MATERIALS SCIENCE

Nanoparticle-enabled gene delivery for pediatric brain tumors

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Pediatric brain tumors are the leading cause of disease-related death in children, yet effective treatment remains a major clinical challenge. These aggressive tumors have limited therapeutic options capable of crossing the blood-brain barrier (BBB) and frequently recur due to genetic or epigenetic alterations and treatment resistance. Gene therapy offers a promising strategy to address these limitations by modifying gene expression, correcting mutations, or introducing functional genes. Nanoparticle (NP) delivery systems facilitate this approach by protecting genetic cargo and enabling BBB penetration and tumor-specific uptake. This review summarizes current genetic engineering strategies and NP-based delivery systems for pediatric brain tumors, including their design principles and delivery mechanisms, highlights key therapeutic approaches under preclinical and clinical investigations, and discusses the major challenges and future directions in NP-mediated gene therapy for pediatric neuro-oncology. By critically evaluating these developments, we aim to underscore the transformative potential of NP-mediated gene therapies in reshaping treatment paradigms and improving outcomes and quality of life for children.

INTRODUCTION

Pediatric brain tumors represent a heterogeneous group of neoplasms originating within the brain and localized structures (*1*). Brain tumors account for 25% of all pediatric cancers and vary widely in cell type, anatomical location, and molecular profile (*2*, *3*). The most common malignant subtypes include high-grade glioma (HGG), medulloblastoma (MB), and ependymoma (EP). Clinical symptoms range from headaches and seizures to motor dysfunction and behavioral changes. The outcomes for these pediatric tumors are generally dismal with 5-year survival rates less than 10% for HGG (*4*), 60 to 80% for MB (*5*, *6*), and ~60 to 70% for EP (*7*).

For children diagnosed with HGG, surgical removal or even debulking of the tumor is often not feasible. This is particularly the case for diffuse midline glioma (DMG), an aggressive and incurable brain tumor that develops in midline structures of the central nervous system (CNS). Due to the critical role of the pons in basic neurological function, surgical intervention is extremely risky and can lead to devastating outcomes such as locked-in syndrome, in which patients are conscious but unable to move or speak because of near-complete paralysis. Moreover, DMG is notoriously resistant to chemotherapy, leaving palliative radiation as the only standard treatment to temporarily slow tumor progression.

The treatment of MB and EP typically involves a combination of surgery, radiation therapy, and, particularly for MB, chemotherapy, which together have improved survival rates in recent years, depending on age and molecular subgroup. However, these intensive treatments often result in severe and lasting side effects. For example, 25 to 40% of children undergoing surgery for MB or EP develop posterior fossa syndrome, characterized by impaired or absent speech along with deficits in motor function, emotional regulation, and behavior (*8*). Radiation therapy, particularly in children under 3 years of age, is a major contributor to long-term neurological damage. As a result, high-dose chemotherapy is predominantly used as an alternative in this age group.

Compounding these issues is the poor penetration of most chemotherapeutics across the blood-brain barrier (BBB), requiring high systemic doses that increase the likelihood of off-target toxicity. For instance, combination regimens routinely include cisplatin, known to be neurotoxic, ototoxic, and nephrotoxic (*9*, *10*), as well as vincristine, associated with neuropathy and cyclophosphamide, known to cause hemorrhagic cystitis, infertility, and myelosuppression (*11*). Distressingly, even after these highly toxic treatments, survival remains poor for some subgroups, such as group 3 MB in infants under 3 years, where 5-year survival rate is less than 50% (*12*).

The advent of gene therapy has opened new avenues for treating brain diseases by genetically engineering target genes, mRNA, or synthesis of an exogenous protein. In 2023, the first-in-human trial combining cytotoxic and immune-stimulatory gene therapy was reported, showing the safety and feasibility of this approach for patients with glioma (*13*). However, despite the potential of gene therapy, effective gene delivery in brain diseases faces substantial hurdles, including rapid systemic clearance, cargo susceptibility, biological barriers, and endosome trapping within target cells. Thus, high-performance systems that can overcome biological barriers and achieve precise and effective gene delivery are urgently needed.

Current viral vector–based gene therapies, which constitute all approved gene therapies as of 2022, present several issues including immunogenicity, insertional mutagenesis, poor selectivity, and inefficiency in delivery (*14*). This has prompted research and development into nonviral systems. Nonviral vectors, particularly nanoparticles (NPs), offer a promising alternative due to their versatile design for cargo loading, ease of functionalization for targeted delivery and

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controlled release, as well as relatively low immunogenicity and toxicity compared with viral vectors (*15*). A variety of NP-based vectors, such as inorganic NPs (gold and iron oxide), polymeric NPs, and lipid NPs (LNPs), have been developed for brain targeting or gene delivery (*16*). Notably, LNP small interfering RNA (siRNA) drug patisiran was approved by US Food and Drug Administration (FDA) for amyloidosis treatment (*17*), highlighting the promising future of NP-based systems in gene therapies.

Here, we review current genetic engineering strategies and synthetic NP–based delivery systems for pediatric brain tumors. We begin by introducing the in vivo gene therapy strategies for pediatric brain tumors, highlighting both permanent and temporary effects, as well as therapeutic approaches now in preclinical and clinical trials. We then discuss the NP design principles aimed at overcoming the BBB. Last, we discuss the key challenges in gene therapy in pediatric neuro-oncology and future directions for research and development. By critically evaluating these parameters, we aim to underscore the transformative potential of NP-based gene-based therapies in improving treatment outcomes and quality of life for children with brain tumors.

GENE THERAPY FOR PEDIATRIC BRAIN TUMORS

In vivo gene therapy encompasses various strategies that promise to play a future role in combating pediatric brain tumors. As shown in Fig. 1, methods are generally split into two broad categories: those designed to have a permanent effect and those designed for a temporary effect.

Permanent gene modulation

Permanent gene modulation can be achieved using approaches including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and, most recently, Nobel prize winning, clustered regularly interspaced short palindromic repeats (CRISPR) (*18*). ZFNs, developed in the early 2000s, represented one of the first programmable nuclease-based systems that allowed for targeted, permanent modifications to the genome (*18*). Next came TALENs that benefited from the rapid design of TALE proteins using a straightforward "protein-DNA code" that links modular DNA-binding repeat domains to specific bases within the target-binding site (*19*). ZFNs and TALENs create double-strand breaks at specific locations in the genome, which are then repaired through the error-prone

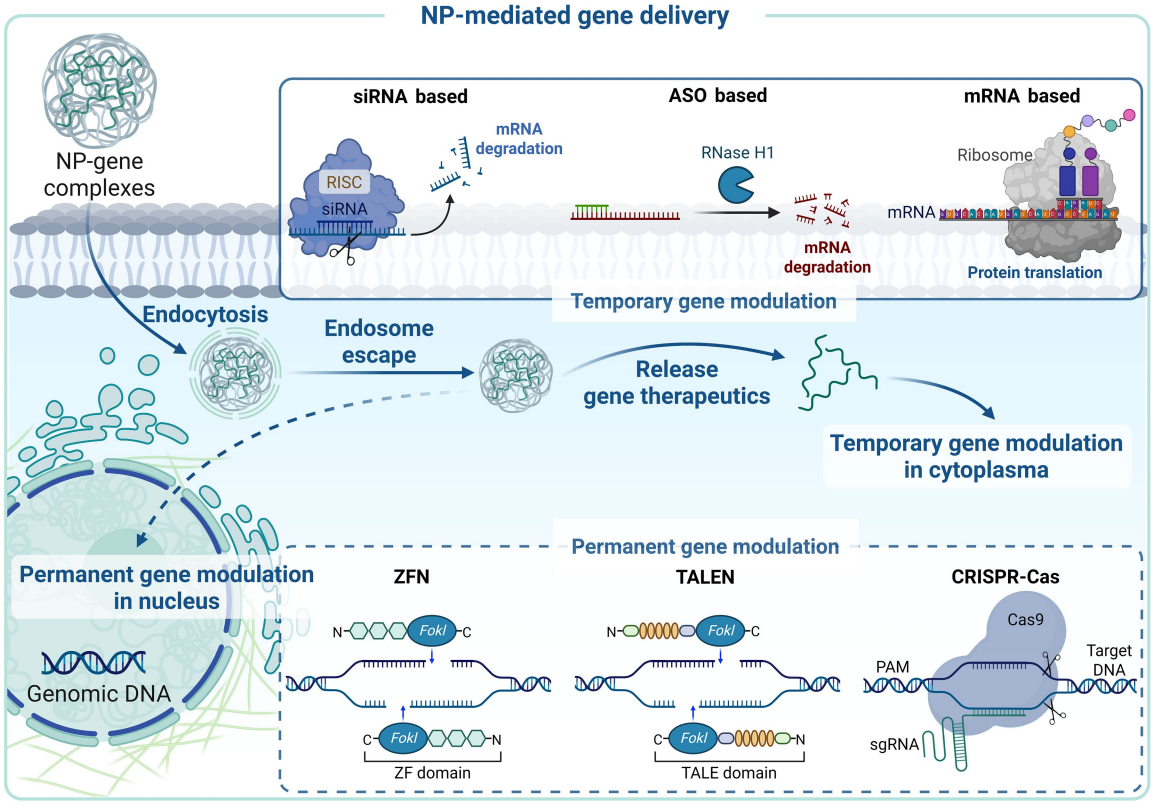


Fig. 1. In vivo gene therapy strategies. Permanent gene modulation includes ZFN, TALEN, and CRISPR systems, which induce targeted modifications to genomic DNA. ZFNs are custom-engineered enzymes that have ZF domains fused with *FokI* cleavage domain. Two ZF domains interact opposite sides of a target DNA site and create a double-strand break to knockout the target gene. TALENs function similar to ZFNs: TALE domains fused with *FokI* domain and bind opposite sides of DNA, creating a double-strand break and leading gene knockout. CRISPR-based gene modulation combines Cas with sgRNA to bind and cut target DNA for gene editing. Temporary gene modulation can be mediated by siRNA, ASO, or mRNA in the cytoplasm. siRNA-based therapy relies on RISC; the antisense strand of siRNA directs sequence-specific cleavage and degradation of complementary target mRNA, resulting in reduced protein translation. ASOs bind target mRNA via Watson-Crick base pairing, creating a hybrid that triggers recognition followed by cleavage by enzyme RNase H. mRNA-based therapy introduces synthetic mRNA into cells to produce specific proteins. ZFN, zinc finger nuclease; TALEN, transcription activator-like effector nucleases; CRISPR-Cas, clustered regularly interspaced short palindromic repeats–CRISPR-associated protein; siRNA, small interfering RNA; ASO, antisense oligonucleotides; sgRNA, single guide RNA; RISC, RNA-induced silencing complex. Created in BioRender. Zhu, J. (2026), https://BioRender.com/4dh2ahp.

nonhomologous end-joining pathway, resulting in small insertions and deletions at the cleavage sites (*20*).

