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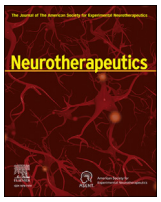

Review

Neurosurgical gene therapy for central nervous system diseases

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ABSTRACT

Viral vector mediated gene therapies for neurodegenerative and neurodevelopmental conditions that require neurosurgical administration continue to expand. We systematically reviewed the National Institutes of Health (NIH) ClinicalTrials.gov database to identify all clinical trials studying in-vivo viral vector mediated gene therapies targeted to the CNS for neurodegenerative and neurodevelopmental diseases. We isolated studies which delivered therapies using neurosurgical approaches: intracisternal, intraventricular, and/or intraparenchymal. Clinical trials primarily registered in international countries were included if they were referenced by an NIH registered clinical trial. We performed a scoping review to identify the preclinical studies that supported each human clinical trial. Key preclinical and clinical data were aggregated to characterize vector capsid design, delivery methods, gene expression profile, and clinical benefit. A total of 64 clinical trials were identified in active, completed, terminated, and long-term follow-up stages. A range of CNS conditions across pediatric and adult populations are being studied with CNS targeted viral vector gene therapy, including Alzheimer's disease, Parkinson's disease, AADC deficiency, sphingolipidoses, mucopolysaccharidoses, neuronal ceroid lipofuscinoses, spinal muscular atrophy, adrenoleukodystrophy, Canavan disease, frontotemporal dementia, Huntington's disease, Rett syndrome, Dravet syndrome, mesial temporal lobe epilepsy, and glutaric acidemia. Adeno-associated viral vectors (AAVs) were utilized by the majority of tested therapies, with vector serotypes, regulatory elements, delivery methods, and vector monitoring varying based on the disease being studied. Intraparenchymal delivery has evolved significantly, with MRI-guided convection-enhanced delivery established as a gold standard method for pioneering novel gene targets.

Introduction

Gene therapy, which broadly refers to the delivery of genetic material to alter a biological function, has grown to encompass a wide range of disease targets, gene products, and interventional platforms [1,2]. As the genetic bases underlying the pathogenesis and morbidity of neurological diseases are delineated, interest in developing clinically viable gene therapies continues to increase [3]. Across pediatric and adult central nervous system (CNS) disorders, there has been dramatic growth in the development of therapies which can introduce, modify, or silence a specific gene target [4,5]. The implementation of these therapies is inextricably linked to the delivery vehicles used to introduce genetic material, known as vectors. Both non-viral and viral vectors have been studied with in vivo and ex vivo configurations, all with distinct benefits and drawbacks [6,7]. Viral vectors, such as adeno-associated viruses (AAV), take advantage of the ability of viruses to enter a cell, enabling direct intracellular gene product delivery [8–10]. Modifications of viral vector subtypes, capsid protein shells, and administration routes have increasingly enabled more controlled tissue targeting and gene expression in the nervous system [11,12]. An example is the development of the only in vivo viral vector therapeutic approved for a neurological disorder in the United States, onasemnogene abeparvovec, an AAV9 based gene therapy for spinal muscular atrophy [13].

Despite preclinical and clinical data demonstrating the relative safety of these in vivo therapies across CNS pathologies, there have been challenges in clinical translation. In human clinical trials, the efficacy of viral vector gene therapies have not translated from animal studies in a completed trial [14–16]. Several hypotheses have been proposed to explain this finding, including differential patterns of human cell targeting, immune response to viral capsids, and variability in gene transduction efficiency [17,18]. In addition, limitations in viral vector packaging capacity, engineered transgenes, drug dosing, and manufacturing purification have been hurdles for in vivo therapeutic development [19–22]. Recent gene therapy pipelines have sought to

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address some of these challenges by deploying technologies like vector capsid engineering to increase tissue uptake and distribution, new gene products like short hairpin ribonucleic acids (shRNA) to overcome payload constraints, scalable production platforms for clinical grade viral-vectors, intra-operative magnetic resonance imaging (MRI) to improve therapy localization, and convection-enhanced delivery (CED) for greater target coverage [23–27]. Given these developments, we sought to examine viral vector gene therapies currently in human clinical trials for neurodegenerative and neurodevelopmental conditions delivered through surgical means. We focused on in vivo CNS targeted approaches, reviewing the strengths, challenges, and outcomes across delivery methods, vector constructs, and clinical trials. When available in the literature, we identified the precedent preclinical data that supported specific studies. Thus, this review seeks to serve as a entry resource for understanding the current translational landscape in the development and delivery of neurosurgical gene therapies.

Methods and Materials

We searched the National Institutes of Health (NIH) ClinicalTrials.gov database to identify all clinical trials testing a viral-vector based in-vivo gene therapy for neurodegenerative and neurodevelopmental conditions up to April 27, 2024. Trials were included if therapies were delivered using a surgical corridor: intracisternal, intraventricular, and/or intraparenchymal. Exclusion criteria were: 1) ex vivo gene transduction, 2) neuro-oncologic diseases, 3) isolated ophthalmic conditions, and 4) primary therapeutic target was not within the CNS. Additional clinical trials registered in international databases were included if they met inclusion/exclusion criteria and were referenced by an NIH registered clinical trial. Peer-reviewed publications of clinical trials were collected if available. Characteristics of each clinical trial were extracted, including location, enrollment, and patient populations studied. Therapies were assessed for viral vector capsid design, packaged gene product, delivery method, and clinical outcomes.

A scoping review was performed to identify preclinical animal model studies examining the vectors and gene products utilized in included clinical trials. Preclinical studies were included if cited by the clinical trial publication or within clinical trial reporting documentation for scientific evidence forming the rationale for human trials. Studies were selected if they tested the same vector-gene construct being utilized in the human clinical trial. Additional preclinical studies were also examined for novel vector and gene constructs, such as engineered capsids or packaged genetic material. We extracted viral vector kinetics, uptake pattern, target coverage, gene expression, and post-treatment symptom change from pre-clinical studies.

Clinical trials

Our search identified 64 clinical trials studying viral vector gene therapy for neurodegenerative or neurodevelopmental indications (Fig. 1A). A list of included trials with corresponding vector constructs can be found in Supplement 1. The two neurological diseases with the greatest number of clinical trials were Parkinson's disease (PD, 20) and Aromatic L-amino acid decarboxylase (AADC) deficiency (6). Trials covered both pediatric and adult populations, enrolling patients from 3 months to 80 years old, with a median number of 12 patients per trial (range: 1–87, Fig. 1B). A majority of included clinical trials were sponsored by private companies versus academic institutions (49/64, 76.6%) and most were in the Phase 1 and/or Phase 2 stage (58/64, 90.6%).

Delivery Routes

There were variable routes of administration for gene therapies based on the conditions targeted, with surgical CNS delivery through intraparenchymal, intraventricular, and/or intracisternal routes (Fig. 2A). Across all clinical trials, patients were excluded if there were anatomical risks linked to the route of therapy delivery. Therapies were administered only once, with repeated dosing precluded by the humoral antibody response against the viral vector and occasionally the T-cell mediated response against the gene product delivered [28,29].

Intraparenchymal delivery

Intraparenchymal therapeutic delivery enables direct delivery to the target structure, bypassing the blood brain barrier. Given the immune privilege of the CNS, this reduces exposure of the viral vector to the

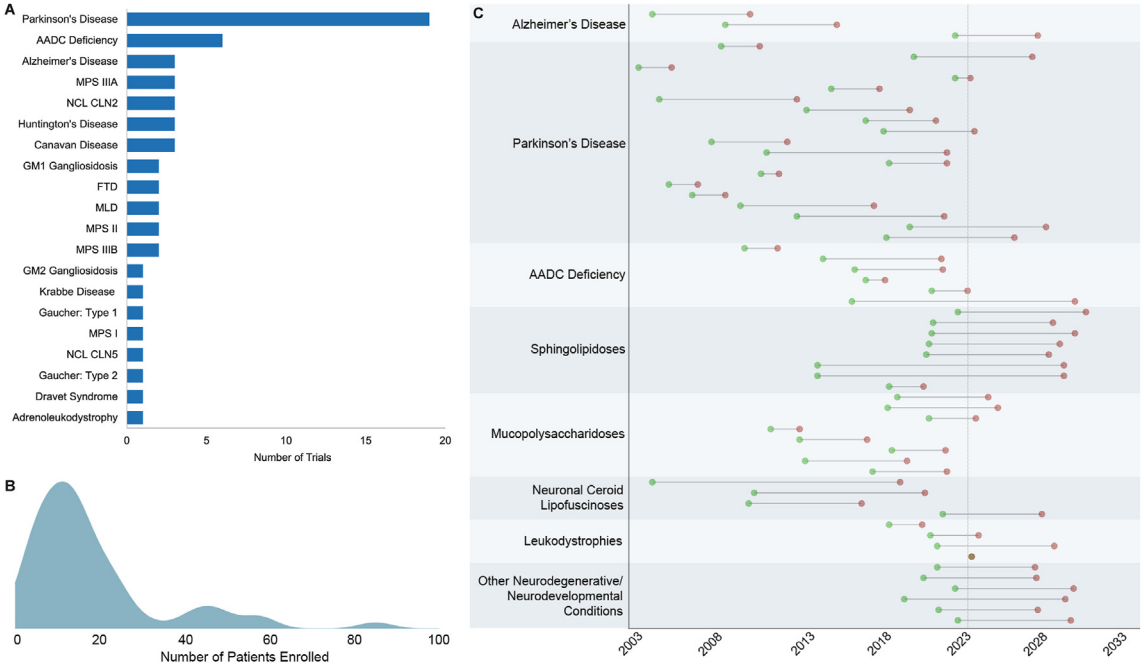

Fig. 1. Neurosurgical Gene Therapy Clinical Trials. A. Number of included clinical trials for neurodegenerative and neurodevelopmental conditions. B. Distribution of the number of patients enrolled across all included clinical trials. C. Timelines of included clinical trials by disease category.

