

Advances and Challenges in Gene Therapy Methods for Central Nervous System Disorders: A Comprehensive Review

Fozilova Sarvinoz ¹, Davronov Sobirjon ², Kabulov Sarvarbek ³, Adilov Abdujabbor ⁴, Abduraimov Kamronbek ⁵

^{1,2,3,4,5} KIMYO International University in Tashkent, Uzbekistan.

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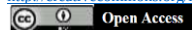
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ABSTRACT

Central nervous system (CNS) disorders of genetic origin remain largely incurable with conventional pharmacotherapy, which in most cases offers only symptomatic relief. Gene therapy aims instead at durable correction of the underlying molecular defect, but its application to the CNS is constrained by the post-mitotic nature of neurons, the architectural complexity of neural circuits, and the blood-brain barrier (BBB). This review summarises the current methodological landscape of CNS gene therapy, compares the available delivery paradigms and vector systems, and identifies the principal translational barriers that still limit clinical adoption. A narrative review of peer-reviewed original studies, clinical trial reports, regulatory approval documents and authoritative reviews published between 2009 and 2024 was performed. Sources were retrieved from PubMed, Scopus and Web of Science using combinations of the keywords listed below, and were organised according to a three-part analytical framework based on the route of vector delivery: ex vivo, in vivo and in situ. Lentiviral ex vivo modification of autologous haematopoietic stem cells has produced durable neurological stabilisation in X-linked adrenoleukodystrophy and metachromatic leukodystrophy through microglial repopulation and enzymatic cross-correction. Adeno-associated virus (AAV) vectors dominate in vivo delivery and underpin the two approved CNS-directed products, onasemnogene abeparvovec and eladocogene exuparvovec, whereas in situ delivery remains largely confined to neuro-oncology. Engineered capsids and non-viral carriers improve BBB traversal, while CRISPR-Cas9, base editing and prime editing extend the field from gene augmentation towards targeted genomic correction. Dose-dependent hepatotoxicity, complement activation, dorsal root ganglion toxicity, pre-existing anti-capsid immunity and insertional mutagenesis remain the dominant safety signals. CNS gene therapy has become a clinical reality for a small number of monogenic diseases. Its broader adoption depends less on the editing chemistry itself than on solving delivery, immunogenicity, long-term safety surveillance and cost-related equity problems.

1. Introduction

Gene therapy is among the most rapidly maturing areas of molecular medicine, offering potentially curative strategies for severe hereditary, congenital and acquired diseases through direct modification of the genetic apparatus. Over the past three decades, progress in functional genomics, viral vector engineering and gene-editing platforms has converted theoretical genetic concepts into licensed clinical interventions.

Disorders of the central nervous system pose a distinctive set of obstacles. Neurons are post-mitotic, so a therapeutic construct must persist for the lifetime of the cell rather than being amplified by division; neural circuits are anatomically complex, so partial or spatially restricted correction may be clinically insufficient; and the blood-brain barrier excludes most systemically administered macromolecules. Where conventional pharmacotherapy fails or provides only palliative benefit, gene therapy seeks durable phenotypic correction at the molecular cause of the disease.

Despite two regulatory approvals and a broad preclinical pipeline, the field still lacks a consolidated methodological comparison of the available delivery routes together with their respective safety profiles. The present review therefore pursues three aims: (i) to classify and compare the delivery paradigms used in CNS gene therapy; (ii) to summarise the vector systems and BBB-crossing strategies on which they depend; and (iii) to define the immunological, ethical and economic constraints that currently limit clinical translation.

2. Materials and Methods

This work is a narrative review of the published literature; no experimental work involving human participants or animals was performed, and no ethical approval was therefore required.

Search strategy. The PubMed, Scopus and Web of Science databases were searched for publications issued between 2009 and 2024. The search combined the terms "gene therapy", "central nervous system", "adeno-associated virus", "lentiviral vector", "blood-brain barrier", "genome editing", "CRISPR", "leukodystrophy" and "spinal muscular atrophy" using Boolean operators. Reference lists of the retrieved reviews were screened manually for additional relevant sources, and the records of the European Medicines Agency and the United States Food and Drug Administration were consulted for approval data.

Selection criteria. Publications were included when they reported original clinical or preclinical data on gene transfer to the CNS, described vector engineering or delivery technology relevant to the CNS, or provided authoritative synthesis of these topics. Publications were excluded when they addressed gene therapy in non-neural tissues only, when they were available as conference abstracts without full text, or when the described methodology could not be assessed from the report.

Analytical framework. The retrieved material was organised according to the route of vector delivery and the dynamics of target-cell transduction, which yields three operational paradigms: *ex vivo*, *in vivo* and *in situ*. Within each paradigm the evidence was examined for vector type, target cell population, therapeutic mechanism, reported clinical outcome and documented adverse events. Cross-cutting themes of vector biology, BBB traversal, genome-editing technology, safety and ethics were then analysed across all three paradigms.

