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In vivo gene therapy for Mucopolysaccharidosis type III (Sanfilippo syndrome): A new treatment horizon

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Abstract

For most lysosomal storage diseases (LSDs) there is no cure. Gene therapy is an attractive tool for the treatment of LSDs caused by deficiencies in secretable lysosomal enzymes in which neither full restoration of normal enzymatic activity nor transduction of all cells of the affected organ are necessary. However, some LSD, such as Mucopolysaccharidosis type III (MPSIII) diseases or Sanfilippo syndrome, represent a difficult challenge because patients suffer severe neurodegeneration with mild somatic alterations. The disease's main target is the central nervous system (CNS) and enzymes do not efficiently cross the blood-brain barrier (BBB) even if present at very high concentration in circulation. No specific treatment has been approved for MPSIII. Here we discuss the adeno-associated viral (AAV) vector-mediated gene transfer strategies currently being developed for MPSIII disease. These strategies rely on the local delivery of AAV vectors to the CNS, either through direct intraparenchymal injection at several sites or through delivery to the Cerebrospinal Fluid (CSF), which bathes the whole CNS, or exploit the properties of certain AAV serotypes capable of crossing the BBB upon systemic administration. Although studies in small and large animal models of MPSIII diseases have provided evidence supporting the efficacy and safety of all of these strategies, there are considerable differences between the different routes of administration in terms of procedure-associated risks, vector dose requirements, sensitivity to the effect of circulating neutralizing antibodies that block AAV transduction, and potential toxicity. Ongoing clinical studies should shed light on which gene transfer strategy leads to highest clinical benefits while minimizing risks. The development of all these strategies opens a new

horizon for the treatment of not only MPSIII and other LSD, but also of a wide range of neurological diseases.

INTRODUCTION

MPSIII disease

Mucopolysaccharidoses (MPSs) are a group of rare inherited lysosomal storage diseases (LSDs) caused by specific lysosomal enzyme deficiencies that lead to intracellular accumulation of partially degraded glycosaminoglycans (GAG, formerly called mucopolysaccharides) within the cells[1,2]. Depending on the nature of stored material and the deficient enzyme, MPSs have been classified in different types[3]. MPS type III (MPSIII), also known as Sanfilippo syndrome, is an autosomal recessive disease characterized by intralysosomal accumulation of the GAG heparan sulphate (HS)[1,3,4]. It is caused by deficiency in one of the four enzymes involved in the lysosomal degradation of HS: deficiency in N-Sulfoglucosamine Sulfohydrolase or sulfamidase (SGSH, EC 3.10.1.1) causes type IIIA (OMIM#252900)[5], in α-N-acetylglucosaminidase (NAGLU, EC 3.2.1.50), type IIIB (OMIM#252920)[6], in acetyl CoA α-glucosaminide acetyltransferase (HGSNAT, (EC 2.3.1.78), type IIIC (OMIM#252930)[7] and in N-acetylglucosamine-6-sulfatase (GNS, EC 3.1.6.14), type IIID (OMIM#252940)[8].

MPSIII is one of the most common types of MPS[4]. MPSIIIA is the most frequent subtype of Sanfilippo syndrome in North-West Europe, North America and Australia[9–14], and type B has the highest prevalence in South-East Europe and Brazil[13,15–19]. MPSIIIC and D are much rarer diseases with few cases reported in the literature[4,9,16,20–22].

Glycosaminoglycans, including HS, play an important role in the mechanical support of tissues, and are also involved in regulating cell growth and development, cell-cell interactions, immunity and defense against viral infections,

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coagulation and lipid metabolism[23]. The excess of HS fragments and HS-derived oligosaccharides that accumulate in lysosomes and that are released into the extracellular medium in MPSIII disease could interfere with all of these processes[3,4]. However, the exact cascade of pathological events that leads to cell dysfunction and death is not completely understood. In addition to HS, neurons from Sanfilippo patients accumulate GM2 and GM3 gangliosides and in some cases also unesterified cholesterol[24–30]. This secondary accumulation also appears to play an important role in the neurological pathology characteristic of the disease[31].