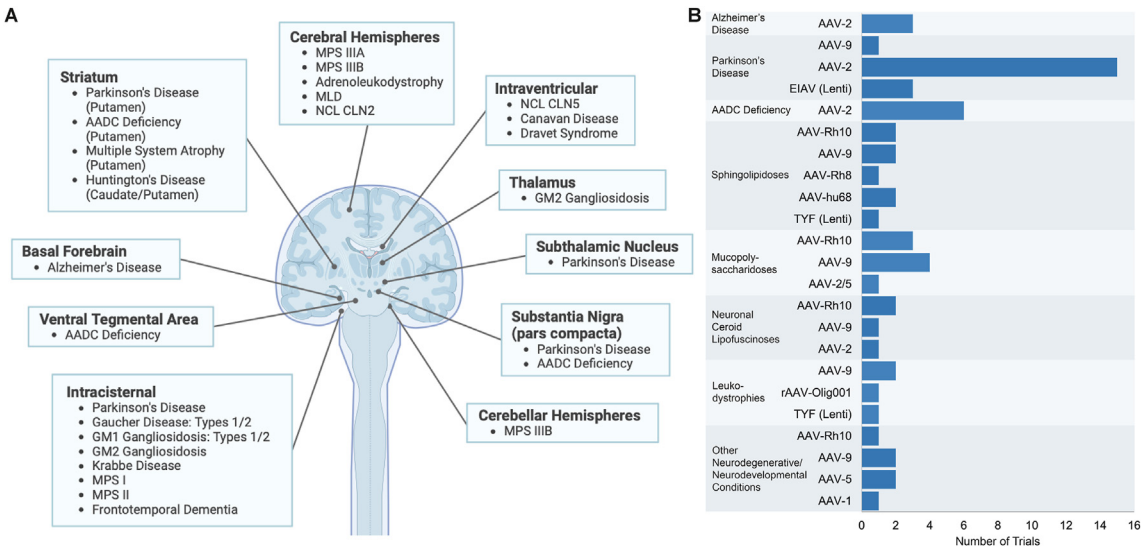

Fig. 2. Neurosurgical Gene Therapy Targets. A. Delivery locations for therapeutic agents by disease. B. Summary of viral-vector serotypes used for gene transfer.

immune system which limits transduction efficiency through a humoral and/or cell-mediated response [30]. Infusions have been performed in the striatum, the basal forebrain, and more diffuse locations such as the white matter in bilateral cerebral hemispheres. Direct stereotactic guided injection is frequently utilized for intraparenchymal delivery. However, the distribution of vector after injection has posed a challenge and is thought to contribute to the variability in clinical efficacy [23]. In rodent and non-human primate (NHP) models with direct intraparenchymal infusion, vector distribution and downstream gene product activity has been shown to be highest around the injection site, with increasing variability at greater distances [31–34].

To improve target penetration and monitor coverage, CED of therapeutics with reflux resistant catheters and real-time image guidance has been shown to improve specificity and delivery efficiency [26,35–39]. CED was developed in the 1990s as a method to enhance the distribution of infused macromolecules by maintaining a pressure gradient during the process of infusion [40]. Used in conjunction with step-design cannulas to prevent reflux despite positive pressure, CED enables the delivery of uniform and broad infusions [41]. Early in the history of intraparenchymal gene therapy, infusions were performed without monitoring of infusate volumes, which resulted in inadequate delivery contributing to initial PD trial failures [23]. However, the development of MRI contrast-spiked infusates which are delivered in interventional or intraoperative MRI scanners has enabled real-time monitoring of infusion volumes, shown in NHP studies to correspond reliably to regions of eventual transgene expression [42,43]. The evolution of catheter technology and real-time MRI monitoring has been reviewed previously, with benefits demonstrated in animal models and humans trials [23,26].

Three clinical trials for PD have used CED for putaminal injections with intraoperative serial MR images (NCT01621581, NCT01973543, NCT03065192). At least 10 trials have used the SmartFrame and SmartFlow cannula (ClearPoint Neuro; Solano Beach, CA), an integrated skull-mounted aiming device and software platform with a stepped cannula designed specifically for this purpose [42].

Intraventricular and intracisternal delivery

Intraventricular and intracisternal delivery is performed with catheter placement in the lateral ventricles or cisterna magna. Delivery to CSF spaces was intended to enable circulation of vector and subsequent expression of gene product throughout the neural axis while bypassing the blood brain barrier. Real-time MRI of contrast delivery in a NHP model demonstrated that intracisternal injections, traditionally achieved by suboccipital puncture, resulted in broad coverage of the brain and spinal cord while intraventricular injection showed brain cortex coverage and minimal spinal cord distribution [44]. Yet, as vector distribution is dependent on distance from CSF spaces, deeper CNS regions generally showed poor viral vector uptake and gene transduction [45]. Preclinical studies of vector constructs demonstrated this location based phenomenon, with variability in transduced tissue gene expression associated with distance from the structure injected. The cerebellum and spinal cord showed the highest level of transduction with intracisternal injections while periventricular regions showed the highest transduction with intraventricular injections [46–50].

The majority of clinical trials using a cerebrospinal fluid (CSF) delivery route focused on patients with conditions that required global CNS gene replacement, such as SLDs or Canavan disease. To avoid risks of medullary injury with intracisternal injection, new methods using intrathecal navigation of vascular microcatheters have been developed. This method was implemented effectively in one trial for GM2 gangliosidosis delivering AAVrh8-HEXA and AAVrh8-HEXB (NCT04669535) [45].

Viral Vectors

In vivo gene transfer is limited by the ability to deliver DNA into the CNS compartment, localization of genetic material, and stable long-term gene expression. Viral vectors such as adenoviruses, AAVs, and lentiviruses have been developed to overcome some of these challenges, with evolution in viral structure and capsids enabling more complex gene delivery schemes. To understand the evolution in vectors utilized across clinical trials, we characterize the construct strategies that have been explored for clinical translation. The majority of trials examined here utilized AAV based vectors (59/64) with a few utilizing lentiviruses (5/64, Table 1, Fig. 2B).

AAV based vectors

AAVs are part of the parvoviridae family, originally discovered as a contaminant in adenovirus preparations [51]. Specifically, they belong to the Dependoparvovirus genus, lacking genes essential for replication and relying upon coinfection with a helper virus to complete their life cycle [51]. AAVs possess a single stranded 4.7 kb genome flanked by 145bp T-shaped inverted terminal repeats (ITRs). Second strand synthesis occurs once the transgene is delivered to the cell nucleus. The transgene generally persists as a double-stranded circular episome, with some level

Table 1
Disease specific vector constructs, gene products, and expression strategies under investigation in human clinical trials.

	Vector Capsid	Gene Product	Delivery Route[a]	Delivery Target(s)[a]	Delivery Adjuncts	Antero-grade[b]	Retro-grade[b]	CNS Cell Tropism	Novel Features
AD	AAV2	BDNF	Intra-parenchymal	Basal forebrain	CED, MRI	–	++	Neurons (+++) Glia (+)	CED and MRI monitoring
	AAV2	NGF	Intra-parenchymal	Basal forebrain	–	–	–	Neurons (+++) Glia (+)	-
PD	AAV2	hAADC	Intra-parenchymal	Striatum (putamen)	CED, MRI	++	–	Neurons (+++) Glia (+)	CED and MRI monitoring
	AAV2	GAD	Intra-parenchymal	Sub-thalamic nucleus	–	++	++	Neurons (+++) Glia (+)	-
	AAV2	NTN	Intra-parenchymal	Striatum + substantia nigra	–	++	++	Neurons (+++) Glia (+)	Dual location approach to improve coverage
	AAV2	GDNF	Intra-parenchymal	Striatum (putamen)	CED, i-MRI	++	+	Neurons (+++) Glia (+)	CED and real time MRI monitoring
	AAV9	GBA1	Intracisternal	Systemic/CNS	–	–	–	Neurons (+++) Glia (++)	-
	EIAV (Lenti)	TH/ AADC/ GTP-CH1	Intra-parenchymal	Striatum	–	–	–	Neurons (+++)	Three transgenes delivered for dopamine synthesis
AADC Def.	AAV2	hAADC	Intra-parenchymal	Striatum (putamen), substantia nigra + ventral tegmental area	CED, MRI	++	–	Neurons (+++) Glia (+)	CED and MRI monitoring
SLD	AAV9	GBA1	Intracisternal	Systemic/CNS	–	–	–	Neurons (+++) Glia (++)	-
	AAVhu68	GLB1	Intracisternal	Systemic/CNS	–	–	–	Neurons (+++) Glia (++)	Ubiquitin C promoter for greater CNS transduction
	AAVrh.10	GLB1	Intracisternal	CNS	–	–	–	Neurons (+++) Glia (++)	–
	AAVrh.8	HEXA, HEXB	Intra-parenchymal + intracisternal	Thalamus + CNS	–	–	–	Neurons (+++) Glia (++)	Two separate vectors co-injected
	AAVhu68	GALC	Intracisternal	CNS	–	–	–	Neurons (+++) Glia (++)	
	AAVrh.10	ARSA	Intra-parenchymal	Cerebral hemispheres	–	++	++	Neurons (+++) Glia (++)	CAG/cu promoter to increase CNS transduction.
	TYF (Lenti)	ARSA	Intra-parenchymal	Cerebral hemispheres	CED	–	–	Neurons (++) Astrocytes (+) Oligo (+)	–
MPS	AAV9	IDUA	Intracisternal	CNS	–	–	–	Neurons (+++) Glia (++)	–
	AAV9	IDS	Intracisternal	CNS	–	–	–	Neurons (+++) Glia (++)	–
	AAVrh.10	SGSH/ SUMF1	Intra-parenchymal	Cerebral hemispheres	–	–	–	Neurons (+++) Glia (++)	–
	AAVrh.10	SGSH	Intra-parenchymal	Cerebral hemispheres	–	–	–	Neurons (+++) Glia (++)	–
	AAV2/5	NAGLU	Intra-parenchymal	Cerebral + Cerebellar hemispheres	–	–	–	Neurons (+++) Glia (+)	–
NCL	AAV2	CLN2	Intra-parenchymal	Cerebral hemispheres	–	++	++	Neurons (+++) Glia (+)	–
	AAVrh.10	CLN2	Intra-parenchymal	Cerebral hemispheres	–	++	++	Neurons (+++) Glia (+)	–
	scAAV9	CLN5	Intraventricular + intravitreal	CNS	–	–	–	Neurons (+++) Glia (++)	Self-complementary genome
Leuko-dystrophies	TYF (Lenti)	ABCD1	Intra-parenchymal	Cerebral hemispheres	–	–	–	Neurons (++) Astrocytes (+) Oligo (+)	–
	AAV9	ASPA	Intravenous, intraventricular	Systemic/CNS, CNS	–	–	–	Neurons (+++) Glia (++)	–
	AAVOlig 001	ASPA	Intraventricular	CNS	–	–	–	Oligo (+++) Neurons (+)	Oligo specific vector capsid
Other Neurodegenerative/ Neurodevelopmental	AAV1	GRN	Intracisternal	CNS	–	–	–	Neurons (+++) Glia (++)	–
	AAV9	GRN	Intracisternal	CNS	–	–	–	Neurons (+++) Glia (++)	–
	AAV5	miHTT	Intra-parenchymal	Striatum	–	++	++	Neurons (+++) Glia (+)	microRNA that binds and silences HTT gene
	AAVrh.10	CYP46A1	Intra-parenchymal	Striatum	–	–	–	Neurons (+++) Glia (+)	–

