REVIEW ARTICLE

Adeno-Associated Virus-Based Gene Therapy for CNS Diseases

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Gene therapy is at the cusp of a revolution for treating a large spectrum of CNS disorders by providing a durable therapeutic protein via a single administration. Adeno-associated virus (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 research on AAV-based CNS gene therapy and to discuss prospects for future development based on a new generation of vectors and delivery.

INTRODUCTION

NEUROLOGICAL DISORDERS are among the most difficult pathologies to treat because of the limited access to brain structures, which are protected by physical barriers such as the blood–brain barrier (BBB); the complexity of the CNS; and cell interactions. Gene therapy is an alternative to traditional pharmacological approaches that has made important advances over the last decade in treating the nervous system.

Experimental use of gene therapy for brain tumors has increasing promise, with a multitude of research programs using recombinant adeno-associated viral (rAAV) gene therapy with systemic or direct administration of therapeutic agents into the tumor site. This work is not considered here, as the goal to influence the proliferation of abnormal cells is not within the scope of this review. The aim of this review is to provide a thorough summary of the most notable advancements in preclinical and clinical research on rAAV gene delivery in treating neurodegenerative, genetic, and acquired diseases affecting the nervous system.

AAV is a small, nonenveloped virus that has been the subject of intense research interest from the gene therapy field. rAAVs 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] because of their safety, nonpathogenic nature, and ability to infect dividing and quiescent cells *in vivo*, particularly neurons. rAAVs have also demonstrated long-term gene expression *in vivo*.[2]

Initial preclinical proofs of concept used first-generation vectors based on AAV serotype 2. They moved from the conceptual stage to clinical trials for 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 AAV serotypes most studied in the CNS have been serotypes 1, 2, 5, 8, 9, and recombinant human (rh)10.[5–8] The effectiveness of a serotype depends on the brain region, the species, and the targeted cell type. These serotypes efficiently transduce neurons;

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Table 1. *Clinical Trials*

	Injection site	Disease	Clinical trial	Inclusion	Serotype	Transgene	Promoter[a]	Dose, min vg	Dose, max vg	Volume, μL	Speed, μL/min	IS	Status	Identifier	Ref.
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 ($n=12$)	MPS IIIA	Phase I/II	4	rh10	SGSH	PGK	7.2×10^{11}		720	0.5	Y	C	NCT01474343	16
	WM ($n=12$)/ Cer ($n=4$)	MPS IIIB	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	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	PeV	SMA I	Phase I/II	15	9	SMN	CAG	6.7×10^{13}	3.3×10^{14} vg/kg	NA	NA	NA	O	NCT02122952	NA
	PeV	MPS IIIA	Phase I/II	9	9	SGSH	U1a	5×10^{12}	1×10^{13} vg/kg	NA	NA	Y	O	NCT02716246	NA

[a]The CAG promoter designation includes the CBA and CB promoter.

Alz, Alzheimer; C, completed; Can, Canavan; Cer, cerebellum; CLN6, Batten CLN6 disease; GAN, giant axonal neuropathy; IS, immunosupressor; IT, intrathecal; IV, intravenous; LINCF, Late infantile neuronal ceroid lipofuscinosis; MLD, metachromatic leukodystrophy; max vg, maximum vector genome; min vg, minimum vector genome; MPS IIIA, mucopolysaccharidosis IIIA; MPS IIIB, mucopolysaccharidosis IIIB; Lom, lombar; N, no; NA, not available; NBM, nucleus basalis of meynert; O, ongoing; Par, Parkinson; PeV, peripheral vein; Put, Putamen; SMA I, spinal muscular atrophy type I; SN, substancia nigra; StN, subthalamique nucleus; Str, striatum; WM, white matter; Y, yes.

