

# Viral gene therapy for paediatric neurological diseases: progress to clinical reality

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## ABBREVIATIONS

AA DC	Aromatic L-amino acid decarboxylase
AAV	Adeno-associated virus
ASO	Antisense oligonucleotide
MPS	Mucopolysaccharidosis
rAAV	Recombinant adeno-associated virus

In the era of genomic medicine, diagnoses of rare paediatric neurological diseases are increasing. Many are untreatable and life-limiting, leading to an exceptional increase in gene therapy development. It is estimated that 20 gene therapy products will have received approval from the US Food and Drug Administration by 2025. With viral gene therapy considered a potential single-dose cure for patients with spinal muscular atrophy type 1 as one example, and contemporaneously tragically resulting in the deaths of three male children with X-linked myotubular myopathy receiving high-dose gene therapy in 2020, what is the current state of gene therapy? What is behind the decades of hype around viral gene therapy and is it high impact, but high risk? In this review, we outline principles of viral gene therapy development and summarize the most recent clinical evidence for the therapeutic effect of gene therapy in paediatric neurological diseases. We discuss adeno-associated virus and lentiviral vectors, antisense oligonucleotides, emerging genetic editing approaches, and current limitations that the field still faces.

The impact of genome and exome sequencing has led to an unprecedented rise in diagnoses of genetic paediatric neurological diseases.<sup>1</sup> This provides an opportunity to understand disease mechanisms and to develop novel therapeutic approaches for significant unmet clinical needs. Gene therapy aims to restore gene function and thus cell function through different approaches, depending on the consequences of the genetic mutation. Therapeutic strategies include: (1) gene supplementation (inherited monogenic disorders), (2) gene silencing (e.g. for Huntington disease), and (3) mutation correction through gene, base, or prime editing (e.g. for Duchenne muscular dystrophy).<sup>2</sup>

The concept of gene therapy was first described in the late 1900s and for decades the promise failed to meet expectation.<sup>3</sup> In the 1990s, serious adverse events in two trials devastated the field. The first was ornithine transcarbamylase deficiency gene therapy delivered by intrahepatic infusion of wild-type adenovirus that resulted in vector-related immune response, multi-organ failure, and death.<sup>4</sup> The second was gamma retroviral correction of X-linked severe combined immunodeficiency, which reconstituted immunity but vector-related leukaemia consequently developed.<sup>5</sup> These highlighted an inadequate understanding of vector properties, and gene therapists have worked tirelessly to engineer recombinant viral vectors to improve safety and efficiency. Recent landmark clinical trials in gene therapy for haemophilia, Leber congenital amaurosis, and spinal muscular atrophy type 1 showcase the profound

therapeutic impact of gene therapy.<sup>6–8</sup> With over 800 gene and cell therapy programmes and eight therapies receiving European Medicines Agency and US Food and Drug Administration approval, gene therapy is now a clinical reality (Fig. 1).<sup>9,10</sup> It is important for all to have an understanding and perspective on emerging new genetic precision medicines from bench to bedside. Here, we provide an overview of viral gene therapy development and review clinical gene therapy trials for paediatric neurological diseases on [clinicaltrials.gov](https://clinicaltrials.gov) as of February 2021 (Tables 1–4).

Viral gene therapies deliver therapeutic genes to affected cells using modified viruses based on adenovirus, retrovirus, and adeno-associated virus (AAV). The main viral vectors used are AAV and lentiviral vectors. These viral vectors transduce both mitotic and post-mitotic cells and hold different properties that lend them to different clinical diseases. A gene therapy construct consists of a promoter, therapeutic transgene, regulatory elements, and signal sequences that enable packaging into a vector. The resulting viral vector is the vehicle used to deliver the therapeutic construct, as a medicine, to the diseased cell target (Fig. 2).

## AAV VECTORS

AAVs were identified in 1960s as contaminants of adenovirus isolates and belong to the *Parvoviridae* family.<sup>2,9</sup> AAVs are naturally endemic to humans with no reported

pathogenicity and require co-infecting helper viruses (adenovirus or herpes simplex virus) for replication. Recombinant AAV (rAAV) is generated by removal of all open reading frames to render it replication-deficient. The remaining sequences allow for the therapeutic construct to be flanked by two inverted terminal repeats. AAVs are small non-enveloped viruses (~25nm) with an icosahedral capsid structure and a cargo capacity of up to 4.65kilobases of single-stranded DNA (halved in their double-stranded, 'self-complementary' configuration [Fig. 1]), restricting application to smaller transgenes. There have been over 200 rAAV clinical trials demonstrating excellent safety profiles due to non-integration and episomal persistence for at least 10 years.<sup>2,9,10</sup> There are over 100 AAV serotypes that show different cellular tropisms and some with superior central nervous system (CNS) transduction (Table 5). AAV2 is the most extensively characterized serotype; it transduces neurons and is delivered by intraparenchymal injection. AAV serotype 9 crosses the blood–brain barrier, transducing both neurons and astrocytes after intravenous delivery.<sup>11</sup> This has resulted in CNS gene therapies delivered by intraparenchymal, intravenous, or cerebrospinal fluid space delivery (Fig. 3).<sup>8–10</sup> Other neurotropic