(*continued on next page*)

Table 1 (*continued*)

	Vector Capsid	Gene Product	Delivery Route[a]	Delivery Target(s)[a]	Delivery Adjuncts	Antero-grade[b]	Retro-grade[b]	CNS Cell Tropism	Novel Features
	scAAV9	eTF-SCN1A + GABAre	Intraventricular	CNS	–	–	–	Neurons (+++) Glia (++)	Self-complementary genome coding for engineered SCN1A transcription factor, regulatory element for selective GABA neuron expression

AD: Alzheimer's Disease, ICP: intracranial pressure, MPS: Mucopolysaccharidoses, NCL: Neuronal Ceroid Lipofuscinoses, Oligo: oligodendrocytes, PD: Parkinson's Disease, SLD: Sphingolipidoses.

[a] Delivery route and location as reported for human clinical trial.

[b] Transduction direction reported if demonstrated in pre-clinical or clinical data.

of genome insertion dependent on host cell processing [52]. Genome integration occurs at a specific site (AAVS1) on the q arm of human chromosome 19 [53–55]. Preclinical and clinical studies have indicated persistent long-term AAV transgene expression, including a decade after initial systemic or direct CNS delivery [56,57]. Propagation of recombinant material only requires the presence of the ITRs, which enables maximal transgene packing and minimizes vector immunogenicity and cytotoxicity [58]. Different AAV serotypes demonstrate variable binding affinities for distinct cell surface receptors, typically glycoproteins, which helps generate their variable tissue tropism profiles [59]. Because of their inability to self-replicate, genetic composition, and differential tissue tropisms, AAVs have quickly become the most widely used viral vector for gene transfer.

Natural AAV capsids

Soon after the initial discovery of AAV in the 1960s, multiple serotypes were identified with variable transduction and receptor binding profiles, prompting the search for AAV variants that might facilitate specific gene therapies [5,51,60–62]. Overall, twelve serotypes and over 100 AAV variants have been isolated in humans and NHPs [63]. Capsid variants naturally found in humans currently in use for CNS clinical trials are AAV1, AAV2, AAV5, AAV9, and AAVhu68, all of which have tropism for CNS tissue. However, the fact that AAVs appear endemic to humans – with 40–80% of the human population demonstrating seropositivity for antibodies against AAV – has motivated isolation of AAV capsids from NHPs in order to mitigate vector immunogenicity [63–65]. Thus far, the NHP derived capsid variants used in CNS clinical trials are AAVrh.8 and AAVrh.10 which are able to cross the blood brain barrier following intravenous delivery and improve CNS transduction while limiting peripheral tissue uptake [66,67]. Efforts are also being undertaken to develop AAV vectors derived from a variety of other vertebrates that could be implemented with the lowest risk of immunogenicity, but these have yet to be incorporated into clinical trials [68–76].

Engineered AAV capsids

Engineering to enhance and customize vector capsids – with regards to their transduction profiles, immunogenicity, or other features – has largely involved three strategies: rational design, directed evolution, and in silico computational approaches. Rational design, in which deliberate and targeted alterations are made to the capsid genome to leverage prior knowledge of capsid structure-function relationships, has led to the development of one AAV variant used in a CNS clinical trial with intraparenchymal delivery: AAV2/5 for mucopolysaccharidosis (MPS) IIIB (NCT03300453) [29]. AAV2/5, which is constructed by inserting AAV2 ITRs into the AAV5 capsid, was designed to limit the humoral response, improve transduction efficiency, and capitalize on specific cell tropism [77–79].

Directed evolution, which involves generating a diverse library of capsids (by methods including error-prone PCR, gene shuffling, and peptide insertion) and applying selective pressure to enrich for variants with desired features, has generated one AAV variant used thus far in a CNS clinical trial with intraventricular delivery: AAVOlig001 for Canavan disease (NCT04833907) [80]. AAVOlig001, which comprises a chimeric mixture of AAV1, AAV2, AAV6, AAV8, and AAV9, was developed by performing capsid shuffling and exerting selective pressure for striatal oligodendrocyte transduction in vivo. Testing in a murine model with intraventricular delivery demonstrated widespread and specific oligotropism [46,81].

In silico computational approaches, in which machine learning based structure-function analyses are utilized to evaluate novel theoretical candidate capsids, have yet to develop AAV variants used in clinical trials. Nevertheless, they show promise as a method to implement the full possible diversity of capsid variants without necessitating complete a priori understanding of capsid biology [17,65,82].

AAV transgene engineering strategies

A challenge in AAV mediated delivery is the payload capacity, limited to 5 kb given the size of the wild-type genome [83]. Various strategies are being explored in clinical trials to circumvent the size limitation, such as truncated gene products or regulatory genetic elements that can modulate gene products (e.g., miRNA for HTT suppression, intraparenchymal delivery, NCT04120493, NCT05243017) [84,85]. Dual AAV vector approaches in which smaller transgenes can recombine to form a larger transgene are also being explored but have not made it to clinical trials for a neurological indication [86].

AAV variants continue to be designed to enhance adequate expression of transgenes at doses that can be safely administered [87]. Self-complementary AAV (scAAV) genomes, where double-stranded transgenes are packaged, are being developed across serotypes to accelerate AAV gene transduction by eliminating the rate limiting step of complementary strand synthesis [88]. While some clinical trials are utilizing scAAV constructs, this effectively cuts the maximum gene size deliverable in half, restricting the types of diseases that can be tackled by such an approach.

AAV transduction

AAVs bind to cell surface receptors with differential affinities based on serotype. After undergoing clathrin-mediated endocytosis, AAVs can undergo varying degrees of anterograde and/or retrograde transport depending on serotype [89]. All AAV serotypes listed above have previously been reported to undergo some level of anterograde and retrograde axonal transport in animal models following systemic or direct CNS delivery [90]. Directed evolution has also been employed to tailor AAV variants such as AAV2-retro and AV-MNM004 with dramatically enhanced retrograde transport after intraparenchymal injection [91,92]. Of the vector constructs currently in clinical trials with intraparenchymal delivery, immunohistochemical analyses in animal models have shown primarily anterograde transport for AAV2-BDNF, AAV2-GDNF, and AAV2-hAADC and both anterograde and retrograde transport for AAV2-NTN, AAV2-GAD, AAV2-CLN2, AAV5--miHTT, AAVrh.10-CLN2, and AAVrh.10-ARSA [27,37,39,93–98].

Human viral transport kinetics have been examined in PD patients across three trials who received intraparenchymal AAV2-NTN (NCT00252850, NCT00400634, NCT00985517). Post-mortem examination of two patients who died approximately one month and three months following putaminal injection showed minimal transduction of the SNpc compared to animal models of PD [99]. However, post-mortem examinations of one patient ten years after putaminal injection and one patient eight years after simultaneous putaminal and nigral injections showed robust transgene expression in both the putamen and substantia nigra. This indicated a time dependency for vector transmission (either by retrograde transport or transduction) from the putamen to substantia nigra (NCT00985517) [56].

AAV vectors also demonstrate the potential for transsynaptic transport. AAV1 and, to a lesser extent, AAV9 have been shown to exhibit anterograde transsynaptic transduction after intraparenchymal delivery [100]. Specifically, AAV1 appears to spread across glutamatergic and GABAergic synapses but not through serotonergic, cholinergic, or noradrenergic projections [101]. Although the mechanism of this transsynaptic spread remains uncertain, it has been postulated to involve synaptic transcytosis, given that endothelial transcytosis has been shown to enable AAV9 to cross the blood-brain barrier [102–104].

