Review

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Gene therapy for neurodegenerative diseases

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Gene therapy is, potentially, a powerful tool for treating neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, Parkinson's disease (PD) and Alzheimer's disease (AD). To date, clinical trials have failed to show any improvement in outcome beyond the placebo effect. Efforts to improve outcomes are focusing on three main areas: vector design and the identification of new vector serotypes, mode of delivery of gene therapies, and identification of new therapeutic targets. These advances are being tested both individually and together to improve efficacy. These improvements may finally make gene therapy successful for these disorders.

Gene therapy: theory and practicalities

In theory, gene therapy is a straightforward process. A disease is treated by delivery of a transgene that either replaces or corrects a defective gene, or generally supports cells in the disease environment. In practice, it is considerably more complex, and a variety of factors need to be optimized. The correct vector needs to be selected, the appropriate mode of delivery optimized, and the transgene chosen. The interaction between the host immune system and the vector or transgene may further complicate therapy. For neurodegenerative diseases, the nature of the target tissue adds an extra layer of complexity.

Gene therapy vectors can be either viral or non-viral. Viral vectors harness the natural ability of viruses to infect cells. Their genomes are modified to remove genes that would allow them to replicate, rendering them translatable for clinical use. For neurodegenerative diseases the two most common viral vectors used are adeno-associated viruses (AAVs; see Glossary) and lentiviruses. AAV and lentiviral vectors have the ability to infect both dividing and non-dividing cells. However, lentivirus integrates into the host genome, while AAV does not. Integration confers stable, long-term expression, but also raises the possibility of integrational mutagenesis. Although AAV is non-integrating, it can still deliver stable gene expression in non-dividing cells [1,2]. Non-viral vectors usually consist of naked plasmid DNA or in complex with cationic lipids or polymers. They have a localized effect and require a higher therapeutic dose than viral vectors. In general, non-viral delivery confers only transient gene expression, which is usually not sufficient for the treatment of chronic neurodegeneration.

The delivery route is an important consideration, especially to the central nervous system (CNS). Remote delivery, via intravenous injection, has the advantage of being non-invasive. However, the blood–brain barrier (BBB) is a significant obstacle hindering the passage of most vectors into the CNS. Thus, the discovery that AAV9 has the ability to cross the BBB was significant [3]. The disadvantages of remote delivery are the increased risk of off-target effects and the need to deliver a greater dose to achieve a therapeutic dose in the target tissue. Direct delivery to the CNS limits off-target effects and reduces the required dose of the gene therapy vector. In the CNS this can be achieved via intraparenchymal injection (directly into the brain or spinal cord) or injection into the cerebrospinal fluid [CSF; either intracerebroventricular (ICV) or intrathecal]. Both intrathecal delivery and direct spinal cord injection have been demonstrated to be safe [4,5], as well as direct injection into the human brain [6,7]. The proposed transgene can target the specific gene causing the disease, if known, or target a pathway or process within the disease. As more is understood about the disease process and progression, additional potential therapeutic targets can be identified.

There have been several gene therapy clinical trials for neurodegenerative diseases. One of the first trials for AAV-mediated gene therapy was for Canavan disease, which is caused by a mutation in the aspartoacylase (*ASPA*) gene. AAV2-*ASPA* was injected into the brain and the patients were monitored for up to 10 years post-surgery [8]. Follow-up showed a slowed progression in brain atrophy with some improvement in seizure frequency [8]. Recently, the first trial to deliver AAV9 intrathecally delivering the gigaxonin gene (*GAN*) (ClinicalTrials.gov registry number NCT02362438) to treat giant axonal neuropathy (GAN) has begun to recruit patients. However, many of the clinical trials have not demonstrated efficacy (Table 1). Efforts in improving vectors, targeting delivery, and expanding the choice of possible transgenes should increase efficacy in gene therapy trials.

Parkinson's disease

PD is a neurodegenerative disease that is characterized by loss of the dopaminergic neurons of the substantia nigra pars compacta (SNc) and reduction of levels of dopamine in the striatum. Symptoms include rigidity, resting tremor, and motor function impairment, including freezing and bradykinesia. The current standards of care include dopamine replacement with drugs such as levodopa, a

