AAV based gene therapy for CNS diseases

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Abstract

Gene therapy is at the cusp of a revolution for treating a large spectrum of central nervous system (CNS) disorders by providing a durable therapeutic protein with a single administration. Adeno-associated viral (AAV)-mediated gene transfer is of particular interest as a therapeutic tool because of its safety profile and efficiency in transducing a wide range of cell types. The purpose of this review is to describe the most notable advancements in preclinical and clinical progress of AAV based CNS gene therapy and discuss prospects for future development using a new generation of vectors and delivery.

Abbreviations

AADC:	L-amino acid decarboxylase
AAV:	Adeno-associated virus
AD:	Alzheimer's disease
ADAR2:	Reduced adenosine deaminase acting on RNA 2
ADK:	Adenosine kinase
ALS:	Amyotrophic lateral sclerosis
AMD:	Alpha-mannosidosis
APOE:	Apolipoprotein E
APP:	Amyloid precursor protein
ASPA:	Aspartoacylase
BBB:	Blood-brain barrier
BDNF:	Brain-derived neurotrophic factor
CBD3:	Ca^{2+} channel-binding domain 3
CLN:	Ceroid lipofuscinosis neuronal
CNS:	Central nervous system
CNTR:	Ciliary neurotrophicfactor
CSF:	Cerebro-spinal fluid
CYP46A1:	24-hydroxylase enzyme
DRG:	Dorsal root ganglion
ECE:	Endothelin converting enzyme
EEG:	Electro-encephalogram
GAN:	Giant axonal neuropathy
GCL	Globoid cell leukodystrophy
GDNF:	Glial cell line-derived neurotrophic factor
GFL:	GDNF-family of ligands
GUSB:	Glucuronidase beta
HD:	Huntington disease
HTT:	Huntingtin
ICV:	Intra cerebro-ventricular

IGF:	Insulin-like growth factor
IL:	Interleukine
INCL:	Infantile neuronal ceroid lipofuscinosis
ISF:	Interstitial fluid
IT:	Intrathecal
IV:	Intravenous
LINCL:	Late infantile neuronal ceroid lipofuscinosis
LSD:	Lysosomal storage diseases
mHTT:	mutant Huntingtin
miRNA:	MicroRNA
MLD:	Metachromatic leukodystrophy
MPS:	Mucopolysaccharidoses
MSD:	Multiple sulfatase deficiency
MSNs:	Medium spiny neurons
NAA:	N-Acetyl-Aspartate
NAGLU:	N-acetyl-alpha-glucosaminidase
NEP:	Neprilysin
NGF:	Nerve growth Factor
NFTs:	Neurofibrillary forming tangles
NHP:	Non human primates
NPD:	Niemann-Pick disease
NPY:	Neuropeptide Y
NTN:	Neurturin
PD:	Parkinson Disease
rAAV:	Recombinant adeno-associated virus
SOD1:	Superoxide dismutase 1
SGSH:	N-sulfoglucosamine sulfohydrolase
SMA:	Spinal muscular atrophy
SMN:	Survival of motor neuron
SNI:	Spared nerve injury
SNP:	Single nucleotide polymorphisms

SUMF1:	Sulfatase-modifying enzyme factor 1
TDP:	TAR DNA Binding
TRPV1:	Vanilloid receptor 1
ZFP:	Zinc finger protein
24S-OHC:	24 Hydroxycholesterol
3NP:	3-Nitropropionic acid

Introduction

Neurological disorders are among the most difficult pathologies to treat due to the limited access to the brain structures protected by physical barriers such as the blood-brain barrier (BBB), the complexity of the central nervous system (CNS) and cell interactions. Gene therapy is an alternative to traditional pharmacological approaches that has made important advances over the last decade in treating genetic disorders and acquired diseases affecting the nervous system.

Experimental use of gene therapy for brain tumors has increasing promise, with a multitude of research programmes using rAAV gene therapy with systemic or direct administration of therapeutic agents into the tumor site. This work has not been considered in this review, as the goal to affect the proliferation of abnormal cells is considered out of scope. The aim of this review is to provide a thorough summary of the most notable advancements in pre-clinical and clinical research of rAAV gene delivery in treating neurodegenerative, genetic and acquired diseases affecting the nervous system.

Adeno-associated virus (AAV) is a small, no enveloped virus that have been the subject of intense research interest from the gene therapy field. Recombinant adeno-associated virus (rAAV) have demonstrated remarkable evidence of efficacy and safety in a large number of animal models. They have become the most commonly used gene therapy vectors for the CNS[1] due to their safety, non-pathogenic nature, and ability to infect dividing and quiescent cells in vivo, particularly neurons. rAAV have also demonstrated very long-term gene expression in vivo[2].

Initial preclinical proofs of concept have used first generation vectors based on AAV serotype 2. They moved from the conceptual stage to clinical trials in several inherited and acquired diseases, such as Parkinson's, Batten's, and Canavan's disease[3] (Table 1). Since then, several other AAV serotypes have been isolated and engineered AAV strains with improved tissue tropisms and biodistribution have been extensively characterized in animal models. The recombinant genome of a given serotype can be packaged into the capsid of another serotype (i.e. rAAV2/5 contains the AAV2 recombinant genome packaged in the capsid proteins encoded by the cap gene of AAV5[4]). The most studied AAV serotypes in CNS have been 1, 2, 5, 8, 9 and rh10[5-8]. The effectiveness of a serotype depends on the brain region, the species, and the targeted cell type. These serotypes efficiently transduce neurons, however transduction of astrocytes, oligodendrocytes or microglia is limited[5, 6, 9] but can be improved by the use of cell specific promoters[10-13]. Transduction efficiency of these different serotypes has been further established in large animal models such as dogs, cats, primates and pigs (Table 2, Table 3 and Table 4), and finally used in therapeutic trials in human patients (Table 1).

Several strategies have been developed to deliver genes to the CNS [14]. Initially AAV gene delivery to the brain relied on intraparenchymal injection(s) in brain regions affected by the considered disease (Figure 1). This is a straightforward strategy for conditions involving a restricted brain region i.e. Parkinson's disease. This approach has also been evaluated in conditions affecting large regions of the brain such as lysosomal storage diseases (LSD) with major successes in terms of preclinical proof of efficacy in numerous animal models (Table 2) and also encouraging results, with excellent safety, in human patients (Table 1) using multiple injection tracks into the brain parenchyma[15-17].

Many research groups are developing cerebro-spinal fluid (CSF)-based delivery using intra cerebro-ventricular (ICV) or cisternal or lumbar intrathecal (IT) administration (Figure 1). These approaches, require high quantities of vector compared to intraparenchymal injections (Table 1, Table 2, Table 3 and Table 4) but are able to safely deliver secreted proteins, such as growth factors, lysosomal enzymes or Apoliprotein E (APOE). However, these strategies are associated with increased leakage of the recombinant vector in the blood circulation and with consequent off-target tissue transduction and expression in peripheral organs, particularly the liver[18, 19]. Moreover strategies will be required to reduce an immune response that may ultimately impair therapeutic benefit in patients [20].

Several studies have focused on evaluating the ability of AAV vectors to cross the BBB in mouse models and Non-human primates (NPH) following IV administration[21-23]. If encouraging results could be obtained in neonate mice, decreased transduction efficiency was observed in the primate brain, with a shift in rAAV9 tropism from neuronal to glial cells[23] throughout the brain and interestingly into the dorsal root ganglia neurons and motor neurons within the spinal cord[21]. A study comparing the ability of different rAAV strains to cross the BBB in mice demonstrates that rAAVrh.10 is at least as efficient as AAV9 vectors in CNS gene transfer following systemic administration[24].

rAAV gene therapy for neurodegenerative diseases

Recent progress in rAAV gene therapy has allowed for novel treatments of both genetic and acquired neurodegenerative diseases such as lysosomal storage diseases, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). Exciting treatment results have been obtained in animal models of the corresponding human diseases and clinical trials have been, or soon will be, launched.

Lysosomal storage disorders

Lysosomal storage disorders are a group of over 50 individual inherited metabolic diseases resulting from the deficiency of a lysosomal function responsible for progressive storage of undigested or partially digested materials leading to cellular and metabolic dysfunction[25]. Although individually rare, they have a collective incidence of approximately 1 per 7,000 live births[26], and at least 70% of all LSDs have a significant CNS component that are typically characterized by neuro-inflammation and neurodegeneration in multiple brain regions[27].