ZFNs have shown promise in treating adult glioblastoma (GBM) by genetically modifying GBM-specific cytolytic T lymphocytes, incorporating a chimeric T cell receptor consisting of an extracellular interleukin-13 (IL-13) domain and a cytoplasmic CD3 domain (IL-13–zetakine) into $CD8^+$ T cells (*21*). Early-stage clinical trial (NCT02208362) (*22*) for patient-specific IL-13–zetakine–expressing $CD8^+$ T cell established the safety of this approach, with promising clinical activity. This provides a foundation for future studies investigating off-the-shelf, zinc-finger–modified, and/or glucocorticoid-resistant chimeric antigen receptor T (CAR T) cells aimed toward brain cancer therapy (*23*). Because of their large size and repetitive nature, delivering TALENs into specific tissues or cells in vivo presents substantial challenges (*24*). Therefore, they tend to be more practical and effective for in vitro/ex vivo applications where they can be controlled more effectively.

In comparison to genome-editing tools ZFNs and TALENs, the CRISPR–CRISPR-associated protein (Cas) system, CRISPR-Cas (Fig. 2), is proposed to provide greater precision, fewer off-target effects, reduced cytotoxicity, and greater versatility in design (*25*). CRISPR-Cas is a technique that combines Cas with single guide RNA (sgRNA) to bind and cut target DNA for gene editing (*26*). This revolutionary approach has considerable promise in pediatric brain pathologies, where a single gene is responsible for driving the pathology. Such devastating conditions include Rett syndrome caused by mutations in gene *MECP2* and vanishing white matter caused by a mutation in genes encoding the eIF2B subunits (*EIF2B1-EIF2B5*) (*26*). For the fatal pediatric brain cancer DMG, CRISPR technology is also providing hope through attempts to correct the driver H3.3K27M mutation or targeting downstream pathways impacted by this mutation (*27*); nevertheless, these studies are still very much preclinical. Meanwhile, some important clinical trials for other cancers have already arisen from using CRISPR technology in cell-based immunotherapy approaches (Table 1). To date, all trials are reliant on ex vivo methods and CRISPR apparatus delivered mostly by viral vectors and are predominantly for treating blood cancers because, right now, viral vectors and cell-based immunotherapy (e.g., CAR T) approaches are proving most effective in these areas. However, with continued safety concerns around the use of viral vectors, their restrictive size as delivery vehicles for CRISPR apparatus, and questions of safety and purity during scale up, it will be encouraging to observe more studies like NCT04213469 (*28*) that have opted to use a nonviral NP, especially given its encouraging results so far. Nevertheless, although it is delivered via a viral vector, one exciting example is Great Ormond Street Hospital for Children's trial of CD19 targeted CAR T cells with CRISPR guides for CD52 and TRAC (NCT04557436) (*29*) for children with pre-B cell acute lymphoblastic leukemia.

While we are beginning to observe greater successes from permanent gene intervention approaches, this is still a relatively new area of research compared with more temporary gene modulation approaches. As such, various temporary approaches have progressed

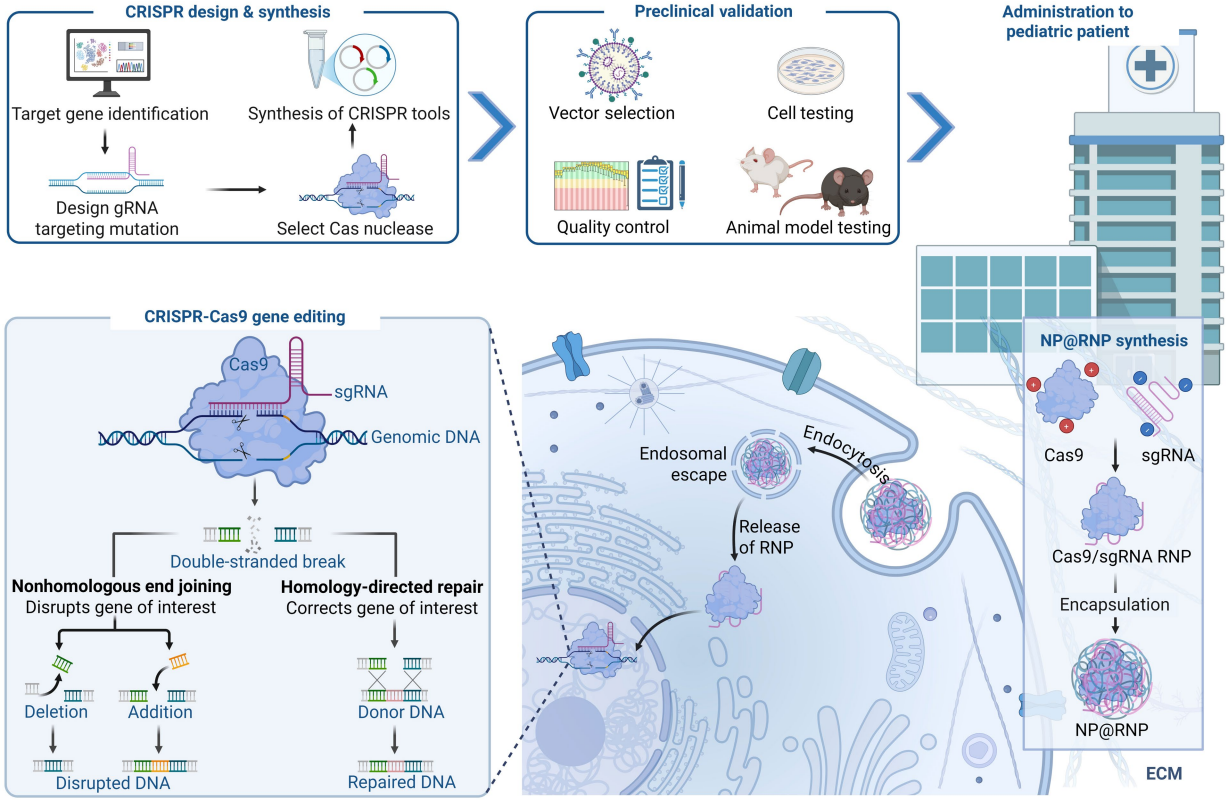


Fig. 2. Overview of CRISPR-NP technology for pediatric patients. CRISPR-Cas9–based gene editing and transcriptional modulation, and major mechanisms include nonhomologous end-joining and homology-directed repair pathway. NP, nanoparticle; RNP, ribonucleoprotein; ECM, extracellular matrix. Created in BioRender. Zhu, J. (2026), https://BioRender.com/4dh2ahp.

much further and are now a valued part of clinical medicine in a range of diseases and disorders. Right now, temporary gene therapy led approaches reside at the pinnacle of in vivo attempts, dedicated to curing the now incurable, including pediatric brain cancers.

Temporary gene modulation

Most common temporary gene modulation methods center around siRNA, antisense oligonucleotides (ASOs), and mRNA-based therapies. All are driving NP-led successful in vivo outcomes for various disorders, infections, and diseases.

Small interfering RNA

siRNA is a short, double-stranded RNA molecule, typically about 20 to 25 nucleotides (nt) long (*30*). Once delivered into the cells cytoplasm, siRNA is incorporated into the RNA-induced silencing complex (RISC), where argonaute2 (Ago2) retains the antisense strand and removes the sense strand. The antisense strand of siRNA binds the target mRNA in a complementary base-pair manner. Complementary binding between siRNA and mRNA signals endonucleolytic protein Ago2 to fragment the mRNA sequence ready for degradation by cytoplasmic exonucleases (*31*). Meanwhile, the siRNA sequence is freed by the RISC ready to be taken up by other resident RISC complexes and enabling further removal of target mRNAs to complete posttranscriptional silencing of the target gene (Fig. 3).

The scientific paradigm of siRNA was first documented in 1998 by Andrew Fire and Craig Mello (*32*). Since then and against a backdrop of endless obstacles associated with the multiple steps involved in successful application, siRNA therapeutics have, in recent years, encountered a dynamic trajectory of substantial life-changing breakthroughs. The first siRNA therapeutic to gain FDA and European Medicines Agency approval in 2018 was patisiran. Patisiran targets

Table 1. Clinical trials using CRISPR-Cas–engineered cell–based immunotherapy. PD1, programmed cell death 1; r/r B-NHL, relapsed/refractory B cell non-Hodgkin lymphoma; TRAC, T cell receptor alpha constant; B-ALL, B cell acute lymphoblastic leukemia; TCR, T cell receptor; RFX5, regulatory factor X5; T-ALL, T cell acute lymphoblastic leukemia; AML, acute myeloid leukemia; β_2M, β_2-microglobulin; DLBCL, diffuse large B cell lymphoma; FL, follicular lymphoma; ALK$^+$ ALCL, ALK-positive anaplastic large cell lymphoma; CMV, cytomegalovirus.