Clinical manifestations appear once HS accumulation causes cellular damage, after which symptoms progressively worsen. MPSIII is generally characterized by severe progressive central nervous system (CNS) degeneration. Affected children appear normal at birth, and the first symptoms are detected around 1-4 years, generally under the form of delayed intellectual development[4,20,22,32,33]. Disease progresses to severe neurological manifestations, including hyperactivity, sleeping problems, loss of speech and epilepsy [4,20,22,32,33]. At later stages of the disease, affected individuals develop profound dementia and progressive loss of motor functions, being most patients wheelchair-bound and fully dependent by the second decade of their lives[4,20,22,32–34]. In contrast to the majority of other MPS disorders, children with MPSIII have relatively mild somatic symptoms[4,35]. Facial dysmorphisms, although usually slight, are detected in most individuals[4,20,22,32,36]. Patients frequently present visceromegaly, mainly hepatosplenomegaly, and mild skeletal alterations[20,32,33,36]. Recurrent ear, nose, throat and chest infections are

commonly found in young patients, as well as frequent diarrhea[4,20,32,33,37]. Death usually occurs in the mid-late teenage years[4,22,32,33,36–38].

Treatment of MPSIII disease

Presently, there is no specific treatment approved for MPSIII. Control of the disease is merely symptomatic, aimed at improving the quality of life of patients and their families[3,39]. Several new therapies are being developed to treat Sanfilippo syndrome, and some of them have already reached clinical stage. Most of these therapies are forms of enzyme replacement therapy (ERT), in which the enzyme is provided periodically as recombinant protein, or a classic example of gene augmentation gene therapy, in which enzyme is endogenously synthetized by cells that have received a correct copy of the mutated gene. Underlying all these strategies is the principle of cross-correction, whereby a soluble lysosomal enzyme present in the extracellular compartment can be taken up by endocytosis though binding to mannose-6-phospate receptors present on the plasmatic membrane of cells[40–43]. Therefore, the enzyme present in the extracellular milieu after recombinant protein administration or produced by engineered/corrected cells can cross-correct other cells[41–43].

All these novel therapeutic approaches need, however, to overcome a major challenge in drug development for LSDs with CNS involvement: the existence of the blood-brain barrier (BBB), which limits the efficient access of compounds to the CNS after systemic administration. To bypass the BBB and allow for repeated drug administrations, the use of a drug delivery device permanently implanted in the intrathecal space for delivery of recombinant proteins directly to the cerebrospinal fluid (CSF) has been tested for MPSIIIA

(www.clinicaltrials.gov; NCT01155778, NCT01299727, NCT02060526 and NCT02350816)[44,45] and MPSIIIB (www.clinicaltrials.gov; NCT02754076 and NCT03784287). Although technically feasible, the permanent implantation of intrathecal devices is associated with complications, such as implant site infections or device malfunction[44–46]. Despite these shortcomings, clinical studies of intrathecal ERT with recombinant human sulfamidase for MPSIIIA (www.clinicaltrials.gov; NCT01155778 and NCT02060526) have consistently demonstrated declines in HS levels in the CSF, although clear evidence of clinically meaningful improvements in neurocognitive function is still missing[44,45]. An investigational intrathecal ERT drug for the treatment of MPSIIIB (NCT02754076) consisting in a recombinant chimeric protein in which human NAGLU is fused to a truncated form of human insulin-like growth factor 2 has shown HS reduction in the CSF and stabilization of development quotient (DQ)[47].

IN VIVO GENE THERAPY FOR MPSIII DISEASES

To overcome the need for periodic administration of the therapeutic protein of classic ERT approaches, gene therapies for MPSIII are being developed to achieve constant production of active enzymes available to cross-correct cells. A key advantage of gene therapy strategies is that correction of the genetic defect in all cells of an organ is not required, since few corrected cells could, in principle, produce sufficient amount of active enzyme to become available to neighboring cells or to other cells within the target organ or non-targeted organs in the body if the enzyme reaches main fluids, e.g. CSF and serum[48–58].

Since the CNS is the most affected organ in MPSIII, and the CNS is anatomically isolated from the rest of the body, a sufficient degree of gene

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correction has to be achieved in this organ. CNS-targeted gene therapy is a very active field of research. Recent years have witnessed the publication of numerous pre-clinical and clinical studies that demonstrate the potential of gene therapy for treating neurological conditions, particularly monogenic hereditary diseases[59,60]. Most of the gene therapy strategies that are being developed for MPSIII are based on *in vivo* gene transfer mediated by adeno-associated virus (AAV)-derived vectors. These vectors have shown high transduction efficiencies *in vivo*, as well as excellent safety profiles in clinical studies[61,62]. In addition, preclinical studies in large animal models as well as clinical data have provided strong evidence supporting long-term expression in the CNS mediated by AAV vectors, in the absence of clinically-significant adverse events[48,49,51,63–67].