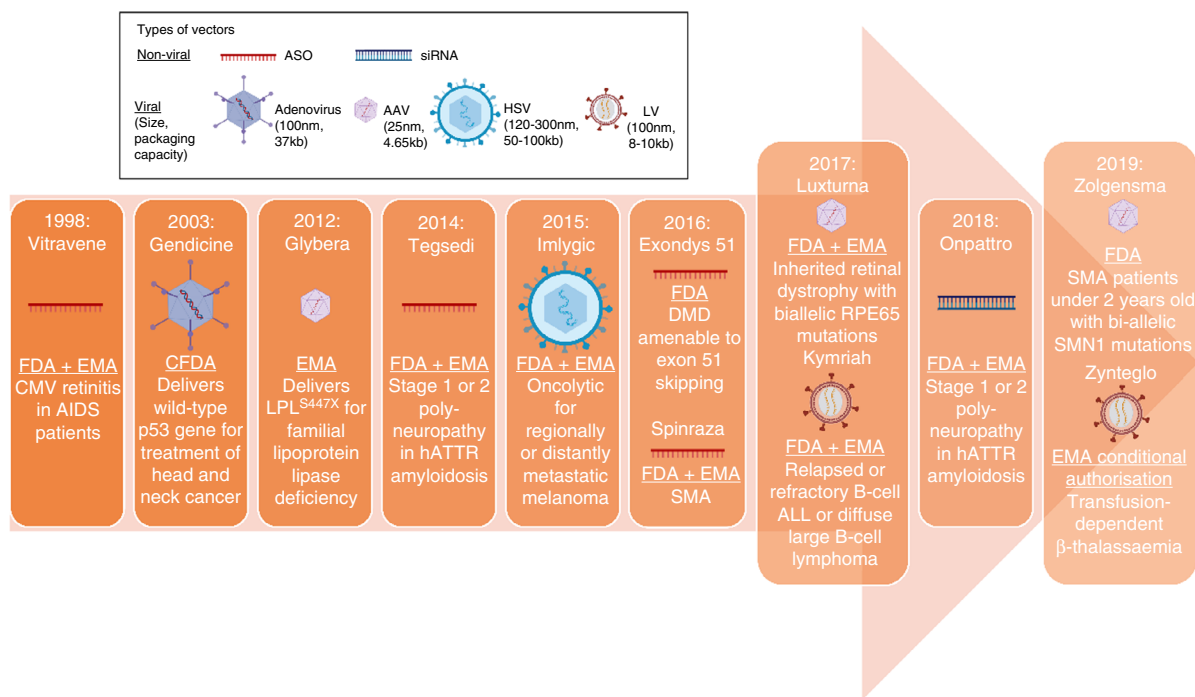
### What this paper adds

- Viral gene therapy development and clinically used transgenes, regulatory elements, capsids, dosage, and delivery routes are summarized.
- Viral gene therapy for 18 childhood neurological disorders involving over 600 children in 40 clinical trials are reviewed.

serotypes used in clinical trials (Table 5), novel serotypes, and engineered rAAV capsid variants are continually under development to improve efficiency.<sup>9</sup>

### LENTIVIRAL VECTORS

Lentiviruses are recombinant retroviral vectors derived from the human immunodeficiency virus with single-stranded RNA genome converted to DNA in the transduced cell by a virally encoded enzyme called reverse transcriptase. Lentiviruses have higher packaging capacity (8–10kilobases) than AAV and efficiently transduce both proliferating and post-mitotic cells including neural precursor, haematopoietic stem cells, neurons, and glia. Lentivirus integrates into the host cell genome, leading to lifelong transgene expression in targeted cells.<sup>12</sup> Advances to improve safety of retroviral and lentiviral vectors have resulted in successful clinical trials in ex vivo correction



**Figure 1:** Timeline showing regulatory approvals of gene therapy products. Ten out of 12 products have been approved in the past decade. Viral-vector-mediated delivery is known to provide long-lasting effects due to episomal persistence or integration, while non-viral approaches such as antisense oligonucleotide (ASO) and small interfering RNA (siRNA) require repeated administration; therefore, it might result in a difference in price per dose in approved products. Vitravene and Glybera have been withdrawn owing to limited patient demands. AAV, adeno-associated viral vector; AIDS, acquired immune deficiency syndrome; ALL, acute lymphoblastic leukaemia; CMV, cytomegalovirus; EMA, European Medicines Agency; FDA, US Food and Drug Administration; hATTR amyloidosis, hereditary transthyretin-mediated amyloidosis; HSCs, haematopoietic stem cells; HSV, herpes simplex virus; LPLD, lipoprotein lipase deficiency; LV, lentivirus; RPE65, retinal pigment epithelium 65kDa; SMA, spinal muscular atrophy; SMN1, survival motor neuron.

**Table 1:** Clinical interventional viral gene therapy studies for paediatric neurological diseases: lysosomal storage diseases

Disease	Promoter. <i>transgene</i>	Vector	Trial phase	Patient number	Total dose	Delivery route	Clinicaltrials.gov ID; study	Duration
LINCL (Batten)	CAG. <i>CLN2</i>	rAAV2	1 (active, not recruiting)	10	$3 \times 10^{12}$ vg	Intracerebral (12 injections)	NCT00151216; Worgall et al. <sup>19</sup>	2004–2020
		rAAVrh.10	1/2 (active)	8	$2.85 \times 10^{11}$ vg	Intracerebral (12 injections)	NCT01161576; NCT01414985	2020–2032 2010–2017
	P546. <i>CLN3</i>	rAAV9	1/2 (recruiting)	7	N/A	Intrathecal	NCT03770572	2018–2023
	CB. <i>CLN6</i>	rAAV9	1/2 (active, not recruiting)	13	$1.5 \times 10^{13}$ vg	Intrathecal	NCT02725580	2016–2021
MPS IIIA	PGK1. <i>SGSH</i> .Ires. <i>SUMF1</i>	rAAVrh.10	1/2 (completed)	4	$7.2 \times 10^{11}$ vg	Intracerebral (6 injections, 12 deposits)	NCT01474343; NCT02053064; Tardieu et al. <sup>20</sup>	2011–2013
	CAG. <i>SGSH</i>	rAAVrh.10	2/3 (active, not recruiting)	20	$7.2 \times 10^{12}$ vg	Intracerebral (6 injections)	NCT03612869	2018–2022
	U1a. <i>SGSH</i>	rAAV9	1/2 (recruiting)	22	$5 \times 10^{12}$ vg/kg $1 \times 10^{13}$ vg/kg $3 \times 10^{13}$ vg/kg	Intravenous	NCT02716246	2016–2022
MPS IIIB	CMV. <i>NAGLU</i>	rAAV9	1/2 (recruiting)	12	$3 \times 10^{13}$ vg/kg	Intravenous	NCT04088734	2019–2023
		rAAV9	1/2 (recruiting)	12	$2 \times 10^{13}$ vg/kg $5 \times 10^{13}$ vg/kg	Intravenous	NCT03315182	2017–2020
	PGK1. <i>NAGLU</i>	rAAV5	1/2 (completed)	4	$4 \times 10^{12}$ vg	Intracerebral (16 injections)	NCT03300453	2013–2019
Tay–Sachs	CBA. <i>HEXA</i> and CBA. <i>HEXB</i>	rAAVrh.8	N/A	2	$5 \times 10^{13}$ vg/kg of brain weight $1 \times 10^{14}$ vg/kg of brain weight	Premedullary cistern or cisterna magna via lumbar spinal cord	Taghian et al. <sup>22</sup>	2019
GM1 and 2	CAG. <i>GLB1</i>	rAAV9	1/2 (recruiting)	45	$1.5 \times 10^{13}$ vg $4.5 \times 10^{13}$ vg	Intravenous	NCT03952637	2019–2024
Gaucher type 1	Promoter not disclosed. <i>GBA</i>	LV	1/2 (recruiting)	16	N/A	<i>Ex vivo</i>	NCT04145037	2019–2022
Fabry	Promoter not disclosed. <i>AGA</i>	LV	1/2 (recruiting)	12	N/A	<i>Ex vivo</i>	NCT03454893	2018–2021
MLD	hPGK. <i>ARSA</i>	LV	1/2 (active, not recruiting)	20	N/A	<i>Ex vivo</i>	NCT01560182; Sessa et al. <sup>27</sup>	2010–2023
	CAG. <i>ARSA</i>	rAAVrh.10	1/2 (active, not recruiting)	5	$1 \times 10^{12}$ vg $4 \times 10^{12}$ vg	Intracerebral (6 injections, 12 deposits)	NCT01801709	2013–2019
	Promoter not disclosed. <i>ARSA</i>	LV	2 (recruiting)	10	N/A	<i>Ex vivo</i>	NCT03392987	2018–2028
MLD + X-ALD	Promoter not disclosed. <i>ARSA.ABCD1</i>	LV	1/2 (recruiting)	10	N/A	Intracerebral (not detailed)	NCT03725670	2018–2020
			1/2 (recruiting)	50	N/A	<i>Ex vivo</i>	NCT02559830	2015–2025