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 various serotypes has been further established in large-animal models such as dogs, cats, primates, and pigs (Tables 2–4), 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) into brain regions affected by the considered disease (Fig. 1). This is a straightforward strategy for conditions involving a restricted brain region, such as, Parkinson's disease. This approach has also been evaluated in conditions affecting large regions of the brain such as lysosomal storage diseases (LSDs), 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 cerebrospinal fluid (CSF)-based delivery, using intracerebroventricular (ICV) or cisternal or lumbar intrathecal (IT) administration (Fig. 1). These approaches require high quantities of vector compared with intraparenchymal injections (Tables 1–4) but are able to deliver secreted proteins, such as growth factors, lysosomal enzymes, or apolipoprotein E (APOE). However, these strategies are associated with increased leakage of the recombinant vector into 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 nonhuman primates (NPHs) after IV administration.[21–23] Even 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 various rAAV strains to cross the BBB in mice demonstrated that rAAVrh.10 is at least as efficient as AAV9 vectors in CNS gene transfer after systemic administration.[24]

Table 2. *Preclinical trials for lysosomal storage diseases*

Model			Injection site	Disease	Serotype	Transgene	Promoter[a]	Dose, min vg	Dose, max vg	Volume, μL	Speed, μL/min	Ref.
Intraparenchymal	Rodent	Mice	Tha/DCN or Hip	GM1	1	BGAL	CAG	4×10^{10}	7.2×10^{10}	4/1	0.2/0.1	38,166
			Str	GM2	2	HEX	NA	9.9×10^{9}	1.4×10^{10}	3	NA	167
			Cor/Hip/Tha	GCL[b]	2/5	GALC	CAG	3.3×10^{7}		12	NA	168
			Cor/Hth/Cer	INCL[b]	5	CLN1	CAG	1.2×10^{10}		12	NA	169
			Str/Hip/Cer	JNCL[b]	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[b]	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
		NHP	WM or Str/Tha	MLD[c]	5	ARSA	PGK	3.8×10^{11}	1.9×10^{12}	120	3	180
			WM	MLD[c]	rh10	ARSA	CAG	2.2×10^{11}	1.1×10^{12}	360	0.5	181
			WM	LINCL[c]	rh10	CLN2	CAG	1.8×10^{12}	1.8×10^{12}	180	1	182
			Str	MPS I[c]	1; 2 or5	IDUA	PGK	1.4×10^{10}		100	NA	183
Intra-CSF	Rodent	Mice	ICV	MPS I[b]	8	IDUA	CAG	2×10^{10}		5	1	184
			IT	MPSI	2	IDUA	CMV	2×10^{9}	4×10^{10}	50–100	*>5 min*	55
			CM	MPS IIIA	9	SGSH	CAG	5×10^{9}	5×10^{10}	5	NA	49
			ICV	MPS IIIA[b]	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	*<30 sec*	185
			IT	MPS VII[b]	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[b]	1; 2 or 5	GUSB	GUSB	1.8×10^{10}		4	NA	42
			ICV	GCL[b]	1	GALC	CMV	6×10^{10}		4	NA	44
			ICV	GM1[b]	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	*>10 min*	186
			ICV	MLD	1	ARSA	NA	2×10^{11}		20	*>10 min*	187
			ICV	MSD[b]	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		1 mL	NA	53
		Dog	CM	MPS I	9	IDUA	CAG	1×10^{12} vg/kg		1–2 mL	NA	156
			CM	MPS IIIA[c]	9	SGSH	CAG	2×10^{13}		1 mL	*<10 min*	49
			CM	MPS IIIB[c]	9	NAGLU	CAG	6.5×10^{12}		NA	NA	50
			CM	MPS VII	9 or rh10	GUSB	CAG	5×10^{12} vg/kg		1–2 mL	*>1 min*	52
			ICV	LINCL	1; 2; 4; 5; 8 or 9	CLN2	CAG	2×10^{12}		NA	*>25 min*	47
		NHP	CM	MPSI[c]	9	IDUA	CAG	3×10^{12} vg/kg		1–2 mL	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[b]	rh10	GALC	CAG	7.6×10^{9}	2×10^{11}	25/10	NA	58,189
			TeV	GCL[b]	2/5	GALC	CAG	1.38×10^{7}		100	NA	168
			JuV	MLD[b]	9	ARSA	CAG	2×10^{12}		100	NA	57
			TeV	MSD[b]	4 or 9	SUMF1	CMV	2×10^{11}		100	NA	188
			TeV	MPS II[b]	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		1 mL	NA	194
		Dog	CeV	MPS VII[b]	9 or rh10	GUSB	CAG	2×10^{13} vg/Kg		1–2 mL	*>2 min*	52
		NHP	CeV	MPS IIIB[c]	9	NAGLU	CMV	1×10^{13}	2×10^{13} vg/Kg	5 mL	NA	195

(*continued*)