This overview will focus on *in vivo* gene therapies for MPSIII. Nevertheless, *ex vivo* gene therapies have also been developed for MPSIIIA and MPSIIIB[68–71]. They are based on the autologous transplantation of hematopoietic stem cells genetically modified using lentiviral vectors to express SGSH or NAGLU for the treatment of MPSIIIA or MPSIIIB, respectively[68–70]. The progeny of transplanted, gene-corrected cells traffics to the brain, bypassing the BBB, to become resident cells that produce the therapeutic protein in the CNS. Proof of concept studies demonstrated normalization of brain HS, secondary GM2 storage and neuroinflammation, and improvement of behavioral deficits, in MPSIIIA and MPSIIIB mice[68–70]. A clinical trial is to be initiated soon in MPSIIIA patients (www.orchard-tx.com). Similar *ex vivo* gene therapy approaches have demonstrated clinical efficacy for the treatment of other neurometabolic storage disorders, such as Adreno and Metachromatic Leukodystrophies, as well as other in indications such as MPS type I[72–78]. These studies are discussed in detail in

the Review by Poletti and Biffi included in this Special Issue of Human Gene Therapy (https://doi.org/10.1089/hum.2019.190)[72].

Routes of administration to deliver AAV vectors to the CNS

MPSs are diseases in which the neurodegenerative process affects the whole CNS, although little information is available from human specimens on whether there are structures that are more affected than others[27,79,80]. Several AAV-based strategies for the delivery of genes to extensive areas of the CNS have been described. Initial efforts administered the AAV through multiple direct injections to the brain parenchyma. Other systems followed that exploited the ability of certain AAV serotypes, such as serotype 9 (AAV9), to cross the BBB after intravenous administration, resulting in widespread transduction of the brain as well as of liver and other peripheral organs through a non-invasive procedure. More recently, in an effort to maximize gene transfer to the most affected organ while minimizing vector dose, the direct delivery of AAV vectors to the CSF has been proposed as an alternative for global CNS gene transfer. Additionally, at least in animal models, this approach results in transduction of the peripheral nervous system and the liver, providing for somatic disease correction. Fig. 1, depicts a schematic representation of potential vector and enzyme biodistribution after AAV vector delivery through these different routes of administration[49,51,54,81–85]. Several clinical trials are currently testing the safety and efficacy of these approaches in MPSIII disease and are listed in Table 1.

Direct administration of AAV vectors to the brain parenchyma

Vectors can be delivered directly to the CNS by intraparenchymal injection, and this approach has been used safely in patients to target the putamen in the context of investigational gene therapies for Parkinson disease[65,86–90]. MPSIII, however, affects the whole of the CNS. Given the limited diffusion of AAV from the site of injection[67,91], the applicability of this method to global neurodegenerative diseases has required the development of a rather complex surgical procedure by which small volumes of vector are simultaneously deposited in the brain white matter at different sites[64,92–94]. There is a limit to the number of injections and to the locations at which these injections can be safely performed[67,92,94]. Moreover, intraparenchymal injection fails to transduce deep CNS structures, a fact that has been considered the culprit of the limited efficacy in previous studies in humans using this approach for other neurodegenerative diseases[64]. In the original procedure, a total of six burr holes were drilled laterally from the midline and vector was administered at 2 depths per site[94]. Intraparenchymal administration of AAVs at multiple sites has been tested in the clinic for MPSIIIA (NCT01474343) and MPSIIIB (NCT03300453), using AAVs of serotypes rh10 and 5, respectively[92,93]. In the case of the Phase I/II trial for MPSIIIA, the vector encoded for SGSH as well as for the Sulfatase Modifying factor 1 (SUMF1) protein. SUMF1 post-translationally generates C-alpha-formylglycine (FGly), the catalytic residue in the active site of eukaryotic sulfatases, from a cysteine[95]. The rationale behind SUMF1 supplementation was to avoid potential toxicity derived from competition for SUMF1 sequestration at sites with very high levels of expression of sulfamidase (e.g. the injection sites). Preliminary results of the one-year follow-up of this trial reported moderate