AAV immunogenicity

A consideration for viral vector mediated delivery of gene products is the immunogenicity of such therapies [105]. While the innate immune response to AAVs is generally mild, the adaptive immune response to AAV capsids and transgene products has been well documented with systemic vector delivery [106]. As humans are a host to AAVs, there is a high prevalence of neutralizing antibodies to certain AAV capsid serotypes circulating in the population. Anti-AAV capsid and transgene antibodies can limit the mode of administration and re-dosing of AAV based therapies. Capsid evolution strategies have attempted to reduce the humoral response to AAVs with varying degrees of efficacy [107]. The development and exposure to anti-AAV antibodies is further dependent on the route of delivery and dose required, with high dose systemic delivery resulting in a greater antibody mediated neutralization of AAV vector versus lower dose direct CNS delivery [108]. In addition to the humoral response, T cell mediated responses to AAV capsids and transgenes have been documented [109]. Human clinical trials with direct CNS delivery have demonstrated mild transient increases in serum anti-AAV antibodies with no detectable antibody mediated response to the transgene. T cell response after CNS delivery was studied in one trial which used an AAV2/5-NAGLU construct: a CD4 and CD8 T cell response to the transgene product NAGLU persisted 5.5 years after injection but had no impact on transgene expression [29,110].

Lentivirus based vectors

Lentiviral vectors are part of the retroviridae family and have a more complex genome compared to AAVs. They have a larger payload capacity compared to AAVs, up to a ceiling of approximately 10 kb for efficient transfer [111]. After binding to cell surface glycoproteins, lentiviruses release their gene product which integrates with the host genome through retroviral integrase enzymes [112–114]. Although integration may facilitate stable transgene expression, there is a risk of inadvertent modification of regulatory genes that could result in cancer by insertional mutagenesis [115–117]. Another safety concern of lentiviral vectors is unintended replication and viral proliferation. To address this, modifications have been made to lentiviral systems to significantly mitigate the risk of generating replication-competent lentiviruses [118–121]. Lentiviruses have particular tropism for neural stem cells, but adjustments to surface proteins and the inclusion of tissue specific promoters enable selective transgene expression in other tissues of interest [114,122,123].

Across the clinical landscape, a majority of lentivirus vectors have been used for ex vivo gene transfer, including the development of chimeric antigen receptor (CAR) T cells for acute lymphoblastic leukemia and transduction of $CD34^{+}$ hematopoietic stem cells for adrenoleukodystrophy [124,125]. For in-vivo treatment of neurological conditions, five trials have employed two different types of lentiviral constructs, TYF and equine infectious anemia virus (EIAV). In pre-clinical rodent and NHP models, TYF constructs have been delivered in the bilateral cerebral hemispheres, showing the highest level of gene transduction around the injection site with no transgene expression detected in peripheral organs [31,126]. Gene expression was robust in neurons, with expression also present in astrocytes and oligodendrocytes, albeit at a reduced level. Further, some animals had reactive neuroinflammation around the injection site, though this was heterogeneous [31,126]. EIAV constructs for PD have been engineered to deliver multiple genes for key enzymes: tyrosine hydroxylase, amino acid decarboxylase, and GTP-cyclohydrolase-1. In NHPs, EIAV vectors injected in the putamen showed restricted gene transduction in the region, largely present in neurons. Minimal reactive neuroinflammation around the injection sites was observed, without gene expression detected in peripheral tissues [127,128].

The pre-existing immune response to lentiviral vectors is lower compared to AAVs given the low prevalence of circulating neutralizing antibodies [129]. Nevertheless, lentiviral vectors can elicit similar humoral and cell-mediated immune responses following vector delivery. In human clinical studies and in a rodent model, direct CNS delivery of a lentiviral vector resulted in a mild transient antibody response against the lentivirus capsid with no response against the transgene [31,130].

Targeted Disease Entities

Given the heterogeneity of CNS conditions targeted with viral vector gene therapy, diseases in our review were categorized into nine groups based on similarities in pathophysiology and transgenes delivered: Alzheimer's disease (AD), PD, AADC deficiency, Sphingolipidoses (SLD; Gaucher disease Types 1 and 2, GM1 gangliosidosis, GM2 gangliosidosis, Krabbe disease, metachromatic leukodystrophy), mucopolysaccharidoses (MPS; Types I, II, IIIA, IIIB), neuronal ceroid lipofuscinoses (NCL), SMA, leukodystrophies (adrenoleukodystrophy, Canavan disease), and other neurological conditions (frontotemporal dementia, Huntington's disease, Dravet syndrome, Rett syndrome, mesial temporal lobe epilepsy, glutaric acidemia type 1). Gene therapies for PD were some of the first to emerge for a CNS indication (Fig. 1C). In recent years, lysosomal storage disorders such as SLD, MPS, and NCL have seen growth in the number of ongoing clinical trials, likely due to monogenetic enzyme-linked deficiencies identified across these diseases [131].

Alzheimer's disease

Three trials have examined therapies for AD with two different vector constructs: AAV2-BDNF (brain-derived neurotropic factor) and AAV2-NGF (nerve growth factor), both of which were delivered to the bilateral basal forebrain: AAV2-BDNF had CED while AAV2-NGF was non-CED. All trials focused on mild to moderate forms of AD in adult patients and a total of 71 patients were targeted for enrollment.

In preclinical studies, delivery of BDNF to the entorhinal cortex reversed neuronal atrophy and cognitive impairment in murine and NHP animal models [132]. A NHP study of AAV2-BDNF reported accurate vector targeting in the entorhinal cortex with CED and intraoperative MRI guidance. Robust BDNF expression was observed in both the entorhinal cortex and hippocampus, confirming retrograde transduction of the vector [39]. NGF delivered to the basal forebrain in a murine model has previously been shown to protect against age related atrophy and improve learning and memory [133]. AAV2 mediated NGF delivery to the basal forebrain was studied in a murine model, showing significant neuroprotection of cholinergic neurons with sustained transgene expression [134].

Results from two clinical trials have been published for the AAV2-NGF construct. AAV2-NGF was targeted bilaterally to the basal forebrain region containing the nucleus basalis of Meynert, infusing 10 or 20 μL per site depending on dose selection (1.2×10^{10}, 5.8×10^{10}, 1.2×10^{11} vg). While there were no major treatment related adverse events, treatment with AAV2-NGF did not significantly slow clinical deterioration compared to untreated AD patients. On imaging, there was no significant difference in posterior cingulate gyrus or hippocampal volume loss between treated and untreated patients with similar FDG-PET uptake in the basal forebrain [135,136]. A subsequent post-mortem analysis of treated patients indicated that while NGF expression persisted for at least seven years after treatment (longest treatment course studied), NGF did not reach areas of cholinergic neurons in the nucleus basalis of Meynert at any of the injected sites. This was attributed to the limited penetration and spread of AAV2-NGF following injection [137]. The ongoing clinical trial for AAV2-BDNF iterates on this finding by using CED and intraoperative MRI to ensure adequate vector targeting (as performed in the preclinical study), aiming to evaluate whether improved target coverage can drive improvement in cognitive function and volume loss.

Parkinson's disease

Twenty trials are being conducted for PD, with all but one trial focused on idiopathic PD (one trial examined GBA induced PD). Patients with atypical or severe forms of PD as well as those with prior surgical intervention for PD were generally excluded. A majority of PD trials enrolled patients who responded to levodopa but were at a maximum tolerated dose that did not control their symptoms. Vector constructs studied for PD include AAV2 delivering hAADC, GAD, NTN, or GDNF, AAV9-GBA1, and EIAV-TH/AADC/GTP-CH1 (lentivirus, ProSavin). Almost all constructs were delivered through intraparenchymal administration, with only AAV9-GBA1 delivered intracisternally. AAV9-GBA1 is further discussed amongst the sphingolipidoses given the original target disease entity for development. CED was utilized for AAV2-hAADC and AAV2-GDNF delivery, while other vector constructs were delivered through non-CED. Almost all vector constructs developed for PD used an AAV2 capsid given the ability for neuronal transgene expression as well as anterograde and retrograde transduction. Gene products delivered by AAV2 for PD can broadly be categorized into two different strategies to enhance dopamine function: enzymatic and neurotrophic.

Aromatic amino acid decarboxylase

AAV2-hAADC is an enzymatic strategy to deliver AADC, a critical enzyme for dopamine synthesis that is depleted in PD, limiting the efficacy of levodopa. Several preclinical studies have examined intraparenchymal AAV2 mediated delivery of hAADC to the striatum, with some using CED to maximize target penetration. Delivery of AAV2-hAADC in NHPs was well tolerated with robust AADC expression confined mostly to neurons that persisted for years after initial injection [138–140]. At a sufficient dose, AADC expression increased response to levodopa and improved motor symptoms in hemi-parkinsonian NHPs [141]. While initial studies reported transgene expression largely confined to the striatum, higher doses of vector in NHPs resulted in anterograde delivery [27]. Initial studies of AAV2-hAADC delivery to the bilateral putamen demonstrated approximately 30% reduction in PD symptoms measured by the Unified Parkinson's Disease Rating Scale (UPDRS) [142–144].

A recent AAV2-AADC clinical program evolved the state of the art in intraparenchymal delivery during the course of the trial (NCT03065192). The first cohorts of patients underwent a traditional targeting approach, via transfrontal trajectories. In the final cohort, to enhance coverage of the posterior putamen and reduce the number of trajectories necessary, a posterior (parieto-occipital) approach with the patient in the prone position was adopted during the course of the trial. Vector infusion volumes also were increased to a maximum of 1800 μL per putamen (2.6×10^{12} vg/mL). Posterior trajectories achieved better coverage of the putamen with a single trajectory and allowed the surgeon to advance the cannula in response to the infusion dynamics visualized during continuous, serial imaging of the infusion (Fig. 3) Since the length of the cannula pass within the putamen was much longer than with a frontal approach, the second cannula step could enter the putamen much earlier in the infusion process and the infusion rate could be increased more rapidly, allowing backflow to increase coverage of the putaminal tail, as shown in NHP studies [49,51]. This methodological evolution resulted in total putaminal coverage exceeding the 50% goal, with mean 76% coverage of the post-commissural putamen [26]. The shift to the posterior approach significantly reduced infusion durations and overall procedure times. At 3 years, PD medications were reduced by 21%–30% in the 2 highest dose cohorts, and standard measures of motor function, global impressions of improvement, and quality of life were stable or improved compared with baseline at 12, 24, and 36 months across cohorts [145–147]. The subsequent phase 2 study, originally sponsored by Voyager Therapeutics, Inc. and then acquired by Neurocrine Biosciences, unfortunately was terminated by the sponsor following placement of an FDA clinical hold, which resulted from a request by the study's data safety monitoring board to pause dosing in order to evaluate non-symptomatic MRI abnormalities observed in trial participants [148]. However, it is likely that adoption of posterior surgical approaches could help achieve improvement in putaminal target coverage for constructs across disease states for which the putamen is a therapeutic target.