rAAV, recombinant adeno-associated virus; ABCD1, ATP binding cassette subfamily D member 1; AGA, alfa-galactosidase A; ARSA, arylsulphatase A; CAG, CMV-chicken  $\beta$ -actin promoter with  $\beta$ -globin splice acceptor; CB, cytomegalovirus enhancer/promoter-chicken  $\beta$ -actin promoter; CBA, chicken  $\beta$ -actin promoter; CLN, ceroid lipofuscinosis; CMV, cytomegalovirus enhancer/promoter; GBA, glucocerebrosidase; GLB1, beta-galactosidase 1; HEXA/B, hexosaminidase A/B; hPGK, human phosphoglycerate kinase 1 promoter; Ires, internal ribosome entry site; LINCL, late infantile neuronal ceroid lipofuscinosis; LV, lentivirus; MLD, metachromatic leukodystrophy; MND, myeloproliferative sarcoma virus enhancer; MPS IIIA/B, mucopolysaccharidosis/Sanfilippo type A/B syndrome; NAGLU, N-acetyl-alpha-D-glucosamine; P546, truncated MeCP2-promoter; PGK1, mouse phosphoglycerate kinase 1 promoter; SGSH, N-sulfoglucosamine sulfohydrolase; SUMF1, sulfatase modifying factor 1; U1a, mouse small nuclear RNA promoter; vg, vector genomes; X-ALD, X-linked adrenoleukodystrophy.

and immune reconstitution for congenital immunodeficiencies.<sup>13,14</sup> CNS applications of lentivirus include human clinical trials for leukodystrophies and adult Parkinson disease.<sup>10</sup>

## NON-VIRAL VECTORS AND ANTISENSE OLIGONUCLEOTIDES

Viral vectors present intrinsic limitations (e.g. potential immunogenicity, packaging restriction, complex bioproduction). Alternative technologies include synthetic non-viral delivery platforms such as cationic liposomes and polymers, or inorganic nanoparticles enclosing nucleic acids. These systems present the advantages of simpler

production techniques, lower immunogenicity, and larger payload capacity. However, clinical translation is limited owing to lower transfection efficiency and potential cytotoxicity caused by cationic surfaces.<sup>15</sup> Preclinical studies have shown promising results in nanoparticle cancer therapy, including improved overall survival in mouse models of paediatric brain malignancies.<sup>16</sup>

Genetic therapies also use RNA as a therapeutic molecule. Three antisense oligonucleotides (ASOs) and one small interfering RNA hold regulatory approval for neurological conditions.<sup>17</sup> ASOs are synthetic, short, single-stranded oligodeoxynucleotides that can alter complementary messenger RNA and relative protein expression

**Table 2:** Clinical interventional viral gene therapy studies for paediatric neurological diseases: leukodystrophies

Disease	Promoter.transgene	Vector	Trial phase	Patient number	Total dose	Delivery route	Clinicaltrials.gov ID; study	Duration
Canavan	NSE.ASPA	rAAV2	1 (recruiting)	13	$9 \times 10^{11}$ vg	Intracerebral (6 injections)	Leone et al. <sup>25</sup>	N/A
X-ALD	MND.ABCD1	LV	2/3 (active, not recruiting)	32	N/A	Ex vivo	NCT01896102	2013–2021
	Promoter not disclosed.ABCD1		1/2 (recruiting)	10	N/A	Intracerebral (not detailed)	NCT03727555	2018–2020
	MND.ABCD1		3 (recruiting)	35	N/A	Ex vivo	NCT03852498	2019–2023

rAAV, recombinant adeno-associated virus; ABCD1, ATP binding cassette subfamily D member 1; ASPA, aspartoacylase; LV, lentivirus; MND, myeloproliferative sarcoma virus enhancer; NSE, human neuron-specific enolase promoter; vg, vector genomes; X-ALD, X-linked adrenoleukodystrophy.

**Table 3:** Clinical interventional viral gene therapy studies for paediatric neurological diseases: neuromuscular disorders

Disease	Promoter.transgene	Vector	Trial phase	Patient number	Total dose	Delivery route	Clinicaltrials.gov ID; study	Duration
SMA1	CB.SMN	rAAV9	1 (completed)	15	$6.7 \times 10^{13}$ vg $2 \times 10^{14}$ vg	Intravenous	NCT02122952; Mendell et al. <sup>8</sup>	2014–2017
			3 (completed)	22	Not disclosed	Intravenous	NCT03306277	2017–2019
			1 (suspended, pending preclinical data review)	51	$6 \times 10^{13}$ vg $1.2 \times 10^{14}$ vg $2.4 \times 10^{14}$ vg	Intrathecal	NCT03381729	2017–2021
			3 (active, not recruiting)	33	Not disclosed	Intravenous	NCT03461289	2018–2020
			3 (active, not recruiting)	30	$1.1 \times 10^{14}$ vg/kg	Intravenous	NCT03505099	2018–2023
			3 (active, not recruiting)	6	$2 \times 10^{14}$ vg	Intravenous	NCT03837184	2019–2021
DMD	CK8.Micro-dystrophin	rAAV9	1/2 (recruiting)	16	Not disclosed	Intravenous	NCT03368742	2017–2021
	MHCK7.Micro-dystrophin	rAAVrh.74	1/2 (active, not recruiting)	4	$2 \times 10^{14}$ vg/kg (10mL/kg)	Intravenous	NCT03375164	2018–2021
	Promoter not disclosed.Mini-dystrophin	rAAV9	1 (enrolling)	15	Not disclosed	Intravenous	NCT03362502	2018–2025
LGMD2D	tMCK.SGCA	rAAV1	1 (completed)	6	$3.25 \times 10^{11}$ vg in 1.5mL	Intramuscular (2–6 injections)	NCT00494195	2008–2011
		rAAVrh.74	1/2 (completed)	6	$1 \times 10^{12}$ vg/kg (single limb) $1 \times 10^{12}$ vg/kg (both limbs) $3 \times 10^{12}$ vg/kg (both limbs)	Intravenous	NCT01976091	2015–2019
XLMTM	Des.MTM1	rAAV8	1/2 (active, not recruiting)	24	$1 \times 10^{14}$ vg/kg $3 \times 10^{14}$ vg/kg	Intravenous	NCT03199469	2017–2024
Charcot-Marie-Tooth 1A	tMCK.NTF3	rAAV1	1/2 (active, recruiting)	9	$2 \times 10^{12}$ vg/kg $6 \times 10^{12}$ vg/kg	Intramuscular (2 injections)	NCT03520751	2020–2023
Giant axonal neuropathy	JeT.GAN	rAAV9	1 (recruiting)	30	$3.5 \times 10^{13}$ vg $1.2 \times 10^{14}$ vg $1.8 \times 10^{14}$ vg	Intrathecal	NCT02362438	2015–2030