Table 2. *(Continued)*

	Model		Injection site	Disease	Serotype	Transgene	Promoter[a]	Dose, min vg	Dose, max vg	Volume, μL	Speed, μL/min	Ref.
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[b]	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[b]	2	GUSB	CMV	3×10^{10}–4×10^{11}		NA	NA	199
	Large	Dog	CeV/CM	MPS VII[b]	9 or rh10	GUSB	CAG	2.8×10^{12}–2×10^{13} vg/Kg		1–2 mL	*>2 min*	52

[a]The CAG promoter designation includes the CBA and CB promoters.
[b]Newborn animals.
[c]The study was performed using wild type model of the animal in order to study the adeno-associated virus for the disease mentioned.
AMD, α-mannosidosis; CeV, cephalic vein; CM, cisterna magna; Cor, cortex; DCN, deep cerebellar nuclei; GM, gagliosidosis; GCL, globoid cell leukodystrophy; Hip, hippocampus; Hth, hypothalamus; ICV, intracerebroventricular; INCL, infantile neuronal ceroid lipofuscinosis; JNCL, juvenile neuronal ceroid lipofuscinosis; LINCL, late infantile neuronal ceroid lipofuscinosis; MLD, metachromatic leukodystrophy; MPS, mucopolysaccharidosis; MSD, multiple sulfatase deficiency; NPD, Niemann-Pick disease; IP, intraparenchymal; JuV, jugular vein; Str, striatum; TaV, tail vein; TeV, temporal vein; Tha, thalamus.

rAAV GENE THERAPY FOR NEURODEGENERATIVE DISEASES

Progress in rAAV gene therapy has allowed for novel treatments of both genetic and acquired neurodegenerative diseases such as LSDs, 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

LSDs are a group of more than 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 7000 live births,[26] and at least 70% of all LSDs have a significant CNS component that is typically characterized by neuroinflammation 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 basis. Cross-correction opens the possibility of genetically modifying a relatively small number of cells so that they express and secrete supraphysiological levels of the deficient enzyme, which 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-phosphate 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 because no deleterious effects are associated with supraphysiological levels of most of the lysosomal enzymes. 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 are summarized below.

Intraparenchymal delivery. Intraparenchymal injection may effectively treat the CNS of patients with neuropathic LSDs because rAAV and secreted enzymes can be transported to areas distal from the injection sites.[29,32] The relative low dose required, compared with 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] Various sites of injection into the brain (cortex, striatum, hippocampus, thalamus, ventral tegument, cerebellum), using various AAV serotypes (1, 2, 5, 9, rh8, and rh10), have been tested in mouse models of neuropathic LSDs such as GM_1 and GM_2 gangliosidosis, globoid cell leukodystrophy (GCL),

Table 3. *Preclinical trials for neurodegenerative diseases*

Model			Injection site	Disease	Serotype	Transgene	Promoter[a]	Dose, min vg	Dose, max vg	Volume, μL	Speed, 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
		Rat	Str	Hun[b]	2	BDNF	CAG	8×10^{8}–8×10^{9}		5	0.15	109
			Str	Hun[b]	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[b]	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
		Mice	➢ **Disease affecting the spinal cord**									
			DCN	ALS	1 or 2	IGF-1	NA	4×10^{10}		6	0.5	125
	Large animal	NPH	➢ **Disease affecting the CNS**									
			Put	Hun[b]	1	siRNA	U6	6.8×10^{10}		68	1	206
			Cau/Put	Par[b]	2	AADC	NA	3.6×10^{11}		180	NA	2
			Put	Par[b]	2	GDNF	CMV	9.9×10^{11}		150	NA	100
			Put/SN	Par[b]	2	GDNF	CMV	8.3×10^{10}–8.3×10^{11}		50–75	NA	99
			Cau/Put	Par[b]	2	NTN	CAG	3×10^{11}		150	2	103
			STN	Par[b]	NA	GAD	NA	6×10^{10}–1.2×10^{11}		20	NA	207
			Put	Par[b]	2	hAADC	CMV	6×10^{9}–5×10^{11}		200	0.1–1	101
			Put	Par[b]	2	hAADC-2	CMV	3×10^{11}		200	1	97
			Str/SN	Par[b]	NA	GDNF	CAG	8.4×10^{10}		21	0.25	98
IntraCSF	Rodent	Mice	➢ **Disease affecting the CNS**									
			ICV	Hun	4	BDNF/ΔB2Noggin	NA	3.7×10^{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}		10	NA	127
								1.6×10^{11} or 6.8×10^{11}		3		
			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
	Large model	Pig	➢ **Disease affecting the spinal cord**									
			IT	SMA[b]	9	SMNI	CAG	3×10^{12}		1500	NA	136
		NHP	CM/IT	SMA[b]	9	SMNI	CAG	2.5×10^{13}		6000	NA	136
			IT	ALS[b]	rh10	miR SOD1	U6/CAG	6×10^{12} vg/kg		300	NA	211