3. Results

3.1. Ex vivo gene therapy

In the *ex vivo* paradigm, autologous somatic cells of the patient — most commonly haematopoietic stem cells (HSCs) or progenitor populations — are harvested, isolated and cultured outside the body. Functional transgenes are introduced by retroviral or lentiviral vectors, and the modified cells are expanded, subjected to quality assurance and re-infused after a conditioning regimen.

Lentiviral HSC gene therapy has proved effective in neurodegenerative metabolic disease of the CNS. In X-linked adrenoleukodystrophy (X-ALD), a fatal demyelinating disorder caused by mutations in *ABCD1* encoding a peroxisomal ATP-binding cassette transporter required for the beta-oxidation of very-long-chain fatty acids, re-infusion of autologous HSCs transduced with lentiviral *ABCD1* produced durable neurological stabilisation (Cartier et al., 2009). The corrective mechanism depends on physiological migration of circulating monocyte progeny across the BBB and their differentiation into tissue-resident microglia expressing functional ABCD1 protein.

The same approach was extended to metachromatic leukodystrophy (MLD), an autosomal recessive lysosomal storage disorder arising from mutations in the arylsulfatase A (*ARSA*) gene. The infantile and juvenile forms feature progressive demyelination, motor decline, cognitive deterioration and early death driven by sulfatide accumulation in oligodendrocytes, microglia and neurons. Transplantation of transduced HSCs in pre-symptomatic infants achieved cross-correction through

secretion of the functional enzyme, prevented neurodegeneration and preserved motor and cognitive development well beyond the age of onset recorded in untreated siblings.

3.2. *In vivo* gene therapy

In vivo gene therapy administers the genetic vehicle directly to the patient by the intravenous, intrathecal or intraparenchymal route, after which the vector transduces CNS cell populations systemically or locally. Adeno-associated virus (AAV) vectors have become the principal vehicle for this purpose because they are non-pathogenic, integrate into the host genome only minimally, and sustain episomal expression in post-mitotic neurons.

Early clinical trials targeted localised monogenic disorders, including Parkinson's disease, late-infantile neuronal ceroid lipofuscinosis and Canavan disease, using first-generation AAV2 vectors delivered by stereotactic intraparenchymal injection. These studies established reliable expression within the targeted nuclei, such as the striatum, but transgene distribution remained restricted to the vicinity of the injection tract. The resulting focal expression pattern was adequate for Parkinson's disease yet clearly insufficient for diffuse neurodegenerative conditions affecting extensive brain regions.

3.3. *In situ* gene therapy

In situ delivery is a localised subclass of the *in vivo* paradigm in which the construct is deposited directly into a defined lesion, tumour mass or discrete neural structure. Its application requires two conditions: direct anatomical accessibility of the target tissue, and high transduction specificity of the vector for the local host cells. The approach is used mainly in neuro-oncology, for example in glioblastoma multiforme, where suicide genes such as herpes simplex virus thymidine kinase or immunomodulatory cytokines are introduced into the surgical resection cavity.

3.4. Vector biology and delivery across the blood-brain barrier

Vector selection is a decisive determinant of outcome. Recombinant AAVs are preferred for neuronal targeting because of their low cytotoxicity and long-term episomal persistence, and individual capsids display distinct tropisms: AAV1 and AAV2 show strong neuronal affinity, whereas AAV8 and AAV9 transduce both neurons and macroglia. Engineered variants such as AAV-PHP.B and AAV-PHP.eB cross the BBB efficiently after systemic intravenous administration in animal models, which has substantially advanced non-invasive delivery (Deverman et al., 2018).

Non-viral platforms — lipid nanoparticles, polymeric nanocarriers and lipid-protein complexes — attract increasing interest because of their essentially unlimited cargo capacity, the absence of pre-existing anti-capsid neutralising immunity and lower manufacturing cost at scale. For both vector classes the tight junctions of the brain microvascular endothelium remain the principal obstacle. Reported strategies for traversal include functionalisation of the carrier surface with ligands for receptor-mediated transcytosis, such as anti-transferrin receptor antibodies, and transient disruption of endothelial tight junctions by magnetic-resonance-guided focused ultrasound in combination with microbubbles.

3.5. Approved therapies and the clinical pipeline

Two products define the current regulatory landscape. Onasemnogene abeparvovec, an intravenous AAV9 vector carrying a functional *SMN1* transgene, is approved for spinal muscular atrophy; a single administration restores survival motor neuron protein expression, prevents alpha motor neuron loss and alters the clinical course of an otherwise fatal infantile neuromuscular disease (Mendell et al., 2017). Eladocagene exuparvovec, an AAV2 vector delivered by stereotactic intraputamenal infusion, received European approval for aromatic L-amino acid decarboxylase deficiency; it delivers a functional *DDC* gene to striatal neurons and thereby restores dopamine synthesis and motor function. Further candidates in development address Huntington's disease, amyotrophic lateral sclerosis (ALS) and lysosomal storage disorders using either gene silencing, by microRNA or antisense oligonucleotides, or gene augmentation.