NCT number	Target antigen	CRISPR target	Cancer subtype	Vector/cell type	Phase
NCT04213469 (*28*)	CD19	PD1 integration	r/r B-NHL	Nonviral/CAR T	1
NCT04227015 (*144*)	CD19/CD22	TRAC, CD52 disrupted	r/r B-ALL	Lentiviral/CAR T	1
NCT03545815 (*145*)	Mesothelin	PD1, TCR disrupted	Mesothelin$^+$ solid tumors	Lentiviral/CAR T	1
NCT04557436 (*29*)	CD19	TRAC, CD52 disrupted	B-ALL	Lentiviral/CAR19 T	1
NCT04538599 (*146*)	CD7	TRAC, CD7, RFX5 disrupted	T-ALL, AML	Allogeneic CAR T	1
NCT04637763 (*147*)	CD19	TRAC, PD1 knockout	r/r B-NHL	Allogeneic CAR T	1
NCT04035434 (*148*)	CD19	TRAC, β_2M disrupted	r/r DLBCL, grade 3b FL	Allogeneic CAR T	1
NCT04767308 (*149*)	CD5	Endogenous CD5 knockout	r/r ALK$^+$ ALCL	Autologous CAR T	1

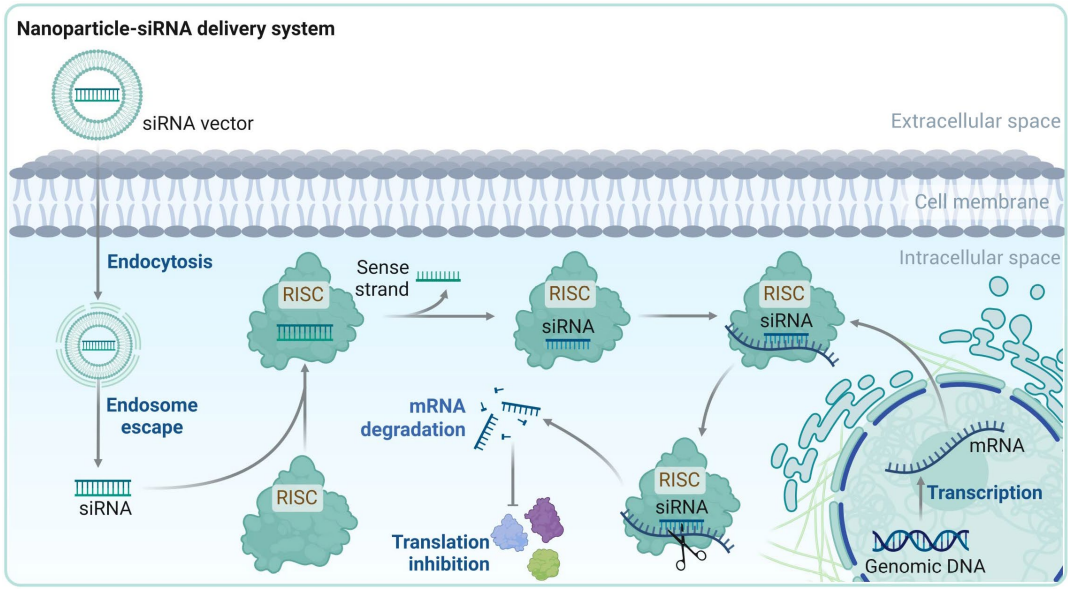


Fig. 3. Schematic diagram of NP-siRNA delivery system. Following cytoplasmic delivery, siRNA incorporates into the RISC, where sense strand is removed to activate the siRNA. The antisense strand then directs sequence-specific cleavage and degradation of complementary target mRNA, resulting in reduced protein translation. Created in BioRender. Zhu, J. (2026), https://BioRender.com/4dh2ahp.

peripheral nerve disease (polyneuropathy) by silencing gene expression of hereditary transthyretin-mediated amyloidosis. Givosiran, used to treat acute hepatic porphyria quickly followed suit after performing well in clinical trials and was commercially available in 2019 (*30*). The years 2020–2021 saw two further additions to the siRNA therapeutics portfolio with regulatory approval of lumasiran for the treatment of primary hyperoxaluria type 1 (*33*) and inclisiran, developed to target hypercholesterolemia or mixed dyslipidemia, lowering low-density lipoprotein cholesterol (*34*). This year vutrisiran received regulatory approval as the second siRNA therapy targeting ATTR (*35*). Meanwhile, Table 2 identifies promising clinical trials aimed at improving cancer treatment. Unfortunately, to date, there are no trials applying siRNA in the treatment of brain cancers. There is, however, a trial underway for the childhood malignancy, retinoblastoma, which may offer a future pathway for children with brain cancer (NCT06424301) (*36*).

Antisense oligonucleotides

ASOs are a class of gene therapeutic that differ to siRNA in that they are single-stranded DNA or chemically modified RNA (~15 to 30 nt) (*37*), moreover, processed differently by cells. ASOs are not reliant on RISC, rather they bind target mRNA via Watson-Crick base pairing, allowing for more specificity even with genetic variants (*38*). Through complementary binding, they silence genes or modify pre-mRNA splicing, which, in turn, facilitates reduction, modification, or restoration of a specific protein (*38*). Gapmers are single-stranded ASOs composed of a DNA core surrounded by chemically modified RNA-like structures that are resistant to ribonuclease (RNase) H degradation (*38*). Gapmers are capable of either approach through specific binding of the target mRNA, creating a hybrid that triggers recognition followed by cleavage by enzyme RNase H (*39*). When ASOs bind to splice sites or regulatory elements in pre-mRNA, splicing machinery will be prevented or redirected (e.g., exon skipping or exon inclusion). But when ASO binds to the 5′ untranslated region or start codon region of mRNA, it can either block or enhance protein translation (*40*). ASOs can also be designed to bind to microRNA resulting in silencing of the encoded genes. While siRNA is considered to be more adept at silencing gene expression, ASOs are considered less likely to initiate mismatched binding, which leads to off-target effects. Moreover, their single-stranded identity makes their delivery and uptake moderately easier. However, although ASOs can travel through the blood, they are poor at penetrating the BBB and entering the brain, so they are now administered via intrathecal or intracerebroventricular injections (*41*). Zhang *et al.* (*42*) took heed of these factors in their diffuse intrinsic pontine glioma (DIPG) study, using ASO to facilitate knockdown of the H3-3A mutant mRNA. ASOs were delivered directly into the cerebrospinal fluid using a stereotactic device in two different orthotopic DIPG mouse models developed via the RCAS (replication-competent avian sarcoma-leukosis virus long-terminal repeat with splice acceptor) vector. The results demonstrated a reduction in tumor growth, promotion of neural-stem-cell differentiation, and increase in survival (*42*).

Tofersen (BIIB067) is an ASO therapeutic for targeting amyotrophic lateral sclerosis (ALS). BIIB067 targets mRNA for *superoxide dismutase 1* (*SOD1*) silencing its expression (*43*). Here, too, BIIB067 is delivered via intrathecal or intracerebroventricular injections. New less invasive methods of administration are warranted to minimize patient discomfort and treatment compliance. NPs are the most effective resource capable of increased delivery efficiency of ASO in a minimally invasive manner. Recent work published in 2025 by Ediriweera *et al.* (*44*) introduced a new calcium-phosphate-LNP delivery system to carry ASOs. The authors have subsequently taken more advanced steps toward maximizing brain accumulation by combining NP delivery with another minimally invasive technique, transcranial focused ultrasound (FUS) to transiently open the BBB. This elegant strategy resulted in a more than 3.5-fold increase in therapeutic NP uptake in the brain compared with the control group of mice without BBB disruption. BBB opening was transient and without structural changes, neuroinflammation, or toxicity to the brain. This work highlights the promise of this multipronged strategy, not only for ALS but also pediatric brain cancers, silencing oncogenic drivers and facilitating more specific delivery to areas of the brain where tumors reside.

mRNA

siRNA and ASOs are designed to reduce gene expression and, therefore, decrease protein translation. Conversely, mRNA is designed to increase protein translation. mRNA is single-stranded RNA manufactured enzymatically using a process called in vitro transcription (*45*). Therapeutic sequences are much longer (~2000 to 3000 nt), although this number is highly specific to the mRNA target. This increased size can make delivery more challenging compared with the two gene silencing methods (*46*) and makes mRNA more sensitive to the effects of hydrolysis (*47*). Once delivered into the cytoplasm via NPs, electroporation or other means, the 5′ cap structure and 3′ poly(A) tail of the mRNA sequence are recognized by the

Table 2. Clinical trials using siRNA-based therapies on solid tumors. NUDT21, nudix hydrolase 21 modulators; LAPC, locally advanced pancreatic cancer; KarsG12D, Kirsten rat sarcoma viral oncogene homolog with a G12D mutation; LODER, Local Drug EluteR; VEGF-A, vascular endothelial growth factor A; KSP, kinesin spindle protein; LNP, lipid nanoparticle; EphA2, ephrin type-A receptor 2; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine; PDAC, pancreatic ductal adenocarcinoma.