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Glossary

Acyl-CoA:cholesterol acyltransferase (ACAT) 1: an endoplasmic reticulum enzyme that modulates build-up of cholesterol in membranes by converting it to cholesterol esters.
Adeno-associated virus (AAV): non-enveloped, non-integrating virus with the ability to infect dividing and non-dividing cells.
Alzheimer's disease (AD): a progressive disorder characterized by problems with memory, thinking, and behavior.
Amyloid β (Aβ): peptide that form the main component of plaques in Alzheimer's disease.
Amyotrophic lateral sclerosis (ALS): a progressive disorder resulting from loss of upper and lower motor neurons in the brain, brainstem, and spinal cord that is usually fatal within 2–5 years from diagnosis. ALS can be familial or sporadic in origin.
Antisense oligonucleotides (ASOs): short fragments of nucleic acid that bind to their target sequence and inhibit translation.
Blood–brain barrier (BBB): composed of endothelial cells of microvessels which form a barrier to the entry of most blood-borne substances to the brain. It excludes toxic substances and maintains a stable environment.
Convection enhanced delivery (CED): a pressurized infusion technique that allows therapies to be delivered to large volumes of the brain.
Deep brain stimulation (DBS): the implantation of electrodes into specific parts of the brain to control movement and affective disorders.
Glycosaminoglycan (GAG): a long unbranched polysaccharide; accumulation can cause mucopolysaccharidosis (MPSI).
Granulocyte colony stimulating factor (G-CSF): a hematopoietic factor important in regulating production of blood cells and in bone marrow stem cell survival.
Hypoxia-inducible factor (HIF1): a core regulatory factor that regulates gene expression during hypoxia.
Insulin-like growth factor 1 (IGF1): a neurotrophic factor that promotes neuronal survival.
Intracerebroventricular (ICV): delivery of a therapeutic factor directly into the ventricles of the brain, bypassing the BBB.
Parkinson's disease (PD): disorder characterized by loss of dopaminergic neurons in the substantia nigra. Symptoms include rigidity, resting tremor, and motor function impairment.
Magnetic resonance imagery (MRI): a technique that uses a magnetic field and radio waves to create detailed images of tissues and organs.
Metachromic leukodystrophy (MLD): is caused by an inherited mutation in arylsulfatase A (*ARSA*). Symptoms results from sulfatide accumulation in Schwann cells, oligodendrocytes and brain neurons.
Nerve growth factor (NGF): a neurotrophic factor that is important for the survival and maintenance of sympathic and sensory neurons, and induces axonal growth.
Neurturin (NRTN): a neurotrophic factor related to GDNF. It enhances dopaminergic neuron survival.
Nonsense-mediated mRNA (NMR) decay: a mechanism for degrading transcripts with a premature termination codon.
Spinal muscular atrophy (SMA): infantile form of motor neuron disease caused by the loss of the *SMN1* gene. Prognosis varies depending on the severity of the disease.
Spinocerebellar ataxia (SCA): a neurodegenerative disease caused by mutations in ataxin genes.
Substantia nigra par compacta (SNc): an area of the brain that serves as an input to the basal ganglia circuit. It supplies the striatum with dopamine.
Superoxide dismutase 1 (SOD1): an enzyme that converts the superoxide radical to either molecular oxygen or hydrogen peroxide. Mutations in the *SOD1* gene can cause ALS.
Survival motor neuron 1 (SMN1): a housekeeping protein that from complexes with gemin proteins in the nucleus. It functions is assembling small nuclear ribonucleoproteins and in pre-mRNA splicing.
Trimethoprim (TMP): an antibiotic with the ability to cross the BBB.
Vascular endothelial growth factor (VEGF): an angiogenic factor with neurotrophic and neuroprotective properties.

dopamine precursor, and deep brain stimulation (DBS). Levodopa can restore some motor function with varying efficiency; however, as PD progresses levodopa becomes less effective and its side effects become more pronounced. DBS has been effective at treating the symptoms of PD but does not target the cause. Moreover, DBS can exacerbate the cognitive and emotional deterioration that can characterize late-stage PD [9].

The possibility of using gene therapy to treat PD has been explored extensively. Several gene therapy trials have fulfilled the Phase I safety criteria and suggested some efficacy [10]. However, when advanced to controlled, blinded Phase II trials, the majority have failed to show improvement beyond the placebo effect. The only trial to show efficacy in a controlled, blinded Phase II trial was delivery of AAV2-glutamic acid decarboxylase (*GAD*) by direct injection into the subthalamic nucleus (NCT00643890) [11]. Patients who received AAV2-*GAD* had improvement in symptoms over control patients; however, this improvement was not greater than that seen with the current standard of care, and the study was terminated. Another trial used AAV2 to deliver neurturin (*NRTN*) (Cere-120) to the putamen to support the dopaminergic neurons (NCT00400634) [12]. Analysis of postmortem brain tissue from Phase II trial patients found that although there was an increase in *NRTN* expression in the injected area of the striatum/putamen, there was no corresponding increase in the substantia nigra [13]. This was thought to be due to failure of retrograde transport of the dopaminergic neurons. To address this issue a Phase IIb trial was undertaken in which both the putamen and the substantia nigra were injected. The dose was also increased with the aim of increasing transduction of the putamen. Preclinical data showed that this strategy significantly increased *NRTN* expression [14,15]; however, results from this Phase IIb trial showed no improvement in clinical outcome for the participants [16]. The trial coordinators hypothesized that the degenerative state of the PD brain may have affected transport of NRTN through the brain. Secondary outcome measures suggested that *NRTN* expression may have improved younger patients treated earlier in their disease course, arguing that growth factor gene therapies need to be delivered before neurodegeneration has progressed extensively.

