaneously across the entire delivery process. On the one hand, improving efficacy requires delivery systems that can protect nucleic acid cargos from extracellular degradation, rapid clearance, and immune recognition, while also overcoming formidable biological barriers such as the blood-brain barrier (BBB), restricted diffusion within the dense neural microenvironment, poor

cellular internalization, and inefficient endosomal escape. On the other hand, the very properties that enhance delivery performance may also increase safety liabilities, as non-viral carriers themselves can induce cytotoxicity, inflammatory responses, or off-target accumulation. Thus, non-viral gene delivery to the CNS is fundamentally limited by the difficulty of simultaneously maximizing delivery efficiency while maintaining an acceptable safety profile. In this review, we focus on this central dilemma and summarize recent strategies from the dual perspectives of safety enhancement and efficacy optimization, with the aim of providing a clearer framework for the development of non-viral vectors and accelerating their translation toward broader clinical applications in neurological diseases (**Fig 1**).

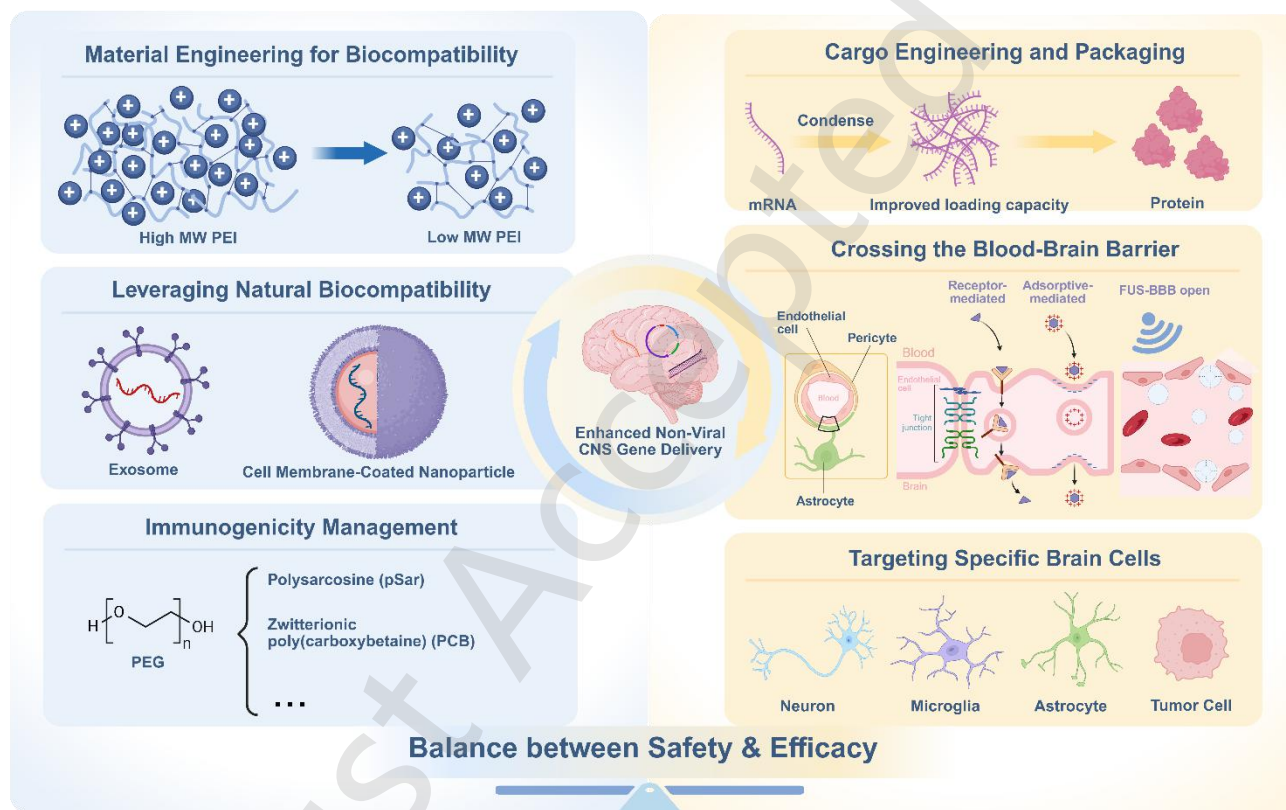


Figure 1. Schematic overview of strategies for enhancing non-viral gene delivery to the CNS through the optimization of safety and efficacy. Figure was drawn by Biorender.

2. Brain-Specific Safety Considerations and Mitigation Strategies

Safety is the foremost consideration in CNS gene delivery, owing to the extreme vulnerability of neural tissues and the limited tolerance of the CNS to inflammation and off-target injury. Beyond general carrier-associated cytotoxicity, non-viral vectors may compromise blood-brain barrier and neurovascular unit integrity, perturb neuronal and glial homeostasis, activate microglia and local neuroinflammatory pathways, or induce unintended transfection of non-target brain cell populations. These risks may be further amplified by prolonged cerebral retention or repeated administration, which can increase cumulative local exposure and immune sensitization. Therefore, improving the safety profile of non-viral carriers is not merely a matter of reducing toxicity, but

a prerequisite for achieving clinically viable CNS gene delivery. Recent advances in materials engineering, biomimetic design, and immune modulation have provided increasingly sophisticated strategies to mitigate these adverse effects. In the following sections, we summarize the major approaches that have been developed to enhance the biosafety of non-viral gene delivery systems for CNS applications.

2.1 CNS-Compatible Material Engineering

For CNS applications, material engineering should prioritize the reduction of neural toxicity while preserving neural-cell homeostasis. Carrier charge, biodegradability, and endosomal-escape properties must therefore be optimized to minimize neuronal and glial stress while maintaining effective cargo delivery. Permanently cationic liposomes are among the earliest non-viral gene delivery systems,

beginning with DOTMA-based lipofection and followed by related formulations such as DOTAP and DC-Chol/DOPE. These lipids carry a constitutively positive headgroup that enables electrostatic complexation with DNA or RNA and promotes adsorption to negatively charged cell membranes. However, the same permanent positive charge that facilitates nucleic acid condensation also underlies their major safety liabilities, including membrane perturbation, reactive oxygen species generation, cytokine dysregulation, and charge-dependent systemic toxicity[18, 19]. As a result, the field progressively shifted away toward ionizable lipids, which remain neutral at physiological pH but become protonated in the acidic endosomal environment, thereby improving tolerability in vivo. mRNA vaccines built on ionizable lipids such as SM-102 and ALC-0315 achieved remarkable success during the COVID-19 pandemic, underscoring the translational potential of this material class[20]. Nevertheless, accumulating evidence indicates that ionizable lipids can still induce inflammatory responses, which further constrains their application for delivery within the brain[21, 22]. Therefore, additional efforts are needed to develop biodegradable ionizable lipids and LNP platforms with improved biosafety profiles[23-25]. Murthy and colleagues introduced an azido-acetal linker that hydrolyzes within minutes in endosomes, enabling the generation of rapidly degradable LNPs (RD-LNPs)[26]. The rapid hydrolysis of RD-LNPs was proposed to promote endosomal disruption and facilitate the cytosolic release of mRNA before lysosomal degradation (**Fig 2A**). Compared with conventional LNPs, RD-LNPs exhibited more rapid intracellular clearance, which may help reduce cellular accumulation and mitigate tissue toxicity. Moreover, RD-LNPs delivered mRNA more efficiently to the brains of mice than conventional LNPs. While improving biosafety, this strategy also preserved delivery efficiency. The same team subsequently extended acid-degradable LNPs (ADP-LNPs) to fetal brain gene editing via in utero intracerebroventricular (ICV) injection, achieving transfection in 30% of brain cells in Ai9 mice without detectable adverse effects on fetal development or postnatal growth[27]. In an Angelman syndrome model, ADP-LNPs induced gene editing in 21% of brain cells within the first 7 days after birth, underscoring