NCT number	Cancer type	Gene target	Gene therapeutics	Age	Phase
NCT06424301 (*36*)	Refractory retinoblastoma	NUDT21	Novel targeting NUDT21 siRNA drug	≥6 months	1
NCT01188785 (*150*)	LAPC	KrasG12D	siG12D-LODER (polymeric matrix encapsulated siG12D)	≥18 years	1
NCT01676259 (*151*)					2
NCT01158079 (*152*)	Advanced solid tumors	VEGF-A, KSP	ALN-VSP02 (LNP encapsulated siRNA)	≥18 years	1
NCT00882180 (*153*)					1
NCT01591356 (*154*)	Advanced/recurrent solid tumors	EphA2	DOPC or liposome encapsulated siEphA2	≥18 years	1
NCT03608631 (*155*)	PDAC with KarsG12D mutation	KrasG12D	iExosomes (exosomes with siG12D)	≥18 years	1

cells' ribosomes. The ribosomes move along the sequence reading its codons, and, as it does so, the codon becomes exposed to tRNAs that bind the exposed codons in a complementary manner (aminoacylation) (*48*). The binding between the tRNA and amino acid sequence signals the ribosome to link the amino acids together through catalytic bonds that are formed at the ribosome's P site (peptidyl site) and the amino acid on the tRNA in the A site (aminoacyl site) (*48*). This dynamic process happens sequentially as the ribosome moves across the mRNA. Having fulfilled its role, the tRNA exits the ribosome and a new tRNA sequence is tasked with identifying the next amino acid in the sequence until a stop codon is reached, signifying the whole protein has been formed (translation). Protein folding comes next, whereby the polypeptide chain folds into its three-dimensional (3D) structure ready for performing its designated role (*48*).

Proteins artificially expressed by mRNA can take on many therapeutic directions. Unmodified mRNA naturally exhibits immunogenicity because it interacts with Toll-like receptors, especially in antigen-presenting cells (APCs) such as dendritic cells (DCs). However, this can be controlled by incorporating modified nucleotides like m5C, m6A, m5U, s2U, or pseudouridine to substantially attenuate mRNA triggered immune activation (*49*). Now, the most widely explored mRNA therapies include amplifying viral and cancer antigen presentation for vaccine development, programming immune cells (e.g., T cells, macrophages, and DCs) to express CARs that bind to mostly cancer-specific antigens for immune clearance and expanding populations of proteins designed to induce apoptosis (programmed cell death) in cancer cells.

mRNA cancer immunotherapies

mRNA technology became a household name following the life saving effects of Moderna and Pfizer's COVID-19 vaccines, and these successes have provided the foundation for vaccine exploration outside of the infectious diseases field. Cancer vaccines are central to the current excitement that surrounds mRNA therapeutics, offering potential as both therapeutics (treat existing cancer) and prophylactics (preventive in the future). Preventative protection is achieved through the development of memory T cells, which recognize the original CAR-target antigen and subsequently eliminate cells that later exhibit heightened expression of that antigen (Fig. 4).

NCT05533697 (*50*) is a Moderna-sponsored phase 1/2 clinical trial evaluating LNP-formulated mRNA-4359. mRNA-4359 encodes immunogenic epitopes derived from programmed death-ligand 1 and indoleamine 2,3-dioxygenase 1, two immune-evasion pathways commonly up-regulated across solid tumors, with the aim of eliciting antigen-specific T cell responses against cells expressing these targets.

Found in abundance following mRNA translation, these immunogenic peptides are readily engulfed by APCs (DCs, macrophage, etc.), which, in turn, activate T cell response. NCT05727839 (*51*) is

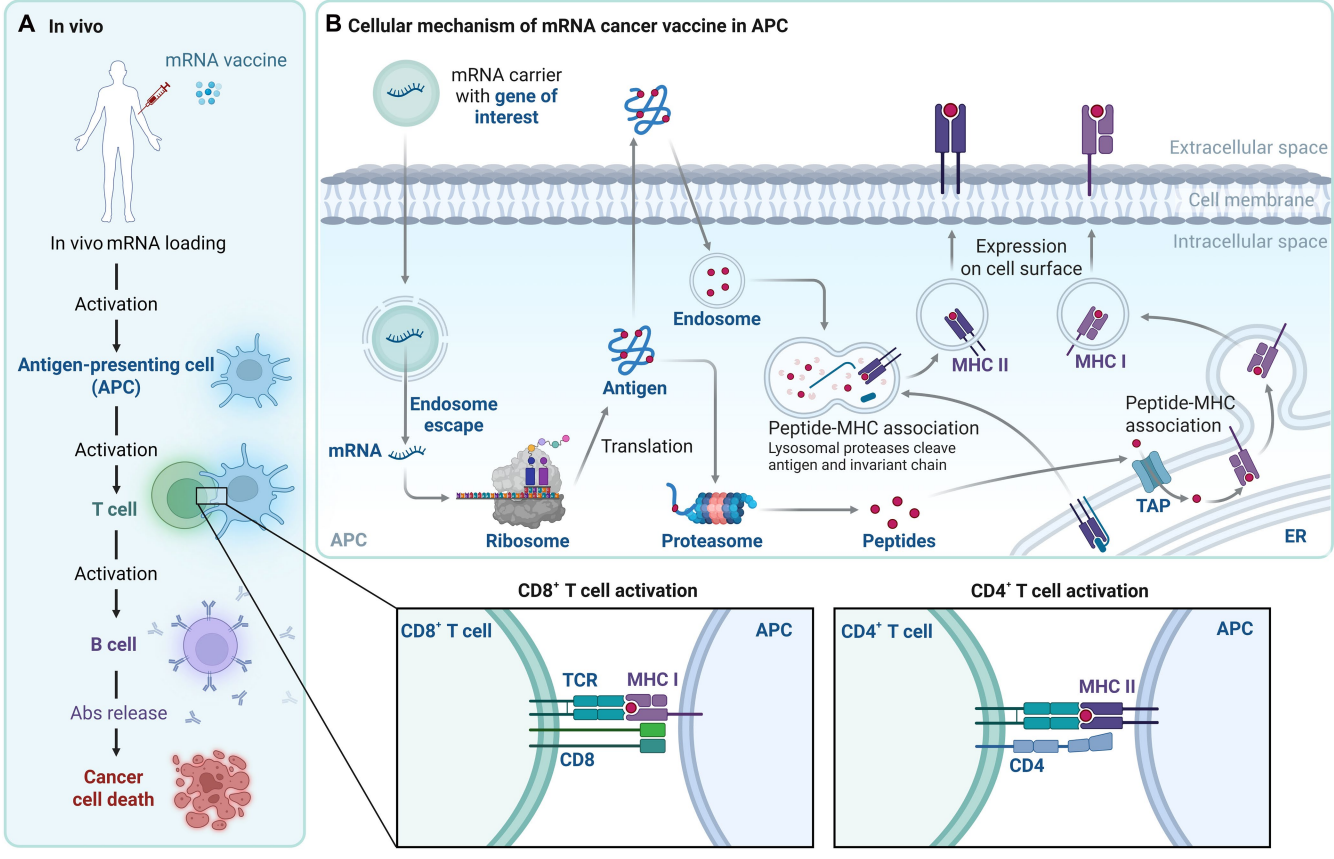


Fig. 4. General principles of mRNA cancer vaccines. (**A**) In vivo delivery strategies: mRNA vaccines are administered directly into the patient, where they are taken up by antigen-presenting cells (APCs), triggering immune activation and leading to cancer cell death. Abs, antibodies. (**B**) Cellular mechanism: Translated mRNA encodes tumor antigens, which are presented on major histocompatibility complex (MHC) molecules by APCs, leading to activation of $CD4^+$ and $CD8^+$ T cells that recognize and eliminate cancer cells. Created in BioRender. Zhu, J. (2026), https://BioRender.com/4dh2ahp.

a phase 1 clinical study sponsored by Immorna Biotherapeutics evaluating JCXH-211, a LNP-encapsulated self-replicating RNA therapeutic encoding engineered human IL-12 for patients with malignant solid tumors. Unlike Moderna's mRNA-4359, which encodes tumor-associated antigens, JCXH-211 is designed to amplify intratumoral IL-12 expression and stimulate antitumor immunity through the combined effects of IL-12 and srRNA-driven immune activation. Considered highly promising, early data have resulted in the FDA granting permission for an investigational new drug application (phase 1/2, multicenter, open-label, dose-escalation, and dose-expansion study) of intravenously delivered JCXH-211. Meanwhile, on the back of this success, Peking University Cancer Hospital and Institute are in trial (NCT05579275) (*52*) with JCXH-212, another self-replicating RNA type of mRNA (target unspecified). Last, autogene cevumeran, a lipoplex NP-mRNA vaccine that delivers up to 20 neoantigens targeting major histocompatibility complex class I and class II, is demonstrating feasibility and tolerability in early trial (NCT03289962) (*53*) and could prove to be a game-changing approach for patients with pancreatic cancer (*54*).

Although novel NP-formulated mRNA vaccines for infectious disease, most notably mNEXSPIKE, which has progressed to market following strong clinical performance in trial NCT05137236 (*55*), demonstrate the rapid clinical maturation of NP-mRNA technologies, translation of these platforms to pediatric brain cancers remains largely at the preclinical stage. Consequently, substantial anticipation surrounds the development of robust in vivo mRNA approaches capable of addressing the unique biological barriers posed by pediatric CNS malignancies.

Ex vivo mRNA-CAR immune cell therapies for pediatric brain cancer

NCT04185038 (*56*) is a first-in-human phase 1 trial for children with DMG using B7 homolog 3 (B7-H3)–specific CAR T cells. The target receptor, B7-H3, is expressed on the surface of pediatric brain cancers, including atypical teratoid rhabdoid tumor, DIPG, DMG, MB, and HGG tumors (*57*). T cells bind to the B7-H3 receptors on the tumor cell membrane, causing T cells to become activated and release cytotoxic perforin and granzymes. Perforin creates pores in the cancer cell membrane, allowing granzymes to enter and induce apoptosis of the tumor cell (*58*). The NCT04185038 (*56*) study comes following previous trials using locoregional CAR T cells targeting either human epidermal growth factor receptor 2 (NCT03500991) (*59*) or epidermal growth factor receptor (NCT03638167) (*60*) where DMG was not included, and these studies remain ongoing to treat pediatric patients with brain cancer. NCT04196413 (*61*) is a clinical trial in the US (Stanford University) exclusively aimed at DIPG and DMG. In this landmark trial, autologous disialoganglioside GD2-directed CAR T cells (GD2-CARTs) are under evaluation; nevertheless, dose-impairing cytokine release syndrome (CRS) was reported as an expected outcome following intravenous administration. Monje *et al.* (*62*) subsequently switched to intracerebroventricular only delivery for second- and third-arm studies. The intracerebroventricular delivery of GD2-CARTs is also evaluated in clinical trial NCT04099797 (*63*) aimed at GD2-expressing brain tumors, with an addition of a constitutively active IL-7 receptor on GD2-CARTs to enhance T cell activity (*64*). In Australia, a similar trial is testing a unique third-generation GD2-CART in combination with bevacizumab in children with DMG (ACTRN12622000675729). Meanwhile, in Belgium, clinical trial NCT04911621 (*65*) exploring adjuvant DC immunotherapy for pediatric patients with HGG or DIPG (ADDICT-pedGLIO) is active. This is one of the first DC-based immunotherapies to reach phase 1/2 clinical trial status. ADDICT-pedGLIO targets protein, *Wilms' tumor gene 1* (*WT1*), found in abundance on DIPG cells relative to healthy brain cells. CAR-DCs that target *WT1* presents another important cancer cell specific strategy, and this trial, like NCT04196413 (*61*), marks another important step toward an effective treatment for a now incurable pediatric brain cancer.