The Prosavin trial targeted the dopamine synthesis pathway, using a tricistronic lentiviral vector to deliver the genes encoding the rate-limiting dopamine biosynthesis enzymes tyrosine hydroxylase, aromatic amino acid dopa decarboxylase (*AADC*) and GTP cyclohydrolase I (*GCH1*) [17,18]. A tricistronic vector is advantageous as cells transduced with the vector will express all three enzymes. The clinical trial entailed a Phase I/II dose-escalation study (NCT00627588 and NCT01856439) targeting the sensorimotor part of the striatum and the putamen. The trial achieved its goal in demonstrating the safely profile of Prosavin. The efficacy data also showed promise, with improvement in motor function in the off-medication state that correlated with increasing dose of Prosavin [18]. However, given that this was an open-label trial, the efficacy results have to been interpreted cautiously. Palfi *et al.* noted that the improvement observed in patients was within the placebo effect range seen in other clinical trials. The investigators intend to optimize the delivery method and then proceed to a double-blinded randomized trial to determine the efficacy of Prosavin.

AADC was also used as the sole therapeutic transgene in two Phase I clinical trials using AAV2 as the viral vector [19,20]. Long-term follow-up determined no adverse safety events and stable *AADC* expression after 4 years. An improvement in the unified PD rating scale (UPDRS) was observed in the first 12 months, but slowly deteriorated

Table 1. Gene therapy trials for neurodegenerative diseases

Disease	Gene therapy	Delivery route	Trial code	Current status
ALS	ASOs to *SOD1*	Intrathecal	NCT01041222	Phase I safety trial successful
Pompe's disease	AAV1-*GAA*	Intramuscular injection targeting the diaphragm	NCT00976352	Phase I/II trials showed treatment well tolerated
Pompe's disease	AAV9-*GAA*	Intramuscularly into the TA muscle	NCT02240407	No results posted
MLD	AAVrh10-*ARSA*	Intracerebrally	NCT01801709	No results posted
MPSIIIA	AAVrh10-*SGSH* and *SUMF1*	Direct brain injection	NCT01474343 NCT02053064	Phase I/II trial. No safety issues with preliminary efficacy data
SMA	AAV9-*SMN*	Intravenous	NCT02122952	Phase I trial started 2014
SMA	ASOs targeting *SMN2* splicing	Intrathecal	NCT0149701, NCT01703988, NCT02052791, NCT02193072, NCT02292537	Phase I–III trials. No study results posted
Alzheimer's disease	AAV2-*NGF*	Injection into the basal forebrain	NCT00876863	Phase I successful, treatment well tolerated. Phase II initiated but no updates posted
Parkinson's disease	AAV2-*GAD*	Injection into the subthalamic nucleus	NCT00643890	Phase II successful, showed improvements over controls. However, not better than current standard of care and trial was terminated
Parkinson's disease	AAV2-*NTRN*	Injection into putamen	NCT00400634, NCT00985517	Phase I safety trials successful. Phase II safety trails did not show efficacy over control groups
Parkinson's disease	Lentivirus-*TH*, *AADC*, and *CH1*	Injection into striatum and putamen	NCT00627588, NCT01856439	Phase I trial successful. Initial efficacy data. Delivery system being optimized before continuing with trial
Parkinson's disease	AAV2-*AADC*	Injection into the putamen	NCT02418598	Phase I safety trial successful Phase II in progress
Parkinson's disease	AAV2-*AADC*	Intrastriatial infusion	NCT00229736	Phase I safety trial successful

in the following years [21]. Following up on these results, researchers have focused on improving delivery with the aim of increasing coverage of the putamen. This has been demonstrated in non-human primates using an MRI-guided delivery system with convection enhanced delivery (CED) [22]. Combining an MRI-guided tracer with the viral vector has allowed the development of a system that can monitor the delivery of the gene therapy in real time. AAV2-*AADC* in non-human primates using CED has been proven to be a safe and effective delivery method [22], and a human trial using iMRI and CED to deliver AAV2-*AADC* is anticipated in the near future.