Several features of LSDs make them ideal candidates for gene therapy. Each LSD is a single-gene recessive disorder with a relatively well understood underlying molecular bases. Cross-correction opens the possibility of genetically modifying a relatively small number of cells so they express and secrete supraphysiologic levels of the deficient enzyme that can then correct a wide range of cell types at a distance[28]. A portion of newly synthesized lysosomal enzymes can be secreted and internalized by neighboring cells via mannose 6-phoshate receptors that are present on the plasma membrane. In the CNS, circulating lysosomal enzymes can diffuse via the ventricular system or be transported from the site of production to distal sites via axonal transport[29]. Levels of induced gene expression are generally not critical. Low levels of residual enzyme activity resulting from missense mutations are often associated with significant impacts on the clinical course of the disease[30]. Enzyme activities of less than 10% of the physiological level may therefore be sufficient to have a clinical impact on the disease course. Tight regulation of gene expression is not required since no deleterious effects are associated with supraphysiological levels of most of the lysosomal enzyme. The identification or development of small and large animal models recapitulating the clinical course of LSDs has been and is instrumental in evaluating therapeutic strategies [31].

Several strategies of rAAV infusion have been developed and tested in animal models of neuropathic LSDs via systemic administration as well as direct injection into the CNS through the brain parenchyma or the CSF. Main preclinical studies are listed in Table 2 and results of this research is summarized below.

Intraparenchymal delivery

Intraparenchymal injection effectively treats the CNS of neuropathic LSDs since rAAV and secreted enzymes can be transported to areas distal from the injection sites[29, 32]. The relative low dose required, compared to systemic or CSF administration[33], in an immune-privileged site like the brain

also limits the impact of a potential preexisting immunity to AAV serotypes [34]. Different sites of injection into the brain (cortex, striatum, hippocampus, thalamus, ventral tegument, cerebellum) using different AAV serotypes (1, 2, 5, 9, rh8 and rh10) have been tested in mouse models of neuropathic LSDs such as GM1 and GM2 gangliosidosis, globoid cell leukodystrophy (GCL), Niemann-Pick (NPD) type A, infantile and late infantile neuronal ceroid lipofuscinosis (INCL and LINCL), metachromatic leukodystrophy (MLD), mucopolysaccharidosis (MPS) type I, III and VII (Table 2). These studies showed that AAV vectors, injected into the parenchyma, are able to lead to widespread enzyme distribution as well as biochemical and histological correction in large regions of the mouse brain resulting in improved behavioral symptoms, motor function and lifespan. These initial data obtained in LSD mice were replicated in large disease animal models in which multiple parenchymal injections or injection into specific CNS structures with high interconnectivity are more representative of clinical transferability. For example, widespread expression of enzyme throughout the brain of MPSI and MPSIIIB mice following injection of rAAV5 in the striatum was also observed in MPSI and MPSIIIB dogs treated with a similar approach[35-37]. Injection into the thalamus and deep cerebellar nucleis of rAAV1 coding for β-galactosidase in GM1 gangliosidosis mice was also successfully transferred to the feline model with an rAAVrh8 leading to long-term clinical improvement and survival[38, 39]. Intraparenchymal injections are particularly applicable to LSDs with severe neurological but moderate systemic involvement and phase I/II clinical trials have been performed in LINCL, MPS IIIA, MPSIIIB and MLD (Table 1).

The first phase I/II trial of AAV intraparenchymal injections was for Canavan disease[40], a pediatric neurodegenerative disorder associated with mutations in the gene encoding for aspartoacylase (ASPA), an enzyme that converts N-acetyl-aspartate (NAA) into acetate and aspartate. Results have proved the feasibility and overall safety of rAAV injection into the brain via multiple sites of vector administration. Humoral immune response against the vector was observed in three of the ten subjects[41]. Long-term follow-up indicated a decrease in substrate levels in the brain of some patients and the stabilization of clinical disease [15]. A phase I clinical trial in 10 LINCL patients was performed through the direct injection into the brain parenchyma of rAAV2 carrying the human CLN2 gene. No safety issue related to the product or the way of administration was reported and there was evidence of slowing disease progression[17]. A Phase I/II clinical trial in LINCL is currently ongoing with a second-generation AAVrh.10-based vector which confers greater enzyme distribution within the brain and a better immunogenicity profile than rAAV2. rAAVrh.10 was also tested for intracranial delivery of N-sulfoglucosamine sulfohydrolase (SGSH) and the sulfatase-modifying enzyme factor 1 (SUMF1) genes in MPS IIIA patients. The administration of the vector was safe and well tolerated by the 4 patients included in the trial after one year. Stabilization in three patients and a possible

improvement in one patient were observed[16]. Additionally, a phase I/II clinical trial for MPS IIIB using AAV5 to express N-acetyl-alpha-glucosaminidase (NAGLU) is ongoing as well as a phase I study using AAVrh.10 for MLD (Table 1). Although further follow-up is necessary, these initial results suggest that intraparenchymal AAV based gene therapy is a realistic option for neuropathic LSDs.

CSF delivery

Direct delivery to the CSF is an alternative route that has been tested using different AAV serotype (1, 2, 4, 5, 8, 9, rh8 and rh10) via ventricles, cisterna magna, or spinal cord into small and large animal models of different LSDs such as GM1 and GM2 gangliosidosis, GLC, LINCL, MLD, multiple sulphatase deficiency (MSD), α-mannosidosis (AMD), MPS I, IIIA, IIIB and VII (Table 2). Initial proof of concept was made in MPS VII newborn mice where intra cerebro-ventricular (ICV) injection of rAAV1, 2 and 5 resulted in the expression of the glucoronidase beta (GUSB) in broad regions of the CNS[42]. Similar observations were reported with rAAV1 in newborn mice models of GLC and GM1 gangliosidosis[43, 44] and with rAAV5 in MPS IIIA newborn pups[45]. Even though broad CNS distribution in newborn mice could be increased by the potential immaturity of the ependymal barrier, or by its disruption favored by the large volume of injection, direct targeting of ependymal cell lining by rAAV4 in adult mice model of MPS VII[46] or by rAAV2 in LINCL dog model, also led to broad distribution of enzyme in the CNS associated with therapeutic benefits[47]. However, because of the rapid turnover rate of the ependymal cells[48], long-term benefits of this approach need to be confirmed. rAAV9 or rAAVrh10 injected into the cisterna magna showed favorable phenotypic outcomes in mouse and dog models of MPS type IIIA and B [49, 50], as well as in large models of alpha-mannosidosis (AMD)[51], MPSVII[52], and MPSI[53]. Potential additional positive effects of this approach on the pathology have been observed but only in absence of serum antibodies against the vector[49]. Intracisternal administration, although easily accessible in animal models, is more problematic to translate in human. A less invasive route into the CSF via lumbar intrathecal injection showed positive results of enzyme distribution in the brain of MPS I and MPS VII mice models using rAAV2[54, 55] but have not yet been described in the brain of larger animal models. A first clinical trial in Batten CLN6 has recently been launched using a self-complementary AAV9 carrying the CLN6 gene administered intrathecally by lumbar puncture (NCT02725580). The minimum calculated dose in this clinical trial (i.e 1,2E+14 gc for a 1 year old child of ~8kg) is already 30 fold higher than the highest dose (4E+12 gc) tested so far in intraparenchymal injection clinical trials (Table 1).

Intravenous delivery

The capacity of AAV9 or AAVrh10 serotypes to cross the BBB[23, 56] has led to the exploration of intravenous (IV) injections as an alternative to treat neuropathic LSD (Table 2). Proof of concept has been shown in neonatal GLC or MLD mice[57, 58]. AAV9 IV administration has shown an impact on the CNS pathology of older animal models in MPS III type A and B[59, 60] and GM1 gangliosidosis [61]. However, the efficiency of CNS transduction seems to be more limited in older mice as evaluated in GM2 gangliosidosis[62]. Moreover, transduction patterns observed in mice can be different from larger animal species[23]. The nature of the storage can also limit the efficacy of this approach, as observed in the MPS VII model where accumulation of sialic acid within the CNS, an inhibitor of AAV9 transduction, precluded any CNS benefit[63]. The first IV phase I/II clinical trials are currently being conducted in spinal muscular atrophy (SMA) (NCT02122952) and MPS IIIA (NCT02716246). Results on the interventions potential beneficial change on the CNS aspects of these diseases will demonstrate whether this approach could be envisaged for other LSDs. A big issue in transition to humans will be linked to the high doses of virus required to have a therapeutic effect in the CNS (i.e doses up to 3.3E+14gc of AAV9/kg are proposed in the SMA clinical trial).