rAAV, recombinant adeno-associated virus; CB, cytomegalovirus enhancer/promoter-chicken  $\beta$ -actin promoter; CK8, muscle creatine kinase promoter/enhancer element; Des, human desmin promoter; DMD, Duchenne muscular dystrophy; GAN, giant axonal neuropathy; JeT, early SV40, human  $\beta$ -actin and ubiquitin C promoter hybrid; LGMD2D, Limb-girdle muscular dystrophy type 2D; MHCK7, hybrid alpha-myosin heavy chain enhancer-/muscle creatine kinase enhancer-promoter; MTM1, myotubularin; SGCA, sarcoglycan alpha; SMA1, spinal muscular atrophy type 1; SMN, survival motor neuron; tMCK, truncated muscle creatine kinase promoter; vg, vector genomes; XLMTM, X-linked myotubular myopathy.

through targeted degradation, translational arrest, inhibition of RNA-binding proteins, splicing modulation, or increased translational activity. Small interfering RNAs are synthetic, double-stranded oligonucleotides that degrade target messenger RNA through RNA interference mechanisms.<sup>18</sup> Chemical modifications to the backbones

(phosphorothioate DNA, phosphorodiamidate morpholino, peptide nucleic acid, tricyclo-DNAs, ribose substitutions, locked nucleic acids) have significantly enhanced pharmacokinetic properties, tolerability profiles, and target-binding affinity. However, these molecules require periodic administration and do not readily cross the blood–brain

**Table 4:** Clinical interventional viral gene therapy studies for paediatric neurological diseases: neurotransmitter disorders

Disease	Promoter/transgene	Vector	Trial phase	Patient number	Total dose	Delivery route	Clinicaltrials.gov ID; study	Duration
AADC deficiency	CMV-hGHi. AADC	rAAV2	1/2 (active, not recruiting)	10	Not disclosed	Intrarenchymal (Putamen)	NCT01395641; Hwu et al. <sup>41</sup> ; Chien et al. <sup>42</sup>	2014–2020
			2 (active, not recruiting)	10	$2.37 \times 10^{11}$ vg	Intrarenchymal (Putamen); 2 injections	NCT02926066	2016–2020
			1 (recruiting)	6	$1.3 \times 10^{11}$ vg	Intrarenchymal (SNc and VTA; 4 injections)	NCT02852213	2016–2021

rAAV, recombinant adeno-associated virus; AADC, aromatic L-amino acid decarboxylase; CMV, cytomegalovirus enhancer/promoter; hGHi, human growth hormone intron; SNc, substantia nigra pars compacta; vg, vector genomes; VTA, ventral tegmental area.

barrier, warranting the need for invasive intrathecal or intracerebroventricular delivery routes.<sup>17</sup>

## FROM BENCH TO BEDSIDE

With the knowledge of genetic defect, cellular/organ target, and gene therapy construct size, a suitable vector is selected to deliver the gene therapy. The vectors are validated in initial proof-of-concept in vitro and rodent studies, and higher animals for biodistribution, safety, and toxicity. The preclinical development to first-in-human study may take 5 to 10 years and beyond, while research continues to optimize gene expression, dose, and delivery routes. Herein, we summarize the results of gene therapy clinical trials in paediatric neurological diseases (Tables 1–4).

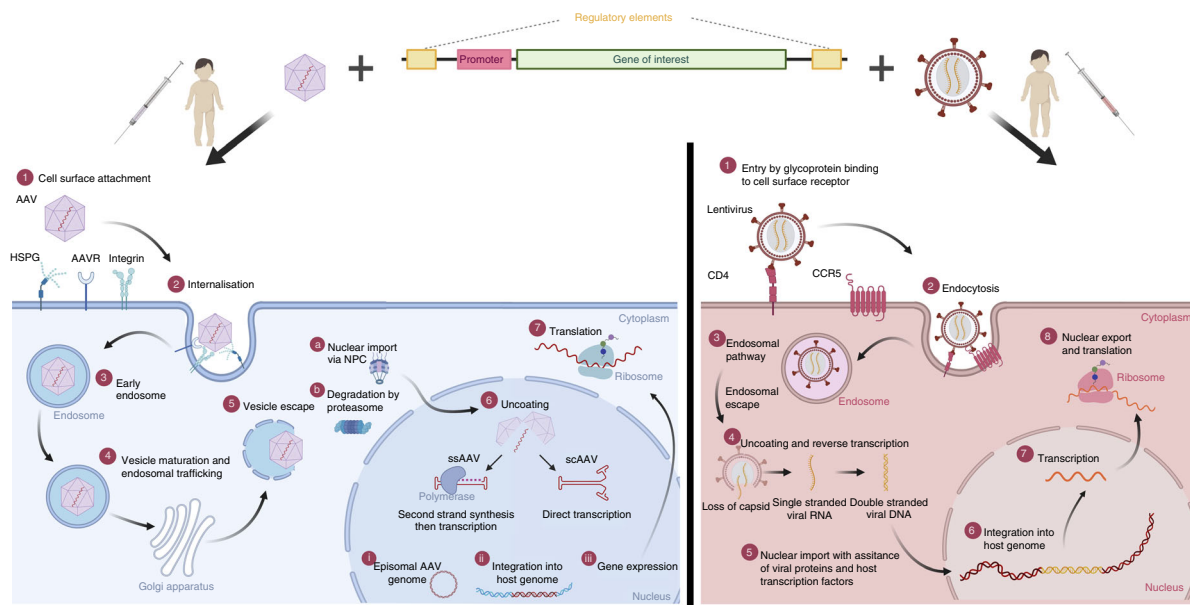
## LYSOSOMAL STORAGE DISEASES

### Neuronal ceroid lipofuscinosis

Late infantile neuronal ceroid lipofuscinosis, Batten disease, is caused by mutations in the *CLN2* gene encoding tripeptidyl peptidase-I. Ten children received rAAV2.*CLN2* delivered through multiple stereotactic intraparenchymal injections, demonstrating safety.<sup>19</sup> A clinical rating scale showed reduced neurological decline for 18 months and quantitative magnetic resonance imaging suggested a trend in maintained brain volume.<sup>19</sup> However, there was inadequate tripeptidyl peptidase-I production due to insufficient diffusion of the gene therapy over the whole brain achievable by the rAAV2 serotype, as disease progression was not halted and survival did not improve. Subsequently, AAV serotypes that spread more widely in the brain were used in follow-on studies, namely intraparenchymal rAAVrh.10 and intrathecal rAAV9 for *CLN3* and *CLN6* (Table 1).