Meanwhile, CAR-DCs have also demonstrated some success in targeting adult cancer (GBM). IO-VAC is an innovative DC approach consisting of DCs loaded with autologous tumor antigens and matured with cytokine cocktail and Newcastle disease virus (*66*). Developed by researchers at the Immuno-Oncology Center in Cologne, IO-VAC is undergoing clinical trials for GBM (*66*). Following the findings of Van Gool *et al.* (*67*) for GBM, they subsequently examined whether IO-VAC may also prove to be a promising attack strategy for DIPG through retrospective analysis. Their results suggest that IO-VAC may prove useful when combined as part of a multimodal immunotherapy in first-line DIPG treatment.

While these trials and preclinical evaluations represent some of the best results, to date, for the incurable brain cancer, DIPG/DMG, there are still substantial areas for improvement in survival time and toxicity. A major safety concern limiting CAR T cell application in CNS malignancies is CRS, a systemic inflammatory toxicity that may occur concurrently with neurotoxicity (*62*, *68*). Neurotoxicity itself can manifest as immune effector cell–associated neurotoxicity syndrome, characterized by endothelial activation, BBB disruption, cytokine-driven neuroinflammation, and neuronal dysfunction (*69*). Additionally, tumor inflammation-associated neurotoxicity has been described in CNS-directed CAR T trials, including GD2-targeted approaches, where intense local immune activation within the tumor site results in transient neurological deficits (*62*, *70*). Beyond toxicity, the collection practices and ex vivo production of CAR T cell therapies is extremely costly, as well as being time and labor intensive (*71*). Understanding how best to manage these toxicities of CAR T therapies is continually evolving, while genome-edited CAR T products, such as those employing CRISPR-Cas modifications, show promise in improving safety and persistence, as in the trials summarized in Table 1.

Among all gene therapy strategies for brain cancer introduced above, a major challenge remains the effective delivery of gene therapeutics to the brain. In vivo nonviral, synthetic NP approaches can tackle this concern, but, unfortunately, substantial hurdles in NP design remain before we reach the scientific summit. For in vivo NP approaches aimed at brain cancers, one of the key design considerations is how to develop NPs that can navigate across the BBB before delivery of their gene therapy cargo at the tumor site.

NP DESIGN CONSIDERATIONS FOR OVERCOMING THE BBB

Studies that focus on in vivo NP-delivered gene therapeutics to the brain must overcome one of the most rigid physiological barriers in the body, the BBB.

Overview of BBB

The BBB is a highly selective and protective barrier that regulates what substances can enter the brain from the bloodstream. In health, the BBB is essential in maintaining the brain's homeostasis and thus routine function (*16*). Many therapeutic drugs that could potentially be

used for brain tumors are unable to cross the BBB due to characteristics including large size (more than 400 Da) and polarity as nonpolar molecules can cross unaided, whereas polar molecules require receptor-mediated access (*72*). Additionally, if drugs have managed to traverse, then the BBB contains a plethora of efflux transporters that rapidly pump drugs and toxins out as quickly as they enter (*73*).

BBB geometry (Fig. 5) is complex, a microvascular network made up of tightly integrated brain-specific endothelial cells (BSECs) with transporters embedded within the luminal and abluminal membranes. Each BSEC is sandwiched together by active intracellular adhesion sites called tight junctions. Tight junctions create high resistance to ions and molecules attempting to avoid cellular uptake when attempting access to the brain. Further regulation comes from the presence of neurovascular units (NVUs) situated along the length of the BSEC vessels. NVUs are composed of pericytes, astrocytes, microglia cell types, and the extracellular matrix (basal membrane). Architecturally, the NVUs form a perivascular complex that encased the BSEC vessels within their core collectively working to provide stability and support to the whole BBB microvascular. Meanwhile, tunneling nanotubes (TNTs) are intercellular structures located within the BBB, which is a fairly recent finding (~year 2000) that facilitate communication and the transfer of materials between cells within the BBB (*74*).

Endosome-mediated transport through the BSECs of the BBB consists of adsorptive-mediated transcytosis (AMT) and receptor-mediated transcytosis (RMT). AMT facilitates endocytosis through electrostatic interactions that occur between the incoming positively charged entity and the negatively charged membrane of the BSECs, resulting in invagination and endosome formation, a process driven primarily by caveolae or caveolin. AMT is the fundamental process manipulated by NPs developed for passive accumulation approaches. Meanwhile, RMT is accomplished using receptors embedded along the BBB with the most frequently occurring being transferrin. This transport portal is activated when the receptor recognizes its ligands or known substrates (transferrin, insulin, etc.) that bind complementary to the receptor. Binding facilitates endocytosis and endosome formation, which, in future, results in exocytosis into the brain parenchyma. Targeted NP approaches use this approach with the expectation that it will lead to more extensive uptake and delivery of the therapeutic cargo. However, this is yet to be successfully proven in clinical practice. While AMTs and RMTs are located on the luminal side, efflux transporters that control the exit of NPs from the brain are located on the abluminal side. Efflux pumps (p-glycoprotein, breast cancer resistance protein, and multidrug resistance-associated proteins) are responsible for removing matter from the brain in attempts to prevent infection and toxicity from manifesting. Drugs and antibodies are regularly hindered in their role by the function of efflux pumps that routinely expel them before they are able traverse into the brain. Although similar limitations can occur with NP delivery systems, NPs are considerably less susceptible to efflux-mediated clearance and, therefore, offer a comparatively improved likelihood of retaining therapeutic cargo within the brain. Nonetheless, achieving

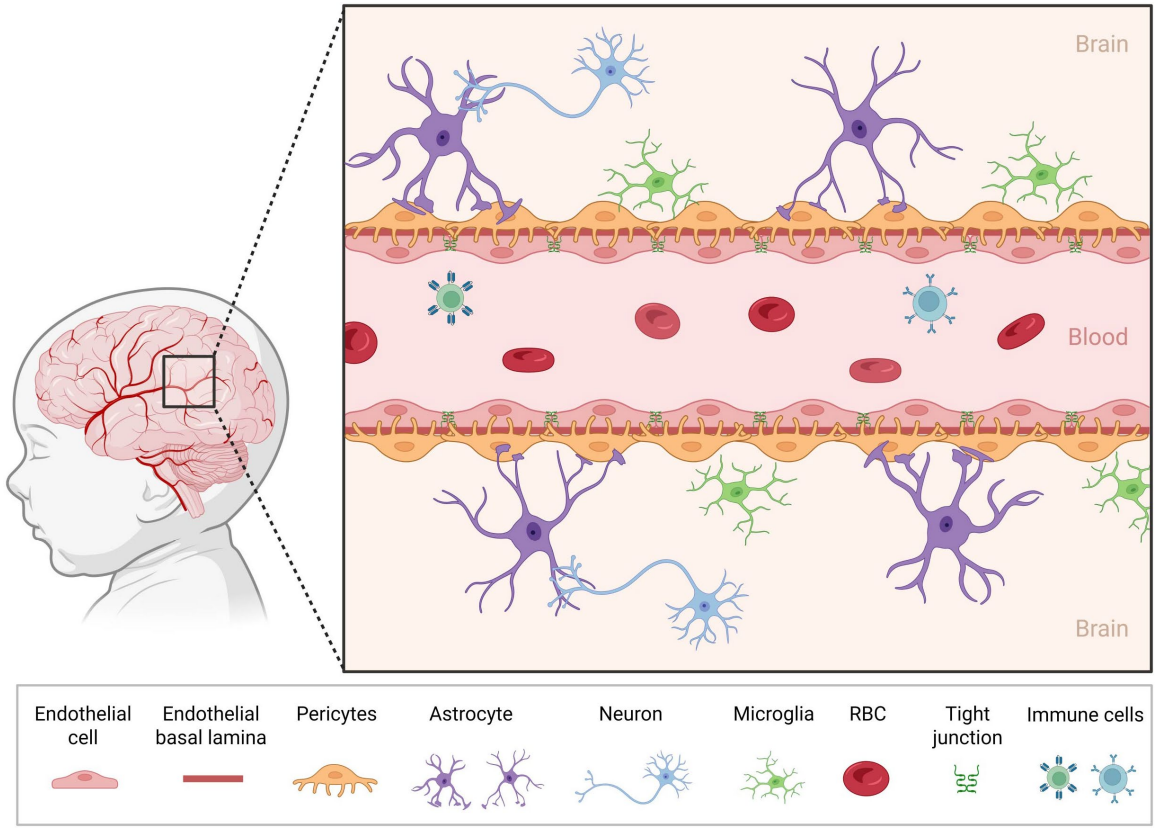


Fig. 5. Overview of the BBB. Endothelial cells embedded within the luminal and abluminal membranes are sandwiched together by tight junctions. NVUs situated along the length of the endothelial cells and are composed of pericytes, astrocytes, microglia, and the extracellular matrix (basal membrane). Created in BioRender. Zhu, J. (2026), https://BioRender.com/4dh2ahp.

sufficiently high intracerebral NP accumulation to exert a meaningful therapeutic effect remains a central and unresolved challenge in the field.