(*continued*)

Table 3. *(Continued)*

Model			Injection site	Disease	Serotype	Transgene	Promoter[a]	Dose, min vg	Dose, max vg	Volume, μL	Speed, μL/min	Ref.
Intravenous	Rodent model	Mice	➢ **Disease affecting the CNS**									
			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	>1 min	130
			TaV	ALS	rh10	miR SOD1	U6/CAG	2×10^{11}		200	NA	211
Intramuscular	Rodent	Mice	➢ **Disease affecting the spinal cord**									
			Qua	ALS	2	IGF-1 GDNF	NA	4×10^{7}–3×10^{10}		NA	NA	123
Intra SP	Rodent model	Mice	➢ **Disease affecting the spinal cord**									
			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

[a]The CAG promoter designation includes the CB and CBA promoter.
[b]The study was performed using wild type model of the animal in order to study the disease mentioned
ALS, amyotrophic lateral sclerosis; Alz, Alzheimer; Cau, caudate; FaV, facial vein; GAN, giant axonal neuropathy; Hun, Huntington; LC, lumbar cord; LSC, lumbar spinal cord; Med Sep, medial septum; Qua, quadriceps; SMA, spinal muscular atrophy; STN, sub-thalamique nucleus; Ven, ventricle; VGM, ventral gray matter; VH, ventral horn.

Niemann-Pick disease (NPD) type A, infantile and late infantile neuronal ceroid lipofuscinosis (INCL and LINCL), metachromatic leukodystrophy (MLD), and mucopolysaccharidosis (MPS) types 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 life span. These initial data obtained in LSD mice were replicated in large-animal disease models in which multiple parenchymal injections or injection into specific CNS structures with high interconnectivity are more representative of clinical transferability. For example, widespread expres-

Table 4. *Preclinical trials for epilepsy and pain*

Model			Injection site	Disease	Serotype	Transgene	Promoter[a]	Dose, min vg	Dose, max vg	Volume, μL	Speed, 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	*>2 min*	148
		Rat	IT	Diabetic NP	5	shRNA anti $Na_v1\times3$	NA	3.5×10^{11}		5	NA	147
			IT	Chronic NP	8	ppβEP and/or IL-10	CMV	3×10^{10}		15	*>1 min*	217
Intra SP	Rodent	Rat	DRG	Chronic NP	6	CBD3	CMV	5×10^{9}		2	*>5 min*	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	*>2 min*	144

[a]The CAG promoter designation includes the CBA promoter.
DRG, dorsal root ganglion; Inf Col, inferior colliculus; NP, neuropathic pain; Pir Cor, piriform cortex; SGM, spinal gray matter; Temp Cor, temporal cortex.