their potential for prenatal treatment of neurodevelopmental disorders (**Fig. 2B**). A similar rationale has also been validated in cationic polymer-based delivery systems, which likewise rely on dense positive charges to complex with nucleic acids and mediate intracellular delivery[28]. For non-degradable cationic polymers, such as PEI and poly(amidoamine) (PAMAM), the major source of toxicity generally arises from the high cationic charge density, which can disrupt cellular membranes. Strategies to mitigate these safety concerns include the use of low-molecular-weight PEI[29], the incorporation of biodegradable linkages such as disulfide bonds to enhance degradability[30], and conjugation or modification with biocompatible polymers or amino acid-derived components, such as poly(ethylene glycol) (PEG) and poly(L-lysine) (PLL)[31, 32]. Borgmann et al employed arginine-modified PEI as a less cytotoxic alternative. Their A5P10 constructs exhibited reduced cytotoxicity and favorable biocompatibility in both primary human astrocytes and neurons, while maintaining efficient pDNA delivery[33]. Similarly, Lee and colleagues developed PG2HR by modifying low-molecular-weight second-generation PAMAM (PG2) with histidine and arginine. Compared with PEI (25 kDa, PEI25k), PG2HR showed lower cytotoxicity and higher gene delivery efficiency in the brain, ultimately leading to pronounced therapeutic benefits in ischemic stroke[34]. In another study, Wang and colleagues demonstrated that PEGylation on the surface of MNP-PLGA-PEI nanoparticles shielded the surface charge and prevented perturbation of intracellular calcium homeostasis, thereby reducing nanoneurotoxicity[35] (**Fig. 2C**). Biodegradable cationic polymers, such as poly(β -amino ester)s (PBAEs), have also emerged as an important option for improving the safety of polymer-based gene delivery, although their reduced delivery efficiency often necessitates careful structural optimization[36]. Wang et al. developed biodegradable highly branched PBAE (HPAESG) that combined high biocompatibility with efficient gene delivery, achieving >90% cell viability and robust transfection in primary astrocytes[37](**Fig. 2D, E**).

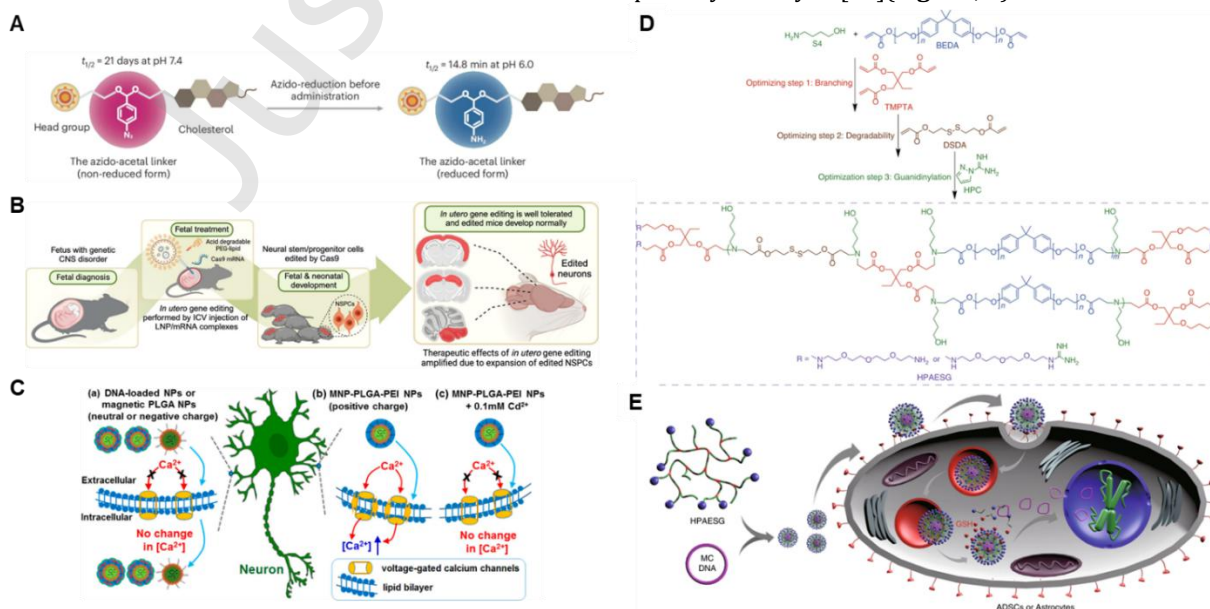


Figure 2. (A) Murthy group present a new acid-degradable linker, termed 'azido-acetal', that rapidly hydrolyses in endosomes but is also stable at pH 7.4. The azido-acetal has a pH 6.0 hydrolysis half-life ($t_{1/2}$) of 14.8 min and a hydrolysis $t_{1/2}$ of 21 days at pH 7.4. Reproduced with permission from Ref.

[26], © Springer Nature Limited 2024. (B) In utero delivery of densely PEGylated LNPs globally transfects the brain in utero with mRNA for gene editing enzymes. Reproduced with permission from Ref.[27], © American Chemical Society Limited 2024. (C) Graphical illustration of intracellular calcium change stimulated by nanoparticles with different surface charge: (a) neutrally or negatively charge DNA-loaded nanoparticles or magnetic PLGA nanoparticles; (b) positively charged magnetic PLGA-PEI nanoparticles, MNP-PLGA-PEI NPs; (c) MNP-PLGA-PEI NPs with addition of 0.1 mM Cd2+. Reproduced with permission from Ref. [35], © American Chemical Society Limited 2019. (D, E) A synergistic gene delivery system for the transfection of ADSCs and astrocytes. Reproduced with permission from Ref. [37], © Springer Nature Limited 2019.

2.2 Leveraging Biomimicry for Selective and Well-Tolerated CNS Delivery

Biomimetic and hybrid carriers can improve compatibility with the CNS microenvironment by reducing nonspecific interactions and enhancing selective uptake. These approaches are particularly attractive for limiting local neuroimmune activation and off-target exposure across neuronal and glial populations, thereby improving the tolerability of CNS-directed gene delivery. Extracellular vesicles (EVs), particularly exosomes and engineered small EVs, are nanoscale membrane-bound carriers with native-like lipid-protein architectures[38, 39]. Owing to their low immunogenicity, ability to cross biological barriers, and capacity to carry diverse therapeutic cargos, including DNA, RNA and proteins, EVs have emerged as attractive non-viral platforms for gene therapy[40, 41]. Andaloussi et al. developed engineered EVs that integrated an engineered mini-intein for active cargo loading and release with fusogenic VSV-G to enhance endosomal escape, thereby enabling efficient intracellular delivery of protein and RNA therapeutics. This platform supported robust in vitro recombination and genome editing after delivery of Cre recombinase or Cas9/sgRNA RNPs, and achieved >40% and 30% recombined cells in the hippocampus and cortex, respectively[42]. Zhang et al. developed an immature dendritic cell (imDC)-derived exosome-based targeted delivery system carrying siRNA targeting SNCA (siSNCA), which effectively downregulated α -syn protein synthesis, inhibited the formation of α -syn aggregates, and improved motor behavior in Parkinson's disease mice[43]. In addition, this engineered 'nanoscavenger' system could also clear immune activation due to its natural imDC-derived exosome coating, underscoring its promise as a multifunctional platform for the treatment of neurodegenerative diseases. Besides dendritic cell-derived exosomes, Levenberg et al. also employed mesenchymal stem cell-derived exosomes (MSC-Exo) to deliver siRNA targeting phosphatase and tensin homolog (PTEN), thereby achieving effective PTEN silencing in the injured spinal cord after intranasal