### Mucopolysaccharidoses

Clinical trials for mucopolysaccharidoses (MPS) are also underway. MPS are characterized by accumulation of glycosaminoglycans due to deficient mucopolysaccharides enzymatic degradation. To address predominant CNS involvement in Sanfilippo type A (MPS IIIA), four patients aged 6 months to 2 years received intraparenchymal rAAVrh.10 to express *N*-sulfo-glucosamine sulfohydrolase (SGSH) and SUMF1 (sulfatase-modifying factor, a catalytic activator of SGSH). The phase 1/2 trial showed safety and cognitive improvement in the youngest patient, suggesting earlier intervention improves therapeutic benefits.<sup>20</sup> The 2018 phase 2/3 study for 20 children (19 treated so far) delivered intraparenchymal rAAVrh.10.SGSH. This is on clinical hold following localized changes on magnetic resonance imaging at the injection sites. A 5-year-old female dosed in 2020 died, but this was not deemed intervention-related. Further investigation is underway and the remaining 18 dosed patients continue follow-up.<sup>21</sup> The intravenous rAAV9 trial is still ongoing for MPS IIIA. There is also a current phase 1/2 study for MPS IIIB (Sanfilippo type B) of intraparenchymal rAAV5 delivering *N*-acetyl- $\alpha$ -D-glucosamine (Table 1).



**Figure 2:** Transduction pathway of adeno-associated virus (AAV) and lentivirus. For AAV (left): details of the trafficking pathways are not fully understood, AAVs are thought to bind to serotype-specific receptors/co-receptors (1) and trigger internalization (2) by endocytosis (3–4), followed by endosomal escape facilitated in the acidic environment of matured vesicles (5). From here, the viral particles are translocated to the nucleus through the nuclear pore complex (a) or undergo degradation by proteasomes (b). In the nucleus, viral DNA is released by uncoating (6) and ‘self-complementary’ AAV can directly undergo transcription and export messenger RNA for translation (iii; 7); ssAAV requires second-strand synthesis using host cell polymerase. AAV genome can also form circular concatemers that persist as episomes in the nucleus (i) or, at a low frequency, integrate into the host genome (ii). For lentivirus (right): attachment of viral surface glycoprotein to specific cellular receptors (1) leads to fusion of the two plasma membranes (2–3). The lentiviral RNA genome is released into the target cell and undergoes reverse transcription into DNA (4), which is then imported into the nucleus (5) and integrated into the host cell genome (6). This results in transcription of the proviral genome using cellular machineries (7) and thus protein synthesis (8). AAVR, adeno-associated virus receptor; CCR5, C-C chemokine receptor type 5; CD4, cluster of differentiation 4; HSPG, heparan sulfate proteoglycan; NPC, nuclear pore complex; scAAV, self-complementary AAV; ssAAV, single-stranded AAV.

## Tay–Sachs disease

Tay–Sachs disease is caused by the accumulation of GM2 ganglioside due to defects in  $\alpha$ - (HEXA) or  $\beta$ - (HEXB) subunits, resulting in loss of function of the  $\beta$ -hexosaminidase A (Hex A) degradation enzyme. A phase 1 study showed safe global CNS transduction following a novel intra-cisterna magna injection in two infants (30mo and 7mo). An intrathecal microcatheter was threaded upwards to the cisterna magna, infusing equimolar rAAVrh.8.HEXA and rAAVrh.8.HEXB. The study showed increase in the Children’s Hospital of Philadelphia Infant Test of Neuromuscular Disorders score, increased cerebrospinal fluid Hex A activity, clinical disease stabilization, with seizure cessation and increased myelination.<sup>22</sup> rAAV therapies for neuronopathic Gaucher disease and Niemann–Pick type C1 disease are also progressing towards clinical trials.<sup>23,24</sup>

## LEUKODYSTROPHIES

### Canavan disease

One of the earliest paediatric rAAV gene therapies was for Canavan disease and reported outcomes at 5 years in 2012.

Canavan is due to aspartoacylase deficiency, resulting in accumulation of *N*-acetylaspartate causing dysmyelination and spongiform CNS degeneration. Intraparenchymal rAAV-V2.ASPA was injected into 28 patients (3mo–8y old). *N*-acetylaspartate levels decreased in all patients with arrest or reversal of brain atrophy, modest motor function and alertness improvement, and decreased seizure frequency. Clinical outcome improved in younger patients compared with those with significant atrophy at intervention.<sup>25</sup>

### X-linked adrenoleukodystrophy

There are two strategies for gene therapy in leukodystrophies, using lentivirus or AAV vectors. Lentiviruses are mainly used ex vivo, where patients’ cells are transduced with vectors carrying the therapeutic gene and transplanted back. Lentivirus-based gene therapies are used for X-linked adrenoleukodystrophy and metachromatic leukodystrophy.<sup>26,27</sup> In 2018, Lenti-D, an investigational gene therapy lentivirus, was granted US Food and Drug Administration ‘breakthrough therapy’ designation and used to treat 17 patients with X-linked adrenoleukodystrophy. Adrenoleukodystrophy protein (encoded by the *ABCD1*

**Table 5:** Adeno-associated virus (AAV) serotypes in use for current clinical trials and approved gene therapy products

AAV serotype	Tropism	Main receptor	Co-receptor
1	Muscle, CNS, heart	Sialic acid	AAVR
2	Liver, CNS, muscle	Heparin	Integrin, FGFR, HGFR, LamR, AAVR
5	CNS, lung, eye	Sialic acid	PDGFR, AAVR
9	All tissues	Galactose	LamR, AAVR
rh.8	Similar to AAV8: Liver, muscle, pancreas, CNS	Unknown	Similar to AAV8: Integrin, LamR
rh.10	Liver, CNS	Unknown	Similar to AAV8: Integrin, LamR
rh.74	Similar to AAV8: Liver, muscle, pancreas, CNS	Unknown	Similar to AAV8: Integrin, LamR