Glutamic acid decarboxylase

AAV2-GAD is an enzymatic strategy designed to reduce parkinsonian symptoms by inhibiting excitatory subthalamic nucleus (STN) output neurons. Glutamic acid decarboxylase (GAD) is the enzyme that catalyzes the decarboxylation of glutamate to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Intraparenchymal delivery of AAV2-GAD to the STN of mice was well-tolerated, with significantly increased GABA release into the STN and decreased excitatory output reported. Treatment resulted in significant reduction in parkinsonian symptoms in rodent models [149,150]. A preclinical NHP study was not reported prior to the first clinical trial, which did not employ CED. In clinical trials, AAV2-GAD delivered to the bilateral subthalamic nuclei resulted in approximately 20% improvement in the UPDRS score, with a reduction in metabolic activity in the subthalamic nuclei that suggested relative inhibition [151–153]. Subsequently, histological analysis of AAV2-GAD in NHPs showed vector confined to the STN [154] while a murine study reported both anterograde and retrograde distribution of AAV2-GAD after STN injection [94]. Results from the ongoing trial of AAV2-GAD (NCT05603312) remain pending to determine if symptom improvement is observed in a larger cohort of patients with this construct.

Lentivirus-GCH1-TH-AADC

ProSavin is a lentiviral vector that encodes the three dopamine biosynthetic enzymes—tyrosine hydroxylase (TH), AADC, and GTP-cyclohydrolase 1 (CH1). ProSavin sought to improve dopamine synthesis by delivering genes for two key enzymes and a cofactor involved in dopamine synthesis. Preclinical studies showed that delivery of all three genes enabled dopamine production in non-native dopamine cells. Intraparenchymal delivery of ProSavin to the striatum of a murine model resulted in long-term multi-gene expression and improvement in motor symptoms. While dopamine production was detected, the relative proportion of dopamine detected in the striatum compared to healthy controls was small [155]. An updated version of ProSavin with greater transgene expression was tested in a NHP model of PD, showing a significant partial restoration in dopamine production. Further, there was long-term improvement in motor symptoms without induction of dyskinesias [156].

Clinical delivery of ProSavin was well tolerated via bilateral putaminal delivery (non-CED), with modest but significant improvement in UPDRS scores compared to pre-treatment baseline. While UPDRS

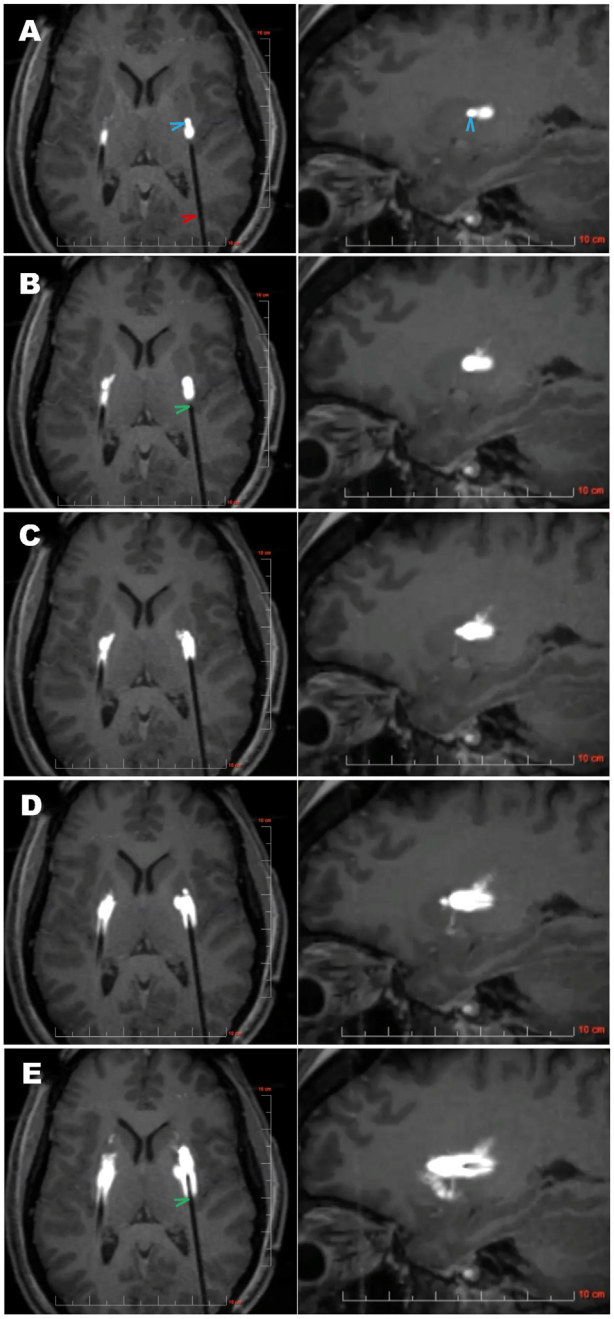

Fig. 3. Technique for Intraparenchymal Delivery. Serial MRI images (axial, left panels; sagittal right panels) demonstrate the technique of progressive advancement of the SmartFlow cannula (red arrow in A) through the putamen from a posterior approach. The blue arrow heads in A demonstrate the start of a new area of infusion after cannula advancement. Panels B–E show progressive time points of the infusion as the cannula is inserted further into the putamen. In B, the green arrowhead demarcates the step in the cannula that is 13 mm above the tip. Parking this step at the putaminal border allows the infusion to fill the posterior putamen as seen in C, after which the infusion remains confined to this boundary as the cannula is advanced further (green arrow in E).

improvement was sustained up to four years, the study noted that this change was similar to UPDRS fluctuations seen in sham surgery groups in previous PD trials [130,157]. A newer generation of this construct is being tested which has been designed to result in more potent dopamine production compared to the original vector [158]. While initial data pointed to a greater improvement in UPDRS, development has since been halted due to resource constraints at the sponsor company [159].

Neurturin

AAV2-NTN is a neurotrophic construct delivering neurturin (NTN), a neuroprotective agent that can help enhance dopaminergic neuron survival. Initial studies examining simultaneous non-CED delivery of AAV2-NTN into the striatum and substantia nigra of NHPs showed that treatment significantly improved motor function and preserved neurons in the substantia nigra. While injections were performed in both locations, there was evidence of AAV2-NTN transduction from the striatum to the substantia nigra [160]. Further studies with striatal injection only of AAV2-NTN in healthy murine and NHP models showed the same transduction pattern in the substantia nigra through anterograde and retrograde movement [161–163]. AAV2-NTN was first studied with non-CED delivery to the bilateral putamen: while the phase 1 study showed improvement in the UPDRS score, a larger phase 2 study showed no significant difference between treated patients and those who received a sham surgery shortly after treatment. At one and half years after therapy, there was a modest but significant improvement in UPDRS scores in treated versus sham surgery patients. Post-mortem studies revealed minimal transport to the substantia nigra [164,165]. To improve coverage in the substantia nigra, a dual injection approach was tested with simultaneous non-CED delivery of AAV2-NTN to the bilateral putamen and substantia nigra. This approach, however, did not result in significant UPDRS score change after treatment. The lack of efficacy was believed to be a result of insufficient delivery and possibly intervening too late in the disease course of treated PD patients [166].

Glial-derived neurotrophic factor

AAV2-GDNF is another neurotrophic construct delivering glial-derived neurotrophic factor (GDNF), a closely related factor to NTN which also enhances dopaminergic tone. Preclinical studies with AAV2-GDNF built on NTN, incorporating CED to the putamen to achieve better target penetration. Intraparenchymal delivery of AAV2-GDNF to the putamen showed robust coverage with greater anterograde versus retrograde transport in healthy NHPs [98]. In NHP models of PD, AAV2-GDNF delivery to the putamen improved functional deficits and led to growth of dopaminergic fibers. GDNF was detected in both the putamen and substantia nigra pars reticulata. This suggests anterograde transport from the putamen is likely responsible for GDNF distribution into the substantia nigra rather than retrograde movement through damaged dopaminergic neurons origination from the SNpc [167,168]. To improve consistency in AAV2-GDNF delivery and putaminal coverage, an interventional MRI-CED platform was validated in NHPs, which enabled real time monitoring of CED and optimized infusion parameters to achieve a more uniform putaminal distribution of GDNF [37].

The NINDS AAV2-GDNF clinical trial then became the first human trial to employ iMRI-CED, delivering vector to the posterior putamen bilaterally through a transfrontal approach. While the therapy was well tolerated and PET uptake suggested a neurotrophic effect, only 26% of the putamen was covered by vector as visualized on MRI. This was reflected in a lack of significant improvement in UPDRS scores [169]. These data suggested that the investigators may not have fully utilized the opportunities provided by real-time visualization to acutely modify infusions and to modify the general infusion strategy over the course of the study. A phase 1 and phase 2 trial respectively (NCT04167540, NCT06285643) are addressing these challenges by altering the trajectory for putaminal injection (posterior approach), increasing the dose delivered, and including patients earlier in the disease course. Results from the phase 1 trial demonstrated 63% putaminal coverage of CED delivered AAV2-GDNF, with no serious adverse events [170].