administration.[44]. This strategy markedly enhanced axonal growth and neovascularization while reducing microgliosis and astrogliosis, and ultimately promoted significant functional recovery in rats with complete spinal cord injury. Nevertheless, EV-based systems still face considerable challenges in large-scale production and efficient cargo loading. To address these limitations, increasing attention has been directed toward engineered exosomes with defined membrane ligands, controllable cargo loading, and standardized manufacturing workflows, aiming to preserve the innate biocompatibility of exosomes while improving potency and reproducibility[45].

Another strategy involves the construction of hybrid vectors, in which a synthetic core mediates nucleic acid condensation, endosomal escape, and cargo protection, while an outer biologically derived shell minimizes adverse immune reactions and imparts tissue- or cell-selective interactions[46-48]. Zhang et al. developed a microglia-targeted plasmid delivery platform--PHSA@PF/pTREM2, consisting of a fluorinated PEI/pTREM2 core wrapped within a human serum albumin-based shell[49]. The albumin coating prolonged circulation and reduced cytotoxicity, thereby improving the safety profile of the system. This platform upregulated TREM2 expression and enhanced A β clearance, underlining its promise as a gene delivery strategy for reversing microglial dysfunction in Alzheimer's disease therapy. Cai et al. developed a polymer-locking fusogenic liposome (Plofsome) for BBB-penetrant delivery of siRNA or CRISPR-Cas9 ribonucleoprotein complexes to glioblastoma (GBM) cells[50] (**Fig. 3**). By incorporating a traceless ROS-cleavable linker as a molecular 'lock', the fusogenic activity of the liposome was selectively triggered in the ROS-rich tumor microenvironment rather than in normal tissues, thereby enhancing the safety of combined RNAi and CRISPR-Cas9 therapy. This platform achieved efficient MDK silencing, attenuated TMZ resistance, and consequently suppressed tumor progression in orthotopic GBM models.

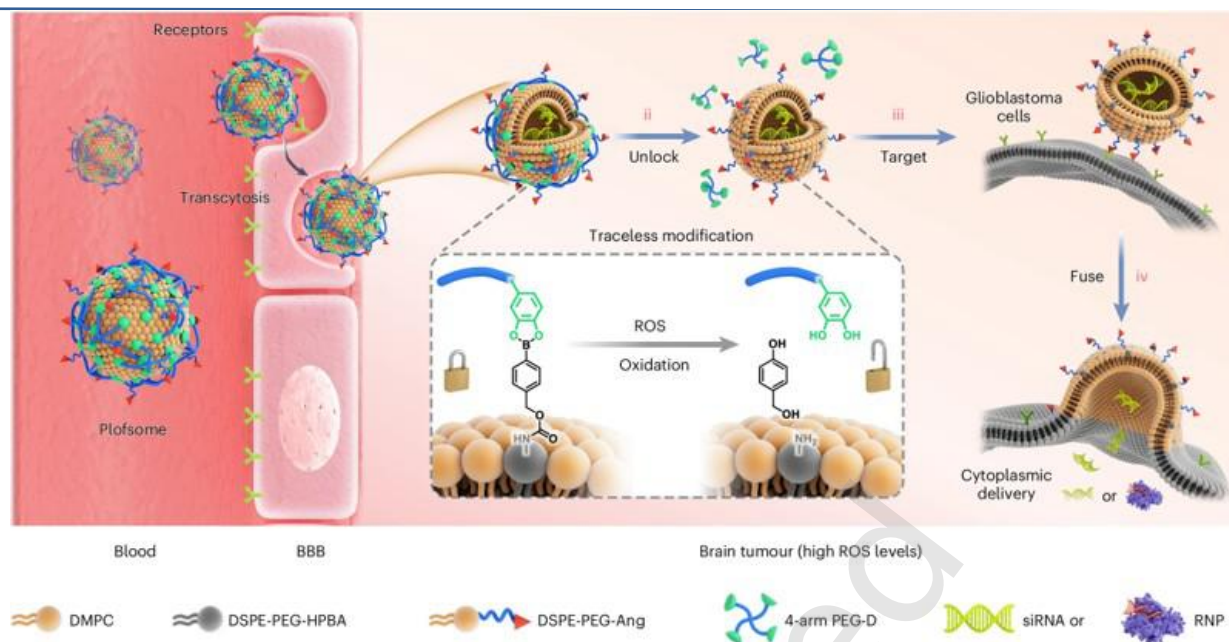


Figure 3. Plosomes for GBM-targeted siRNA delivery and CRISPR-Cas gene editing. Reproduced with permission from Ref. [50], © Springer Nature Limited 2024.

2.3 Controlling CNS Immune Reactivity and Cumulative Exposure

Immune compatibility in the CNS is a critical determinant of the safety of CNS-directed non-viral gene delivery, particularly for formulations intended for repeated administration. In the brain, carrier-induced innate and complement responses may amplify microglial reactivity, disturb local tissue homeostasis, and compromise long-term tolerability. Even when direct cytotoxicity is limited, non-viral carriers may still fail in the CNS because of immune activation. Carrier can be recognized as foreign substances, thereby triggering innate immune signaling, cytokine release, and complement activation, all of which may alter biodistribution, destabilize the carrier, and amplify local inflammatory risk[51, 52]. This issue is particularly critical in the brain, which is exceptionally vulnerable to inflammatory perturbation and has limited tolerance for immune-mediated injury. The use of PEG represents one of the major challenges in immunogenicity management[53, 54]. Although PEG has been widely employed to prolong circulation time and reduce immune recognition, PEGylated gene carriers may induce the production of anti-PEG antibodies, such as IgM and IgG, which may arise either from prior exposure to PEG-containing consumer products or from *de novo* induction following initial administration[55]. These anti-PEG antibodies can exert multiple adverse effects on nanomedicines, including complement activation, inflammatory reactions, and accelerated blood clearance (ABC), as well as altered biodistribution, destabilization of the carrier structure with premature release of genetic cargo, and even hypersensitivity reactions such as anaphylaxis[56, 57].