CNS, central nervous system; AAVR, adeno-associated virus receptor; FGFR, fibroblast growth factor receptor; HGFR, hepatocyte growth factor receptor; LamR, laminin receptor 1; PDGFR, platelet-derived growth factor receptor.

gene) could be detected in all patients and, at interim analysis, 15 out of 17 patients were alive and free of major functional disease phenotype.<sup>26</sup> However, the therapy was ineffective in one patient who subsequently died from disease progression, and another patient withdrew to undergo standard allogeneic stem-cell transplantation, which resulted in transplant-related death.<sup>26</sup>

### Metachromatic leukodystrophy

Metachromatic leukodystrophy is a lysosomal storage disease-related leukodystrophy caused by loss of function of the arylsulfatase A (*ARSA*) gene resulting in sulfatides accumulation and myelin destruction centrally and peripherally. Phase 1/2 trial interim data on nine patients with metachromatic leukodystrophy (seven presymptomatic) treated with haematopoietic stem cells modified with a lentivirus encoding *ARSA* showed *ARSA* activity in both peripheral haematopoietic cells and cerebrospinal fluid of all patients. Disease progression was halted in six presymptomatic patients, but was ineffective in symptomatic patients at treatment.<sup>27</sup> A total of 20 patients will be treated by 2023. Another ongoing study uses intraparenchymal rAAVrh.10 to deliver *ARSA* to five children with metachromatic leukodystrophy (Table 2).

## NEUROMUSCULAR DISORDERS

### Spinal muscular atrophy

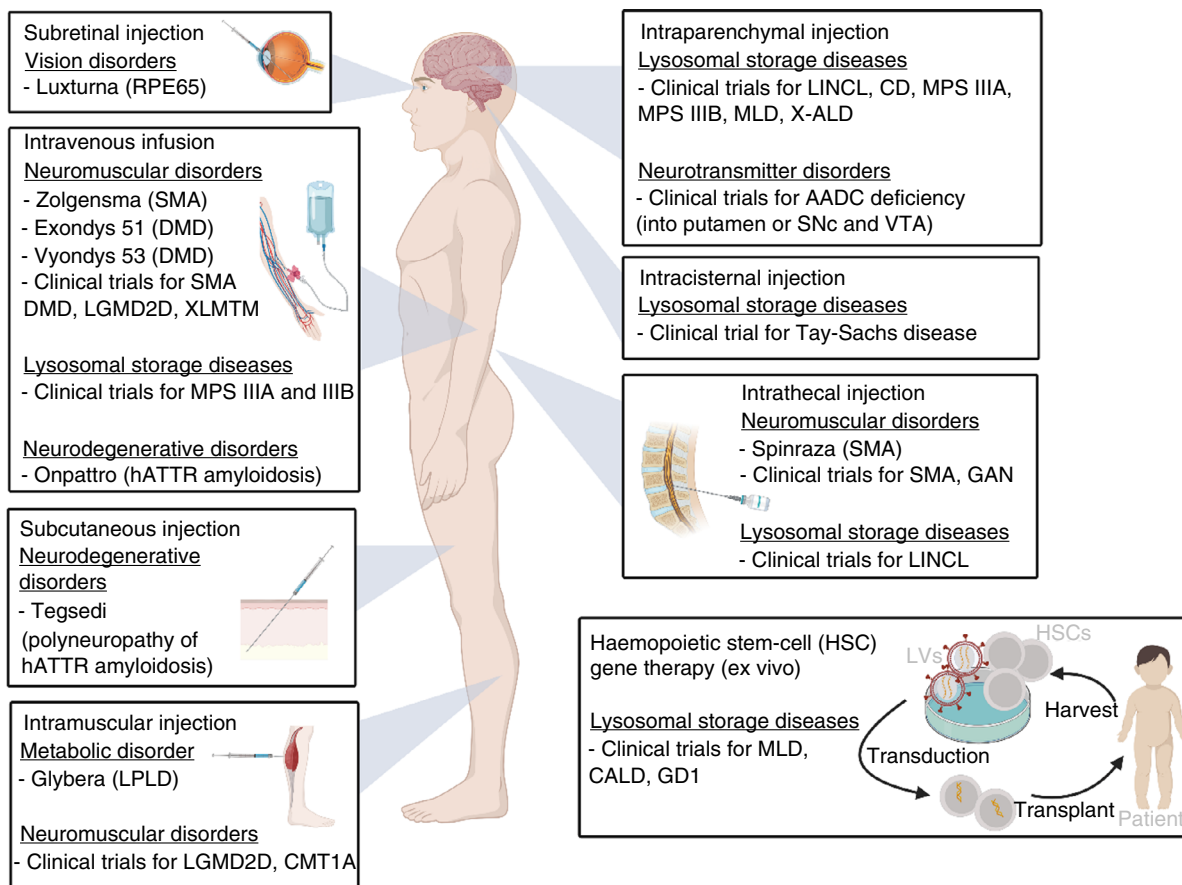
Classic proximal 5q SMA is a progressive motor neuron disorder, and is the most common genetic cause of childhood mortality. About 60% are severe, infantile-onset type 1, developing profound limb and trunk weakness before 6 months, and failing to rollover or achieve independent sitting. Nusinersen (Spinraza) is a 2'-*O*-methoxyethyl phosphorothioate-modified ASO drug.<sup>28</sup> Spinraza alters splicing of *SMN2* pre-messenger RNA and increases the functional survival motor neuron protein levels that are deficient in

SMA. Patients receiving a 12mg dose showed incremental improvement in Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders scores and increased compound muscle action potential amplitude compared with baseline.<sup>28</sup> This is delivered by intrathecal delivery every 4 months and needs to be delivered lifelong. Managed access agreements for access to Spinraza are available on public healthcare in more than 40 countries as of August 2019.<sup>29</sup> This success stimulated the development of a personalized ASO (Milasen) for a patient with Batten disease.<sup>30</sup>

Viral gene therapy for spinal muscular atrophy type 1 has resulted in a fundamental change in rAAV gene therapy. All 15 infants who received intravenous rAAV9.*SMN* were alive and ventilator-free at 20 months old. Of 12 patients receiving a higher dose, 11 had Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders scores over 40 attaining head control and unassisted sitting, and two could crawl, stand, and walk independently.<sup>8,31</sup> Transient hepatic transaminitis in two patients was controlled with oral prednisolone, and trials extended to over 100 patients including those with other SMA types (Table 3). Two patients' deaths were recently reported and attributed to progression of underlying disease.