Engineering nanocarriers for enhanced BBB penetration

Overcoming the restrictive nature of the BBB remains the major challenge in delivering gene therapeutics to pediatric brain tumors. For nanotherapeutics delivery, nanocarriers can be precisely engineered to enhance BBB penetration. The types of core organic and inorganic NP materials and compositions of FDA approved or now under therapeutic investigation have been explained in thousands of reviews over the years and thus are not included here; however, examples of each can be found in the following publications (*15*, *75*). Additionally, NPs under evaluation in clinical trials for drug delivery in pediatric cancers can be found in the following publication (*76*).

Here, we have focused on the latest examples and concepts that relate to the adaptable properties of the NPs themselves and explore how these properties shape the latest engineering strategies for crossing the BBB and entering cancer cells. The physicochemical characteristics of NPs including size, shape, and surface chemistry (zeta potential, surface coatings, and targeting moieties) are mutable properties that are well established in their ability to influence BBB penetration. More recently, surface roughness, stiffness, and viscoelastic properties have joined the list of characteristics favorable to enhanced penetration and cellular uptake by cancer cells.

Size

The size of NPs is one of the most critical factors influencing BBB penetration, especially when attempting AMT (*77*). In 2023, Wu *et al.* (*78*) concluded in their review that nanomaterials larger than 200 nm exhibited negligible BBB transport, and, in 2024, Asimakidou *et al.* (*77*) suggested a hydrodynamic size in the range of 10 to 100 nm may be more optimal for BBB penetration as small NPs may potentially facilitate BBB penetration through nanomaterial-induced endothelial leakiness, whereby inorganic NPs can disrupt VE-cadherin junctions between adjacent endothelial cells, opening up the paracellular route of the endothelium (*79*, *80*). Meanwhile, NPs of ∼50 nm are often observed to achieve the highest cellular uptake (*15*), a necessary attribute for successful AMT approaches, suggesting that this may actually be a more effective size. When opting for RMT, slightly larger NPs can be successfully applied. For instance, Thirumurugan *et al.* (*81*) fabricated angiopep-2–decorated microemulsion formed titanium-alloy core-shell magnetic NPs (Ti@FeAu-Ang) of ∼130 nm in size, determining that conjugation with angiopep-2 successfully facilitated crossing of the BBB and more effective targeting of GBM cells upon alternating magnetic field stimulation. Meanwhile, Kim *et al.* (*82*) in their attempts to better target GBM cancer stem cells (CSCs) developed even larger NPs of ∼200 nm.

While these suggest that larger NPs can be successfully used during RMT and given the reasonable assumption that larger NPs can carry more payload, the current consensus, as reported by Arms *et al.* (*83*), indicates that, similar to AMT, NPs of ∼50 nm remain the most effective size for both AMT and RMT. However, the 2023 publication by Tylawsky *et al.* (*84*) would suggest that there is still some latitude to this 50-nm benchmark. Based on a prior study suggesting that 97% of BBB transport is through RMT (*85*), Tylawsky *et al.* (*84*) developed ∼80-nm NPs from sodium bicarbonate polysaccharide nanoprecipitation. Fucoidan inclusion targeting endothelial P-selectin to induce caveolin-1–dependent transcytosis across the BBB together with incorporation of the Sonic hedgehog (SHH) effector smoothened inhibitor, vismodegib (FiVis), provided functionality and therapeutic capacity. Using a genetic mouse model of SHH pathway activated MB (SHH-MB), with an intact BBB, the authors reported effective delivery of FiVis to the tumor microenvironment (TME) and improved therapeutic index for FiVis (*84*). Meanwhile, Infante *et al.* (*86*) demonstrated even smaller size NPs (∼22 nm) through self-assembly when demonstrating micelles delivering hydrophobic SHH inhibitor, Glabrescione B.

Shape

Consistent with the impact of size, the shape of NPs also determines their ability to cross the BBB. Generally, NPs form a spherical shape during self-assembly or fabrication; however, they can also be specifically designed to take on a discoidal, rod- or cube-shaped morphology (*87*). The geometrical shape affects not only their interaction with endothelium but also the clearance rate from systemic circulation. Compared with spherical, nonspherical NPs tend to show lower uptake from peripheral phagocytes, suggesting a higher probability of the NPs reaching the brain (*88*). Meanwhile, rod-shaped NPs have shown to lead to greater endothelial adhesion and transcytosis, facilitating greater accumulation in the brain parenchyma (*77*).

Exploiting access to the brain via TNTs and capacity to leverage cell connectivity over long distances is a markedly new concept in NP development and where shape appears important. Sierri *et al.* (*89*) determined that discoidal NPs composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine, cholesterol, and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[amino(polyethylene glycol)-2000] (molar ratio, 35:40:25) display greater BBB permeability and transfer efficiency between GBM TNTs than spherical and deformable counterparts. These are impressive early indicators; however, we look forward to future studies where they have moved beyond transwell hCMEC/D3 single-cell BBB models as an indicator of permeability. That being said, the GBM tumoroid model was an excellent approach to demonstrating NPs to be transferred between cells via GBM synthesized TNTs (*89*).

Surface charge

Surface charge plays a crucial role in BBB penetration. NPs with near-neutral or mildly positive surface charge tend to have better interactions with the negatively charged cell membranes of the BBB, potentially enhancing their uptake and transcytosis through AMT (*90*). Meanwhile, a too positive charge is associated with increasing nonspecific binding and toxicity (*91*). Contrary to positive, negative charges are suggested to limit cellular interactions 100-fold because of electrostatic repulsion (*92*). Striking a balance is integral to navigating the BBB effectively.

Surface coating

Surface coatings are a key determinant of BBB penetration. NP coatings with an affinity for water (hydrophilicity) or, conversely, lack thereof (hydrophobicity) have contrasting but equally important benefits. Hydrophilicity enhances circulation time by reducing nonspecific protein binding (fouling). This approach can be witnessed in clinically approved NP formulation whereby polymer, poly(ethylene glycol) has been included in the surface modification step (PEGylation) (*93*). While PEG is one of the most established hydrophilic additions to coating for NPs, reported issues surrounding its potential to produce systemically reactive anti-PEG antibodies have led researchers to investigate alternatives. Zwitterionic amino lipids (*94*) and polyzwitterions (PZPs) have received notable attention in this respect. PZPs display excellent resistance to fouling due to their ability to form stable hydration layers through electrostatic interactions with water

molecules. They achieve this through having side chains containing both a positively charged group (amino group or a quaternary ammonium group found in betaines) and a negatively charged (carboxylate, phosphoryl, or sulfonate) group. Other approaches have included sulfoxide-containing polymer poly(2-(methylsulfinyl)ethyl acrylate) (*95*) and polyoxazoline (*96*) surface coatings to excellent, albeit preclinical effect.

Meanwhile, hydrophobicity is suggested to promote more effective interactions with cell membranes and, through this, to facilitate greater endocytosis or transcytosis, potentially aiding BBB crossing. However, excessive hydrophobicity also promotes rapid clearance and aggregation and, potentially, increases toxicity. Fluoropolymer inclusion is an established way of creating more hydrophobic NPs. Hydrophobicity stems from strong carbon-fluorine bonds that make them stable, biocompatible, and, through reduction in fouling, less immunogenic (*97*). Collectively, this has made fluoropolymers a popular addition during NP development aimed at cancer diagnostics (*98*), drug delivery (*99*), and especially temporary gene therapies aimed at pediatric brain cancer (*100*, *101*). Notably, the positioning of the fluoropolymer chain on the NP is considered an important consideration in the NPs ability to effectively deliver siRNA in group 3 MB (*101*). Additionally, fluoropolymers appear to be important additions to innovative CRISPR-Cas therapies in GBM (*102*).

Inspired through nature (biomimicry), approaches have been gaining traction in attempts to enhance biocompatibility. More transparent and united alliances between biologists and chemists have led to the creation of polyzwitterionic polymers that mimic phosphoserines (PSs), apoptotic signaling lipids that translocate to the outer surface of the cell membrane in dying cells. PS serves as signals for phagocytic clearance, but, additionally, PS also modulates phagocytic cells, generating immune tolerance. These characteristics have made them an effective coating substance for reducing fouling and immunomodulatory effects and particularly interesting for delivery of an immunogenic payload (*103*). Another nature-inspired idea is the use of cell membranes as camouflage for systemic delivery of NPs in a bid to improve circulatory times, targeted delivery, and immune evasion (*104*). Red blood cells (*105*), platelets (*106*), mesenchymal stem cells (*104*), and cancer cells (*107*) are all considered viable options for this approach.

Functional groups and targeting moieties

Functional groups and targeting moieties not only substantially influence crossing of the BBB and targeting pediatric cancer cells (*108*), particularly through the RMT route, but also play a role in improving stability in circulation, reducing recognition and clearance by the immune system, and facilitating targeted delivery to brain tissues. The inclusion is, much like all other engineering strategies, a balancing act. While a high density increases the likelihood of cell uptake, when density is too high, the diffusion coefficient decreases and endocytic mediator clathrin can be obstructed (*109*).