A series of PEG alternatives has been developed to address immunogenicity-related concerns[58-60]. Among them, polysarcosine (pSar), a polypeptoid derived from the endogenous amino acid sarcosine (N-methyl glycine), has attracted considerable attention because it exhibits stealth-like properties comparable to those of PEG both in vitro and

in vivo[61]. BioNTech previously demonstrated that pSar-functionalized LNPs enhanced mRNA delivery in vivo relative to PEGylated formulations, while reducing cytokine release and complement activation in vitro[62]. Zhang et al. developed pSar-lipids as PEG alternatives and showed that their chain length and lipid carbon tail length critically govern the balance between transfection efficiency and circulation[63]. Notably, 2.5% C14-pSar2k-containing lipoplexes achieved efficient mRNA delivery and the highest protein expression in the zebrafish brain after intraventricular injection, underscoring the potential of pSar-lipids to replace PEG-lipids for controlled mRNA expression in the CNS. Zwitterionic poly(carboxybetaine) (PCB), derived from naturally occurring glycine betaines, has emerged as a promising PEG alternative because of its superhydrophilicity and strong stealth properties[64]. Unlike PEG, which relies primarily on hydrogen bonding for hydration, PCB exhibits much stronger hydration through electrostatic interactions with water molecules, thereby conferring low immunogenicity, and favorable biocompatibility. Jiang et al. developed PCB-lipids as replacements for PEG-lipids in mRNA formulations. These PCB-LNPs not only enhanced mRNA delivery efficiency but also exhibited a safer immunotoxicity profile, enabling repeated intravenous administration in mice without efficacy loss[65].

Moreover, accumulating evidence indicates that the immunogenicity of non-viral carriers arises from multiple components, including polymers, ionizable lipids and helper phospholipids[54, 66, 67]. Nevertheless, the CNS application of their alternative materials remains relatively limited. For CNS settings that require repeated administration, effective restraint of excessive host reactivity and reduction of carrier-associated adverse effects may further enhance the efficiency and translational potential of non-viral gene delivery.

3. Efficiency Enhancement Strategies I: Cargo Engineering and Packaging

For non-viral gene delivery to the CNS, delivery efficiency is

determined not only by carrier composition, but also by how the nucleic acid cargo is packaged and molecularly engineered. At the packaging level, effective condensation can prevent nucleic acids from nuclease degradation, reduce hydrodynamic size, modulate surface charge, and facilitate uptake, and intracellular release. In addition, pre-condensation of the genetic cargo before encapsulation into lipid or polymeric carriers can further enhance loading capacity, thereby reducing the amount of vector required for effective delivery. At the sequence level, optimization of the payload itself can enhance translation efficiency per delivered molecule, thereby reducing the amounts of both carrier and nucleic acid required to achieve a therapeutic threshold. This dose-sparing effect is particularly important in the CNS, where the BBB, the negatively charged extracellular matrix (ECM), and the narrow neuroinflammatory safety window impose exceptionally stringent physiological constraints, making it essential to maximize the efficiency of gene cargo release under these conditions

3.1 Nucleic Acid Condensation Strategies

Condensation is an important step in the formulation of DNA, mRNA, siRNA, and oligonucleotide therapeutics. Nucleic acids are large, polyanionic, and structurally fragile, they typically require association with cationic peptides, polymers, lipids, or coordination-inducing components to form nanoscale complexes with acceptable stability and transport properties. In the CNS setting, the ideal condensation strategy must achieve a careful balance: the complex should be stable enough to survive extracellular barriers, yet labile enough to dissociate intracellularly and release the active cargo at the proper subcellular site.

Protamine is one of the earliest and most biologically intuitive nucleic acid condensing agents[68]. As an arginine-rich polycationic peptide already used clinically as a pharmaceutical excipient, protamine can spontaneously assemble with nucleic acid through electrostatic neutralization of the phosphate backbone to generate nanoparticles across a tunable size range[69, 70]. Csaba et al. developed a gene delivery system based on low-molecular-weight protamine (LMWP) and dextran sulfate, and demonstrated its efficacy in glioblastoma models, including multiple glioblastoma cell lines, three-dimensional (3D) spheroids, and zebrafish. The formulation exhibited negligible toxicity, efficient cellular internalization, and consistent transgene expression[71]. Cationic polymers remain among the most versatile nucleic acid-condensing materials, owing to the tunability of their charge density, topology, and buffering capacity[72]. In addition to the representative synthetic cationic polymers discussed above, such as PEI, PAMAM, and PBAEs, naturally derived cationic polymers, including chitosan and cyclodextrin-based materials, have also been explored for gene cargo condensation. Chitosan, a biodegradable linear amino polysaccharide derived from the deacetylation of chitin, is particularly attractive because of its low toxicity even at relatively high concentrations, supporting its potential for CNS gene delivery[73-75]. Multiple sclerosis (MS) is a chronic demyelinating and neurodegenerative disease of the

central nervous system. To improve brain delivery of miR-219, which promotes remyelination, Aliomrani et al. developed a glutathione-targeted non-viral polyplex by conjugating chitosan with tragacanthic acid[76]. The resulting nanoparticles enhanced brain accumulation of miR-219, promoted remyelination, reduced neuroinflammation, and improved regenerative responses in a cuprizone-induced MS model. For Huntington's disease (HD), O'Driscoll, et al. developed cyclodextrin-based nanoparticles for ASO delivery and showed that modified γ -cyclodextrins, particularly when functionalized with the brain-targeting peptide RVG, enhanced cellular uptake and mutant huntingtin (mHTT) silencing, highlighting their potential for HD gene therapy[77].

Metal ions have also emerged as promising candidates for nucleic acid condensation, owing to their ability to efficiently complex therapeutic cargos such as siRNA, miRNAs, and mRNA through electrostatic interactions and coordination bonding[78, 79]. Caruso et al. developed a versatile noncationic metal-organic nanoparticle platform in which metal ions mediated PEG-polyphenol network formation and served as key structural components for mRNA assembly and nanoparticle stabilization[80] (**Fig 4**). By tuning the nanoparticle composition, they obtained highly biocompatible formulations that supported efficient mRNA transfection in vitro and in vivo, with intravenous administration enabling protein expression and gene editing in the brain, liver, and kidney. Biodistribution and related assessments further supported the favorable safety and biocompatibility of this platform. However, given that metal ion accumulation in the brain may pose additional risks of neurological disorders[81], careful consideration should be given to the incorporated metal ion content, together with its clearance profile and metabolic fate, in the design and application of such strategies.

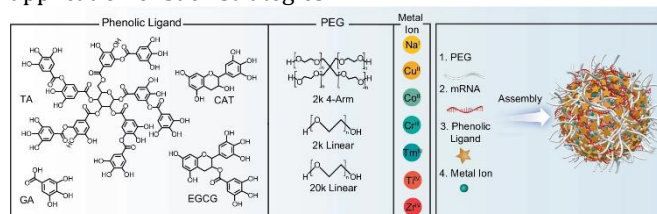


Figure 4. Formulation screening and optimization of mRNA-MPN NPs. Reproduced with permission from Ref. [80], © Springer Nature Limited 2024.

3.2 Payload Sequence Optimization

Beyond carrier design, optimization at the payload sequence level provides an additional route to improve the efficacy of non-viral gene delivery in the CNS. In the case of mRNA, the transcript is structured with a 5' cap, a poly(A) tail, a coding sequence, and untranslated regions (UTRs), all of which together regulate translation efficiency and intracellular stability[82, 83] (**Fig 5**).

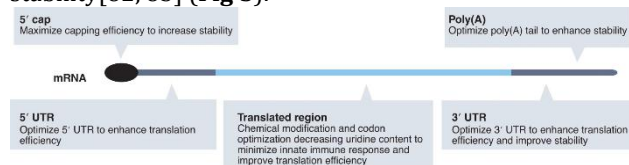


Figure 5. Structural Optimization and Modification of mRNA.