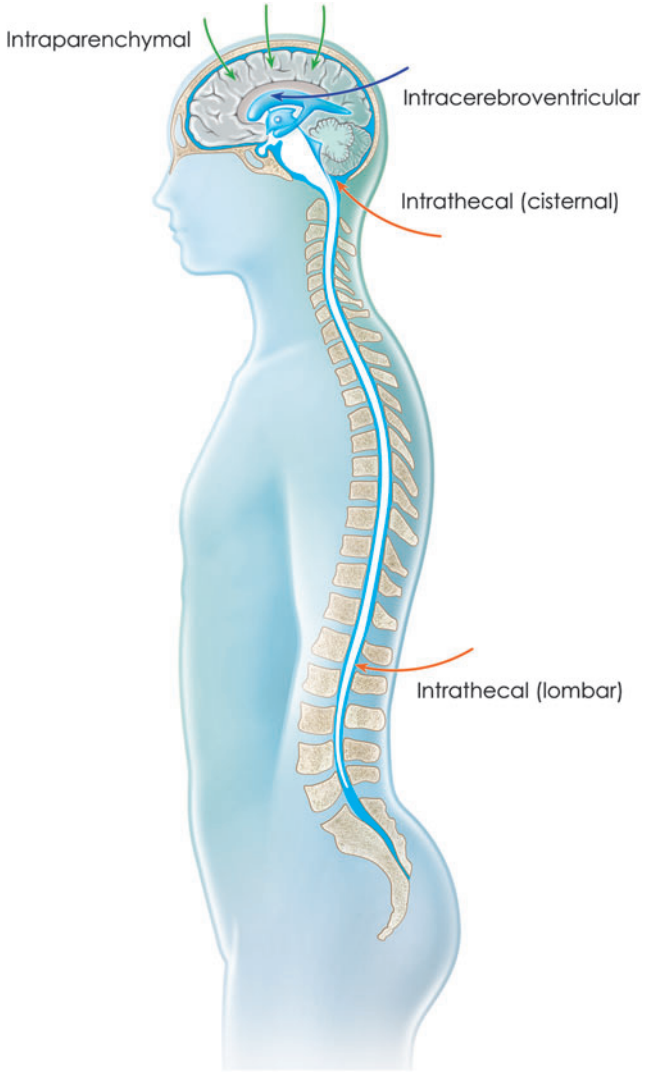

Figure 1. Routes of administration targeting the CNS by direct injection into the parenchyma or by injection into the cerebrospinal fluid via the intracerebroventricular or intrathecal (cisternal or lumbar) route.

sion of enzyme throughout the brains of MPS I and MPS IIIB mice after injection of rAAV5 into the striatum was also observed in MPS I and MPS IIIB dogs treated by a similar approach.[35–37] Injection of rAAV1 encoding β-galactosidase into the thalamus and deep cerebellar nuclei of GM_1 gangliosidosis mice was also successful in the feline model, with 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, MPS IIIB, 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 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 3 of the 10 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 patients with LINCL was performed by direct injection into the brain parenchyma of rAAV2 carrying the human *CLN2* gene. No safety issue related to the product or the manner 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 that 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 patients with MPS IIIA. The administration of the vector was safe and well tolerated by the four patients included in the trial after 1 year. Stabilization in three patients and a possible improvement in one patient were observed.[16] In addition, a phase I/II clinical trial for MPS IIIB, using AAV5 to express *N*-acetyl-α-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 with various AAV serotypes (1, 2, 4, 5, 8, 9, rh8, and rh10) via ventricles, cisterna magna, or spinal cord into small- and large-animal models of various LSDs such as GM_1 and GM_2 gangliosidosis, GCL, LINCL, MLD, multiple sulfatase deficiency (MSD), α-mannosidosis (AMD), and MPS I, IIIA, IIIB, and VII (Table 2). Initial proof of concept was made in newborn MPS VII mice where ICV injection of rAAV1, 2, and 5 resulted in the expression of β-glucuronidase (GUSB) in broad regions of the CNS.[42] Similar observations were reported with rAAV1 in newborn mouse models of GCL and GM_1 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 the adult mouse model of MPS VII,[46] or by rAAV2 in the 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 animal models of MPS types IIIA and B,[49,50] as well as in large-animal models of AMD,[51] MPS VII,[52] and MPS I.[53] Potential additional positive effects of this approach on the non-CNS pathology have been observed but only in the absence of serum antibodies against the vector.[49] Intracisternal administration, although easily accessible in animal models, is more problematic to translate into humans. 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 mouse 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 been launched with a self-complementary AAV9 carrying the *CLN6* gene administered intrathecally by lumbar puncture (NCT02725580). The minimum calculated dose in this clinical trial (i.e., 1.2×10^{14} genome copies [GC] for a 1-year-old child of $\sim$8 kg) is already 30-fold higher than the highest dose (4×10^{12} GC) tested so far in intraparenchymal injection clinical trials (Table 1).