Significant progress has been made in global CNS targeting using rAAV with evidence of therapeutic potential for neuropathic LSD. Preclinical studies demonstrate that a variety of serotype and injection routes can be used to improve neuropathological and functional deficits. Phase I/II clinical trials of multiple intraparenchymal AAV injections showed the safety of this approach that can delay the onset or progression of the disease. Studies using larger numbers of patients will be required to confirm these observations.

Alzheimer's disease

Key neuropathological hallmarks of AD are extracellular amyloid plaques produced from the metabolism of the Amyloid Precursor Protein (APP) and intracellular accumulation of abnormally phosphorylated Tau protein, a microtubule assembly protein, forming neurofibrillary tangles (NFTs)[64-66]. The mechanisms underlying these neuropathological changes remain unclear. There is today no treatment able to stop the progression of the disease. Several gene therapy strategies have been evaluated, targeting different pathways involved in AD physiopathology, some with encouraging results.

Strategies to reduce the amyloid accumulation by increasing amyloid degrading proteases, such as Neprilysin (NEP) and endothelin converting enzyme (ECE)[67] or by delivering anti-Aβ single chain antibody into the corticohippocampal regions of AD mice using AAV vectors were shown to decrease plaques and Aβ in the cortex and hippocampus of AD mice model[68-70], with no sign of neurotoxicity[70].

The non-amyloidogenic pathway[71] of APP metabolism prevents the production of amyloid toxic forms and enables the release of the soluble APPsα which is thought to be responsible for the important physiological functions of APP[72]. Loss of the neuroprotective APPsα could contribute to development of AD. APPsα levels are decreased in the CSF of AD patients, in both genetic and sporadic forms, which is correlated with poor memory[73, 74]. APPsα inhibits tau phosphorylation through GSK3β modulation[75]. Increasing physiological APP pathway is thus an interesting strategy to treat AD and APPsα overexpression could alleviate AD related symptoms. APPsα overexpression by the mean of AAV virus in hippocampal neurons of AD mice[76] rescued spatial memory defects, restored synaptic plasticity and spine density, and decreased soluble Aβ and amyloid plaques. This was associated with microglial activation and amyloid plaques phagocytosis.

Administration of neurotrophic factors are good candidates as neuroprotective strategies but these factors may cause off-target adverse effects, necessitating a targeted delivery strategy to control their localization and spread in the brain[77]. Nerve Growth Factor (NGF), that stimulates the function of basal forebrain cholinergic neurons undergoing early degeneration in AD[78] was evaluated in early disease onset in animal models. Encouraging preclinical data and the first clinical trial based on modified fibroblast implantation resulted in a phase 1 clinical trial in which NGF was administered in ten patients who received rAAV2-NGF into the basal forebrain region (NCT00087789). A dose escalation protocol was used, 1.2E+10 to 1.2E+11 vector particles[79] (Table 1). The brains of patients, that were examined, exhibited a trophic response to NGF in the form of axonal sprouting toward the NGF source. Cholinergic neuronal hypertrophy occurred on the NGF-treated side. Activation of cellular signaling and functional markers was present in the 2 patients who underwent rAAV2-NGF gene transfer. An overall lower rate of cognitive decline and increased cortical glucose uptake were reported. A phase II multi-centre, sham-surgery-controlled trial of NGF in AD is ongoing in 49 patients with mild to moderate AD based on a single administration of AAV-NGF vector that encodes the gene for nerve growth factor (CERE-110) or an appropriate sham (placebo) surgery control treatment[80]. Insulin-like growth factor (IGF) 2 that plays a critical role in memory consolidation in rodents was administered using an AAV2 vector in the hippocampus of aged wild-type mice and in APP Tg2576 mice. IGF-2 expression enhanced memory, promoted dendritic spine formation of WT mice, decreased amyloid levels and rescued behavioural deficits in AD mice, suggesting that IGF-2 may act as an Aß scavenger[81].

Increasing evidence demonstrate the role of inflammation in AD. Anti-inflammatory cytokine signaling may play an emerging role as neurotransmitters, neuromodulators, and neurohormones in the brain. Targeting the inflammatory pathway was thus evaluated using interleukin (IL) delivery. Expression of IL-4 or IL-10 in the brain of AD mice both resulted in reduced astro/microgliosis, Aß deposition, increased neurogenesis and improved spatial learning[82, 83].

The role of lipid metabolism is increasingly evidenced in AD. APOE, a regulator of lipoprotein metabolism in the CNS that plays several important roles such as cholesterol transport, neuroplasticity, and inflammation and Aβ clearance and aggregation[84] is the major risk factor for late onset AD. A gene transfer approach to bathe the cortex of amyloid plaque–bearing transgenic mice with APOE was used by injecting into the lateral ventricles of AD mice an AAV vector expressing the various human APOE alleles to transduce the ependymal layer. Human APOE proteins diffused into the CSF and interstitial fluid (ISF). Human APOE isoforms affected the concentrations of soluble oligomeric Aβ in the ISF, the pace of Aβ fibrillization and deposition, and the extent of peri-plaque neurotoxic effects. Increase in soluble Aβ, exacerbation of synaptic loss, and the increased number of dystrophic neurites around each deposit was observed in AD mice receiving APOE4, whereas a relative protective effect was observed with APOE2. These results suggest that therapeutic approaches aimed at decreasing APOE4 may be beneficial in AD[85].

In AD, altered cholesterol metabolism seems to play a pivotal role in the formation of amyloid plaques and in tau pathology[86]. The major exportable form of brain cholesterol is 24-hydroxycholesterol (24S-OHC) generated by the neuronal cholesterol 24-hydroxylase enzyme (CYP46A1). Overexpression of CYP46A1 by administrating in the brain an AAV vector carrying the CYP46A1 in rodent models of AD reduced the number of amyloid plaques and improved spatial memory in amyloid models [87] and improved cognitive deficits, impaired long-term depression and spine defects in mice with tauopathy[88] suggesting that CYP46A1 is a relevant target for AD.

All the approaches described have shown good preclinical efficacy in mouse models of AD and raised hopes for clinical applications in human patients. However, the main hurdle of gene therapy for AD is the diffusion of the lesions in the brain. AD impacts a number of anatomical sub-regions of the brain that are involved in learning and memory. AAV-based gene therapy strategies for AD should thus rely on safe neurosurgical protocols able to target efficiently early affected regions by the mean of improved delivery methods and use of serotypes with good diffusion properties (anterograde and retrograde transport). The development of modified AAV vectors able to efficiently cross the BBB should improve such strategies in the mid future [1, 89-92].

Parkinson's disease

Many neuroprotective treatments have been developed these past few years for PD which is the second most common neurodegenerative disorder. In addition to deep brain stimulations and cell therapies, gene therapies have recently emerged as promising alternatives. For example, it has been shown that the lysosomal hydrolase glucocerebrosidase activity is reduced in PD. Increasing it by gene transfer in mouse and rat models of PD showed neuroprotective effects against dopaminergic neuron degeneration[93]. Protecting these dopaminergic neurons from Parkinson degenerative processes have also been shown by the AAV-mediated overexpression of the transcription factors Nurr1 and Foxa2[94]. As for other diseases such as epilepsy, the glial cell line-derived neurotrophic factor (GDNF) has been overexpressed in rats and monkeys with lesions of the dopamine system and showed amelioration of lesion-induced behavioral deficits[95, 96]. NHPs are indeed of interest to validate the clinical utility of AAV infusion in brain structures affected by PD such as striatum and putamen [97] and GDNF overexpression into the striatum and substantia nigra of marmoset monkeys showed behavioral and anatomical efficacy[98]. Clinically relevant effects were also obtained in rhesus macaques where GDNF overexpression promotes restoration of the dopaminergic system in these regions[99, 100]. Another strategy often used in gene therapies for PD consists in overexpressing the enzyme aromatic L-amino acid decarboxylase (AADC). Indeed, AADC is in charge of converting the L-Dopa, the main medication for PD, to dopamine and is decreased in PD patients. AAV-mediated overexpression of AADC showed 50% of improvement in L-Dopa responsiveness[101] and persistent results for at least eight years[2]. CERE-120, an AAV2 coding for the Neurturin (NTN) has been shown to protect dopaminergic neurons in pre-clinical studies[102, 103]. Injection of CERE-120 in the putamen alone or both putamen and substantia nigra in more than a hundred people with PD (Table 1) showed no adverse effect for up to 5 years[104]. However, efficacy results were modest with no significant improvement in primary outcome measures[105, 106]. To date, several clinical trials are on-going to evaluate the safety and effectiveness of rAAV-GDNF or rAAV-AADC injection (Table 1). Due to the recent lack of efficacy of several drugs in phase III such as creatine and ubiquinone, rAAV-gene therapy for PD seems promising and could offer an interesting alternative.