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Mauger et al. demonstrated that mRNA secondary structure, particularly within the coding sequence, is an important determinant of protein expression. Using modified nucleotides and rationally designed reporter constructs, they showed that a highly structured coding region can enhance protein output by prolonging functional mRNA half-life[84]. Building on a similar rationale, Das et al. established the PERSIST-seq and In-line-seq platforms to define design principles for therapeutic mRNAs, showing that in-cell stability is a strong determinant of protein output. They further demonstrated that highly structured 'superfolder' mRNAs, especially when combined with pseudouridine modification, can simultaneously enhance mRNA stability and protein expression[85].

More recently, computational sequence design has emerged as a powerful strategy for improving the performance of therapeutic mRNAs, thereby complementing advances in non-viral delivery systems. Xie et al. developed RiboDecode, a deep learning-based platform for context-aware codon optimization of therapeutic mRNAs[86]. By improving translational efficiency across different cellular environments and mRNA formats, RiboDecode markedly enhanced in vitro protein expression and in vivo efficacy, enabling optimized NGF mRNA to achieve equivalent neuroprotection at one-fifth the dose of the unoptimized sequence. Huang et al. developed LinearDesign, a principled computational framework for joint optimization of codon usage and mRNA secondary structure[87]. This strategy substantially improved mRNA stability and protein output, and further translated into markedly enhanced in vivo immunogenicity in SARS-CoV-2 and varicella-zoster virus (VZV) vaccine settings, with antibody titers increased by up to 128-fold in mice relative to conventional codon-optimized designs. Sequence optimization can also be synergistically integrated with carrier engineering in CNS gene delivery. Brain-derived neurotrophic factor (BDNF) has long been explored as a therapeutic factor for neurodegenerative diseases[88]. Mao et al. combined PBAE-mediated mRNA delivery with BDNF sequence engineering, using LinearDesign to enhance mRNA stability and translation, while further engineering the 3'UTR with neuron-specific miR-124 target sites to suppress neuronal overexpression and preserve astrocyte-dominant BDNF production[89]. As a result, astrocytes released BDNF to support neurons, thereby improving memory in APP/PS1 double-transgenic AD model mice.

For pDNA, the ultimate expression output is governed by the coordinated effects of multiple regulatory elements, including the promoter/enhancer, 5'UTR/Kozak context, intron design, codon composition of the coding sequence (CDS), 3'UTR/poly(A) signals, and the overall CpG content of the plasmid[90-92]. Lu et al. highlighted 5'UTR engineering as an effective strategy for enhancing transgene expression in plasmid-based systems[93]. By combining high-throughput design, screening, and optimization of a large 5'UTR library, they identified three synthetic 5'UTRs that outperformed commonly used non-viral gene therapy plasmids in protein expression, underscoring the value of

UTR optimization for improving the potency of engineered cell and gene therapies. Overall, payload sequence optimization serves as an important complement to carrier engineering for improving the performance of non-viral gene delivery in the CNS. By rationally tuning coding and noncoding elements in mRNA or pDNA cargos, these strategies can enhance expression efficiency, enable dose sparing, and potentially broaden the therapeutic window.

4. Efficiency Enhancement Strategies II: Overcoming Biological Barriers

During CNS delivery, non-viral gene vectors must interact with the biological system at multiple levels, and their ultimate therapeutic efficacy depends on the ability to sequentially overcome several distinct biological barriers. At the tissue level, the highly restrictive and densely organized blood-brain barrier (BBB) represents the first major obstacle to CNS entry. After reaching the brain parenchyma, the vectors must then be efficiently internalized by the target neural or glial cell populations. Therefore, the capacity to navigate these barriers in a stepwise manner is an important consideration in the rational design of non-viral delivery systems for CNS applications. In the following sections, we discuss the major strategies that have been developed to improve delivery efficiency at each of these stages.

4.1 Strategies for Crossing the BBB

Building on the BBB features described in the Introduction, this section focuses on endothelial transport pathways that can be leveraged for intravenous non-viral gene delivery to the brain. The BBB endothelium contains selective receptors, nutrient transporters, and efflux systems that regulate molecular exchange between the circulation and the CNS, providing potential entry routes for brain-targeted delivery. Accordingly, current strategies can be broadly divided into four categories: receptor-mediated transcytosis (RMT), which exploits endogenous transport receptors on brain endothelial cells to shuttle cargos across the barrier; adsorptive-mediated transcytosis (AMT), which is primarily driven by electrostatic interactions between cationic ligands and the endothelial membrane; carrier-mediated transport (CMT), which harnesses endogenous nutrient transport systems on the BBB, such as those responsible for glucose and amino acid uptake, to ferry suitably engineered cargos or transporter-targeting moieties across brain endothelium; and focused ultrasound combined with microbubbles for transient blood-brain barrier opening (BBBO). In the following sections, we discuss recent advances in innovative non-viral gene delivery systems developed on the basis of these mechanisms for brain delivery following intravenous administration.

RMT: Among receptor-mediated transcytosis-based strategies, the transferrin (tf) receptor is commonly targeted by peptides such as T7 (HAIYPRH), T12 (CGGGTHRPPMWSPVWP), TBP (GGGHKYLRLW), and B6 (GHKAKGPRK), whereas peptide-22 (Ac-(cMPRLRGC)-NH₂) and related CMPRLRGC-derived ligands are used to engage the low-density lipoprotein receptor (LDLR); in parallel, angiopep-2 (Ang, TFFYGGSRGKRNNFKTEEYC) serves as a

representative ligand for lipoprotein receptor-related protein 1 (LRP1)[94], and RVG29 (YTIWMPENPRPGTPCDIFTNSRGKSRASNG) is widely employed to target the nicotinic acetylcholine receptor (nAChR)[95, 96], collectively providing a molecular basis for receptor-guided transport. A representative example is an angiopep-2-functionalized, disulfide-cross-linked nanocapsule for systemic delivery of anti-Polo-like kinase 1 (PLK1) Cas9/sgRNA to the brain, where angiopep-2 promoted BBB crossing via LRP1-mediated transcytosis. After intravenous administration, this nearly neutral and ultrasmall platform enabled efficient targeting of the brain and orthotopic glioblastoma, with reductive shell cleavage facilitating intracellular cargo release and effective gene editing[97]. Subsequently, Tan et al. also developed an Angiopep-2-modified LNP platform for systemic gene therapy against GBM. By optimizing the LNP formulation and incorporating Angiopep-2 to enhance BBB penetration and brain accumulation, this system enabled effective delivery of PLK1 siRNA; notably, the optimized formulation significantly inhibited GBM growth in mice and prolonged the median survival of tumor-bearing animals by 2.18-fold[98]. RVG29 is also a commonly used functional ligand for the surface modification of LNPs or polymeric carriers[99]. Taking advantage of the transient BBB disruption after traumatic brain injury (TBI), Kwon et al. developed RVG-functionalized mRNA-loaded LNPs for intravenous delivery to the injured brain and showed that administration at 24 h post-injury achieved both effective brain access and transfection activity[100]. RVG modification increased the proportion of transfected neurons by 6.4-fold compared with untargeted LNPs. Tf is also a widely used BBB-targeting ligand that has been broadly incorporated into liposomes and other nanocarriers for the treatment of brain tumors and neurodegenerative diseases[101, 102].