Intravenous delivery. The capacity of the AAV9 or AAVrh10 serotype 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 GCL or MLD mice.[57,58] AAV9 IV administration has shown an impact on the CNS pathology of older animal models in MPS III types A and B[59,60] and GM_1 gangliosidosis.[61] However, the efficiency of CNS transduction seems to be more limited in older mice as evaluated in GM_2 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 of the intervention's potential beneficial effect on the CNS aspects of these diseases will demonstrate whether this approach could be envisaged for other LSDs. A significant issue in the transition to humans will be linked to the high doses of virus required to have a therapeutic effect in the CNS (i.e., AAV9 doses up to 3.3×10^{14} GC/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, which 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 an AD mouse model[68–70] with no sign of neurotoxicity.[70]

The nonamyloidogenic 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 the development of AD. APPsα levels are decreased in the CSF of patients with AD, in both genetic and sporadic forms, which is correlated with poor memory.[73,74] APPsα inhibits tau phosphorylation through glycogen synthase kinase 3β (GSK3β) modulation.[75] Increasing the physiological APP pathway is thus an interesting strategy to treat AD, and APPsα overexpression could alleviate AD-related symptoms. APPsα overexpression by means of AAV in the hippocampal neurons of AD mice[76] rescued spatial memory de-

fects, restored synaptic plasticity and spine density, and decreased soluble Aβ and amyloid plaques. This was associated with microglial activation and amyloid plaque phagocytosis.

Neurotrophic factors are good candidates in 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), which 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 I clinical trial in which NGF was administered to 10 patients, who received rAAV2-NGF into the basal forebrain region (NCT00087789). A dose escalation protocol was used: 1.2×10^{10} to 1.2×10^{11} vector particles[79] (Table 1). The brains of patients, which 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 two patients who underwent rAAV2-NGF gene transfer. An overall lower rate of cognitive decline and increased cortical glucose uptake were reported. A phase II multicenter, 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, which plays a critical role in memory consolidation in rodents, was administered via an AAV2 vector into the hippocampus of aged wild-type mice and in APP Tg2576 mice. IGF-2 expression enhanced memory, promoted dendritic spine formation in wild-type mice, decreased amyloid levels, and rescued behavioral deficits in AD mice, suggesting that IGF-2 may act as an Aβ scavenger.[81]

Increasing evidence demonstrates the role of inflammation in AD. Antiinflammatory 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 resulted in reduced astro/microgliosis, reduced Aβ 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 periplaque neurotoxic effects. Increase in soluble Aβ, exacerbation of synaptic loss, and an increased number of dystrophic neurites around each deposit were observed in AD mice receiving APOE4, whereas a relatively 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(*S*)-hydroxycholesterol (24S-OHC) generated by the neuronal cholesterol 24-hydroxylase enzyme (CYP46A1). Overexpression of CYP46A1 by administering to the brain an AAV vector carrying CYP46A1 in rodent models of AD reduced the number of amyloid plaques and improved spatial memory in amyloid models,[87] and improved cognitive deficits and 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 some 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 subregions 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 means 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 future.[1,89–92]

Parkinson's disease

Many neuroprotective treatments have been developed for PD, which is the second most common neurodegenerative disorder. In addition to deep

brain stimulations and cell therapies, gene therapies have emerged as promising alternatives. For example, it has been shown that 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] Protection of these dopaminergic neurons from Parkinson degenerative processes has also been shown by 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] Nonhuman primates are indeed of interest to validate the clinical usefulness of AAV infusion in brain structures affected by PD such as striatum and putamen,[97] and GDNF overexpression in 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 promoted 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 l-dopa, the main medication for PD, to dopamine and is decreased in patients with PD. AAV-mediated overexpression of AADC showed 50% improvement in L-dopa responsiveness[101] and persistent results for at least 8 years.[2] CERE-120, an AAV2 encoding neurturin (NTN), has been shown to protect dopaminergic neurons in preclinical studies.[102,103] Injection of CERE-120 into the putamen alone or into both the putamen and substantia nigra in more than 100 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 ongoing to evaluate the safety and effectiveness of rAAV-GDNF or rAAV-AADC injection (Table 1). Because of the 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 gene (*HTT*) exceeding approximately 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 ligands (GFLs), that is, glial cell line-derived neurotrophic factor (GDNF), NTN, and ciliary neurotrophic factor (CNTF), have also been shown to support striatal neurons viability *in vitro* and *in vivo*. When administered via rAAV2/1 vector in mice with a quinolinic acid (QA) 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 to rAAV4-BDNF/noggin-treated R6/2 mice resulted in delayed deterioration of motor function and increased survival.[110] rAAV2-GDNF was also administered 2 weeks before a 3-nitropropionic acid (3NP) lesion was induced in rats[111] or in the HD mouse model (N171-82Q mice),[112] resulting in improved performance and reduced neuronal atrophy. 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 with control groups.[113] CNTF was shown to support striatal neuron 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 (rAAV2-CNTF at 2.7×10^{12} vector genomes [VG]/ml, 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 pre-