improvements in behavior, attention and sleep disturbances[92]. Nevertheless, these patients have remained under long-term immunosuppression since receiving AAV, and the potentially beneficial effect of immunosuppression on the observed clinical benefit cannot be ruled out because neuroinflammation is a main player in disease pathology[92]. The ongoing long-term follow-up of these MPSIIIA patients (NCT02053064) should provide further information regarding efficacy. Recently, a new open-label, single-arm, multi-center Phase II/III clinical trial has started in MPSIIIA patients (NCT03612869) administered a simplified version of the therapeutic vector which does not include the SUMF1 gene present in the original vector[85]. As a technical improvement to the approach used for the orginal MPSIIIA study, a more recent trial for MPSIIIB was conducted with two additional deposits in the cerebellum, resulting in vector deposition simultaneously in 16 sites (eight burr holes)[93]. Four young MPSIIIB patients have been treated thus far using this method, with concomitant immunosuppression[93]. The procedure was well-tolerated and initial evidence of improved cognitive outcomes is suggestive of efficacy, with the same caveat than for the MPSIIIA trial regarding the use of chronic immunosuppression[93]. Interestingly, a similar intraparenchymal approach using a new AAV vector variant with putative better pharmacokinetic profile, AAV-TT, is being developed for MPSIIIC, although in this case the deficient enzyme is not secretable and cross-correction cannot occur[96]. Treatment with AAV-TT-HGSNAT was able to correct the neurological phenotype of a mouse model of MPSIIIC[96]. A clinical trial has been planned to test the approach in MPSIIIC patients (www.phoenixnestbiotech.com).

Intravascular delivery of AAV vectors to treat MPSIII

Of the several ways of delivering genes to the CNS, the simplest approach exploits the ability of certain AAV serotypes, such as AAV9, to cross BBB and reach the CNS when delivered intravenously (IV)[97,98]. Systemic delivery of AAV9 has proven successful in the treatment of Spinal Muscular Atrophy type 1 (SMA1). SMA1 is caused by a mutation in the survival motor neuron 1 (*SMN1*) gene that leads to severe degeneration and loss of lower motor neurons, resulting in muscle atrophy[99]. Death occurs during infancy (before 2 years of age)[100]. Intravascular AAV9-*SMN1* gene therapy delivered the *SMN1* gene to motor neurons of SMA1 patients, was safe and resulted in longer survival, superior achievement of motor milestones and better motor function than in historical cohorts[99]. The highly positive results obtained in these clinical studies (NCT02122952 and NCT03955679)[99] have led to the recent marketing approval by the Food and Drug Administration (FDA) of the new gene therapy product Zolgensma (www.zolgensma.com).

Treatment of MPSIII disease requires targeting of the whole CNS, and this may be more difficult to achieve after systemic delivery of AAV9 vectors than targeting of motor neurons, as in the case of SMA. Proof of concept studies in mouse models of MPSIIIA and MPSIIIB following IV delivery of AAV9 vectors encoding for the deficient enzymes have shown reduction in brain GAG accumulation and neuroinflammation, as well as correction of behavioral deficits[82,84,101,102]. Based on the demonstrated efficacy in rodent models, clinical studies are presently underway for MPSIIIA and MPSIIIB. In an open-label, dose-escalation phase I/II gene transfer trial, MPSIIIA patients were intravenously administered self-complementary AAV9 vectors carrying the hSGSH gene under

the control of the ubiquitous U1a promoter (scAAV9.U1A.hSGSH) (NCT02716246). All subjects received immunosuppression, consisting in a tapering course of prophylactic enteral prednisone or prednisolone. It has been shown in several clinical trials that systemic exposure to high doses of AAV vectors may trigger the activation of CD8+ T cell responses directed against the viral capsid in a dose-dependent manner[103–106]. Short-term (few weeks) steroid treatment is used to prevent the immune-mediated elimination of vector-transduced cells, resulting in long-term expression of therapeutic genes[52,103]. No product-related serious adverse events have been reported to date in this MPSIIIA trial. Efficacy data have not been published yet, but some interim data have been made available by the sponsor Abeona Therapeutics. A dose-dependent and sustained reduction in the levels of heparan sulfate in the CSF has been reported for all three cohorts of patients treated with low ($5x10^{12}$ vg/kg), medium ($1x10^{13}$ vg/kg) or high ($3x10^{13}$ vg/kg) vector doses. Moreover, the three youngest patients enrolled in the high dose cohort (cohort 3) –14-26 months of age at dosing – continued to track within normal age equivalent development 12-18 months post treatment (www.investors.abeonatherapeutics.com/press-releases/detail/159/abeona-therapeutics-announces-positive-interim-data-from). Based on these encouraging results, the same sponsor has initiated a new trial using the IV AAV9 approach for the treatment of MPSIIIB (NCT03315182). This is an open-label, dose-escalation, Phase I/II trial in MPSIIIB patients aged 6 months to 2 years, or older than 2 years with a minimum cognitive development quotient of 60 or above. In this case, the AAV9 vector carried the h*NAGLU* gene under control of the strong viral Cytomegalovirus promoter (AAV9.CMV.hNAGLU). All dosed subjects have received immunosuppression,

consisting in oral prednisolone from the day prior to gene transfer until at least 60 days after[107]. Two doses of AAV vectors are being tested, $2x10^{13}$ vg/kg (cohort 1) and $5x10^{13}$ vg/kg (cohort 2), which are higher than those administered in cohort 1 and 2 of the MPSIIIA trial. Dosing of cohort 1 has been completed and the first patient in cohort 2 has received the vector (www.investors.abeonatherapeutics.com/press-releases/detail/155/abeona-therapeutics-treats-first-patient-in-second-cohort).