Huntington's disease

HD is an autosomal dominant neurodegenerative disorder characterized by chorea, dystonia, progressive cognitive deterioration and psychiatric disturbances evolving to dementia. The disease

usually occurs in mid-life, followed by progressive aggravation of symptoms and death within 10-20 years[107, 108]. The genetic cause of the disease is a CAG (cytosine-adenine-guanine) triplet repeat expansion in the huntingtin (HTT) gene exceeding ~ 40 copies. Consequent polyglutamine expansion within the amino-terminal region of the HTT protein causes abnormal folding and accumulation of mutant HTT (mHTT) aggregates in cells. Neuroprotective agents have been tested in animal models to counter the toxic cellular effects of mHtt and improve neuronal survival. Brain-derived neurotrophic factor (BDNF), because of its functional interaction with HTT, has been evaluated in animal models of HD. GDNF-family of ligands (GFLs), GDNF (Glial-cell-line-derived neurotrophic factor), neurturin (NTN) and CNTF (Ciliary neurotrophicfactor) have also been shown to support striatal neurons viability in vitro and in vivo. When administered using rAAV2/1 vector in mice with QA-toxin lesion, BDNF resulted in reduced motor impairment and striatal damage but toxicity was observed with weight loss and seizure activity, showing that lower concentrations of BDNF were necessary for neuroprotection without side-effects[109]. Single ICV delivery of BDNF and noggin via rAAV4-BDNF/noggin treated R6/2 mice showed delayed deterioration of motor function and increased survival[110]. rAAV2-GDNF was also administered 2 weeks prior to a 3-nitropropionic acid (3NP) lesion in rats [111] or in the HD mouse model (N171-82Q mice)[112] resulting in improved performances and reduced neuronal atrophy. Neurturin (NTN), shown to protect striatal projection in excitotoxic models of HD, improved the performance of N171-82Q transgenic HD mice (rotarod and clasping tests) after rAAV2-NTN (i.e. CERE-120) injection at 5 weeks of age but did not improve the weight of transgenic animals or their performance in the cognitive radial arm water maze task compared to control groups[113]. CNTF was shown to support striatal neurons viability in vitro and in vivo. However a study has shown that long-term expression of CNTF using the AAV2 vector increases pathology in the brain of R6/1 transgenic mice[114]. These results may be due to the high dose (2.7E12 vg/ml rAAV2-CNTF injected unilaterally or bilaterally). Even neurotrophic factors have already been safely used in clinical trials. Altogether these results indicate that caution must be used to establish doses when considering viral delivery of neurotrophic factors to protect against toxic effects of mHTT while avoiding potential side effects.

Direct therapeutic strategies have been developed to target the causative mHTT and reduce the synthesis of mutant protein and potentially prevent cellular damage. RNA interference strategies have shown that decreasing mHTT improves the HD phenotype in mouse and rat models[112, 115-117]. Using AAV vectors, different approaches have been developed using mHTT mRNA (SNP)-specific microRNAs and single hairpin RNAs to block formation of the mutant protein, and most recently, allele-specific blockage of transcription. Allele-specific methods selectively silencing the mHTT are the most attractive option for HD gene therapy. However, this approach is challenging, since the

mutant gene only differs from the wt allele by the number of CAG repeats, making both alleles vulnerable. Importantly, some toxic effects of shRNA in vivo were suppressed when placed into artificial microRNA (miRNA) expression systems and miRNA-based approaches may provide more appropriate biological tools for expressing inhibitory RNAs in the brain[118]. Repression of mHTT production was also tested with zinc finger proteins (ZFPs), using long artificial ZFP chains designed to bind longer CAG repeats more effectively than shorter repeats. This reduced chromosomal expression of the mutant gene. In vivo, striatal rAAV2/1 delivery in R6/2 mice revealed repression of mHTT in the brain resulting in protein aggregate reduction and some improvement of motor and behavior performance establishing a preliminary proof-of-principle for synthetic transcription factor repressors in the brain[119].

Cholesterol metabolism is impaired in HD and restoring cholesterol metabolism is thus an interesting therapeutic target. In HD, a decrease of plasma 24S-OH cholesterol, the oxysterol produced by brain specific 24 Cholesterol hydroxylase (CYP46A1) follows disease progression proportionally to motor and neuropsychiatric dysfunction and MRI brain atrophy, together with lanosterol and lathosterol precursors (markers of cholesterol synthesis). Increased accumulation of cholesterol was observed in striatal neurons together with reduced levels of cholesterol metabolic precursors. CYP46A1 was recently shown to be decreased in the putamen of HD patients and R6/2 mice. Moreover, striatal injection of AAVrh10-CYP46A1 vector in R6/2 mice decreased neuronal atrophy, decreased HTT aggregates and improved motor deficits, as assessed by rotarod and clasping behavioral tests. It also restored levels of cholesterol and lanosterol and increased levels of desmosterol that were found in vitro to protect striatal neurons expressing mHTT from death[120]. These results strongly confirm that restoring cholesterol metabolism through CYP46A1 overexpression is a relevant therapeutic strategy in HD.

Amyotrophic Lateral Sclerosis

ALS is a rapidly progressive neurodegenerative disorder affecting motor neurons in the spinal cord, brainstem and cortex. All voluntary controlled muscles can be affected and most ALS patients die from respiratory failure 2 to 5 years from the onset of symptoms. 10-20% of ALS cases are inherited. The most studied mutations linked to these familial forms are located in the Cu/Zn superoxide dismutase 1 gene (SOD1)[121]. Thereby, SOD1-transgenic animals in which motor neurons degenerate and animals die shortly after onset of symptoms, are often used as human-like ALS models[122]. rAAV coding the IGF-1 was injected into respiratory and motor limb muscles of SOD1G93A mice taking

advantage or the vector retrograde transport from presynaptic terminals of projecting neurons to the projecting cell nucleus. Vector was transported up to motor neurons of the spinal cord which allowed to extend survival and delay motor decline[123]. Lepore et al. used the same strategy but injected into lumbar spinal cord parenchyma of $SOD1^{G93A}$ mice. The long-term expression of IGF-1 was followed by a delayed disease onset and an extended survival but only in male SOD1G93A mice[124]. The neuroprotective effect of IGF-1 in ALS was associated with decreased glial cell–mediated release of tumor necrosis factor-α and nitric oxide[125]. The same group also achieved promising results with a rAAV injection into the lateral and 4th ventricles of SOD1G93A mice[126]. More recently, ICV or IT injections of AAV vectors encoding microRNA against SOD1 improved disease outcome in SOD1G93A mice, with preservation of muscle innervation and neuroprotection[127]. The abundant evidence showing the importance of trophic factors to motor neurons as well as these preclinical results put forward a reliable strategy which could be brought to the clinic. In addition, IL-10 overexpression using rAAV injection in the spinal cord of the SOD1G93A mice produces an immune modulatory effect with longer survival[128]. More than 80% of ALS cases are sporadic and most patients do not carry the SOD1 mutation. Aizawa et al. showed in 2010 a molecular link between reduced adenosine deaminase acting on RNA 2 (ADAR2) activity and TAR DNA-binding protein (TDP-43) pathology whose loss from the nucleus and positive cytoplasmic inclusions in motor neurons are characteristics of sporadic ALS[129]. Yamashita et al. showed that AAV-ADAR2 delivery in motor neurons of ADAR2 knockout mice (AR2) enabled the restoration of ADAR2 activity and also prevented the progression of motor dysfunction and neuronal death[130]. A few clinical trials using gene therapy in ALS have been started/completed (NCT00748501, NCT02039401, NCT01041222) but none with rAAV. Despite this, positive results obtained with preclinical studies abovementioned may lead to novel promising studies in the clinic.