In addition to the well-established receptor-mediated pathways described above, a number of emerging LNPs built around novel ionizable lipids have demonstrated intrinsic capacity to cross the BBB. Such ionizable lipids are typically identified through large-scale *in vitro* or *in vivo* screening

campaigns to pinpoint candidates with superior penetrating performance. In line with this direction, Mitchell et al. developed a high-throughput BBB transwell screening platform (HTS-BBB) tailored for mRNA LNPs, which simultaneously assesses endothelial transport and transfection activity[103]. By screening a library of LNPs formulated with structurally diverse ionizable lipids, the study identified lead candidates with enhanced BBB-penetrating performance, and these candidates were subsequently confirmed to achieve measurable mRNA expression in the mouse brain following intravenous injection. Another representative recent study provided a new perspective for BBB-penetrating LNP design by constructing a library of 72 BBB-crossing lipids, generated through conjugation of diverse BBB-permeable small-molecule motifs with amino lipids to formulate BBB-crossing LNPs (BLNPs). Through *in vitro* and *in vivo* screening, followed by structural and formulation optimization, Dong et al. identified MK16 BLNPs as a lead candidate that substantially outperformed conventional MC3 LNPs, as well as clinically used ALC-0315- and SM-102-based formulations, for mRNA delivery to the brain after intravenous administration[104]. Mechanistically, MK16 BLNPs appeared to traverse the BBB through caveolae- and γ -secretase-associated transcytosis, and functionally enabled mRNA expression in multiple brain cell types, particularly neurons and astrocytes, across broad brain regions with good tolerability. The versatility of this platform was further demonstrated by successful delivery of Cre and Δ FosB mRNA, as well as therapeutic Pten mRNA in an orthotopic GBM model, where tumour growth was suppressed and survival was prolonged. Subsequently, the same group further extended this strategy by incorporating the 5-HT₃ receptor ligand SR-57227 into ionizable lipid design, yielding OS4T LNPs that achieved over 50-fold higher brain mRNA translation than Onpatro LNPs after systemic administration and exhibited therapeutic efficacy in an orthotopic GBM model when loaded with IL-12 mRNA[105].

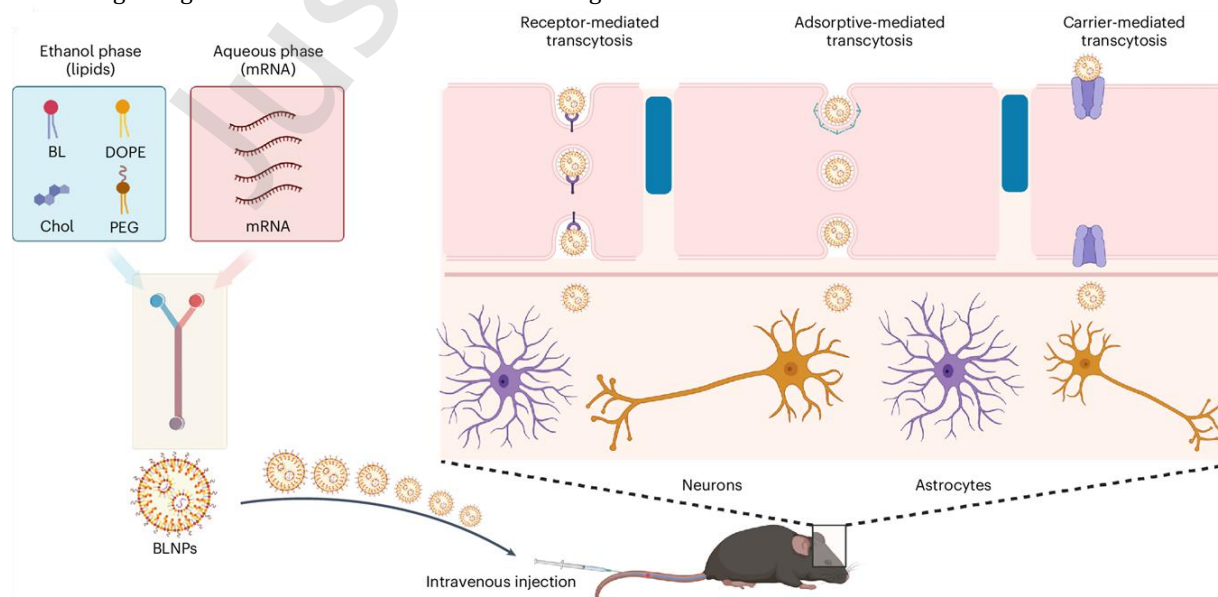


Figure 6. Design of BBB-crossing LNPs. Reproduced with permission from Ref.[104], © Wang C. et al. under exclusive licence to Springer Nature

AMT&CMT: Cell-penetrating peptides (CPPs) are one of the representative ligands used to exploit the AMT pathway for BBB transport[106, 107]. Their cationic nature enables electrostatic adsorption to brain endothelial cells, thereby facilitating translocation across the BBB without disrupting its integrity[108]. One such design integrated PEG and the CPP-derived LIMK2 nucleolar translocation signal peptide into a dendrigraft poly-L-lysine scaffold to construct the DGL-PEG-LNP vector for plasmid delivery. This material engineering strategy markedly improved BBB penetration and tumor-site gene transfection[109]. CMT enables BBB crossing by harnessing endogenous nutrient transporters expressed on brain endothelial cells, thereby improving the brain delivery of nucleic acid therapeutics. Among them, GLUT1 is particularly attractive because of its abundant expression at the BBB and its central role in glucose transport, making it a widely explored target for transporter-guided brain delivery[110]. Building on this concept, a glycosylated “triple-interaction” stabilized polymeric nanomedicine was developed for systemic delivery of siBACE1 in APP/PS1 transgenic AD mice. By incorporating galactose into the polymeric carrier, the resulting Gal-NP@siRNA achieved high blood stability and efficiently crossed the BBB through glycemia-controlled GLUT1-mediated transport. This design enabled effective BACE1 silencing in the brain, modulated related pathogenic pathways, and ultimately ameliorated cognitive impairment without evident side effects[111]. Along the same line, Miura and colleagues further extended this strategy to systemic ASO delivery by constructing glucose-coated polymeric nanocarriers for BBB transport. Glucose decoration with optimized ligand density enabled stable ASO encapsulation and rapid brain accumulation after intravenous administration, leading to significant target knockdown in multiple brain regions, including the cortex and hippocampus[112].

BBBO: Focused ultrasound-mediated blood-brain barrier opening (FUS-BBBO) has emerged as a safe, non-invasive, and spatially precise strategy for brain delivery[113]. When combined with intravenously administered microbubbles (MB), low-intensity FUS induces controlled cavitation within cerebral microvessels, generating mechanical stimuli that transiently loosen endothelial junctions, enhance transcytosis, and promote sonoporation, thereby enabling localized and reversible BBB opening without surgical disruption. Early studies directly employed cationic microbubbles as nucleic acid carriers for CNS delivery, but this approach was limited by relatively low delivery efficiency[114, 115]. Recent strategies more commonly rely on the co-administration of microbubbles and gene-loaded nanoparticles via systemic injection, thereby combining the BBB-opening capability of microbubbles with the superior cargo-loading and delivery performance of nanoscale carriers[116, 117]. Ilovitsh and colleagues combined FUS-BBBO with LNPs to enable non-invasive RNA delivery to the brain. By optimizing ultrasound parameters and using LNPs formulated with ionizable lipids such as SM-102 and Lipid-14, they achieved efficient trans-BBB delivery of both siRNA

and mRNA cargos[118]. This strategy markedly enhanced brain delivery and expression, including about 10-fold higher siRNA-LNP accumulation, 12-fold greater mRNA expression in normal brain, and a 6.7-fold increase in siRNA-LNP delivery in glioblastoma. Suk and colleagues further showed that FUS-BBBO can achieve markedly greater efficacy when paired with rationally engineered nanocarriers[119]. By combining BBBO with long-circulating, serum-stable, biodegradable PBAE nanoparticles, they enabled the systemic delivery of pDNA, mRNA, and even CRISPR/Cas9 components into the FUS-targeted brain region, leading to localized transgene expression and genome editing in astrocytes and neurons (**Fig 7**).