In the area of proof-of-principle testing, there are several new therapies in development. Targeting dopamine synthesis, an AAV5 vector expressing tyrosine hydroxylase (*TH*) and *GCH1* has been tested in rat and nonhuman primate models of PD [23]. While results were promising in the rat model, non-human primate studies did not show efficacy. This was attributed to lack of expression of *TH* in the caudate putamen [24]. The efficacy of cerebral dopamine neurotrophic factor (*CDNF*) has been assessed by two groups in rat models of PD [25,26]. Both reported functional improvement but only one, Ren *et al.*, reported protection of TH-producing neurons [25]. These studies are too preliminary to adequately assess the efficacy of *CDNF*. The study of Back *et al.*, in particular, is difficult to interpret because the gene therapy was administered 2 weeks before induction of PD [26].

Achieving control over transgene expression could allow a more nuanced approach to gene therapy. This is relevant where continuous expression of the transgene could lead to unwanted side effects. In the case of glial derived neurotrophic factor (*GDNF*), it has been found to be effective in animal models of PD. However, side effects from constitutive expression of *GDNF* include aberrant axonal sprouting, downregulation of TH and increased turnover of dopamine [27–30]. Therefore a system where *GDNF* would be delivered and expression controlled is needed for PD therapy [31]. A lentiviral vector expressing *GDNF* fused to the destabilizing domain (DD) of *E. coli* dihydrofolate reductase has been developed. Trimethoprim is a drug which can cross the BBB, and binds to the DD domain. This regulates the stability of the DD domain and, by extension, the expression of GDNF [32]. When tested in a rodent model of PD, it protected neurons and improved motor function [31]. However, it is important to note that the gene therapy was delivered before PD induction in these animals. Further study on animals already displaying PD symptoms will be necessary to determine its therapeutic efficacy.

Amyotrophic lateral sclerosis

ALS (also known as Lou Gehrig's disease) is a progressive, fatal neurodegenerative disease. It results from a loss of both upper and lower motor neurons in the brain, brainstem, and spinal cord. It is usually fatal within 2–5 years. ALS can be either sporadic or familial in origin, and a variety of genes are linked to disease development [33]. The only FDA-approved treatment for ALS is Rilutek, which delays disease progression by 3–6 months. In the case of familial ALS, gene therapy for ALS can be approached by targeting a specific mutation. For sporadic

ALS, neurotrophic factors can be delivered to support motor neurons.

For familial ALS, altering the expression of the affected gene has proven effective. This approach has primarily been tested for the superoxide dismutase 1 (*SOD1*) gene because mutations in *SOD1* were among the first to be identified as causing ALS. Delivering antisense oligonucleotides (ASOs) to *SOD1* intrathecally progressed to a Phase I trial (NCT01041222), with some patients receiving repeat treatments. Efficacy was not expected, as most patients received a single dose, but the safety profile of this treatment was established [34]. One disadvantage of administering ASOs on their own is the need for constant infusion or repeat dosing. Using a viral vector to deliver an ASO or a short hairpin (sh) RNA circumvents this issue. Two proof-of-principle studies have used AAV9-*SOD1*-shRNA to knock down *SOD1* gene expression in rat models. Delivery involved either direct injection into the motor cortex [35] or temporal or tail vein injection [36]. Both studies showed delay of disease onset and extended survival. Foust *et al.* went on to demonstrate successful intrathecal delivery and suppression of *SOD1* expression in non-human primates [36], illustrating the possibilities for translating this therapy to the clinic. A third mechanism tested was the intrathecal delivery of AAV9 expressing a single-chain antibody against misfolded SOD1. This approach delayed disease onset and extended lifespan in transgenic mice overexpressing a mutated form of SOD1 [37]. Although the studies described have been focused on *SOD1*, they have the potential to be applied to other known ALS-causing mutations.