Spinal Muscular Atrophy

SMA is an autosomal recessive neurodegenerative disease leading to infant mortality caused by a mutation in the gene survival of motor neuron (SMN). One approach to mitigating the effects of SMN loss of function is exogenous expression of its gene by introducing viral vectors into motor neurons. Several groups provided strong evidence for effective AAV-mediated gene therapy on SMA transgenic mice. AAV9-mediated gene delivery to replace the SMN protein at day one after birth in SMA mouse resulted in elevated levels of SMN expression and extended survival[131]. rAAV8-SMN was injected bilaterally into the lateral ventricles and the upper lumbar spinal cord in SMA mice allowing improvements in behavioral tests indicating a functional neuromuscular junction and increased

survival from 15 to 50 days[132]. rAAV9 coding a codon-optimized version of the SMN injected into the facial vein at day one after birth resulted in a phenotypic correction, a significant increase in survival[133], complete correction of motor function and a major increase of survival from 27 to over 340 days[134].

As for SMA, gene therapy has been used to rescue the phenotype of SMA with respiratory distress type 1 mice. This disease is caused by a mutation in the IGHMBP2 gene. rAAV9-IGHMB2 injection restored protein levels, rescued motor function and increased life span[135].

As for ALS or neuropathic pain, intrathecal injection is a promising strategy and proof of concept to transduce motor neurons in juvenile farm pigs and monkeys have already been shown[136] supporting the use of gene therapy in clinical studies. To date, one clinical trial is actually ongoing to evaluate safety and efficacy of intravenous delivery of self-complementary rAAV9-SMN as a potential treatment (Table 1).

rAAV gene therapy for other disorders affecting the nervous system

Epilepsy

Epilepsy is mainly characterized by seizures caused by an imbalance between excitation and inhibition of electrical activities between neurons. Even if the cause of most cases of epilepsy is unknown, some result from a brain lesion inducing mostly focal seizures, whereas others are associated with genetic defects resulting in generalized seizures. The identification of genetic cases highlighted several ion channels that helped to understand and investigate epilepsy with the development of antiepileptic drugs targeting several ion channels. Seizures are often not sufficiently controlled, with current therapeutics, and new therapies are needed[137]. rAAV gene therapy has been used to control seizures in several animal models, mostly based on the overexpression of the neuropeptide Y (NPY). NPY is a 36 amino-acid member of the pancreatic polypeptide family which is an endogenous modulator of epileptic activity. AAV-mediated gene transfer to overexpress NPY in rats with electro-encephalogram (EEG) seizures induced by intrahippocampal or intracerebroventricular kainic acid injection, allowed delayed seizure onset and a reduction in seizures by 50-75%, 8 weeks after rAAV injection[138]. Because NPY activates many receptor subtypes and may result in unwanted side effects, Foti et al. developed a rAAV expressing a truncated form of NPY that primarily activates the NPY Y2 receptor and obtained a significantly prolonged latency of

limbic seizures, one week after kainic acid injection[139]. AAV-mediated gene transfer of NPY was also shown to safely improve anticonvulsant activity in kainic acid treated rats without any glia activation or humoral immune response[140]. Other transgenes have been used to attenuate seizures. Galanin, a neuropeptide with anticonvulsant properties combined with a secretory signal, was overexpressed with beneficial effects in kainic acid treated rats[141]. GDNF overexpression also leads to a suppression of seizures in rat models of temporal lobe epilepsy[142]. Downregulation of adenosine kinase (ADK) in mice via AAV-mediated RNA interference completely abolished spontaneous recurrent seizures in ADK-transgenic mice[143]. Thus antiepileptogenic and antiseizure effects of these gene therapy approaches are promising via a decrease of excitatory signals or an increase of inhibitory signals. To date, no clinical trials with AAV-mediated gene transfer are currently on going but all these preclinical studies support their application in the clinic.

Neuropathic pain

Even if neuropathic pain is often accompanied by tissue injury, it is a complex state that seems to have no obvious cause. Analgesic agents are recommended as first-line treatments but analgesia has limitations such as a short half-life, lack of cellular specificity and undesired potential off-site effects. Tissue injury and thus the associated pain, is often chronic and localized and thereby suitable for the use of AAV-mediated gene therapies. A few studies have been carried out using AAV vectors to deliver several gene targets and treat neuropathic pain. In a model of sciatic nerve injury in WISTAR rats, AAV vector overexpressing the BDNF was injected in the dorsal horn of the spinal cord. One week after injection a rescue in several behavioral tasks was observed such as an alleviation of tactile allodynia and thermal hyperalgesia[144]. Another approach used injection of rAAV expressing the Ca^{2+} channel-binding domain 3 (CBD3) into the lumbar dorsal root ganglion (DRG) in rats before the spared nerve injury (SNI). All injected rats presented a significant attenuation of behavioral changes in pain behavior such as hyperalgesia after touch with a pin or sensitivity to acetone stimulation[145]. The knockdown of the voltage-gated sodium channel Nav1.3 via rAAV expressing shRNA was also evaluated in rats with SNI. rAAV injections were performed into the DRG; a partial attenuation of nerve injury-induced mechanical allodynia was observed[146]. Intrathecal injection to treat diabetic neuropathic pain in diabetic Sprague Dawley rats also showed promising results with efficient transduction of DRGs and reduction of nociceptive hyperexcitability and neuropathic tactile allodynia associated with diabetic neuropathic pain[147]. This method is rapid, less invasive than DRG injection and clinically applicable. Intrathecal injection of AAV vector encoding shRNA against vanilloid receptor 1 (TRPV1) into the subarachnoid space of mice, three weeks after a SNI surgery showed a

significant attenuation of thermal hyperalgesia in response to 50°C heat stimulation was observed from 7 days after treatment[148]. These encouraging results should prompt evaluation in human patients with chronic/diabetic neuropathic pain.

Perspective

There is today a tremendous amount of preclinical data demonstrating the relevance and the feasibility of AAV-based gene therapy to treat CNS disorders, not only for rare genetic diseases but also opening the door to new therapeutic strategies for more common severe complex conditions. These have already led to a number of phase I/II clinical applications in human patients with encouraging results that should allow further developments to treat larger numbers of patients. Further steps will need to focus on the development of new tools improving safety, efficacy and simplicity of gene delivery to the brain.

A potential immune response to AAV vectors is still a matter of debate. Direct intraparenchymal delivery uses considerably lower doses and is less likely to have an impact on a potential immune response. The development of IT, ICV and eventually IV injection procedures will need much higher vector doses and will raise the question of immune reactions. Following IV delivery, systemic exposure to high doses of AAV vectors can trigger the activation of CD8+ T-cell responses directed against the viral capsid in a dose-dependent manner as observed in hemophilia clinical trials[149]. Many approaches are currently developed to address this crucial issue, immunological modulation in patients, de-targeting vectors using specific promoters and miRNA sequences[150-154] and induction of immune tolerance[155-157].

Improved efficacy of AAV vectors will benefit from the characterization of new serotypes that progressively emerge from the screening of AAV capsid libraries developed by DNA shuffling of existing AAV capsid genes[89, 158-160]. This should allow to design customized tools, targeted to specific brain regions and for specific cell types. Intravenous delivery remains imperfect by the limited efficacy of AAV vectors to cross the BBB. The development of new tools to transiently increase permeability of the BBB and of new serotypes with higher crossing capacity[90-92] will undoubtedly modify further strategies for safe and efficient CNS gene delivery. However, this will raise the consequent and challenging question on the capacity to produce sufficient amounts of vectors for future clinical applications in a large number of patients [161].

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Illustration of routes of administration targeting the CNS by direct injection into the parenchyma or injection into the CSF via intracerebroventricular or intrathecal (cisternal or lombar) routes.