Although clinical evidence supports that systemic administration of AAV vectors results in therapeutic benefit for MPSIII patients, this route of administration may not be of choice to reach the CNS in the subset of patients that are seropositive for anti-AAV antibodies, which can greatly limit the efficacy of *in vivo* gene transfer upon systemic administration[104,108,109]. Serum neutralizing antibodies (NAbs) to AAV are highly prevalent in humans[110]. The prevalence and magnitude of seropositivity, however, varies with the AAV serotype. While about 60% of the adult population has anti-AAV2 NAbs at high titers, only about 30% of healthy individuals have detectable anti-AAV9 antibodies[110]. Moreover, there is a pattern of seroconversion with age since children under 1 year are seronegative, or seropositive at very low titers, but then titers progressively increase up to around 5–6 years of age, reflecting an increase in socialization, which favors natural infection by wild-type AAVs[111,112]. The same tendency was observed in healthy and MPSIIIA and MPSIIIB-affected children. Serum NAb titers against AAV2 were generally higher than those against AAV9, and they tended to increase with age[51,101].

Aside from the limitations imposed by anti-AAV pre-existing immunity and the potential risk of cytotoxic CD8+ T cell responses, the consistently high doses

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required for brain transduction and efficacy following IV administration of AAV vectors[38,82,84,113,114] may suppose a manufacturing challenge from the technical and economic points of view, although great efforts are being devoted to scaling up and improving the yield of AAV manufacturing by different platforms.

Intra-CSF administration of AAV vectors to transduce the CNS

As an alternative to local delivery of vectors to the CNS, several groups begun to administer AAV vectors -particularly AAV9- directly to the CSF and achieved efficient CNS transduction in different animal species (mice, rats, cats, dogs, pigs and non-human primates (NHP)) at relatively lower vector doses, when compared to those used when the vector is injected systemically[49,51,53,54,56,115–119]. Upon administration to the CSF, the vector is diluted in a fluid that circulates through CNS compartments, bathing the whole encephalon and spinal cord, allowing for widespread vector distribution, including deep structures[51,119]. As a result, the pattern of vector transduction differs considerably from that obtained following intraparenchymal injection at multiple sites, which results in uneven distribution of vector genomes, with high copy numbers at the point of injection quickly decreasing with distance[83,91,120]. When the vectors carry genes encoding for secretable proteins, such as in MPSIIIA and IIIB, transduced cells secrete these proteins to the CSF at relatively high levels[49,51], which could further increase efficacy by distribution of the therapeutic protein throughout the whole CNS. Furthermore, at least in animal models, upon intra-CSF AAV9 delivery a portion of the vector escapes to the circulation and transduces the liver, which can secrete the therapeutic protein to the bloodstream[49,51,53,54,56,116]. Whole-body correction of lysosomal storage disease

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was observed following delivery of AAV9 vectors to the CSF of several MPS mouse models[49,51,53,54,56]. In all cases, administration of the therapeutic vector mediated clearance of accumulated GAGs, resolution of lysosomal pathology and neuroinflammation in the brain, correction of behavioral deficits and significant extension of lifespan.

Proof-of concept studies in dogs and pigs have shown that the delivery of vectors to the CSF is scalable to larger brains[49,51,119]. In most of these studies vectors were administered to the cisterna magna, a large subarachnoid space between the caudal part of the cerebellum and the medulla oblongata, filled with CSF produced in the fourth ventricle that will distribute the vectors to the whole CNS[121]. Intracisternal injection, however, is not a common route of administration to the CSF in clinical pediatric practice, because of the relatively smaller size of the cisterna magna in humans compared with animals and its proximity to vital centers, which adds risk to the procedure in case of accidental puncture[121]. An alternative route for CSF delivery is lumbar puncture. Although this is a common clinical procedure, limited distribution of products in widespread CNS areas is achieved through this route[122,123]. Pre-clinical data in pigs show that AAV9-derived gene expression following local lumbar intrathecal administration remains restricted to areas of injection, and that widespread spinal cord transduction requires several administrations of vector at the cervical, thoracic and lumbar regions, suggesting that vector penetrated mostly at the vicinity of the catheter tip used for delivery[122]. Global CNS gene delivery following intrathecal AAV administration was reported in cynomolgus macaques, a NHP of small size (~3-7 kg), using AAV vectors diluted in a hyperosmotic buffer[116]. Although this study

provides evidence that the approach is potentially feasible, detailed confirmatory studies in larger animal models are needed.