For sporadic ALS, a more general neuroprotective approach needs to be adopted. One method of achieving this is by delivering growth factors to support the motor neurons. Growth factor delivery has shown promise in preclinical testing but little efficacy in clinical trials. However, there is potential for therapeutic efficacy from growth factors. Vascular endothelial growth factor (*VEGF*) was linked to ALS when mice with a deletion in the *VEGF* promoter region developed motor neuron disease similar to *SOD1* mice [38,39]. Gene delivery experiments resulting in increased *VEGF* expression have been tested in ALS animal models. One of these studies delivered AAV4-*VEGF* and AAV4-insulin-like growth factor (*IGF1*) into the lateral and 4th ventricles of *SOD1* mice [40]. Administered individually both factors delayed motor decline and extended survival; however, when delivered in combination the therapeutic effect was not cumulative. An alternative approach might be to increase endogenous *VEGF* production. Delivery of a plasmid expressing a zinc-finger protein that results in upregulated endogenous *VEGF* expression was tested in *SOD1* rats via a series of eight weekly injections into the gastrocnemius muscle [41]. There was localized improvement in motor function, but, because only one muscle group was targeted, there was no effect on overall weight or lifespan [41]. Granulocyte colony stimulating factor (*GCSF*/*CSF3*) is another growth factor that has also been tested, via intramuscular injection and direct injection into the spinal cord of *SOD1* mice. Unlike *VEGF, GCSF* only showed efficacy when injected into the spinal cord [42].

Other potential transgenes that are being assessed in preclinical studies include those involved in the control of RNA expression. *miRNA 155* was identified as having increased expression in *SOD1* mice and in human ALS spinal cord samples [43]. Inhibition of *miRNA 155* expression in *SOD1* mice, via an oligonucleotide repressor, resulted in extended survival and disease duration [44]. Another potential target is upframeshift protein 1 (*UFP1*), which plays a role in nonsense-mediated mRNA (NMR) decay [45]. ALS was induced in mice via injection of AAV9 expressing TAR DNA binding protein TDP-43 (*TARDBP*) on postnatal day 1, and mice were treated on the same day with AAV9-*UFP1*. Treated mice had improved motor scores [46]. Results from this study need to be interpreted cautiously given the early intervention; however, identifying *UFP1* and NMR as potential therapeutic targets in ALS is useful in increasing options for researchers.

Alzheimer's disease

AD is a progressive neurodegenerative disorder that is characterized by problems with memory, thinking, and behavior. Disease progression is associated with degeneration of cholinergic neurons and buildup of amyloid β (Aβ) plaques [47,48]. Familial AD results from mutations in the amyloid β (A4) precursor protein (*APP*) gene, or in proteases that cleave the precursor protein into fragments [49]. The current treatment for AD includes cholinesterase inhibitors which can provide some symptomatic relief for some patients [50].

A Phase I clinical trial of AAV2-nerve growth factor (*NGF*) injected into the basal forebrain has been completed. The therapy was well tolerated, with follow-up data for up to 2 years. Analysis of postmortem tissue identified expression of NGF in an active form [7]. These data supported the initiation of a controlled Phase II trial to rigorously assess the effectiveness of AAV2-*NGF* (NCT00876863); however, the sponsor recently announced that the Phase II trial failed to meet its primary endpoints.

Preclinical studies have targeted a variety of processes in AD, including inhibiting plaque formation, apoptosis, and using growth factors. One strategy for inhibiting plaque formation is to decrease levels of Aβ and tau in the brain. An AAV expressing a miRNA to knockdown acyl-CoA:cholesterol acyltransferase 1 (*ACAT1*) was shown to reduce Aβ levels in a triple transgenic mouse model of AD [51,52]. ASOs were used to inhibit microtubule-associated protein tau (*MAPT*) expression in wild type mice; however, this needs to be assessed in an animal model of AD to determine if it can affect disease progression [53]. Neprilysin, one of the enzymes involved in brain Aβ catabolism, is decreased in the hippocampus of early-stage AD patients [54]. Two independent studies delivering the neprilysin/membrane metallo-endopeptidase (*MME*) gene in an AAV9 vector via either intracardiac administration [55] or direct injection into the right anterior cortex and hippocampus [56] were published. Both studies found reduction in Aβ levels in the brain, while Iwata *et al.* also reported improvement in memory and learning [55]. Leptin is another factor that has the ability to lessen the symptoms and pathology of AD [57–59]. A lentiviral vector containing the leptin gene (*LEP*) was injected ICV into double-transgenic AD mice, resulting in reduced Aβ load, decreased tau

phosphorylation, and improved synaptic density [60]. The reelin pathway is a potent suppressor of tau phosphorylation [61], and has also been recognized as a therapeutic target for AD [62,63]. Reduction in reelin expression has been linked to early pathophysiology in AD [63,64]. A lentiviral vector expressing the reelin homolog F-spondin (*SPON1*) was injected into the dentate gyrus of the hippocampus of AD mice, resulting in improved memory and learning, and decreased levels of Aβ plaque deposits [65].