	Injection site[1]	Disease[2]	Clinical trial	Inclu-sion	Sero-type[3]	Transgene	Promo-ter[3,4]	Dose[3] min vg	Dose[3] max vg	Volume µL[3]	Speed µL/min[3]	IS[3,5]	Status[3,6]	Identifier[3]	Ref[3]
Intraparenchymal	WM (n=6)	Can	Phase I	13	2	ASP	NSE	9.10^{11}		900	2	*NA*	C	*NA*	15
	WM (n=12)	LINCF	Phase I	11	2	CLN2	CAG	$1,8.10^{12}$	$3,2.10^{12}$	600	2	*NA*	C	NCT00151216	17
	WM (n=12)	LINCF	Phase I/II	16	rh10	CLN2	CAG	$2,85.10^{11}$	9.10^{11}	1800	2	*NA*	O	NCT01414985	*NA*
	WM (n=12)	MPSIIIA	Phase I/II	4	rh10	SGSH	PGK	$7,2.10^{11}$		720	0,5	Y	C	NCT01474343	16
	WM(n=12)/ Cer (n=4)	MPSIIIB	Phase I/II	4	5	NAGLU	PGK	4.10^{12}		960	0,5	Y	O	ISRCTN19853672	*NA*
	WM (n=12)	MLD	Phase I/II	5	rh10	ARSA	CAG	1.10^{12}	4.10^{12}	*NA*	*NA*	*NA*	O	NCT01801709	*NA*
	StN (n=2)	Par	Phase II	16	2	GAD	CAG	2.10^{12}		70	0,23	*NA*	C	NCT00643890	162
	Str (n=4)	Par	Phase I	10	2	AADC	CMV	9.10^{10}	3.10^{11}	200	1	N	C	NCT00229736	163
	Put (n=8)	Par	Phase I&II	70	2	NTN (CERE-120)	CAG	$1,3.10^{11}$	$5,4.10^{11}$	80	2	*NA*	C	NCT00252850 NCT00400634	106, 164
	Put (n=6)/ SN (n=4)	Par	Phase I/II	57	2	NTN (CERE-120)	CAG	$9,4.10^{11}$	$2.4.10^{12}$	360	2/3	*NA*	O	NCT00985517	165
	Str (n=2)	Par	Phase I	24	2	GDNF	CMV	9.10^{10}	3.10^{12}	*NA*	*NA*	*NA*	O	NCT01621581	*NA*
	Str (n=2)	Par	Phase I	10	2	AADC	*NA*	$7,5.10^{11}$	$1,5.10^{12}$	*NA*	*NA*	*NA*	O	NCT01973543	*NA*
	Put (n=4)	Par	Phase I/II	6	*NA*	AADC	*NA*	3.10^{11}	9.10^{11}	200/600	3	*NA*	O	NCT02418598	*NA*
	Put (n=2)	Par	Phase I	10	2	AADC	*NA*	*NA*		*NA*	*NA*	*NA*	O	NCT01395641	*NA*
	NBM (n=4/6)	Alz	Phase I	10	2	NGF (CERE-110)	CAG	$1,2.10^{10}$	$1,2.10^{11}$	40/80	2	*NA*	C	NCT00087789	79
	NA	Alz	Phase II	25	2	NGF (CERE-110)	CAG	2.10^{11}		*NA*	*NA*	*NA*	NA	NCT00876863	*NA*
IT[7]	*NA*	GAN	Phase I	20	9	Gigaxonin	JeT	*NA*		*NA*	*NA*	*NA*	O	NCT02362438	*NA*
	Lom	CLN6	Phase I/II	6	9	CLN6	CAG	$1,5.10^{13}$ vg/kg		*NA*	*NA*	*NA*	O	NCT02725580	*NA*
IV[8]	PeV	SMA I	Phase I/II	15	9	SMN	CAG	$6,7.10^{13}$ vg/kg	$3,3.10^{14}$ vg/kg	*NA*	*NA*	*NA*	O	NCT02122952	*NA*
	PeV	MPSIIIA	Phase I/II	9	9	SGSH	U1a	5.10^{12} vg/kg	1.10^{13} vg/kg	*NA*	*NA*	*NA*	O	NCT02716246	*NA*

[1] WM: White Matter; Cer: Cerebellum; StN: Subthalamique nucleus; Str: Striatum; Put: Putamen; SN: Substancia Nigra; NBM: Nucleus Basalis of Meynert; Lom: Lombar; PeV: Peripheral vein; [2] Can: Canavan; Par: Parkinson; Alz: Alzheimer; GAN: Giant Axonal Neuropathy; CLN6: Batten CLN6 disease; SMA I: Spinal Muscular Atrophy type I; [3] *NA* = not available; [4] The CAG promoter designation includes the CBA and CB promoter; [5] IS: Immunosupressor; Y= Yes; N=No; [6] O=Ongoing; C=Completed; [7] IT: Intrathecal; [8] IV: Intravenous.

	Model		Injection site[1]	Disease	Serotype	Transgene	Promoter[2,3]	Dose min vg / Dose max vg	Volume[2] µL	Speed[2] µL/min	Ref
Intraparenchymal	Rodent	Mice	Tha/DCN or Hip	GM1	1	BGAL	CAG	4.10^{10}-$7,2x10^{10}$	4/1	0,2/0,1	38, 166
			Str	GM2	2	HEX	*NA*	$9,9.10^{9}$ -$1,4x10^{10}$	3	*NA*	167
			Cor/ Hip/ Tha	GCL*	2/5	GALC	CAG	$3,3.10^{7}$	12	*NA*	168
			Cor/ Hth /Cer	INCL*	5	CLN1	CAG	$1,2.10^{10}$	12	*NA*	169
			Str/ Hip /Cer	JNCL*	rh10	CLN3	CAG	3.10^{10}	0,5	1	170
			Tha/Cer	LINCL	2 or 5	CLN2	CAG	$3,6.10^{9}$	18	0,5	171
			Str	MPS I	2 or 5	IDUA	PGK	1.10^{9}	5	0,5	36
			Str	MPS IIIA	rh10	SGSH/SUMF1	PGK	$7,5.10^{9}$	2,5	0,5	172
			Str	MPS IIIB	2 or 5	NAGLU	PGK	1.10^{9}	5	0,5	35
			Cor/ Cer	MPS IIIB*	5	NAGLU	CAG	$1,8.10^{10}$	12	*NA*	173, 174
			Str	MPS VII	5	GUSB	*NA*	$2,4.10^{9}$	5	0,5	175
			Cor/Hip/Str	MPS VII	1;9 or rh10	GUSB	GUSB	$1,2.10^{10}$ - $1,3.10^{10}$	1	0,5	176
			Str or Cor	MLD	rh10	ARSA	CAG	$2,3.10^{9}$ - 5.10^{10}	1/2	0,2	177
			Hip/Hth/Cor/ Str/Cer	NPD	2	ASM	CMV	$1,2.10^{11}$	24	0,5	178
	Large animal	Cat	Tha/ DCN	GM1	1 or rh8	BGAL	CAG	3.10^{11} - $1,2.10^{13}$	94	2	39
			Tha	GM2	1 or rh8	HEX A	CAG	3.10^{11} - $4,2.10^{12}$	70	2	179
		Dog	Str/WM	MPS I	5	IDUA	PGK	5.10^{11} - $2,1.10^{12}$	320	2	37
			Str/WM	MPS IIIB	5	NAGLU	PGK	5.10^{11} - $2,1.10^{12}$	320	2	37
			WM or Str/Tha	MLD[#]	5	ARSA	PGK	$3,8.10^{11}$ - $1,9.10^{12}$	120	3	180
		NHP	WM	MLD[#]	rh10	ARSA	CAG	$2,2.10^{11}$ - $1,1.10^{12}$	360	0,5	181
			WM	LINCL[#]	rh10	CLN2	CAG	$1,8.10^{12}$ - $1,8.10^{12}$	180	1	182
			Str	MPS I[#]	1 ; 2 or5	IDUA	PGK	$1,4.10^{10}$	100	*NA*	183
Intra-CSF	Rodent	Mice	ICV	MPS I*	8	IDUA	CAG	2.10^{10}	5	1	184
			IT	MPSI	2	IDUA	CMV	2.10^{9} - 4.10^{10}	50-100	*>5min*	55
			CM	MPS IIIA	9	SGSH	CAG	5.10^{9} - 5.10^{10}	5	*NA*	49
			ICV	MPS IIIA*	5	SGSH/SUMF1	CMV	$1,2.10^{10}$ - 6.10^{10}	2	*NA*	45
			CM	MPS IIIB	9	NAGLU	CAG	3.10^{10}	*NA*	*NA*	50
			CM	MPS IIIB	2	NAGLU	CMV	1.10^{10} - 5.10^{10}	15	*<30sec*	185
			IT	MPS VII*	2	GUSB	CMV	$1,5.10^{11}$ - 5.10^{11}	30-100	*NA*	54
			ICV	MPS VII	4	GUSB	RSV	1.10^{10}	10	0,5	46
			ICV	MPS VII*	1 ; 2 or5	GUSB	GUSB	$1,8.10^{10}$	4	*NA*	42
			ICV	GCL*	1	GALC	CMV	6.10^{10}	4	*NA*	44
			ICV	GM1*	1	BGAL	CAG	$3,3.10^{11}$	4	*NA*	43
			ICV	MLD	9 or 1	ARSA	CAG	$1,1.10^{11}$ - $2,3.10^{11}$	20	*>10min*	186
			ICV	MLD	1	ARSA	*NA*	2.10^{11}	20	*>10min*	187
			ICV	MSD*	4 or 9	SUMF1	CMV	$1,2.10^{10}$	6	*NA*	188
	Large animal	Cat	CM	AMD	1 or 9	MANB	GUSB	1.10^{13}	*NA*	*NA*	51
			CM	MPS I	9	IDUA	CMV/CAG	1.10^{12}vg/kg	1mL	*NA*	53
			CM	MPS I	9	IDUA	CAG	1.10^{12}vg/kg	1-2mL	*NA*	156
		Dog	CM	MPS IIIA[#]	9	SGSH	CAG	2.10^{13}	1mL	*<10min*	49
			CM	MPS IIIB[#]	9	NAGLU	CAG	$6,5.10^{12}$	*NA*	*NA*	50
			CM	MPS VII	9 or rh10	GUSB	CAG	5.10^{12}vg/kg	1-2mL	*>1min*	52
			ICV	LINCL	1 ;2 ;4 ;5 ;8 or 9	CLN2	CAG	2.10^{12}	*NA*	*>25min*	47
		NHP	CM	MPSI[#]	9	IDUA	CAG	3.10^{12}vg/kg	1-2mL	*NA*	156
Intravenous	Rodent	Mice	TaV	GM1	9	BGAL	CAG	1.10^{11} - $3,0.10^{13}$	200	*NA*	61
			TaV	GM2	9	HEX	*NA*	$3,5.10^{13}$ vg/Kg	100	*NA*	62
			TaV	GCL*	rh10	GALC	CAG	$7,6.10^{9}$ - 2.10^{11}	25/10	*NA*	58, 189
			TeV	GCL*	2/5	GALC	CAG	$1,38.10^{7}$	100	*NA*	168
			JuV	MLD*	9	ARSA	CAG	2.10^{12}	100	*NA*	57
			TeV	MSD*	4 or 9	SUMF1	CMV	2.10^{11}	100	*NA*	188
			TeV	MPS II*	5	IDS	CMV	1.10^{11}	*NA*	*NA*	190
			TaV	MPS II	8	IDS	EF1α	1.10^{11}	200	*NA*	191
			TaV	MPS IIIA	rh74	SGSH	U1a	5.10^{12} vg/Kg	150-200	*NA*	192
			NA	MPS IIIA	9	SGSH	CAG	1.10^{12}	200	*NA*	60
			TaV	MPS IIIB	9	NAGLU	CMV	1.10^{13}vg/Kg	150-200	*NA*	59