Ventriculostomy is a standard surgical procedure to deliver drugs (oncology drugs, antibiotics, antifungal agents, etc) to the CSF for the treatment of different diseases, or for the management of hydrocephalus[123,124]. Although intracerebroventricular (ICV) access does require unilateral trepanation of the skull, the trajectory to reach the ventricle is well defined and goes through “mute” areas of the brain[123]. Using a surgical procedure that has been in place for many years has the advantage of providing a solid safety record, as well as a well-characterized list of potential complications and protocols for their management. It also simplifies clinical translation, as the technique is known to pediatric neurosurgeons worldwide and would not require specific training. Compared with intraparenchymal administration, another advantage of ICV administration is that by delivering the vector to the CSF fluid a relatively large volume of vector can be supplied within a brief period of time, thus shortening the duration of the surgery and providing flexibility in terms of vector concentration and formulation. In dogs, ICV administration demonstrated to be highly efficacious in delivering AAV vectors to the whole encephalon and spinal cord [51]. An open-labelled, dose escalation, Phase I/II clinical trial was initiated recently in MPSIIIA patients older than 2 years, which uses the ICV procedure to deliver AAV9 vectors carrying the human SGSH gene (AAV9-CAG-cohSGSH) into the CSF (EU Clinical Trials Register 2015-000359-26). This is a safety, tolerability and initial efficacy clinical trial.

Although it has been suggested that the BBB is disrupted in patients affected by LSD [41], asymmetrical distribution of anti-AAV NAbs across the BBB

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of patients suffering from Sanfilippo Syndrome is preserved even in severely affected children [51]. The evidence showing that very low titers of NAbs are sufficient to completely block transduction upon systemic administration [104,108,109] prompted several labs, including ours, to evaluate CNS transduction after intra-CSF delivery in large animals with anti-AAV pre-existing immunity. In NHPs with serum AAV9 NAb titers of 1:128 successful CNS gene transfer was observed after intra-CSF administration of vectors[116]. Similarly, in healthy dogs pre-immunized by IV administration of non-coding AAV9 vectors leading 1 month later to high anti-AAV9 NAb serum titers (1:100-1:1000), intra-CSF delivery of AAV9 vectors encoding for GFP resulted in efficient transduction of the brain and spinal cord, at levels comparable to those achieved in animals naïve to AAV9[51]. These findings were further confirmed following intra-CSF administration of a therapeutic transgene (canine*Naglu*) to seropositive dogs with high AAV9 NAb titers in plasma (1:100-1:1000)[49]. Again, similarly high levels of NAGLU activity were detected in the CSF of both naïve and seropositive animals upon intra-CSF AAV-mediated gene transfer[49]. These studies suggest that CNS efficacy would not be compromised in seropositive patients when vectors are delivered to the CSF. As expected, transduction of the liver is completely abolished following vector delivery to the CSF of animals with circulating NAbs[51]. Given that the main target organ for MPSIII is the CNS, the lack of peripheral efficacy would still allow for a significant degree of disease correction. Further clinical studies should establish the safety of gene transfer to the CSF of seropositive individuals.

Concluding remarks

Several AAV vector-mediated *in vivo* gene therapy approaches for the treatment of MPSIII have been developed in recent years, some of which are already under clinical investigation. Different therapeutic strategies use different routes of vector administration to reach the CNS, the most affected organ. Studies in small and large animal models of MPSIII diseases have provided evidence supporting the efficacy and safety of these strategies, and are expected to be predictive of possible efficacy in humans. Results from the ongoing clinical trials should confirm or not these expectations. Although direct comparisons of the clinical outcomes of these studies cannot be made, due in part to differences in inclusion criteria, protocol design, or vector manufacturing, the ongoing studies should shed light on which gene transfer strategy maximizes efficacy in the CNS while minimizing delivery-associated risks, leading to higher clinical benefits. Of importance, clinical trials in which the vector is delivered directly to the CNS (IP or intra-CSF) should inform on the potential to achieve amelioration of somatic disease in humans, a fact that would become more relevant as patients live longer due to improved neurological outcome. Finally, these studies should provide information on the tolerability, safety, and efficacy of AAV-mediated CNS gene transfer not only for the treatment of MPSIII but also of other neurodegenerative diseases.

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FB, VH and SM are co-inventors on patent applications for the use of AAV vectors for the treatment of MPS III.