### Duchenne muscular dystrophy

The dystrophin gene is 11.5kilobases and subject to abbreviated iterations with the limited packaging capacity of rAAV. Lentivirus vectors have been considered but show limited widespread skeletal muscle transduction. A phase 1 study has used rAAV9 to deliver the mini-dystrophin gene through intravenous infusion in males aged 4 to 12 years. Another phase 1/2 trial has delivered micro-dystrophin with rAAVrh.74 intravenously to six males aged 3 months to 3 years in one cohort, and six males aged 4 to 7 years in another (Table 3). With about 93% sequence identity to AAV8, rAAVrh.74 was chosen because of lower capsid-related immunogenicity. The 1-year review of the first four males treated met the primary outcomes of safety and tolerability with minimal treatment-related adverse events (vomiting and raised  $\gamma$ -glutamyltransferase that resolved with corticosteroids). Secondary outcomes found robust, targeted micro-dystrophin expression on gastrocnemius muscle biopsy. Exploratory functional outcomes included improved North Star Ambulatory Assessment scores and reduced serum creatine kinase levels compared with baseline.<sup>32</sup> Another phase 1/2 study is investigating intravenous rAAV9.micro-dystrophin. Alternative approaches include eteplirsen (Exondys 51), an ASO that skips exon 51 to enable dystrophin transcription and functional translation, but is mutation-specific, limiting general application. Gene editing with clustered regularly interspaced short palindromic repeats-cas9 (CRISPR-Cas9) delivered by two rAAV9 restored dystrophin expression in a canine model of Duchenne muscular dystrophy<sup>33</sup> and could be a more suitable genetic approach although delivery, off-target



**Figure 3:** Clinical delivery routes of gene therapies used in paediatric neurological disorders. Currently, adeno-associated viral vectors (AAVs) and anti-sense oligonucleotides (ASOs) are the two most commonly applied therapeutic agents. Depending on the target tissue, AAV products are mostly delivered by local injection (e.g. subretinal or intraparenchymal) to restrict the distribution of transgenes; however, direct delivery to the cerebrospinal fluid by intrathecal, intracerebroventricular, or intracisternal routes is used to achieve gene transfer in the central nervous system. Zolgensma is delivered by intravenous infusion as AAV9 crosses the blood–brain barrier. ASOs and small interfering RNAs are chemically modified to improve stability and target-binding affinity; depending on their efficiency. These are delivered intravenously or injected intrathecally or intramuscularly. Lentiviral vectors are used ex vivo and by intraparenchymal injection. AADC, aromatic L-amino acid decarboxylase; CALD, cerebral adrenoleukodystrophy; CD, Canavan disease; CMT1A, Charcot-Marie-Tooth neuropathy type 1A; DMD, Duchenne muscular dystrophy; GAN, giant axonal neuropathy; GD1, Gaucher disease type 1; hATTR amyloidosis, hereditary transthyretin-mediated amyloidosis; HSC, haematopoietic stem cell; LGMD2D, Limb-girdle muscular dystrophy type 2D; LINCL, late infantile neuronal ceroid lipofuscinosis; LPLD, lipoprotein lipase deficiency; LV, lentiviral vector; MLD, metachromatic leukodystrophy; MPS IIIA/B, Sanfilippo type A/B syndrome; RPE65, retinal pigment epithelium 65kDa; SMA, spinal muscular atrophy; SNc, substantia nigra pars compacta; VTA, ventral tegmental area; X-ALD, X-linked adrenoleukodystrophy; XLMTM, X-linked myotubular myopathy.

effects, and immunogenicity remain barriers to clinical translation.

### Limb-girdle muscular dystrophy

Limb-girdle muscular dystrophy type 2D is caused by  $\alpha$ -sarcoglycan (*SGCA*) deficiency. A phase 1 study of muscle-specific rAAV1.*SGCA* through intramuscular injection to the extensor digitorum brevis muscle showed safety, moving to a phase 1/2 trial of rAAVrh.74.*SGCA*.<sup>34</sup> This was delivered through femoral artery by isolated limb perfusion to transduce lower limb muscles only. This resulted in increased fibre diameter in two patients and improved

muscle strength in the knee extensors. However, functional parameters were unchanged or declined as per natural history. This suggests improvement in target muscles, but widespread delivery is required with an intravenous clinical trial underway.<sup>34</sup>

### X-linked myotubular myopathy

X-linked myotubular myopathy is caused by mutations in the *MTM1* gene coding myotubularin. Males with x-linked myotubular myopathy have severe muscle weakness, respiratory insufficiency, and half die by 18 months.<sup>35</sup> A phase 1/2 study treated nine males between 8 months and 6 years



old with intravenous rAAV8.*MTM1*. Initial results showed all treated males could sit independently, increase in Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders score, and increase in maximal inspiratory pressure. Two males could stand with support and one could crawl at 4 to 48 weeks post-gene transfer. All patients showed significant reductions in ventilator use, with three off ventilation.<sup>35</sup> Tragically, three males who received high-dose ( $3 \times 10^{14}$  vector genomes per kilogram) developed severe hepatotoxicity and died. The precise mechanism of toxicity is under investigation. Two of the three patients who died experienced bacterial sepsis, and all three had pre-existing hepatobiliary disease. These patients were at the older end of the age cut-off and lower range of normal body weight.<sup>36,37</sup>

## EPILEPSY

Pharmacoresistant epilepsies represent a major unmet clinical need worldwide, and novel therapies are sorely needed. Preclinical studies have demonstrated expression of different genes to attenuate seizures. These include expressing seizure-suppressing neuropeptides (neuropeptide Y, dynorphin), potassium channel Kv1.1, or designer receptors.<sup>38</sup> These promising preclinical results are on the brink of first-in-human trials. Dravet syndrome is a severe epileptic encephalopathy caused mainly by heterozygous loss-of-function *SCN1A* gene mutations indicating haploinsufficiency. A preclinical study used rAAV9 to deliver single guide RNA to increase *Scn1a* gene expression in vitro and in vivo.<sup>39</sup>

## NEUROTRANSMITTER DISEASES

Gene therapies for Parkinson disease have evaluated different genes to improve neuronal survival such as neurotrophic factors (neurturin), glial-cell-derived neurotrophic factor, and glutamic acid decarboxylase showing safety but not superior to current treatments.<sup>10</sup> Some clinical trials have delivered genes to supplement dopamine synthesis using rAAV2 to deliver aromatic L-amino acid decarboxylase (AADC), and lentivirus to deliver the three genes tyrosine hydroxylase, AADC, and GTP cyclohydrolase-1 (ProSavin).<sup>10</sup> These are relevant in children with neurotransmitter disease. In 2012, a compassionate clinical trial of rAAV2.*AADC* was conducted in children with AADC deficiency.<sup>40,41</sup> AADC is involved in the synthesis of monoamine neurotransmitters dopamine and serotonin, and its deficiency results in life-limiting pharmacoresistant movement disorders. Four children received intraputaminally rAAV2.*AADC* and showed growth, and motor and cognitive improvements.<sup>41</sup> A phase 2/3 trial treating 10 patients re-affirmed safety and efficacy.<sup>42</sup> Another clinical trial has used magnetic resonance imaging convection-enhanced delivery to transport rAAV2.*AADC* to dopaminergic neurons in the midbrain (Table 4).