vent 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, various approaches have been developed using mHTT mRNA (single-nucleotide polymorphism)-specific microRNAs (miRNAs) and small hairpin RNAs (shRNAs) to block the formation of the mutant protein, and most recently, allele-specific blockage of transcription. Allele-specific methods selectively silencing mHTT are the most attractive option for HD gene therapy. However, this approach is challenging, because the mutant gene differs from the wild-type allele only by the number of CAG repeats, making both alleles vulnerable. Importantly, some toxic effects of shRNA *in vivo* were suppressed when placed into artificial 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 in plasma 24S-OHC, the oxysterol produced by brain-specific cholesterol 24-hydroxylase (CYP46A1), follows disease progression proportionally to motor and neuropsychiatric dysfunction and brain atrophy, as indicated by magnetic resonance imaging (MRI), 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 shown to be decreased in the putamen of patients with HD 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 patients with ALS die from respiratory failure 2 to 5 years from the onset of symptoms. Ten to 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] In SOD1-transgenic animals, motor neurons degenerate and the animals die shortly after the onset of symptoms; these animals are often used as human-like ALS models.[122] rAAV encoding IGF-1 was injected into the respiratory and motor limb muscles of $SOD1^{G93A}$ 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 extended survival and a delay in motor decline.[123] Lepore and colleagues used the same strategy but directed the injection into the lumbar spinal cord parenchyma of $SOD1^{G93A}$ mice. The long-term expression of IGF-1 was followed by delayed disease onset and extended survival but only in male $SOD1^{G93A}$ 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 an rAAV injection into the lateral and fourth ventricles of $SOD1^{G93A}$ mice.[126] More recently, ICV or IT injection of AAV vector encoding microRNA against SOD1 improved disease outcome in $SOD1^{G93A}$ 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 that could be brought to the clinic. In addition, IL-10 overexpression, using rAAV injection in the spinal cord of $SOD1^{G93A}$ 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 and colleagues 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 and colleagues 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, and NCT01041222) but none with rAAV. Despite this, positive results obtained in the above-mentioned preclinical studies 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 in SMA transgenic mice. AAV9-mediated gene delivery to replace the SMN protein on day 1 after birth in SMA mice 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 of SMA mice, allowing improvements in behavioral tests indicating a functional neuromuscular junction and increased survival from 15 to 50 days.[132] rAAV9 encoding a codon-optimized version of SMN injected into the facial vein on day 1 after birth resulted in a phenotypic correction, a significant increase in survival,[133] complete correction of motor function, and a major increase in survival from 27 to more than 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 the 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 has already been shown,[136] supporting the use of gene therapy in clinical studies. To date, one clinical trial is actually ongoing to evaluate the 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 characterized mainly 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 researchers understand and investigate epilepsy, leading to 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, based mostly on the overexpression of neuropeptide Y (NPY). NPY, a 36-amino acid member of the pancreatic polypeptide family, is an endogenous modulator of epileptic activity. AAV-mediated gene transfer to overexpress NPY in rats with electroencephalogram (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 and colleagues developed an rAAV expressing a truncated form of NPY that primarily activates the NPY Y2 receptor and obtained significantly prolonged latency of limbic seizures, 1 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 glial 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 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 in excitatory signals or an increase in inhibitory signals. To date, no clinical trials with AAV-mediated gene transfer are currently ongoing, 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 therefore suitable for the use of AAV-mediated gene therapies. A few studies have been carried out with AAV vectors to deliver several gene targets and treat neuropathic pain. In a model of sciatic nerve injury in Wistar rats, AAV vector overexpressing BDNF was injected into the dorsal horn of the spinal cord. One week after injection, 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, 3 weeks after SNI surgery, showed significant attenuation of thermal hyperalgesia in response to 50°C heat stimulation 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 feasibility of AAV-based gene therapy to treat CNS disorders, not only for rare genetic diseases but also potentially 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 the 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. After 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 being developed to address this crucial issue, immunological modulation in patients, detargeting 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 the design of 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 a consequent and challenging question concerning the ability to produce sufficient amounts of vectors for future clinical applications in a large number of patients.[161]

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AUTHOR DISCLOSURE

M.H., S.P., and L.G. are full-time employees of Lysogene. N.C. is a founder and owner of founder equity in BrainVectis Therapeutics. M.A. declares no competing interests.

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