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FIGURE LEGEND

Figure 1. Possible routes of vector administration and subsequent enzyme biodistribution *in vivo* AAV-mediated gene therapy strategies for Sanfilippo syndrome. Based on results obtained in proof-of-concept studies in small and large animal models of MPS III[49,51,53,54,56,82–84,91,96,97,99,102,117–120,125–127], a schematic representation of the possible biodistribution of the gene therapy product in patients is presented. (**A**) The intraparenchymal route of administration consists in multiple direct injections into the brain parenchyma through 6 or 8 burr holes (3/4 per hemisphere) at two different depths, per site of injection. This route of administration has limited AAV diffusion in the CNS with very high local concentrations of vector genomes at the site of injection that progressively decrease with distance. Cells around the sites of injection can be corrected by locally expressed enzyme but release of the expressed protein into the CSF is low. Vector transduction of peripheral organs, e.g. the liver, is very low. (**B**) Systemic administration via the intravenous route is a non-invasive technique to efficiently transduce somatic organs, especially the liver, depending on the serotype of AAV used. Among them, AAV9 has demonstrated the ability to cross the blood brain barrier and transduce the CNS. Administration of very high vector doses is required since injection of lower doses results in fractional delivery of vector to the CNS, insufficient to support therapeutically meaningful levels of enzyme production in the brain. CNS efficacy is expected to be abolished by the presence of pre-existing immunity against the AAV, as serum neutralizing antibodies (NAb) would block the entrance of the vector to the CNS. (**C**) The delivery of AAV9 vectors to the cerebrospinal fluid (CSF) through unilateral administration to the lateral ventricle or via the cisterna magna, leads to

widespread and even distribution of AAV vectors throughout the whole brain and spinal cord, as the CNS is bathed by the CSF. Moreover, some of the AAV9 vector reaches the circulation, leading to the transduction of the liver. As a result of this profile of vector distribution, enzyme activity increases throughout the CNS, in the CSF and in serum, being the liver the most important source of circulating enzyme. Moreover, studies in seropositive animal models suggest that CNS efficacy would not be compromised by the presence of anti-AAV9 NAbs in serum.