Targeting apoptosis in AD has been approached by both knockdown and overexpression of different genes. One of the toxic effects of Aβ is the generation of reactive oxygen species and induction of apoptosis [66]. A lentiviral vector expressing a shRNA against caspase 3 (*CASP3*) was tested in mice that received an injection of aluminum into the brain which induced apoptosis, and as a result models AD. Lentiviral shRNA against *CASP3* resulted in reduced levels of caspase 3 and cell death, and improvements in learning and memory [67]. AAV-mediated overexpression of hypoxia inducible factor 1, α subunit (*HIF1A*) was found to both diminish the effects of Aβ neurotoxicity and decrease apoptosis [68]. Using growth factors to provide neurotrophic support has also been evaluated for AD. A variety of growth factors have shown promise in animal studies but have failed at clinical trial, suggesting that the idea still has merit, but needs refinement and better targeting. Both AAV8-*IGF2* and lentiviral *GDNF* have been tested. Although overexpression of GDNF had no effect on levels of either Aβ or tau, it did preserve learning and memory in a triple-transgenic AD mouse model [69]. AAV8-*IGF2* delivery resulted in significant reduction in Aβ levels in a transgenic mouse model overexpressing human *APP* [70]. This result is interesting as the authors also showed that IGF2 levels were reduced in the hippocampus of AD patients, indicating a potential link between IGF2 levels and AD progression [70]. The identification of these new targets for AD is promising, but ultimately it is difficult to assess their true potential until they are fully tested in clinical trials.

Spinal muscular atrophy

SMA is a childhood form of motor neuron disease and is the leading genetic cause of infantile death. It results from the loss of the survival motor neuron 1 (*SMN1*) gene [71]. Humans have a second gene, *SMN2*, which is highly homologous to *SMN1*. The differences in *SMN2* lead to alternative splicing at exon 7, which results in the production of a truncated, unstable protein. A small fraction of the *SMN2* transcript is properly spliced, producing a full-length transcript. The severity of the disease rests on the number of copies of *SMN2* and the amount of full-length SMN generated from *SMN2* [72]. As the cause of SMA has been identified as a single gene, it makes SMA an ideal candidate for gene therapy. Replacement of *SMN1* has shown efficacy in mouse models of SMA [73–76]. These studies used both intravenous and intrathecal delivery of AAV9 to achieve *SMN1* expression in the spinal cord. They also demonstrated that both modes of delivery were translatable by testing the mode of delivery in non-human primates. A Phase I trial (NCT02122952) testing intravenous delivery of AAV9-*SMN* in SMA patients was initiated in April 2014. This phase is testing the safety and efficacy of increasing doses of AAV9-*SMN* and is scheduled to end in 2017. This study represents a significant advance in the potential to treat SMA. In addition, it is necessary to bear in mind that the severity of SMA and the timing of the intervention will have a large impact on the study outcomes. The issue of timing of AAV9-*SMN* delivery was addressed by Duque *et al.* using a pig model of SMA, where the disease is induced by knockdown of *SMN1* expression [77]. *SMN1* gene expression was restored by intrathecal delivery at different time-points. Gene delivery of *SMN1* in pigs pre-symptomatically did not develop disease symptoms or limb weakness. These animals also had correction of pathological changes associated with SMA. Animals treated at symptom onset showed delayed progression of disease but only partial improvement in motor neuron numbers. Intervention at the symptomatic stage was effective if initiated without delay. These results have clear implications for treating SMA patients and clinical trial design [77].

The second therapeutic option for SMA is to target splicing of *SMN2* to promote the synthesis of full-length transcripts. This can be achieved using ASOs that target exon 7 and promote its inclusion in the mRNA transcript. The efficacy of this approach has been demonstrated in two separate preclinical studies on mouse models of SMA [78,79]. Both studies reported improved motor function and survival. Targeting of *SMN2* mRNA splicing by ASOs has already been tested in a clinical trial. ASOs delivered intrathecally to SMA patients using multiple-dosing paradigms were tested: single dose (NCT0149701) and multiple doses (NCT01703988, NCT02052791), but to date no results have been published. Two separate Phase III trials (NCT02193072 and NCT02292537) have been initiated but no results have been published.

Spinocerebellar ataxia

Spinocerebellar ataxia (SCA) describes a heterogeneous family of neurodegenerative diseases. They result from expanded CAG repeats in one of the ataxin genes. Symptoms are caused by cerebellum and brainstem dysfunction [80]. Because SCA is a monogenic disorder, much of the focus in preclinical studies has been on either knockdown or inhibition of the affected gene. This has been achieved in several different animal models of SCA. The majority of studies have focused on SCA3, which is also known as Machado–Joesph disease. RNAi, encoded by either a lentiviral or AAV vector, has been used to suppress ataxin 3 (*ATXN3*) expression after delivery via direct brain injection. Results from these studies showed improvement both in tissue pathology and in behavioral testing [81–83]. SCA1 and SCA7 have been targeted in a similar manner [84,85]. Results from these studies show great promise for RNAi as a treatment for multiple forms of SCA. However, because some of the therapeutics were administered as SCA was induced, further study is needed to determine how effective RNAi would be when administered post-symptomatically.