			TaV	MPS IIIB	2	NAGLU	CMV	4.10^{11}	100-150	*NA*	193
			NA	MPS VII	9	GUSB	*NA*	1.10^{12}	*NA*	*NA*	63
	Large model	Cat	CeV	MPS I	8	IDUA	TBG	5.10^{12} vg/Kg	1mL	NA	194
		Dog	CeV	MPS VII*	9 or rh10	GUSB	CAG	2.10^{13} vg/Kg	1-2 mL	*> 2min*	52
		NHP	CeV	MPS IIIB#	9	NAGLU	CMV	1.10^{13} - 2.10^{13} vg/Kg	5mL	*NA*	195
IP & CSF	Large	Cat	Tha/ICV	GM2	rh8	HEX	CAG	8.10^{11} - 1.10^{12}	340	2 or 15	196 197
IV & CSF	Rodent	Mice	TeV/ICV	MSD*	4 or 9	SUMF1	CMV	$1,2.10^{10}$ -2.10^{11}	103	*NA*	188
			TaV/CM	MPS IIIB	2	NAGLU	CMV	5.10^{10} - 4.10^{11}	5-20	*> 2 min*	198
			TeV/IT	MPS VII*	2	GUSB	CMV	3.10^{10} - 4.10^{11}	*NA*	*NA*	199
	Large	Dog	CeV/CM	MPS VII*	9 or rh10	GUSB	CAG	$2,8.10^{12}$ -2.10^{13} vg/Kg	1-2mL	*> 2 min*	52

[1]Tha:Thalamus; Hth: Hypothalamus; DCN: Deep Cerebellar Nuclei; Hip: Hippocampus; Str: striatum; Cor: Cortex; Cer:Cerebellum; WM: White Matter; ICV: Intracerebroventricular; CM: Cisterna Magna; TaV: Tail Vein; TeV: Temporal Vein ; JuV: Jugular Vein ; CeV: Cephalic vein ; IT: Intrathecal ; IP: Intraparenchymal ; [2] *NA* : not available ; [3] The CAG promoter designation includes the CBA and CB promoters; # The study was performed using wild type model of the animal in order to study the AAV for the disease mentioned ;* Newborn animals.

	Model		Injection site[1]	Disease[2]	Serotype	Transgene[3]	Promoter[3,4]	Dose[3] min vg	Dose[3] max vg	Vol[3] µL	Speed[3] µL/min	Ref
Intraparenchymal	Rodent	Mice	➢ **Disease affecting the CNS**									
			Hip/ Cor	Alz	5 or 9	NEP/IDE	*NA*	*NA*		*NA*	*NA*	68-70
			Hip/ Cor	Alz	5	ECE	CAG	4.10^9		4	0,5	69
			Hip/ Cor	Alz	2	ScFv59	CAG	2.10^9		8	1	70
			Cor &/or Hip	Alz	5	CYP46A1	PGK	$2,4.10^9$		4	0,2	87, 88
			Hip	Alz	9	AAPsα	Synapsin	2.10^{10}		4	0,2	76
			Hip	Alz	8	IGF-1/IGF-2	EF1α	*NA*		1	0,2	81
			Hip	Alz	2/1	IL-10	*NA*	3.10^9		2	*NA*	82
			Hip	Alz	1/2	IL-4	*NA*	2.10^9		2	*NA*	83
			Hip	Alz	1/2	CCL2 mutant	CAG	2.10^{10}		2	*NA*	200
			Str/Hip/ SN	Par	2 or 5	GBA1/ α-Synuclein	Synapsin	2.10^{10}		10	1	93
			SN	Par	2	Nurr1 &/or Foxa2	CMV	1.10^9		1	0,66	94
			SN	Par	1/2	shRNA anti ROCK2 or LIMK1	Synapsin	$2,5.10^7 – 1.10^8$		1	0,5	201
			Str	Par	2	NTN	CAG	4.10^9		4	0,2	102
			Str	Hun	1	DN-TNF	CAG	2.10^{10} - $4,2.10^{10}$		6	0,5	202
			Str	Hun	1	mRNAs anti-Htt	CAG	$1,8.10^{10}$		4	0,5	117
			Str	Hun	1	mRNAs anti-Htt	mU6	4.10^{10}		10	*NA*	203
			Str	Hun	2	NTN	CAG	4.10^9		4	2	113
			Str	Hun	2	CNTF	CAG	$2,7.10^9$		1	0,5	114
			Str/Cer	Hun	1	shHD2.1	CMV	6.10^{10}		12	0,25	116
			Str	Hun	1/2	ZF11xHunt	CAG	$4,4.10^9$		6	0,25	119
			Str	Hun	5 or rh10	shCYP46A1	CAG or PU6	6.10^9		4	0,2	120
			Str	Hun	2	GDNF	CAG	4.10^9		4	0,2	112
			Str	Hun*	2	BDNF	CAG	8.10^8-8.10^9		5	0,15	109
			Str	Hun*	1/2	BDNF	CAG	4.10^9		4	*NA*	204
			Str	Hun	2	GDNF	CAG	$1,2.10^{10}$		4	0,5	111
			Str	Hun*	1/2	HD70/HD20/HDh8/shHD2	NSE	3.0^9		3	0,1	115
			Med sep	Alz	NA	NGF	NSE	6.10^9		2	*NA*	205
			➢ **Disease affecting the spinal cord**									
		Mice	DCN	ALS	1 or 2	IGF-1	*NA*	4.10^{10}		6	0,5	125
	Large animal	NPH	➢ **Disease affecting the CNS**									
			Put	Hun*	1	siRNA	U6	$6,8.10^{10}$		68	1	206
			Cau/Put	Par*	2	AADC	*NA*	$3,6.10^{11}$		180	*NA*	2
			Put	Par*	2	GDNF	CMV	$9,9.10^{11}$		150	*NA*	100
			Put/ SN	Par*	2	GDNF	CMV	$8,3.10^{10}$ - $8,3.10^{11}$		50-75	*NA*	99
			Cau/ Put	Par*	2	NTN	CAG	3.10^{11}		150	2	103
			STN	Par*	NA	GAD	*NA*	6.10^{10} - $1,2.10^{11}$		20	*NA*	207
			Put	Par*	2	hAADC	CMV	$6.10^9 – 5.10^{11}$		200	0,1-1	101
			Put	Par*	2	hAADC-2	CMV	3.10^{11}		200	1	97
			Str/SN	Par*	NA	GDNF	CAG	$8,4.10^{10}$		21	0,25	98
IntraCSF	Rodent	Mice	➢ **Disease affecting the CNS**									
			ICV	Hun	4	BDNF/ ΔB2Noggin	*NA*	$3,7x10^9$		3	*NA*	110
			➢ **Disease affecting the spinal cord**									
			CVL	ALS	9	amiR SOD1	CAG	5.10^{10}		2	*NA*	208
			ICV	ALS	6 or 9	miRNA αSOD1	CMV or GFAP	$2,4.10^{12}$ $1,6.10^{11}$ or $6,8.10^{11}$		10 3	*NA*	127
			IT	ALS	rh10	amiR-SOD1	Pol II	$1,2.10^{12}$ vg/Kg		8	*NA*	209
			ICV	ALS	4	IGF-1 or VEGF or IGF-1+VEGF	Hb9	4.10^{10} - 8.10^{10}		20	0,5	126
			CM	GAN	9	GAN	CMV	$7,2.10^{10}$		10	*NA*	210
			ICV	SMA	9	SMN	CAG	$2,7.10^{12}$ - $3,3.10^{13}$ vg/Kg		5	*NA*	19
			ICV	SMA	8	SMN	CAG/GUSB	5.10^{10}-$1,7.10^{10}$		12	*NA*	132
			ICV	SMA	9	SMNI	CAG	1.10^9 - 5.10^{10}		6	*NA*	136
	e m		➢ **Disease affecting the spinal cord**									