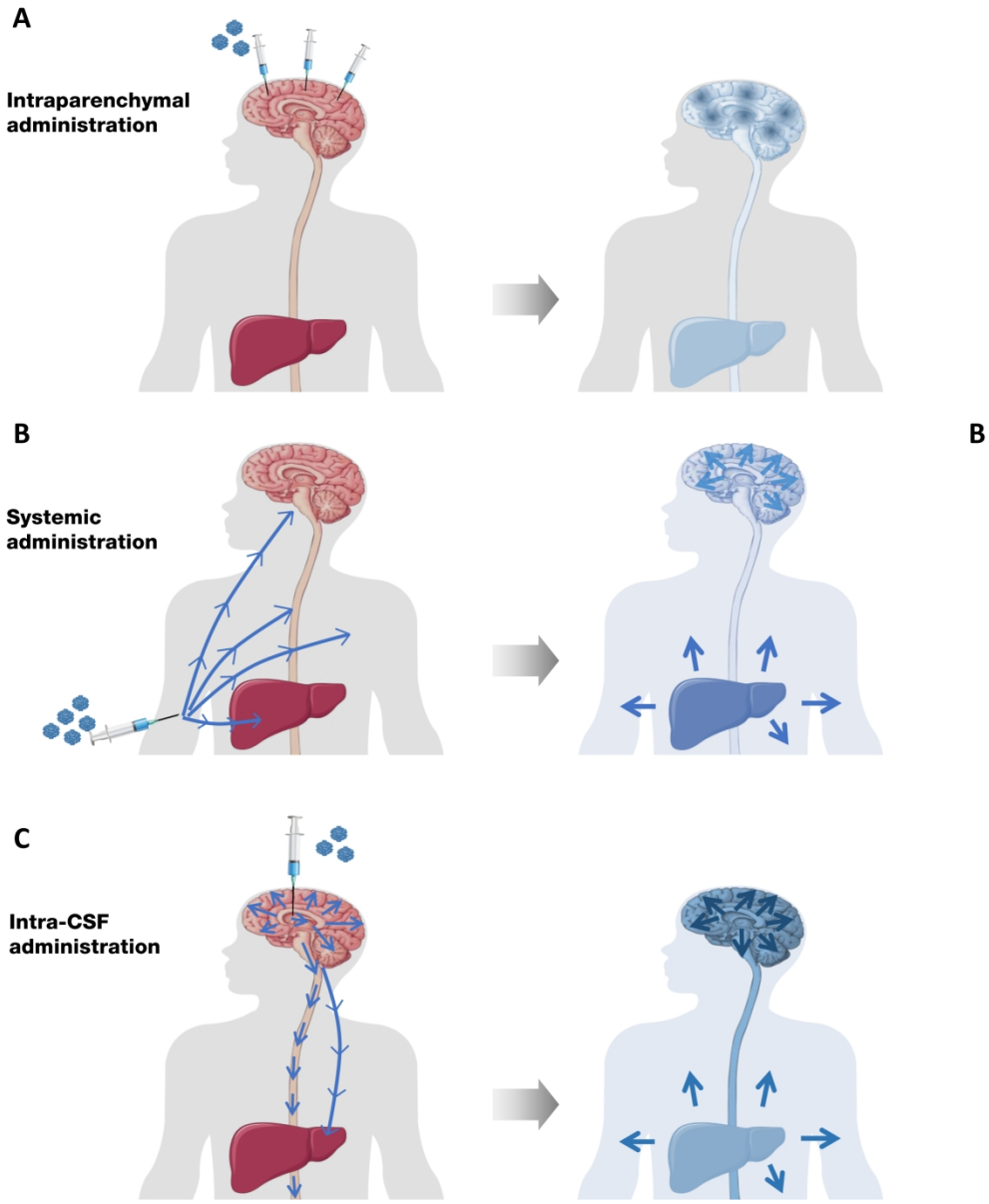

Figure 1. Possible routes of vector administration and subsequent enzyme biodistribution in vivo AAV-mediated gene therapy strategies for Sanfilippo syndrome. Based on results obtained in proof-of-concept studies in small and large animal models of MPS III[49,51,53,54,56,82–84,89,94,95,97,100,115–118,123–125], a schematic representation of the possible biodistribution of the gene therapy product in patients is presented. (**A**) The intraparenchymal route of administration consists in multiple direct injections into the brain parenchyma through 6 or 8 burr holes (3/4 per hemisphere) at two different depths, per site of injection. This route of administration has limited AAV diffusion in the CNS with very high local concentrations of vector genomes at the site of injection that progressively decrease with distance. Cells around the sites of injection can be corrected by locally expressed enzyme but release of the expressed protein into the CSF is low. Vector transduction of peripheral organs, e.g. the liver, is very low. (**B**) Systemic administration via the intravenous route is a non-invasive technique to efficiently transduce somatic organs, especially the liver, depending on the serotype of AAV used. Among them, AAV9 has demonstrated the ability to cross the blood brain barrier and transduce the CNS. Administration of very high vector doses is

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Table 1. ***In vivo*** **gene therapy clinical trials for Sanfilippo patients.**

Disease	Study phase	Route of administration	Type of vector	Outcome	Sponsor	Clinical Trial Identifier	Publications
MPSIIIA	Phase I/II (Completed)	Intraparenchymal	AAVrh10-hSGSH-SUMF1	- Safe and well tolerated - Moderate behavioural improvement	Lysogene	NCT01474343[$] NCT02053064[$] (LT)	Tardieu, 2014
	Phase II/III (Recruiting)	Intraparenchymal	AAVrh10-hSGSH	*NA*	Lysogene	NCT03612869[$] 2018-000195-15[#]	-
	Phase I/II (Recruiting)	Intravenous	scAAV9-hSGSH	*NA*	Abeona Therapeutics	NCT02716246[$] 2015-003904-21[#]	-
	Phase I/II (Active)	Intra-CSF	AAV9-hSGSH	*NA*	Esteve	2015-000359-26[#]	-
MPSIIIB	Phase I/II (Active)	Intraparenchymal	AAV5-hNAGLU	- Safe and well tolerated - Sustained enzyme production in the CSF - Improved neurocognitive progression	UniQure Biopharma B.V./Institut Pasteur	NCT03300453[$] 2012-000856-33[#]	Tardieu, 2017
	Phase I/II (Recruiting)	Intravenous	AAV9-hNAGLU	*NA*	Abeona Therapeutics	NCT03315182[$] 2014-001411-39[#]	-
MPSIIIC	Phase I/II (In preparation)[1]	Intraparenchymal	AAV-TT-hHGSNAT*	*NA*	Phoenix Nest	Not yet registered	-

* Information based on Tordo, 2018; and [1]http://www.phoenixnestbiotech.com/
* Information based on
[$] Clinical trial registered in www.clinicaltrials.gov
[#] Clinical trial registered in www.clinicaltrialsregister.eu
LT= Long-term follow-up
NA= non-available