Lysosomal storage diseases

Lysosomal storage diseases encompass over 50 different individual diseases. They arise from defective catabolism

of macromolecules and their accumulation within lysosomes [86]. The most common form of treatment is for enzyme replacement therapy [87]. However, owing to the inability of large molecules to cross the BBB, this treatment is ineffective for lysosomal storage diseases that have a neurological component. Gene therapy treatments have been tested and some have advanced to clinical trial for particular lysosomal storage diseases.

Pompe's disease is caused by deficiency in lysosomal acid α-glucosidase (GAA), which leads to glycogen accumulation in muscle and motor neurons [88]. There are currently two clinical trials testing AAV-mediated gene therapy for Pompe's disease. The first trial (NCT 00976352) is delivering AAV1-*GAA* to the diaphragm via intramuscular injection. Preclinical data demonstrated that both systemic and directed delivery of AAV1-*GAA* had therapeutic benefit [89,90]. Results from the initial Phase I/II safety trial showed that the treatment was well tolerated and safe. After 180 days the unassisted tidal volume in patients had significantly increased. However, maximal inspiratory pressure was not improved [91]. The researchers are hoping that an earlier intervention will provide functional benefit. The second trial (NCT02240407), which will be conducted by the same research group, is a Phase I safety trial, injecting AAV9-*GAA* intramuscularly. Patients that will be treated will have late-onset Pompe's disease. Preclinical data showed intramuscular injection of AAV1-*GAA* and AAV9-*GAA* to be therapeutically effective, with AAV9 showing better targeting of motor neurons [92]. Recent preclinical studies have shown functional improvements with alternative sites to target for therapy such as intrapleural [93] and spinal cord injection [94]. A further study addressed the problem of an immune response to the gene therapy. An anti-CD4 monoclonal antibody was administered together with the AAV-*GAA* gene therapy and was found to help to control the humoral response [95].

Metachromic leukodystrophy (MLD) is caused by an inherited mutation in arylsulfatase A (*ASA*). Symptoms result from sulfatide accumulation in Schwann cells, oligodendrocytes, and brain neurons [96]. There is no current treatment. A Phase I clinical trial is underway delivering AAVRh10-*ARSA* intracerebrally to early or presymptomatic MLD patients (NCT01801709). No results have been published for this trial, but the proof-of-concept study in a mouse model of MLD (expressing a disrupted form of ASA) showed that delivery of a single injection of AAVRh10-*ARSA* corrected brain sulfatide accumulation and associated brain pathology in the injected hemisphere [97]. An alternative to direct brain injection was explored in a mouse knockout model of MLD, where AAV9-*ASA* was administered via the jugular vein in neonatal mice. Long-term gene expression was detected together with inhibition of accumulation of sulfatide [98]. This form of delivery could be feasible in early forms of MLD.

Mucopolysaccharidosis type III A (MPSIIIA), also known as Sanfilippo type A, is caused by a mutation in the lysosomal heparan-*N*-sulfamidase gene (*SGSH*). Symptoms include cognitive delay, abnormal behavior, and seizures [99]. There are two Phase I/II trials (NCT01474343 and NCT02053064), one a long-term follow-up of the first, looking at the effectiveness of direct brain injection of AAVRh10-*SGSH* and sulfatase-modifying factor (*SUMF1*), which activates the catalytic site of SGSH. In the trial patients received bilateral injections into the white matter anterior, medial, and posterior to the basal ganglia. There were no safety issues associated with the vector, and there were some preliminary efficacy data with stability in regard to brain atrophy for some patients and possible improvements in behavior and sleep [100]. Several preclinical studies have tested both different AAV serotypes and different modes of delivery to correct MPSIIIA [101–103]. All report widespread transgene expression, correction of GAG accumulation and storage, and prolonged survival.