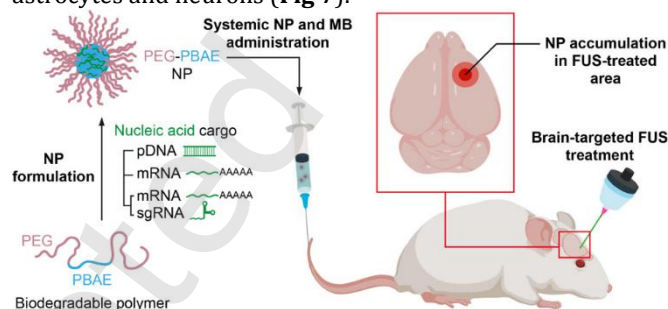


Figure 7. Brain nucleic acid delivery and genome editing enabled by material optimization and ultrasound-induced blood-brain barrier opening. Reproduced with permission from Ref. [119], © American Chemical Society Limited 2024.

4.2 Optimization of Administration Routes

In addition to intravenous approaches for CNS gene delivery, a range of BBB-bypassing administration routes have been investigated, including intranasal delivery and direct CNS administration through injection into the cerebrospinal fluid (CSF) compartment. Intrathecal and intracerebroventricular injection are among the commonly used CSF-based routes. In the following section, we summarize recent progress in the optimization of these administration strategies, with particular emphasis on their combination with advanced carrier design to enhance delivery efficiency.

Intranasal delivery offers a non-invasive route by partially bypassing the BBB through direct nose-to-brain transport pathways[120-122]. However, its application is still limited by the low dosing volume of the nasal cavity, rapid mucociliary clearance, and often insufficient or variable brain bioavailability. Recent strategies have combined distinct material platforms, including extracellular vesicle-like nanoparticles, polymer-based nanocarriers, and LNPs, to improve delivery efficiency[123, 124]. Kuroda and colleagues used GLP2-functionalized ascorbic acid-derived exosome-like nanoparticles to deliver Cas9/gRNA ribonucleoproteins, achieving in vivo editing of C9orf72 and demonstrating therapeutic potential for ALS and frontotemporal dementia[125]. In addition, synergistic amine lipid nanoparticles were used to intranasally deliver si-circATF7IP into the hippocampus, alleviating depressive-like behaviors in a mouse model of major depressive disorder[126]. Richter et al. systematically optimized a panel of tyrosine-modified PEI/PPI-based polyplexes and lipopolyplexes for intranasal siSNCA delivery in a

Parkinson's disease model[127]. Among the tested formulations, LP10Y lipopolyplexes and PPI-Y polyplexes showed the most favorable balance of brain distribution, neuronal uptake, knockdown efficacy, and biocompatibility, enabling delivery to regions including the olfactory bulb, prefrontal cortex, and substantia nigra after nose-to-brain administration. Importantly, these optimized carriers significantly reduced both SNCA mRNA and α -synuclein protein levels without inducing overt neuroinflammation, highlighting that the therapeutic performance of intranasal RNA delivery depends not only on the route itself but also critically on carrier engineering.

Intra-CSF administration represents an important BBB-bypassing route for CNS gene delivery, offering relatively broad intracranial distribution, the possibility of repeated dosing, reduced therapeutic dose requirements, and lower peripheral off-target exposure[128, 129]. This strategy mainly includes intracerebroventricular administration, which delivers cargos directly into the ventricular system, and intrathecal administration, which introduces therapeutics into the subarachnoid space for broadly distributed CSF-based delivery. Dong and colleagues developed brain-targeting LNPs for intrathecal mRNA delivery to the CNS[130]. The lead TD5 formulation outperformed MC3-based LNPs and enabled broad transgene expression across the brain after a single intrathecal injection, with efficient delivery to both neurons and astrocytes. The same platform further supported Cre mRNA expression and Cas9 mRNA/sgRNA-mediated genome editing in multiple brain regions, while maintaining a safety profile comparable to that of MC3 LNPs. In addition, Li et al. used high-throughput in vivo screening to identify the biodegradable ionizable lipid P3B and the resulting P3B-LNPs enabled widespread genome editing across multiple brain regions after intrathecal delivery of Cas9 mRNA/sgRNA or adenine base editor components[131]. In a more instructive example, Byrnes and colleagues evaluated ASO-loaded LNPs for intracerebroventricular delivery to the brain[132]. Although LNP encapsulation enhanced ASO activity by up to 100-fold in primary brain cells in vitro, it failed to achieve widespread mRNA downregulation in vivo after intracerebroventricular injection, likely because the particles accumulated predominantly in ventricular-lining and vascular-associated cells. These findings underscore that administration route and carrier design do not always work as expected, and that efficient CNS gene delivery often requires coordinated optimization and integration of both.

4.3 Improving Cell-Type Specificity

Cell-type specificity is increasingly recognized as another important strategy for improving the efficiency of CNS gene therapy. The central nervous system is composed of multiple functionally distinct cell populations, and the optimal therapeutic target therefore depends on the underlying disease biology and the intended mechanism of action. Neurons remain indispensable targets for disorders driven by cell-autonomous neuronal dysfunction, whereas astrocytes and microglia are highly attractive in diseases dominated by metabolic imbalance, excitotoxicity, neuroinflammation, or defective immune surveillance[133,

134]. In brain tumors, direct targeting of glioma cells is also critical for overcoming recurrence and therapeutic resistance[135].

Several neuronal surface receptors have been explored to improve cell-selective gene delivery. Among them, trisialoganglioside receptors such as GT1b are particularly attractive because they are highly enriched on neuronal membranes and can mediate selective association of ligands such as Tet1, while high-affinity neurotensin (NT) receptors provide an additional entry point for targeting dopaminergic neurons. In addition, neurotrophin-related receptors, especially TrkA and p75NTR, have been investigated for directing gene vectors toward NGF-responsive neuronal populations, including sensory neurons[136]. Using a neuron-targeted NT-PEI/plasmid delivery system together with an implantable Aulo-UCNP neural interface, Chen et al. achieved localized opsin expression, fiber-free deep-brain optogenetic stimulation, and simultaneous electrophysiological recording in vivo, thereby establishing a stable non-viral platform for closed-loop neuromodulation[137]. Zhang and colleagues underscored the importance of rational targeting design by developing a dual-ligand fusion peptide with both BBB-penetrating and neuron-targeting functions, in which Tet1 was used as the neuron-binding motif[138]. This strategy improved delivery efficiency to AD-related brain lesions and ultimately alleviated multiple pathological features, including tau hyperphosphorylation, axonal transport impairment, and microgliosis, while improving cognitive function.