AADC deficiency

AADC deficiency results in impaired dopamine synthesis, leading to developmental delays and motor symptoms. Six trials have studied the reconstitution of AADC activity using AAV2-hAADC via intraparenchymal delivery. Two different intraparenchymal delivery

strategies have been employed: delivery to the bilateral putamen or simultaneous delivery to the bilateral substantia nigra pars compacta and ventral tegmental areas.

One of the first clinical trials examining non-CED bilateral putaminal delivery of AAV2-hAADC (200802042D - Taiwan) enrolled 4 pediatric patients (5×10^{11} vg/ml, 80 μl per target, 2 targets per putamen) and demonstrating significant improvement in motor function with increase in homovanillic acid, a marker of increased AADC function [171]. A subsequent larger cohort of 10 pediatric patients (non-CED bilateral putaminal delivery, 5.67×10^{11} vg/ml, 80 μl per target, 2 targets per putamen, NCT01395641) similarly tolerated the vector well, with improvement in motor function and homovanillic acid as well as robust PET tracer uptake in the putamen. Anti-AAV2 antibodies were detected, peaking at three to six months after delivery [172]. Long-term follow up of these cohorts (NCT02926066) showed sustained improvement in motor and cognitive function over at least five years, with elevated homovanillic acid levels and PET tracer uptake. Notably, younger patients treated closer to disease onset showed greater and faster response to treatment older patients [173]. Another group studied AAV2-hAADC in patients with a different genetic background compared to the above trials (UMIN000017802 - Japan). Six patients were enrolled with non-CED bilateral putaminal delivery of vector (5×10^{10} vg/ml, 50 μl per target, 2 targets per putamen). Motor function was improved in this patients up to two years after treatment, with persistent PET tracer uptake. Stratifying by patient age further corroborated findings from the above trials, with younger patients showing a greater treatment response [174].

Dual delivery of AAV2-hAADC to the bilateral substantia nigra pars compacta and ventral tegmental area using MRI guidance and CED was also well tolerated in 7 pediatric patients (NCT02852213) [175]. Two doses were tested (1.3×10^{11} vg, 4.2×10^{11} vg, 80 μl infusion per side) with significant vector coverage reported (98% in the substantia nigra pars compacta, 70% in the ventral tegmental area). Similar to the isolated putaminal delivery trials above, there was significant increase in detected homovanillic acid and improvement in motor and behavioral function [175].

Sphingolipidoses

Nine trials are developing neurosurgical therapies for sphingolipidoses, a subset of lysosomal storage disorders which lead to neuron cell death, cognitive impairment, and early mortality. Specific SLDs being studied include Gaucher disease types 1 and 2 (AAV9-GBA1), GM1 gangliosidosis types 1 and 2 (AAVhu68 or AAVrh.10 delivering GLB1), GM2 gangliosidosis (AAVrh.8-HEXA + AAVrh8-HEXAB), Krabbe disease (AAVhu68 delivering GALC), and metachromatic leukodystrophy (AAVrh.10 or TYF lentivirus delivering ARSA). Clinical trials have enrolled both pediatric and adult populations with genetically confirmed mutations associated with a particular SLD.

Gaucher disease is caused by biallelic mutations in GBA1, resulting in a deficiency in the lysosomal enzyme GCase. AAV9-GBA1 was initially developed for a non-idiopathic from of PD triggered by monoallelic deficiency in GBA. These patients tend to present at an earlier age with rapidly progressive motor symptoms [176]. A preclinical study with intravenous AAV9-GBA1 delivered to a murine model of PD-GBA showed an increase in GCase activity, the product of GBA expression. There was reduction in neuroinflammation and improvement in motor symptoms that persisted for months after initial treatment [177]. The AAV9-GBA1 construct is now besting tested via intracisternal delivery for patients with Gaucher disease.

GM1 gangliosidosis is a result of mutations in GLB1 leading to deficiency of beta-galactosidase and progressive neurodegeneration. AAVhu68-GLB1 was developed with an optimized ubiquitin C promoter to drive more effective expression of GLB1 in the CNS. Intraventricular injection of this construct in a murine model showed significant transgene expression both in the CNS and in peripheral organs (heart, liver, spleen) while reducing neurological symptoms and prolonging survival [178]. AAVrh.10-GLB1 has been tested in several preclinical models, including rodents (bilateral thalamic non-CED or intraventricular delivery), cats (bilateral thalamic non-CED, intraventricular, or intracisternal delivery), and NHPs (intracisternal) [179]. Intracisternal injection in NHPs led to the greatest CNS expression of beta-galactosidase. A significant humoral response against AAVrh.10 was observed in all treated animals after intracisternal injection (titer level $\geq$1:10,240), indicating a peripheral response to the vector.

GM2 gangliosidosis can be caused by mutations in the HEXA and/or HEXB gene resulting in deficiency of beta-hexosaminidase. The accumulation of lipids from beta-hexosaminidase deficiency leads to neurodegeneration and is almost always fatal. Intraparenchymal non-CED co-delivery of HEXA and HEXB was tested with an AAVrh.8 vector in sheep and NHP models. A 1:1 ratio of AAVrh.8-HEXA and AAVrh8-HEXB was injected both intraparenchymally (thalamus, non-CED) and intraventricularly to maximize CNS transgene expression. Expression was seen across the brain more than one year after injection, with low expression in the spinal cord. Treatment delayed disease progression, extending lifespan by 50%, eliminating seizures as well as vision-loss, and protecting normal social behavior in the sheep model. Further, it attenuated the neuroinflammatory sequelae of GM2 gangliosidosis. However, excessive HEXA and HEXB expression proved to be toxic: high dose therapy resulted in dyskinesias and motor deficits, highlighting the need for further dose finding studies [180,181].

Krabbe disease is linked to mutations in the GALC gene leading to an accumulation of lipids which result in demyelination and motor function impairment. Initial studies combined intraparenchymal (non-CED), intraventricular, and intravenous delivery of an AAVrh.10-GALC construct in a murine model, restoring GALC expression in the CNS and peripheral organs. This injection scheme delayed the onset of motor symptoms, improved myelination, and prolonged survival [182]. However, there were challenges in modulating and driving robust GALC expression with the level of systemic doses required to achieve CNS transduction. Given this, an alternative approach was developed with AAVhu68-GALC. Intracisternal delivery of this construct sought to increase CNS GALC expression more than what could safely be achieved with systemic vector administration. Compared to intravenous delivery, intracisternal AAVhu68-GALC in a canine model preserved myelination, prevented neurological decline, and increased survival. Off-target expression remained present, with elevated GALC detected in the heart in an NHP model after intracisternal delivery, though at lower levels compared to the CNS [48].

MLD is caused by a deficiency in arylsulfatase A (ARSA), leading to widespread demyelination and neurodegeneration. AAVrh.10-ARSA was developed with a CAG/cu promoter to try and increase ARSA transduction. This construct was initially tested with single location intraparenchymal delivery (striatum, non-CED) in a murine model and showed significant ARSA expression with partial reversal of neuropathology in the injected hemisphere. ARSA expression was highest in the striatum but also showed significant expression in other regions, including both directly connected (anterograde/retrograde from striatum) and poly-synaptically connected regions such as the cerebellum and brainstem [93]. Subsequent studies in NHPs showed that vector delivery to the white matter at multiple sites (non-CED) further resulted in the greater CNS ARSA coverage and remained well-tolerated [32,183]. Dose escalation studies, ranging from a total dose of 2.85×10^{10} vg to 1.5×10^{12} vg divided across 12 sites with non-CED intraparenchymal injection, established a safe maximum dose (2.85×10^{10} vg total, 2.4×10^{9} vg per site) that did not lead to neuroinflammatory sequalae from vector delivery [184]. An intraparenchymal lentivirus approach, TYF-ARSA, was developed as an alternative to address the immunogenicity associated with AAV mediated transfer of ARSA [185]. CED injection in the external capsule of NHPs resulted in widespread CNS coverage, likely through diffusion along white matter tracts and perivascular spaces. Neurons, astrocytes, and oligodendrocytes were transduced, with ARSA expression

highest at the injection site. However, CNS coverage remained less than what was seen with multiple injection sites of AAVrh10-ARSA [126].

As almost all clinical trials for SLDs are ongoing, preliminary safety and efficacy data is only available through company press releases and conference abstracts. For GM1 gangliosidosis, intracisternal AAVhu68-GLB1 and AAVrh.10-GLB1 were reported to be safe, with robust GLB1 expression and early signs of developmental improvement in patients who received AAVhu68-GLB1 [186,187]. However, the AAVrh.10-GLB1 trial was subsequently discontinued as treatment did not show biochemical or clinical efficacy and the sponsoring company was delisted and liquidated. Additionally, all patients developed antibodies again the AAVrh.10 vector [186]. For GM2 gangliosidosis, development of the dual vector AAV8-HEXA + AAV8-HEXAB was halted due to the sponsor reporting significant financial losses and the need to implement "a significant headcount reduction to conserve capital." [188] The sponsor later filed for dissolution and liquidation [189]. For Krabbe disease, development of intracisternal AAVhu68-GALC also was stopped also due to the sponsor requiring a refocus in strategy to "extend [the] cash runway" and "decrease operating expenses." [190] Finally, for MLD, intraparenchymal AAVrh.10-ARSA resulted a serious treatment related adverse event in one patient (seizures). While the construct increased ARSA activity, treated patients had similar clinical deterioration compared to untreated patients [191]. The difficulty in translation from pre-clinical models to clinical trials for SLDs may reflect the fact that while global CNS gene transduction can be achieved through intraventricular or intracisternal means, significant challenges remain in localizing and titrating expression of gene products to significantly modify the disease course.

Mucopolysaccharidoses

Eight trials are studying therapies for mucopolysaccharidoses, a set of lysosomal storage disorders that lead to progressive neuronal damage and characteristic physical symptoms. Vector constructs have been developed to deliver the deficient gene for each MPS subtype: MPS I (AAV9-IDUA, intracisternal), MPS II (AAV9-IDS, intracisternal), MPS IIIA (AAVrh.10-SGSH/SUMF1, AAVrh.10-SGSH, intraparenchymal non-CED), and MPS IIIB (AAV2/5-NAGLU, intraparenchymal non-CED). Trials have been conducted with both pediatric and adult populations and requires a genetic diagnosis of an MPS subtype.