## DISCUSSION

There has been outstanding progress in treatments for paediatric neurological diseases over the past decade, with

more than 40 clinical trials involving over 600 children (Tables 1–4) in viral gene therapy alone. These studies have demonstrated clinical impact, particularly in neuromuscular disorders, and they provide positive results of safety and efficacy for several untreatable life-limiting paediatric neurological diseases. The trials demonstrate the role of different capsids and delivery routes for disease application, but much is still to be learnt.

Access to the CNS has always been a barrier to treating neurological diseases, and multiple intraparenchymal injections of gene therapy are associated with neurosurgical procedure risk. Thus, AAV9 delivered intravenously or intrathecally is attractive but requires high vector doses and risks off-target effects, hepatic transaminitis, and host immune response. Severe adverse events have occurred with high-dose intravenous rAAVs for neuromuscular disorders. There was clinical hold on NCT03368742 for Duchenne muscular dystrophy (November 2019–July 2020). One child receiving a high dose ( $2 \times 10^{14}$  vector genomes per kilogram) experienced complement activation, thrombocytopenia, decreased red blood cell count, acute kidney injury, and cardio-pulmonary insufficiency. Neither cytokine- nor coagulopathy-related abnormalities were observed.<sup>43</sup> The deaths of patients with x-linked myotubular myopathy receiving high-dose rAAV8.*MTM1* ( $3 \times 10^{14}$  vector genomes per kilogram) associated with hepatotoxicity and sepsis highlight the importance of understanding mechanisms of immune activation against rAAV, the impact of vector manufacture, and purification methods to improve the safety of rAAV approaches. Before these, high-dose rAAV studies had the highest safety profile in viral gene therapy. It has been postulated that pre-existing immunity to rAAV, or rapidly accumulating antibodies to vector, transgene, or product, could contribute.<sup>36</sup> The precise pathophysiological mechanisms underlying the toxicity related to high-dose intravenous rAAV are under investigation.

Other confounders on gene therapy efficacy are the therapeutic window and the stage of disease at which a child may be treated. Detailed natural histories to best define optimal treatment windows, delivery routes, and doses are vital. The encouraging results from SMA, Tay–Sachs disease, and Canavan disease suggest earlier treatment improves efficacy<sup>22,25,31</sup> and highlight the role of newborn screening for these disorders.

Infants are now being treated with rAAV vectors; whether the vector will express throughout the child's lifetime or re-dosing will be required are unknown. Re-dosing with the same capsid will meet host immune response with neutralizing antibodies or memory T-cell responses, and host immune response to foreign protein may also be elicited.<sup>44</sup> Preclinical studies of novel AAV serotypes and engineered capsids to identify increased transduction efficiency and evasion of host immune response are underway.<sup>9</sup> Moreover, gene expression in off-target tissues or supraphysiological overexpression in target cell types may cause cytotoxicity. For example, mutations in the neuronal transcriptional regulator methyl-CpG-binding protein 2

(*MeCP2*) gene cause Rett syndrome in females, but duplications also cause a developmental disorder with intellectual impairment in males. In a preclinical mouse model lacking *Mecp2*, rAAV-mediated *MeCP2* overexpression resulted in hepatotoxicity that was reduced by modifications of the expression cassette's regulatory elements to control transgene expression.<sup>45</sup> Continual efforts are underway to develop cell-type selective promoters, tissue-specific capsids, and vector de-targeting by modifications to the 3'-untranslated region incorporating binding sites for tissue-specific microRNAs.<sup>2,45</sup> It is conceivable with the advances in precision medicine that patients will be treated with rAAV and ASO, for example.

Another safety concern is potential integration as wild-type AAV integrates into host genome.<sup>46</sup> While rAAV primarily remains episomal, integration events have been observed in mice after neonatal intravenous rAAV.<sup>47</sup> A long-term safety study of rAAV factor VIII in dogs with haemophilia showed integration events across the canine genome. There was no evidence of liver dysfunction or hepatocellular carcinoma on necropsy.<sup>48</sup> It is unlikely that rAAV integration in post-mitotic neurons will result in oncogenesis; however, intravenous rAAV CNS gene therapies will reach the liver via the bloodstream. The US Food and Drug Administration stipulates that patients treated with rAAV need 5 years' monitoring for hepatocellular carcinoma.

Gene supplementation is unsuitable for genetic disorders owing to gain of function or dominant negative effects, and mutation correction strategies have been developed. CRISPR systems use RNA-guided endonucleases (most commonly Cas9) to generate double-stranded breaks at a precise location determined by a single guide RNA.<sup>49</sup> Genomic editing is achieved through two repair mechanisms: non-homologous end joining (preferred for gene silencing) or the template-guided homology directed repair (preferred for mutation correction). Currently, these systems are prone to off-target double-stranded breaks and insertion-deletions. To avoid erroneous double-stranded breaks, newer machineries involve the fusion of a catalytically inactive variant of Cas (dCas) to different effectors, namely deaminases to correct the base (base editing), or engineered reverse transcriptases

for prime editing, and transcriptional activators, repressors, or epigenetic modifiers for gene amplification or repression. Although these offer exciting opportunities for correcting or regulating mutations without altering the genome, challenges such as off-target effects, immune response, risks of random integration, and delivery efficiency of Cas9 and single guide RNA remain obstacles, but some CRISPR phase 1 trials are underway.<sup>49</sup>

This review illustrates the tremendous amount of clinical data exhibiting gene therapy's progress to clinical reality, with safety demonstrated in hundreds of patients. However, the recent deaths in a high-dose rAAV8.*MTM1* trial have intensified the field's efforts to understand the immune responses to rAAV, transgene, transgene product, and, ultimately, to maximize safety. Research efforts will continue to address delivery, immune response, and scalability of vector production, but additional efforts are required to reduce costs and optimize manufacturing to meet demands. Practical, technical, ethical, and economic barriers must be overcome by the scientific, clinical, and biopharmaceutical community to enable gene therapy to be accessible to all patients who would clinically benefit. Future gene therapies will become increasingly sophisticated to meet the needs of diverse and complex diseases. The new vectors in development will be selective for diseased cell targets, fully evasive of host immune response, and modifiable whereby gene therapy responds to disease state.

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## DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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