Mucopolysaccharidosis type III B (MPSIIIB) is caused by mutation in the gene encoding lysosomal enzyme α-*N*-acetylglucosaminidase (*NAGLU*). This disrupts the degradation of heparin sulfates, leading to their accumulation in lysosomes, with cells of the CNS being the most affected [104]. Gene therapy to increase *NAGLU* expression has been tested preclinically. Delivery of AAV9-*NAGU* via either an intravenous injection [105] or direct CSF delivery [106] to MPSIIIB mice resulted in correction of pathology in the CNS and in peripheral tissue. The neurological benefits were long-term, and survival in these mice was significantly extended. Delivery of AAV9-*NAGLU* to non-human primates resulted in no adverse events associated with the vector, but there was an antibody response to both the vector and the transgene. The level of the anti-NAGLU antibody correlated with a loss of circulating enzyme, but did not affect levels of enzyme in the tissue [107]. Delivery in dogs, both naïve and with preexisting immunity to AAV9, resulted in stable levels of transgene expression [106].

Mucopolysaccharidosis type 1 (MPSI), also known as Hurler disease, is a lysosomal storage disease that results from mutations in the α-L-iduronidase (*IDUA*) gene. The enzyme is involved in the degradation of glycosaminoglycans (GAG) and mutation results in the accumulation of partially degraded compounds in lysosomes. This leads to skeletal abnormalities, cardiac and pulmonary disease, and progressive neurological disease [108]. Delivery of AAV8-*IDUA* via ICV injection [109] or AAV9-*IDUA* via intraventricular delivery [110] to MPS1 mice resulted in increased enzyme levels in the brain in both studies. Janson *et al.* reported a reduction in neuronal inclusions [110]. Wolf *et al.* reported that gene delivery prevented the accumulation of GAG and the emergence of neurocognitive dysfunction [109]. A large-animal study in a feline model of MPS1 reported that intrathecal delivery of AAV9-*IDUA* resulted in almost complete correction of both biochemical and histological markers of the disease. There was an immune response which reduced the ability to detect the enzyme, but this did not appear to affect the efficacy of the treatment [111].

Sandhoff's disease is a lysosomal storage disease that results from mutations in the *N*-acetyl-β-hexosaminidase β subunit (*HEXB*) gene. These lead to accumulation of GM2 in the lysosomes in the CNS, and currently there is no effective treatment [112]. Therapeutic success was seen in a *hexb* knockout mouse model of Sandhoff's disease. AAV2-*HEXB* was injected into the right striatum. This was found

to prevent decline in neuronal density and this correlated directly with an increase in lifespan [113]. In a feline model of Sandhoff's disease, AAV8Rh8 expressing β-hexosaminidase α and β subunits (*HEXA*/*HEXB*) was injected into the striatum and delivered ICV [114] and bilaterally into the thalamus and deep cerebellar nuclei [115]. Both studies reported an increase in enzyme activity and improved neurological function. McCurdy *et al.* reported that there was a humoral response to the vector but this did not appear to affect the therapeutic outcome [115].

Concluding remarks and future perspectives

Gene therapy has the potential to significantly advance the treatment of neurodegenerative diseases. However, success in bridging the gap between promising proof-of-principle concepts to therapeutic efficacy in clinical trials has remained elusive. Initial trials have demonstrated that delivery of gene therapies to the CNS is safe and well-tolerated [4,18,116]. To improve delivery, new vectors have been identified and developed. An example is AAV9, which can cross the BBB and has a strong neuronal tropism. Although it must be noted that the transduction pattern of AAV9 has been shown to change from neurons to astrocytes in mice depending on their age at administration [117]. In addition, the manufacturing costs to provide sufficient AAV9 for an intravenous delivery in human trials are significant. This restricts the number of instances in which AAV9 could be delivered systemically. Another noteworthy advance in viral vectors is the tricistronic lentiviral vector being used in the Prosavin trial. A further area where progress is being made is in the methods of delivery for gene therapies to the CNS. Delivery methods such as ICV, intrathecal, and direct injection into the brain and spinal cord are being developed and refined. Improvements in vectors and delivery methods will only show an effect if the therapeutic gene selected is efficacious. The most significant area of development is in the identification and testing of new therapeutic genes. This is based on a better understanding of disease initiation and progression. Better understanding of the etiology of neurodegenerative diseases will also likely lead to earlier diagnosis, which will allow intervention before the targeted cells are lost. One area where this is being pursued is in the identification and validation for biomarkers of neurodegenerative diseases [118–121]. This will allow earlier diagnosis, which will lead to earlier interventions. Another advantage of biomarkers is they allow the therapeutic effect of any intervention to be monitored. Given the results seen in the PD trials, delivering therapies before significant onset of symptoms, before the targeted cells are lost, will likely have a huge impact on the efficacy of these therapies. The combination of these advances will help to translate new gene therapies to the clinic, yielding true improvements in treating these devastating diseases.

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