MPS I is caused by deficiency in iduronidase, a lysosomal enzyme encoded in the IDUA gene. AAV9-IDUA was tested through various routes of administration in murine models to determine whether IDUA could be expressed throughout the CNS. Robust IDUA expression was observed across intravenous, intrathecal, and intraventricular administration. Intravenous and intraventricular administration showed more global CNS coverage while intrathecal administration showed lower IDUA expression that was more restricted to the spinal cord and hindbrain. However, across all groups, IDUA expression was restored to supraphysiological levels. Both intraventricular and intrathecal treatment with AAV9-IDUA restored motor and cognitive function back to that of control rodents [192,193].

MPS II is caused by mutations in the iduronate-2-sulfatase (IDS) gene. AAV9-IDS delivered intraventricularly in a murine model resulted in sustained supraphysiological IDS, similar to that seen with AAV9-IDUA. Reconstitution of IDS prevented neurocognitive deterioration. Vector was also detected in peripheral organs with no significant adverse effects [50].

MPS IIIA is caused by deficiency in N-sulfoglucosamine sulfohydrolase (SGSH), with various vector and transgene co-delivery strategies proposed as treatments. Intraparenchymal non-CED AAVrh.10 delivery of SGSH has been tested in isolation and with co-delivery of SGSH + SUMF1 in murine models, with SUMF1 functioning to enhance SGSH activity. AAVrh.10-SGSH/SUMF1 delivery was safe with robust transgene expression near the delivery location (striatum). Though lysosomal markers of MPS IIIA decreased near injection sites, broader SGSH expression was hypothesized to be needed for clinical improvement [194]. AAVrh.10-SGSH sought to address this by swapping SUMF1 with a stronger promoter, driving increased SGSH expression. Delivery of this construct in murine models at a higher volume than AAVrh.10-SGSH/SUMF1 resulted in more widespread SGSH expression and was more effective at correcting lysosomal pathology [34,195].

MPS IIIB is caused by deficiency in N-acetyl-alpha-glucosaminidase (NAGLU), with a hybrid AAV2/5 vector used to deliver NAGLU. Intraparenchymal non-CED delivery of AAV2/5-NAGLU in a canine model was safe, with multiple injection sites resulting in NAGLU coverage across the brain. Significant reduction in biochemical and histological parameters linked to MPS IIIB was also observed [196].

Clinical trial data has been published for MPS IIIA and IIIB cohorts, with preliminary data available for MPS I and MPS II. For MPS I, intracisternal delivery of AAV9-IDUA has been well tolerated in five patients to date, with increased serum and CSF IDUA activity and a positive efficacy signal [197]. Similarly, intracisternal delivery of AAV9-IDS for MPS II has been safe with a dose dependent reduction in substrates catalyzed by IDS expression. Treated patients with MPS II have also shown improvement in neurodevelopmental function and caregiver reported outcomes [198]. For patients with MPS IIIA, intraparenchymal non-CED delivery of AAVrh.10-SGSH/SUMF1 was well-tolerated, but its efficacy was mixed as there was no clear trend of cognitive improvement in treated patients [199]. The next iteration, intraparenchymal non-CED AAVrh.10-SGSH, was therefore tested to improve therapeutic efficacy. However, only MPS IIIA patients less than two and half years old treated with this construct had an improvement in development [200]. Across the cohort, AAVrh.10-SGSH did not slow brain atrophy or other adaptive functioning impairments. Finally, for MPS IIIB, intraparenchymal non-CED AAV2/5-NAGLU was safe, with robust NAGLU expression detected following delivery. However, there was only a small efficacy signal, with only one treated patient with a milder form of the disease showing significant cognitive improvement compared to untreated patients [29]. Additional analyses are needed to determine MPS patient profiles that may be most responsive to specific therapeutics.

Neuronal ceroid lipofuscinoses

Four trials are examining variants of NCL, a lysosomal storage disorder that leads to neurodegeneration. Three different AAV vectors serotypes were used to deliver the gene product deficient in the respective variant of NCL: AAV2 for CLN2 (intraparenchymal, non-CED), AAVrh.10 for CLN2 (intra-parenchymal, non-CED), and scAAV9 for CLN5. The trial for NCL-CLN5 is pairing intraventricular with intravitreal delivery to address the progressive retinal atrophy seen in NCL.

Preclinical studies have tested vector constructs for NCL across a range of animal models. AAV2 and AAVrh.10 serotypes were examined for delivery of CLN2. AAV2-CLN2 non-CED delivery to the striatum in murine and NHP models showed significantly elevated expression of the downstream gene product (TPP-I) largely confined to neurons. Stable expression of the gene product was also observed in certain cortical regions, thalamus, and substantia nigra, consistent with anterograde and retrograde movement of AAV2 [96]. To achieve more widespread distribution across the brain and improve efficacy, AAVrh.10-CLN2 was developed. This construct produced greater and faster anterograde and retrograde spread after non-CED striatal delivery compared to AAV2-CLN2 in murine and NHP models. Low concentration of vector was detected in the lung, spleen, and liver with neutralizing antibodies detected after injection. Treatment with the AAVrh.10 vector significantly improved motor and behavioral functioning while also prolonging survival [33,97]. Intraventricular injection of scAAV9-CLN5 in a sheep model had a similar effect, halting disease progression in the brain and improving motor function [201]. For NCL CLN5, treated sheep still had a decline in visual function from retinal degeneration as no vector was introduced into the vitreous space [201].

While the NCL-CLN5 trial remains ongoing at time of publication, data on AAV2-CLN2 and AAVrh.10-CLN2 have been published. Non-CED intraparenchymal administration of AAV2-CLN2 led to some slowing of NCL CLN2 progression but there was no difference in brain volume loss between treated patients and the natural history cohort [202]. Similar to preclinical results, non-CED intraparenchymal AAVrh.10-CLN2 showed greater efficacy compared to the AAV2 construct (NCT01161576) [203]. There was robust transgene expression with slowing of brain atrophy as well as improvement in motor function and language. However, this construct was not as effective as the current standard of care, enzyme replacement therapy with direct intraventricular delivery of TPP-1 (the product of CLN2). In addition, T2 hyperintensities at injection sites were observed on MRI without clinical symptoms. The dose was subsequently lowered to address this potential safety concern (reduced from total dose of 9×10^{11} vg to 2.85×10^{11} vg, administered across 12 sites). Seizures and dyskinesias occurred in a few treated patients, but it remained unclear whether this could be attributed to the therapy [203]. Together, these findings may indicate that the dose and/or expression strategy of CLN2 may need to be optimized to drive sufficient expression while minimizing potential safety risks.

Leukodystrophies

Four trials are developing therapies for two different leukodystrophies: adrenoleukodystrophy (ALD, one trial) and Canavan disease (three trials). For ALD, an intraparenchymal non-CED TYF lentivirus construct is being used to deliver the ABCD1 gene in pediatric and/or adult patients. For Canavan disease, AAV9 (intraventricular + intravenous) and AAVOlig001 (intraparenchymal, non-CED) constructs are being used to deliver the aspartoacylase (ASPA) gene in pediatric patients, with AAV9 constructs using an optimized expression transgene to boost gene transduction.

ALD is an X-linked disorder caused by mutations in the ABCD1 gene that leads to impaired peroxisomal function. This leads to the accumulation of very long chain fatty acids (VLCFAs) and a progressive inflammatory demyelinating process. A TYF-ABCD1 lentivirus construct iterated on results seen from initial AAV9 experiments by performing direct non-CED intraparenchymal delivery of ASPA in a murine model. Gene transduction occurred mainly around the injection site with no vector detected in peripheral tissues. There was no significant neuroinflammatory reaction and treated rodents showed a decrease in VLCFA levels with improvement in motor symptoms [31].

Canavan disease occurs due mutations in the ASPA gene in oligodendrocytes which prevents catabolism of N-acetylaspartate. This interferes with myelination, resulting in significant white matter loss. Given the diffuse CNS nature of Canavan disease, intravenous delivery of AAV9-ASPA was initially studied to achieve diffuse gene transduction. In a murine model, intravenous delivery restored ASPA levels in the brain with robust expression in neurons and astrocytes. The vector construct was detected in peripheral tissues with no major symptoms. AAV9-ASPA prolonged survival and delayed disease progression [204]. To boost expression of this construct, an optimized ASPA transgene was developed [205]. In a murine model, intravenous delivery of the optimized ASPA transgene showed greater histopathologic normalization of brain tissue, reduction in brain edema, normalization of NAA levels, and sustained improvement in motor function compared to the unenhanced ASPA transgene [205]. An alternative approach was taken with an AAVOlig001 construct to specifically target ASPA delivery to oligodendrocytes. Intraventricular delivery in a murine model showed the greatest number of cells transduced across all brain regions studied (cortex, deep white matter, striatum, cerebellum) compared to intraparenchymal (non-CED), intracisternal, or intrathecal routes of administration. The majority of cells transduced were oligodendrocytes, followed by a small proportion of neurons. There was minimal vector detected in peripheral tissue, mainly confined to the liver and kidneys. Treatment with AAVOlig001-ASPA significantly normalized brain histopathology, reduced NAA levels, and improved motor symptoms [46]. While final clinical trial results have not been published, preliminary trial results for the AAV9-ASPA construct with an optimized ASPA transgene indicated a significant reduction in CSF and urine NAA in four treated patients with Canavan disease [206].

Other neurological conditions

Ten trials are examining the remainder of neurologic conditions for which neurosurgical gene therapy is being employed: frontotemporal dementia (FTD, three trials), Huntington's disease (HD, three trials), Dravet syndrome (one trial), Rett syndrome (one trial), mesial temporal lobe epilepsy (MTLE, one trial), and glutaric acidemia type 1 (one trial).