Receptors actively targeted for RM-NPs aimed at drug and permanent gene intervention approaches include transferrin, insulin receptor, low-density lipoprotein receptor–related protein 1, lactoferrin receptor, and angiopep-2 (*26*, *110*). Transferrin receptors (TfRs) are especially attractive targets, particularly transferrin receptor 1 (*111*). TfRs are in abundance on the surface of BBB endothelial cells and routinely overexpressed by an order of magnitude in aggressive pediatric brain tumors relative to healthy cells, making them an excellent target for RMT and tumor targeting combined. Liposomes and polymeric and inorganic NPs have all been modified with targeting moieties (antibodies, ferritin, and targeting peptides), aimed at RMT through TfRs. For instance, engineered liposomes functionalized with amphiphilic-cationic-cell–penetrating peptide-penetratin (Tf-Pen) peptide were developed by Lakkadwala *et al.* (*112*), with doxorubicin and erlotinib encapsulated within. Tf-Pen liposomes demonstrated ~12- and 3.3-fold increase in doxorubicin and erlotinib accumulation in the brain of mice with orthotopic GBM tumors relative to free drug. Moreover, by exploiting transferrin targeting, Lakkadwala *et al.* were able to use negatively charged NPs and take advantage of the longer circulatory times afforded by the charge. Despite the widespread interest in TfR targeted NPs, challenges remain to be met before delivering on the promise for improving NPs designed to target pediatric brain cancers. Specifically, these include the expression of transferrin in healthy tissues that could instigate off-target toxicity and potential for over saturation leading to BBB damage (*113*).

Meanwhile, adopting an alternative receptor-mediated approach, a recent MB study used NPs to disrupt intracellular cholesterol regulation, which is implicated in activating the SHH pathway. Using microfluidic technology, Kim *et al.* (*114*) developed high-density lipoprotein-mimetic NPs (eHNPs) with apolipoprotein A1 (Apo1) and anti-CD15, a carbohydrate antigen found on SHH-MB cells as targeting moieties and SHH inhibitor, LDE225, encased within the hydrophobic core (*115*). Using SmoA1 mice crossed with Math1–green fluorescent protein (GFP) to generate homozygous SmoA1$^{+/+}$: Math1-GFP$^{+/+}$ strain that spontaneously develops SHH-MB within ~4 to 6 months with an intact BBB, Kim *et al.* were able to demonstrate that eHNPs cross the BBB following systemic delivery using Apo1 to target scavenger receptor class B type 1. However, through addition of anti-CD15, internalization increased fourfold compared with NPs with Apo1 as a single targeting moiety. Moreover, CD15 effectively targeted CSC populations, as identified through NP presence in CSC niches within brain tumor tissue.

Stimuli responsiveness. Stimuli-responsive NPs are engineered to preserve their structure during circulation only releasing their payload or effect when exposed to one or more specific stimuli. Such stimuli include temperature (*116*), pH (*117*), redox (*118*), enzymes (*119*), ultrasound (*120*), and magnetic field and light (*121*). This is an attractive approach for brain cancers, offering a more targeted effect and safer systemic delivery as demonstrated by Mansur *et al.* (*122*), who developed peptide-functionalized carboxymethylcellulose conjugated with a fluorescent Ag-In-S semiconductor quantum dot core. Polyamidoamine (PAMAM) dendrimers, meanwhile, can release a cargo through pH changes, whereby an acidic microenvironment leads to protonation of amino groups that facilitate the controlled release of a cargo (*39*). β-Cyclodextrin (β-CD)–coated PAMAM-entrapped gold NPs Au (DENPs-β-CD) delivering two therapeutic siRNA, B cell lymphoma/leukemia-2 (BCL-2) siRNA (siBCL-2) and vascular endothelial growth factor (VEGF) siRNA (siVEGF), were recently explored by Qiu *et al.* (*123*). Their findings for DENPs-β-CD delivery of siBCL-2 and siVEGF are promising and may warrant further investigation in DIPG following studies demonstrating the promising effects of inhibiting BCL-2 (*124*) and VEGFR (*125*).

Chemodynamic therapy is an NP-only approach that uses NPs made from transition metals (iron oxide, manganese II, and copper) to catalyze hydrogen peroxide (H_2O_2) into hydroxyl radicals through a Fenton or Fenton-like reaction. These highly reactive and toxic species benefit from low cost, and pH responsiveness. While this is an elegant approach that leans into TME conditions well, its effectiveness

relies on sufficient H_2O_2 concentration, a reduction in pH, and high levels of glutathione. Attempts to overcome these potential shortfalls include the production of copper peroxide and calcium peroxide. Nevertheless, some of the most promising studies have come from combining this approach with chemotherapy, photothermal therapy, sonodynamic therapy, gene therapy, and immunotherapy (*126*, *127*). While not routinely observed within the pediatric cancer realm, this combination approach has been shown to be relatively safe and effective and, thus, could have substantial influence in future endeavor, especially if one nanoagent can be used for two independent attack strategies.

Mechanical properties

The mechanical properties of NPs encompass surface roughness, stiffness, and viscoelasticity, all of which play pivotal roles in modulating interactions between NPs and BBB. Virus-like or spiky NPs demonstrate a morphology of uniform nanospikes akin to the spikes found on viral particles. These spikes can lead to enhanced adhesion to endothelial cells, increasing the likelihood of RMT or transient opening of tight junctions and improving permeability across the BBB (*128*, *129*). In a recent study by Feng *et al.* (*129*) comparing smooth surface (60 nm) Stöber silica NPs with rough surface virus-like silica NPs (VSNP), VSNP showed greater cellular uptake and were more effective at opening tight junctions in in vitro cellular models. Nevertheless, how they perform in vivo remains to be seen, especially how they are affected by protein corona formation, immune surveillance, and clearance.

Viscoelasticity of NPs represents their ability to exhibit both liquid-like (viscous) and solid-like (elastic) response to deformation (*130*). Viscosity describes a material's tendency to undergo time-dependent, permanent deformation when subjected to a constant stress, resulting in continuous flow without shape recovery upon stress removal (*130*). In contrast, elasticity refers to recoverable deformation and is commonly represented as stiffness in the context of nanomedicine. Many studies adopt stiffness as a simplified descriptor of viscoelastic behavior, primarily because it is more readily measurable (e.g., via atomic force microscopy). Stiffness can be reported as a static value, the Young's modulus, although stiffness alone does not capture time-dependent deformation (*92*).

Stiffness is a critical parameter influencing NP biological behavior (*131*). Soft NPs are suggested to exhibit prolonged blood circulation compared with rigid counterparts and, due to greater deformation of their shape, produce a large contact area that facilitated better adhesion to the cell membrane (*132*). Additionally, mechanobiological interactions with the mononuclear phagocyte system in combination with enhanced deformability under macrophage-induced forces reduce mechanotransduction cues that are necessary for efficient phagocytic uptake, diminishing sequestration and enhancing systemic retention (*131*). Further, decreasing NP elasticity has been shown to attenuate recognition and uptake by macrophages, facilitating increased accumulation within the TME (*133*). Intratumorally, NPs with elastic properties are said to exhibit superior penetration afforded through their ability to use deformation to traverse narrow intercellular spaces and infiltrate deeper into tumor cores. Meanwhile, stiff NPs may be restricted to peripheral regions of the tumor (*133*).

When considering the effect of stiffness on BBB penetration and cancer cell uptake, Robles-Fernández *et al.* (*134*) demonstrated that soft LNPs enhanced translocation across the BBB in vitro, while rigid LNPs showed higher internalization and treatment efficacy by GBM cells (U87MG). These contrasting findings highlight how the different cell NP gene therapies encounter in vivo may respond differently to the relative stiffness of NPs and may adopt different pathway mechanisms for uptake. This is demonstrated by Guo *et al.* (*135*), who suggest that soft NPs are mostly taken up via fusion while stiff NPs favor clathrin-mediated uptake.

Challenges for post-BBB brain and tumor distribution

For brain cancer treatment, NP-based gene therapy can be delivered systemically or locally (intracerebroventricular, intrathecal injection, and intranasal administration). Although local delivery may partially bypass the BBB, these approaches can be more invasive and may have limitations in achieving adequate and homogeneous distribution throughout tumors (*136*). They may also require specialized procedures and expertise, limiting their practicality for repeated treatment. Consequently, systemic delivery remains an important strategy in preclinical NP development, and BBB remains the major barrier for brain cancer treatment.

Crossing the BBB represents only the first step in effective NP-mediated gene delivery to the brain. Following entry into the CNS, NPs must achieve adequate distribution throughout the brain parenchyma and, in the case of brain tumors, penetrate the heterogeneous TME to reach sufficient numbers of target cells. This represents a distinct challenge from BBB traversal, and effective NP-mediated gene delivery is also restricted by the transport of nucleic acid through extracellular matrix and into the cell, including cellular uptake, intracellular trafficking and endosomal escape, NP degradation, and nucleic acid release, as reviewed by Yang *et al.* (*137*). These processes are strongly influenced by NP physicochemical properties, including size, morphology, surface charge, composition, and surface chemistry. Thus, optimal nanocarrier design for brain gene therapy requires consideration of both BBB transport and post-BBB tissue distribution, although the present review focuses primarily on the strategies for overcoming the BBB as it is the major challenge to systemic CNS delivery.

PRECLINICAL INVESTIGATIONS OF IN VIVO GENE THERAPIES DEVELOPED FOR CHILDREN'S BRAIN CANCERS

Developing clinically viable gene therapies for pediatric brain cancers remains a major challenge, yet the potential to boost immune defense or precisely silence or eliminate the genetic drivers of these aggressive tumors presents clear advantages over existing treatments. Specificity toward a target is one of the greatest attributes that gene therapy has relative to pharmaceutical drugs, offering the ability to effectively target individual cancer driving mutations or pathways and minimizing damage to healthy brain tissue.