Baigude and colleagues reported a series of glia-targeted non-viral delivery strategies for CNS disorders[139]. By formulating adenosine-conjugated or ionizable lipids, they developed Ad4 and DA6 LNPs for astrocyte-targeted delivery of siTLR4 or brain-derived neurotrophic factor (BDNF) mRNA, which respectively reduced neuroinflammation/BBB disruption and improved cognitive recovery in TBI models[140, 141]. They further constructed mannose-functionalized DoGo lipid nanoparticles (M3) for microglia-targeted siTLR4 delivery, which suppressed pro-inflammatory polarization and promoted a reparative phenotype. Zhang et al. also developed an M2 microglia-targeting LNP platform for selective delivery of IL-10 mRNA to the ischemic brain, thereby promoting protective microglial polarization and treating ischemic stroke[142].

Beyond commonly used tumor-targeting ligands such as cyclic RGD (cRGD)[143, 144], alternative strategies have also been developed to target brain tumor cells[145]. Huang et al. found that ApoE3-reconstituted high-density lipoprotein exhibited markedly higher uptake in glioblastoma cells than in normal primary astrocytes, and further revealed that this preferential accumulation was largely dependent on Ras-driven macropinocytosis[146]. Based on this mechanism, they developed an ApoE3-rHDL nanostructure carrying ATF5 siRNA, which combined BBB penetration with glioblastoma-selective uptake and ultimately induced marked RNA interference and tumor growth inhibition.

5. Application of Safety and Efficacy Strategies in Clinical Trials

Although substantial progress has been achieved in the

preclinical development of non-viral vectors, their translation into clinical studies for CNS gene delivery remains relatively limited. In current clinical practice, viral vectors continue to serve as the principal platform, largely because of their well-established in vivo transduction capacity and more extensive clinical experience[147, 148]. In comparison, the registered clinical landscape of non-viral gene delivery for CNS disorders remains comparatively narrow with the majority of studies focusing on brain tumors. Among the listed non-viral platforms that have entered clinical trials (**Table 1**), liposomal systems and lipid particles constitute the predominant class. One representative example is SGT-53, a tumor-targeted cationic liposomal nanocomplex encapsulating wild-type P53 plasmid DNA and designed to restore p53 function in malignant cells. In recurrent glioblastoma, SGT-53 has been evaluated in combination with temozolomide in an intravenous phase II study (NCT02340156). This platform has also been extended to pediatric CNS malignancies, including an earlier trial conducted with conventional radiotherapy and/or chemotherapy (NCT03554707), as well as the more recent JACOB study combining SGT-53 with radiation and nivolumab (NCT07017816). Another example has been advanced by investigators at the University of Florida, who developed a series of intravenously administered RNA-lipid particle (RNA-LP) vaccines for glioma[149]. This particle consists of onion-like multilamellar aggregates formed by lipids and tumor antigen-encoding mRNA, in which the RNA cargo is organized within a multilayered lipid architecture rather than a conventional single-core nanoparticle format. The registry records indicate that these studies are designed primarily to evaluate safety, feasibility, dose determination, and immunologic activity in the relevant patient populations (NCT04573140, NCT05660408). In the recurrent adult

glioblastoma study, participants are described as receiving two study drug products, including a pp65 RNA-LP vaccine and a second RNA-LP formulation that contains pp65 mRNA together with tumor RNA from each patient's tumor tissue (NCT06389591). Early first-in-human data reported rapid cytokine and chemokine release together with systemic immune activation[149]. Taken together, these registered trials show that intravenously administered RNA-LP formulations have already advanced into early clinical testing for both newly diagnosed and recurrent glioma settings.

By comparison, Polymeric carriers and EV-based systems have not yet emerged as preferred choices in current clinical trials of CNS non-viral gene delivery, likely owing to distinct translational constraints associated with these two platforms. For polymeric systems, transfection efficiency is often closely linked to cationic charge, making it difficult to simultaneously achieve high delivery performance and acceptable biocompatibility[28]. For EV-based systems, this may be attributable to the lack of standardized production protocols, insufficiently defined cargo composition, and limited scalability[16].

It is worth noting that several enabling technologies discussed above have already achieved clinical validation. LNP-based nucleic acid delivery has been established by the FDA approvals of the mRNA COVID-19 vaccines, while MR-guided focused ultrasound has also received FDA approval for CNS indications including essential tremor and Parkinson's disease. These precedents support the translational potential of both advanced nucleic acid carriers and externally controlled delivery platforms, and we anticipate that continued advances in rational design and carrier selection will enable more non-viral vectors to be delivered safely and effectively into the brain for the treatment of neurological disorders.

Table 1. Representative registered clinical trials of non-viral gene delivery for CNS diseases.

Start Year	Drug name	Non-viral vector	Disease	Trial register	Status
2014	SGT-53 and temozolomide	Cationic liposome	Recurrent GBM	NCT02340156	Terminated
2017	NU-0129	Spherical nucleic acid gold nanoparticle	Recurrent GBM / Gliosarcoma undergoing surgery	NCT03020017	Completed
2021	RNA-LP	Lipid particles	Newly diagnosed pediatric high-grade gliomas (pHGG) / adult GBM	NCT04573140	Active, not recruiting
2022	SGT-53 in conjunction with conventional radiotherapy and/or chemotherapy	Cationic liposome	Children with recurrent or progressive CNS malignancies	NCT03554707	Unknown status
2024	RNA-LP	Lipid particles	Recurrent adult GBM	NCT06389591	Active, not recruiting
2025	RNA-LP	Lipid particles	Pediatric recurrent intracranial malignancies	NCT05660408	Active, not recruiting
2025	JACOB: TP53 cDNA + checkpoint + RT	Cationic liposome	Recurrent, progressive or	NCT07017816	Not yet recruiting

6. Conclusion and Future Perspectives

In summary, non-viral gene delivery has emerged as a highly versatile and increasingly promising strategy for the treatment of CNS disorders. Recent advances in material design, carrier engineering, cargo optimization, BBB-crossing strategies, administration route selection, and cell-type-specific targeting have substantially expanded the therapeutic potential of non-viral platforms.

Future advances are likely to shift non-viral CNS gene delivery from conventional carrier optimization toward precision and programmable therapeutic systems. Next-generation vectors should integrate biodegradable and biomimetic materials with cargo-specific packaging, BBB-penetrating strategies, and cell-selective targeting to enable efficient delivery at lower and safer doses. Disease-responsive designs and externally controlled approaches may further provide spatial and temporal precision that is difficult to achieve with conventional administrations. Meanwhile, predictive human-relevant models, computational formulation design, and standardized manufacturing workflows will be essential to improve reproducibility and accelerate clinical translation. Ultimately, successful non-viral CNS gene therapy will require delivery platforms that are not only potent, but also adaptable, repeat-dose compatible, and sufficiently safe for the highly sensitive neural environment.

Data availability

Not applicable.

Acknowledgements

H. W. acknowledges funding support from NIH Maximizing Investigators' Research Award (National Institute of General Medical Sciences 1R35GM147408), Robert A. Welch Foundation Grant (No. F-2084-20240404), UT Austin Portugal Exploratory Research Projects Grant, Alpha-1 Foundation Pilot and Feasibility Grant, National Science Foundation (NSF) CAREER award (2340964), Craig H. Neilsen Foundation Pilot Research Grant, The Levy-Longenbaugh Fund and Texas Biologics

Declaration of competing interest

The authors declare no competing financial interest.

Author contribution statement

X. M.: Writing – review & editing. H. W.: Writing – review & editing, funding acquisition. All authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

Not applicable.

Use of AI statement

None.

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