Various vector constructs and transgenes have been developed for each condition. For FTD, AAV1 and AAV9 constructs are being used to deliver the granulin (GRN) gene intracisternally or intraparenchymally (CED, MRI guided) in adult patients with a pathogenic GRN mutation. AAV5 with miRNA targeting the huntingtin (HTT) gene (AAV5-miHTT, intraparenchymal) and AAVrh.10-CYP46A1 (intraparenchymal) are being explored in adult patients with early manifesting HD and greater than forty repeats in the huntingtin gene without significant striatal volume loss. Both HD vector constructs are targeted to the bilateral striatum. An intraventricular AAV9-eTFSCN1A with GABA regulatory element is being examined for Dravet syndrome in patients who have a pathogenic SCN1A variant with a convulsive seizure before 15 months of age. For Rett syndrome, intraventricular delivery of an AAV-9 vector carrying methyl-CpG-binding protein 2 (MECP2) is being tested in female pediatric patients with confirmed MECP2 mutation. AAV9 with miRNA targeting the glutamate ionotropic receptor kainite type subunit 2 (BRIK2) gene is being examined through intraparenchymal MRI guided delivery (non-CED) in adult patients with refractory MTLE with concordant unilateral hippocampal imaging findings and seizure focus. Finally, an AAV9 vector has been developed for intraventricular delivery of glutaryl-CoA-dehydrogenase (GCDH) for pediatric patients diagnosed with glutaric acidemia type 1 who have biallelic GCDH mutations.

Frontotemporal dementia

FTD caused by a single mutation in the GRN gene is a result of lysosomal dysfunction from low GRN levels [207]. In NHPs, intracisternal delivery of AAV1, AAV5, and AAVhu68 serotypes were first tested. While all treated groups showed elevated GRN expression, AAV1 showed the highest level of GRN transduction and expression. Gene transduction in the brain parenchyma was roughly equivalent between AAV1 and AAVhu68, with AAV5 showing minimal transduction. However, AAV1 showed much higher transduction of ependymal cells lining the ventricles versus AAVhu68, contributing to the higher GRN expression. Though there was a mild lymphocytic infiltrate in the meninges, the therapy was well tolerated with no adverse events [208]. The AAV1-GRN construct was therefore selected for human trials. A separately developed AAV9-GRN construct was tested in both murine and NHP models. Intraventricular delivery of this construct was well tolerated with broad biodistribution, increase in progranulin levels, and decrease in neuroinflammatory markers [209]. Finally, a recent clinical trial has begun testing intraparenchymal CED delivery of AAV9-GRN to the bilateral thalamus with MRI guidance. In preclinical murine (non-CED), sheep (CED), and NHP (CED) models, delivery of AAV9-GRN to the thalamus demonstrated robust GRN expression to physiological levels [210].

Huntington's disease

Fully penetrant HD can be triggered by forty or more repeats in the HTT gene, resulting in a mutant HTT protein that causes neuronal death. An AAV5-miHTT construct was created to express a miRNA that bind to HTT mRNA and suppress its translation [211]. In murine and porcine models of HD, striatal delivery (non-CED) of the AAV5-miHTT construct

showed effective transgene expression in the striatum, with dose-dependent retrograde cortical distribution. Interestingly, AAV5-miHTT delivery to the thalamus (non-CED) showed dose-dependent anterograde and retrograde distribution to the cortex and striatum [95]. There was significantly reduced mutant HTT protein and neuronal dysfunction in the striatum with minimal evidence of neuroinflammation following injection. Intraparenchymal injection also resulted in minimal vector detected in peripheral organs [95,212]. A longer-term preclinical study showed sustained vector DNA and miHTT expression in both the striatum and cortex after non-CED striatal delivery of AAV5-miHTT in a murine model. There was continued lowering of HTT protein with no safety concerns. Treatment further showed functional benefits and improvement in survival [85]. However, there is a concern that sustained excessive lowering of HTT, including normal HTT, could be detrimental. In a murine model, long-term suppression of both normal and mutant HTT with AAV5-miHTT showed signs of impaired motor coordination, supporting the need to develop an optimal dosing scheme for this construct [213]. AAVrh.10-CYP46A1 is another construct being tested for HD, targeting abnormal cholesterol metabolism in HD to prevent excess reduction of normal HTT protein. Specifically, CYP46A1, an enzyme which converts cholesterol to its metabolites, is decreased in the putamen of HD patients and produces striatal neurodegeneration in knockout animal models. Intraparenchymal striatal delivery (non-CED) of AAVrh.10-CYP46A1 in a murine model showed transgene expression in the striatum, localized predominantly to neurons. Delivery and expression of CYP46A1 delayed the deterioration of motor function and helped normalize cholesterol levels [214]. In human clinical trials, interim results for AAV5-miHTT indicate that the drug is well tolerated with a significant decrease in CSF HTT protein in treated patients versus untreated patients [215].

Dravet syndrome

The phenotype of Dravet syndrome is driven by heterozygous loss of function of SCN1A which codes for voltage gated sodium channels that are predominantly expressed on GABA interneurons. This impairs inhibitory function, driving prolonged seizures. As the SCN1A gene exceeds the payload capacity of AAVs, the AAV9-eTFSCN1A construct for Dravet syndrome also employs novel gene modification approaches. eTFSCN1A is an engineered transcription factor which upregulates endogenous SCN1A expression. This is paired with a GABA regulatory element to selectively target expression in GABA inhibitory interneurons. In a murine model of Dravet syndrome, intraventricular injection of this construct led to increased SCN1A expression and voltage gated sodium channels in GABA interneurons with low off target expression in excitatory neurons [216]. This significantly decreased seizures and prolonged survival. In healthy non-human primates, intraventricular delivery of AAV9-eTFSCN1A showed robust expression across the brain and low transgene expression in the liver and cervical lymph nodes. Overall, the vector was well tolerated with no adverse events [216]. The United Kingdom-based clinical trial (NCT06283212) with this vector construct remains ongoing.

Rett syndrome

The X-linked loss of MECP2 function in Rett syndrome impairs transcriptional regulation of DNA, leading to diffuse neuronal dysfunction and stereotypical symptoms such as repetitive hand wringing.

While several different AAV-MECP2 constructs have been tested in preclinical models with systemic delivery, there were concerns surrounding elevated MECP2 expression and associated toxicity [217]. In murine models, intraventricular delivery of AAV9-MECP2 showed greater brain transduction efficiency while also increasing rodent survival and improving behavioral symptoms [218,219]. A recent clinical trial (NCT05898620) has paired intraventricular AAV9-MECP2 with a proprietary system that controls gene expression in the effort to limit dose toxicity, with enrollment ongoing and supporting NHP safety data, according to the sponsor's press release [220].

Mesial temporal lobe epilepsy

Aberrant response from glutaminergic kainate receptors are thought to contribute to temporal lobe seizure foci [221]. Kainic acid animal models have reliably been studied as models for human temporal lobe epilepsy, further implicating kainate receptors [222]. An important subunit of these receptors is encoded by the GRIK2 gene (GluK2), therefore serving as a potential target for a vector mediated therapy. These principles were tested in a preclinical murine model of drug-resistant temporal epilepsy: non-CED intraparenchymal delivery of AAV9-miGRIK2 into the hippocampus significantly downregulated the GluK2 subunit while suppressing interictal-like epileptiform discharges and seizures [223]. In NHPs, CED MRI guided delivery into the hippocampus further demonstrated knockdown of GRIK2 with no safety events [224]. Enrollment is underway for the clinical trial (NCT06063850) assessing this construct.

Glutaric acidemia type 1

Glutaric acidemia type 1 is an inherited metabolic disorder that most commonly presents in infants or early childhood. It is linked to biallelic mutations in GCDH which impact amino acid processing, causing encephalopathic crises, striatal injury, and dyskinesias [225]. In a murine model of glutaric acidemia type 1, intraventricular injection of AAV9-GCDH resulted in functional GCDH expression and was protective against mortality [226]. Patient recruitment recently began in an inaugural trial (NCT06217861) to test this construct.

Identification of the genetic bases underlying neurodegenerative and neurodevelopmental diseases has led to rapid growth in vector mediated gene therapies. The earliest iterations of viral vector delivery showed that such a platform can be safe and well-tolerated in humans across age groups. Through improved preclinical modeling and larger clinical trials, there has been systematic evolution of viral vector gene therapies across the therapeutic development pipeline. New capsid serotypes continue to improve CNS targeting, both through direct CNS and systemic delivery. Directed capsid engineering has opened a new frontier with cell type specificity (e.g., AAVOlig001 and targeted oligodendrocyte expression). Packaging strategies through condensed transgenes, promoter localization, and multi-gene expression has broadened the types of transgene constructs that can be delivered. Development of vector delivery approaches, specifically convection enhanced delivery and real-time intraoperative monitoring of vector distribution has improved CNS regional localization and transgene expression. Packaged regulatory elements have shown the ability for additional cell type specificity as well as fine-tuning of gene product dose. Finally, the introduction of microRNA and engineered transcription factor transgenes has opened up a new dimension on the diseases that can be modified through a vector-based approach. The impacts of these avenues of investigation can be seen in the clinical data, with many trials showing robust gene expression years after initial treatment and significant clinical benefit. Viral-vector gene therapeutics have therefore gone from an emerging conceptual entity to a validated platform at scale, holding significant potential in addressing the range of neurological conditions that so critically need effective treatment options.

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Author's Contributions

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mark Richardson reports a relationship with uniQure biopharma BV that includes: consulting or advisory. Mark Richardson reports a relationship with AviadoBio that includes: consulting or advisory. Mark Richardson reports a relationship with ClearPoint Neuro that includes: consulting or advisory. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurot.2024.e00434.

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