While not without side effects, radiation therapy remains a critical and effective component in the treatment of many pediatric patients with brain cancer (>3 years of age); however, cancer cells can become resistant to radiation therapy over time. Radiation therapy works by initiating oxidative free radical formation that damages the cell's DNA. DNA lesions (abasic sites) are created alongside fragmentation of deoxyribose that blocks any further replication within the cell (*138*). Resistance to radiation therapy lesion formation is through activity of DNA repair enzyme, apurinic/apyrimidinic endonuclease 1 (Ape1), that recognizes and repairs abasic sites. Ape1 is routinely up-regulated in pediatric malignancies, MB, and EP; therefore, switching off Ape1 presents a way of improving the anticancer activity of radiation, which is a method explored by Liu *et al.*

(*139*). Gold NPs were coated with PEG, chitosan, and Au-CP-PEI. Chitosan provided covalent attachment sites for PEG (hydrophobicity) and polyethyleneimine, which provided a cationic charge for electrostatic binding of siRNA-targeting Ape1. Liu *et al.* demonstrated effective gene silencing of Ape1 and subsequent suppression of abasic lesion repair, thereby diminishing resistance to radiation therapy in MB cells.

Poly(beta-amino ester) (PBAE) is suggested to contain cell-type–specific properties and thus able to selectively transfect tumor tissue over healthy tissue in the brain (*140*). Choi *et al.* (*141*) used this property for their recent pediatric brain cancer gene therapy study. A panel of PBAE-NPs was developed via Michael addition reactions between amines and diacrylates to deliver a DNA plasmid for cancer herpes simplex virus I thymidine kinase (HSVtk). The PBAE-NPs contained primary, secondary, and tertiary amines with hydrolytically cleavable ester bonds that allowed for effective DNA binding and encapsulation resulting in complex formation (PBAE-HSVtk) of between 100 and 200 nm. Pediatric brain tumor cell lines, rhabdoid tumor cells (BT-12) and MB cells (D425), were transfected with PBAE-HSVtk in vitro and evaluated, and the most effective was adopted for further investigation in vivo in orthotopic mouse xenograft models of BT-12 and D425, respectively. Applying convection-enhanced delivery that uses a pressure gradient to directly infuse medication into the brain tissue, Choi *et al.* (*141*) were able to deliver 2 μg of the DNA plasmid per transfusion (total of 3). Using Mantel-Cox log-rank tests, both rhabdoid tumor and MB mice showed significantly increased survival (20 and 63%, respectively), which is a particularly impressive achievement for D425 tumors given the aggressive nature of this group 3 MB model. Through developing different PBAEs, the results elegantly demonstrate that subtle changes such as the monomer distribution in PBAE polymer structure can lead to substantial alterations in both uptake and transfection efficiency.

This actuality was also identifiable in our recent MB gene therapy study where we observed that the topology of hydroxy-terminated perfluoropolyether containing polymers within the polymer unit also containing dimethylaminoethyl acrylate (DMAEA) and oligoethylene glycol acrylate (OEGA) monomers had a substantial impact on self-assembly and was essential to the successful delivery of therapeutic siRNA for *Polo-like kinase* (*Plk1*) to D425 cell in vitro and in an orthotopic D425 tumor model (*101*). In contrast to Choi *et al.*, we opted for a hybrid, theranostic NP platform by wrapping 3-nm iron oxide NPs with a polymer coat that allowed binding of siPLK1 through electrostatic interaction with DMAEA and used OEGA as a hydrophilic, antifouling alternative to PEG-PFPE adding hydrophobicity for enhanced cellular uptake. Fluoropolymer-engineered iron oxide NPs (FNPs) showed an excellent pharmacokinetic profile, demonstrated T_2 enhancement to orthotopic tumors, and were able to slow tumor growth following regimes consisting of 3-day consecutive and 8-day, every other day, systemic administration FNP-siPLK1 (10 and 3 mg/kg, respectively). However, we failed to eliminate tumors, suggesting that either siPLK1 therapy was not powerful enough as a single agent to destroy every cell or that FNP-siPLK1 complexes were not able to cross in sufficient quantities to elicit the desired therapeutic effect. However, pairing complementary approaches could provide a way of enhancing the benefits of lowly toxic temporary gene therapy approaches, which is potentially explored by Guo *et al.* (*142*).

Guo *et al.* (*142*) synthesized lipid-polymer hybrid NPs to deliver SMO-targeting siRNA to transgenic SHH-MB mice (SmoA1-Math1) that spontaneously develop tumors and maintain an intact BBB. To maximize BBB penetration, they paired this gene therapy with a custom-built magnetic resonance–guided FUS instrument able to temporarily open the BBB, showing >10-fold increase in delivery to the FUS-targeted brain areas of immunocompetent mice without notable adverse effects (*142*).

CHALLENGES AND FUTURE DIRECTIONS

In terms of NP design and development, although considerable effort has been placed on establishing the most favorable physicochemical properties of NPs for BBB penetration and gene therapeutics delivery to pediatric brain cancers, we are yet to yield a viable contender. NP carrier design may remain tumor-subtype agnostic at the level of overcoming shared delivery barriers of brain tumors. However, existing studies typically evaluate engineered NP systems in a single brain tumor model, with promising therapeutic efficacy demonstrated within that specific model, which may limit their broader applicability and clinical translation. Further efforts should, therefore, prioritize the development of NP platforms with broader applicability across pediatric brain tumor subtypes, which could facilitate scalable manufacturing, streamline clinical development, and reduce the complexity associated with developing an entirely separate NP formulation for each molecular subtype.

BBB integrity is heterogeneous across the brain and among tumor types and molecular subgroups, which further complicates the development of a broadly applicable NP system. To address this challenge, exploring noninvasive delivery strategies for NP-mediated gene therapeutics that can bypass BBB, such as intranasal delivery, could be a promising alternative method. The safety considerations are another challenge during the development of these systems. NPs used for gene delivery can be broadly categorized into inorganic (e.g., gold and iron oxide) and organic (e.g., polymeric and LNP) systems. Although LNP-based systems have achieved clinical translation, inorganic NPs remain subject to additional safety and translational concerns, particularly regarding clearance and potential long-term accumulation. For example, Soenen *et al.* (*143*) evaluated multiple parameters to assess the safety of IONP formulations in vitro, highlighting that cytotoxicity can arise through diverse mechanisms and varies widely between formulations. This finding underscores the challenges associated with the clinical translation of inorganic NPs, although numerous inorganic NPs have achieved preclinical success in treating pediatric brain tumors. The therapeutic genetic modality itself represents another important safety consideration, particularly in pediatric patients with a developing and highly plastic brain. Temporary gene modulation (e.g., siRNA, ASO, and mRNA based) does not intentionally introduce permanent changes to the genomic DNA and, therefore, generally reduces concerns associated with irreversible genomic alteration. However, these approaches are not risk free and may still cause off-target effects, unintended changes in gene expression or innate immune activation. Because their effects are transient and diminish as the therapeutic nucleic acid is degraded, repeated doses may be required to maintain therapeutic activity. In pediatric patients, ongoing cellular development and turnover may further influence the duration and consistency of therapeutic effects. Repeated administration may consequently introduce additional concerns like immunogenicity. Permanent genome-editing approaches, in contrast, offer the potential for durable therapeutic effects but introduce greater concerns regarding irreversible genomic alterations. Unintended off-target editing or unintended on-target genomic consequences in

nontarget cells could result in persistent biological effects that cannot readily be reversed. These concerns are particularly relevant in pediatric patients because the brain continues to undergo substantial structural and functional maturation during development. Unintended genomic alterations in developing neural cells could potentially affect neuronal maturation, connectivity, or circuit function, with adverse neurological, cognitive, motor, or behavioral deficits that may not manifest until years later. Therefore, prolonged posttreatment monitoring is necessary for NP-mediated gene therapies.

While numerous preclinical NP studies have been focused on improving brain cancer treatment, most of these studies have focused on adult GBM, and research based specifically on pediatric brain tumors remains relatively modest, especially regarding gene therapies. This limitation is largely attributed to smaller patient numbers in comparison to adult cases. In addition, pediatric brain tumors often exhibit distinctly different biological and molecular characteristics compared with their adult counterparts. Pediatric brain tumors frequently arise in the lower parts of the brain, including the cerebellum and brainstem, whereas adult tumors are more prone to develop in the upper regions. These anatomical differences mean that NP therapies optimized for adult tumors may not achieve comparable efficacy in pediatric brain cancers. Addressing this disparity requires increased preclinical investigation using pediatric brain tumors, including molecularly characterized pediatric tumor cell lines and relevant in vivo models, to identify and explore the biological determinants of NP delivery and therapeutic response in pediatric brain tumors. Given the limited availability of pediatric tumor specimens, coordinated international collaboration and data sharing should be encouraged to maximize the scientific value of limited biopsy samples and enable more comprehensive molecular and functional characterization of pediatric brain tumors. Nanomedicine researchers could also collaborate closely with clinical specialists (e.g., pediatric neurosurgery, pediatric neuro-oncology, and pediatric neuropathology) to improve understanding of pathological, molecular, and cellular features that can inform NP design.

An additional issue lies in effective disease modeling, although now no single model can fully replicate human tumors and the complex growth environment that they exist and thrive in. Most preclinical studies investigating NP-delivered gene therapies rely on 2D tumor cell models, which primarily capture cell-intrinsic responses to the therapeutic cargo but do not adequately reproduce the multistep barriers governing NP delivery in vivo. Consequently, therapeutic responses observed in 2D models may not translate to animal models, where NP-delivered gene therapeutics must undergo systemic distribution and clearance, BBB penetration, intracellular trafficking, and endosomal escape and, ultimately, be released at a sufficient concentration to achieve target engagement. Future studies should use more age-appropriate mouse models and prioritize patient-derived 3D organoids. Such models can more faithfully recapitulate tumor architecture, cellular heterogeneity, and cell-cell and cell-matrix interactions, thereby providing more predictive platforms for evaluating NP-delivered gene systems and generating more robust preclinical evidence to support subsequent clinical investigation and translation.

To date, there are no registered clinical trials involving nanoenabled gene therapies for pediatric brain tumors. While this could be related to ethical constraints and recruitment challenges, it is more likely due to a lack of robust preclinical data supporting progress toward trials. Adopting the methods described and establishing well-defined translational criteria for NP development could help bridge the gap more effectively between preclinical investigations and clinical trials and encourage greater success rates.

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