3.6. Genome-editing platforms

Precision genome editing extends the field beyond gene augmentation towards targeted correction. The CRISPR-Cas9 platform uses single guide RNAs to induce double-stranded breaks at defined loci, permitting gene knockout or homology-directed repair, and thus allows silencing of dominant toxic alleles such as mutant huntingtin in Huntington's disease or *SOD1* mutations in familial ALS.

To avoid the genomic instability associated with double-stranded breaks, base editing and prime editing have been developed. Base editors combine Cas9 nickases with cytidine or adenosine deaminases and install precise single-nucleotide transitions (C to T, or A to G) without cleaving both DNA strands. Prime editing employs a reverse transcriptase-guide RNA complex and enables arbitrary insertions and deletions as well as all twelve possible base conversions (Anzalone et al., 2020). Both platforms offer, in principle, permanent and scarless correction of hereditary neurodegenerative disease, although CNS delivery of the editing machinery remains the limiting step.

3.7. Immunogenicity, safety and toxicity

The reported risks are consistent across programmes. High systemic doses of viral vectors, particularly intravenous AAV9, can provoke severe systemic inflammatory responses, complement activation, acute hepatotoxicity and dorsal root ganglion toxicity presenting as sensory neuropathy. Pre-existing neutralising antibodies against AAV capsids exclude a proportion of otherwise eligible patients and preclude re-administration of the same vector.

For integrating vectors such as retroviruses and lentiviruses, insertional mutagenesis is an additional concern, since vector insertion near proto-oncogenes may cause genomic instability or malignant transformation. Mitigation strategies reported in the literature include engineering of immune-evading capsids, the use of tissue-specific promoters such as synapsin-1 for neuron-restricted expression, and transient immunosuppression before vector administration.

4. Discussion

Comparison of the three paradigms indicates that the choice of delivery route is dictated primarily by disease biology rather than by technical preference. The *ex vivo* approach succeeds in the leukodystrophies because the pathology is metabolic and cell-autonomous correction is not required: microglia derived from transduced HSCs act as a distributed source of the missing enzyme, and cross-correction compensates for incomplete transduction of the neural parenchyma. Where the defect must be corrected within the affected neuron itself, this mechanism is unavailable and *in vivo* delivery becomes necessary.

The *in vivo* results in turn show that the field's central constraint is spatial rather than molecular. Stereotactic AAV2 delivery reliably produces expression, but only near the injection tract, which explains why the approved intraparenchymal product treats a disorder with a well-defined anatomical target while diffuse conditions remain untreated. Engineered BBB-penetrant capsids and ultrasound-assisted opening of the barrier address precisely this limitation, and their translation is arguably more consequential for the field than further refinement of the editing chemistry itself. The same logic applies to genome editing: base and prime editing solve the problem of precision, not the problem of reaching the target cell.

An unresolved tension runs between efficacy and safety. Broad CNS transduction after systemic administration requires high vector doses, yet dose is also the main driver of hepatotoxicity, complement activation and dorsal root ganglion toxicity. Cell-type-restricted promoters, immune-evading capsids and localised routes of administration are therefore not merely technical refinements but the mechanism by which the therapeutic window is widened. The ethical and economic dimension is inseparable from the scientific one. Approved gene therapies frequently cost between two and three million United States dollars per patient dose, which creates substantial inequity in access and places a heavy burden on health

systems. Additional considerations include the distinction between somatic and germline modification, the need for safety monitoring over decades rather than years, the difficulty of obtaining informed consent in paediatric populations with fatal disease, and the absence of regulatory harmonisation across jurisdictions. Transparent legal frameworks and international coordination are required if rapid innovation is to be reconciled with rigorous patient protection.

Limitations. This review is narrative rather than systematic: the search was not registered, no formal risk-of-bias instrument was applied, and no quantitative synthesis was attempted. The literature itself is biased towards positive outcomes in small, often single-arm cohorts of rare diseases, and published follow-up is short relative to the intended lifelong duration of effect. The findings summarised here should therefore be read as an account of the direction of the field rather than as an estimate of effect size.

Future directions. The most informative next steps are likely to be comparative trials of BBB-penetrant capsids in humans, standardised reporting of anti-capsid seroprevalence, validated strategies for vector re-administration, and long-term registries capable of detecting delayed oncogenic or immunological events.

5. Conclusion

Gene therapy for CNS disease has moved from an experimental concept to a validated clinical modality capable of altering the course of previously intractable disorders. Sophisticated viral capsids, non-viral carriers and precise gene-editing tools now allow intervention at the genetic level of the disease mechanism. The remaining obstacles, however, are predominantly those of delivery across the blood-brain barrier, control of vector immunogenicity, demonstration of long-term safety and resolution of cost-related inequity. Progress will depend on sustained collaboration between molecular biologists, neurosurgeons, bioengineers and regulatory authorities.

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