	Pig	IT	SMA*	9	SMNI	CAG	3.10^{12}	1500	*NA*	[136]
	NHP	CM/IT	SMA*	9	SMNI	CAG	$2,5.10^{13}$	6000	*NA*	[136]
		IT	ALS*	rh10	miR SOD1	U6/CAG	6.10^{12}vg/kg	300	*NA*	[211]
Intravenous	Rodent model	➢ **Disease affecting the CNS**								
	Mice	JuV	Hun	9	HTT-specific RNAi	U6	$6,3.10^{11} – 3.10^{12}$	50-300	*NA*	[212]
		➢ **Disease affecting the spinal cord**								
		FaV	SMA	9	SMN	CAG	5.10^{11}	*NA*	*NA*	[131]
		FaV	SMA	9	SMN	CMV	1.10^{11}	10	*NA*	[133]
		TeV	SMA	9	SMN	PGK	$4,5.10^{10}$	70	*NA*	[134]
		FaV	SMA	9	SMN	CAG	5.10^{11}	100	*NA*	[135]
		NA	SMA	9	SMN	CAG	$3,3.10^{14}$vg/kg	50	*NA*	[19]
		TaV	ALS	9	ADAR2	SYN1	$1,5.10^{11}$ - $2,1.10^{12}$	*NA*	>1min	[130]
		TaV	ALS	rh10	miR SOD1	U6/CAG	2.10^{11}	200	*NA*	[211]
Intra-muscular	Rodent	➢ **Disease affecting the spinal cord**								
	Mice	Qua	ALS	2	IGF-1 GDNF	*NA*	4.10^{7} - 3.10^{10}	*NA*	*NA*	[123]
Intra SP	Rodent model	➢ **Disease affecting the spinal cord**								
	Mice	VGM	ALS	2	IGF-1	CAG	$4,8.10^{10}$	6	0,75	[124]
		LSC	ALS	2	IL-10	CAG	NA	1	*NA*	[128]
	Rat	VH	ALS	2	IGF-1	CAG	$4,1.10^{10}$	5	*NA*	[213]

[1] Hip: Hippocampus; Str: Striatum; SN: Substancia Nigra; DCN: Deep Cerebellar Nuclei; VGM: Ventral gray matter; Put: Putamen; Cau: Caudate; Cor: Cortex; Med Sep: Medial Septum; STN: Sub-Thalamique Nucleus; Ven: Ventricle; LC: lumbar cord; CM: Cisterna magna; ICV: IntraCerebroVentricular; JuV : Jugular Vein; TaV: Tail Vein; FaV: Facial vein; TeV: Temporal vein; Qua: Quadriceps; LSC: Lumbar Spinal Cord; VH: Ventral Horn; [2]Alz: Alzheimer; Hun: Huntington; Par: Parkinson; GAN: Giant Axonal Neuropathy; ALS: Amyotrophic Lateral Sclerosis; SMA: Spinal Muscular Atrophy; [3] *NA*: Not available; [4] The CAG promoter designation includes the CB and CBA promoter; *The study was performed using wild type model of the animal in order to study the disease mentioned

	Model		Injection site[1]	Disease	Serotype[2]	Transgene	Promoter[3]	Dose min vg	Dose max vg	Volume µL	Speed[2] µL/min	Ref
➢ **Epilepsy**												
Intraparenchymal	Rodent	Mice	Hip	Epilepsy	8	ADK	gfaABC1D	2.10^9		2	1	143
		Rat	Hip	Epilepsy	1 and 2	NPY	NSE	6.10^{10}		6	0,2	214
			Hip	Epilepsy	1	NPY	CAG	$6,2.10^{10}$		12	1	215
			Hip	Epilepsy	2	GDNF	CAG	$4,1.10^9$ - $6,3.10^9$		3 or 6	0,2	142
			Pir Cor	Epilepsy	2	NPY	CAG	$1,2.10^{10}$ - $1,3.10^{10}$		4	*NA*	139
			Hip	Epilepsy	1/2 or 2	NPY	NSE	$6,4.10^9$ - $1,3.10^{10}$		6 or 12	*NA*	138
			Inf Col	Epilepsy	*NA*	FIB-GAL or GAL	CMV	$1,75.10^7$-1.10^9		1	*>9 min*	141
			Inf Col or Temp Cor	Epilepsy	*NA*	NRIA or PINA	CMV	1.10^9		1	*>9 min*	216
➢ **Neuropathic pain (NP)**												
Intra-CSF	Rodent	Mice	IT	Chronic NP	9	shRNA anti TRPV1	hU6	2.10^{13}		10	*>2min*	148
		Rat	IT	Diabetic NP	5	shRNA anti $Na_v1.3$	*NA*	$3,5.10^{11}$		5	*NA*	147
			IT	Chronic NP	8	ppβEP and/or IL-10	CMV	3.10^{10}		15	*>1min*	217
Intra SP	Rodent	Rat	DRG	Chronic NP	6	CBD3	CMV	5.10^9		2	*>5min*	145
			DRG	Chronic NP	2	shRNA anti $Na_v1.3$	hU6	$2,7.10^{10}$ - $1,4.10^{11}$		2	0,4	146
			SGM	Chronic NP	*NA*	BDNF	CMV	5.10^5 IU		1	*>2min*	144

[1] Hip: Hippocampus; Pir Cor: Piriform Cortex; Inf Col: Inferior Colliculus; Temp Cor: Temporal Cortex; DRG: Dorsal Root Ganglion; SGM: Spinal Gray Matter; [2] *NA* = not available; [3] The CAG promoter designation includes the CBA promoter

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Figure 1