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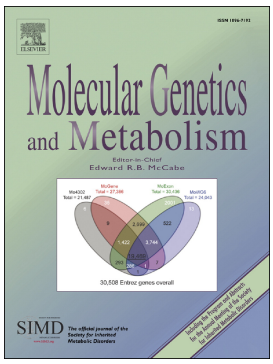

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Treatment of brain disease in the mucopolysaccharidoses

Maurizio Scarpa[a,*], Paul J. Orchard[b], Angela Schulz[c], Patricia I. Dickson[d], Mark E. Haskins[e], Maria L. Escolar[f], Roberto Giugliani[g]

[a]Department of Paediatric and Adolescent Medicine, Helios Dr. Horst Schmidt Kliniken, Center for Rare Diseases, Wiesbaden, Germany and Department for Womand and Child Health, University of Padova, Padova, Italy;
[b]Department of Pediatrics, Division of Pediatric Blood and Marrow Transplantation, University of Minnesota, Minneapolis, MN, USA;
[c]Department of Pediatrics, Children's Hospital, University Medical Center Hamburg-Eppendorf, Hamburg, Germany;
[d]Department of Pediatrics, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA;
[e]Departments of Pathobiology and Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA;
[f]Department of Pediatrics, Program for Neurodevelopment in Rare Disorders, Children's Hospital of Pittsburgh, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA;
[g]Department of Genetics, UFRGS & Medical Genetics Service, HCPA, and INAGEMP, Porto Alegre, RS, Brazil.

* Corresponding author:
Maurizio Scarpa
Department of Paediatric and Adolescent Medicine
Helios Dr. Horst Schmidt Kliniken, Center for Rare Diseases
Ludwig-Erhard-Strasse 100
65199 Wiesbaden, Germany
Tel: +49 611432314
Fax: +49 611433197
Email: Maurizio.Scarpa@helios-kliniken.de

AAV: adeno-associated viruses, AE: adverse event, BBB: blood-brain barrier, CLN2: ceroid lipofuscinosis neuronal type 2, CNS: central nervous system, CSF: cerebrospinal fluid, DQ: developmental quotient, ERT: enzyme replacement therapy, g7: 7-aminoacid glycopeptide, GAG: glycosaminoglycans, HSCT: hematopoietic stem cell transplantation, ICV: intracerebroventricular, IDDD: IT drug delivery device, IQ: intelligence quotient, IT: intrathecal, LAMP-1: lysosomal-associated membrane-1, MPS: mucopolysaccharidosis, MRI: magnetic resonance imaging, SCC: spinal cord compression, SGSH: N-sulfoglucosamine sulfohydrolase, SRT: substrate reduction therapy, SUMF: sulfatase modifying factor

Abstract

The mucopolysaccharidosis (MPS) disorders are a group of lysosomal storage diseases caused by lysosomal enzyme deficits that lead to glycosaminoglycan accumulation, affecting various tissues throughout the body based on the specific enzyme deficiency. These disorders are characterized by their progressive nature and a variety of somatic manifestations and neurological symptoms. There are established treatments for some MPS disorders, but these mostly alleviate somatic and non-neurological symptoms and do not cure the disease. Patients with MPS I, II, III, and VII can present with neurological manifestations such as neurocognitive decline and behavioral problems. Treatment of these neurological manifestations remains challenging due to the blood-brain barrier (BBB) that limits delivery of therapeutic agents to the central nervous system (CNS). New therapies that circumvent this barrier and target brain disease in MPS are currently under development. They primarily focus on facilitating penetration of drugs through the BBB, delivery of recombinant enzyme to the brain by gene therapy, or direct CNS administration. This review summarizes existing and potential future treatment approaches that target brain disease in MPS. The information in this review is based on current literature and presentations and discussions during a closed meeting by an international group of experts with extensive experience in managing and treating MPS.

Keywords: mucopolysaccharidoses; enzyme replacement therapy; gene therapy; transplantation; blood-brain barrier

1. Introduction

Lysosomal enzyme deficiency in the different mucopolysaccharidosis (MPS) disorders leads to progressive glycosaminoglycan (GAG) accumulation and a variety of somatic and neurological manifestations. Despite existing treatments for some MPS disorders, it remains challenging to effectively treat neurocognitive deterioration and behavioral problems in patients with MPS I, II, III, and VII [1]. Central nervous system (CNS) damage tends to be irreversible, and the selective permeability of the blood-brain barrier (BBB) limits the extent to which systemic treatments can penetrate the CNS and prevent neurodegeneration. Moreover, treatment outcomes for brain disease are difficult to measure, as neuroimaging will provide mainly structural information [2] and there are limited biomarkers reflecting CNS disease, and their correlation to clinical outcomes is difficult to establish.

The BBB acts as a protective barrier to preserve CNS homeostasis and keep out neurotoxic substances. The main cellular elements of this barrier are endothelial cells, connected through tight junctions, pericytes, and perivascular glial processes, all surrounded by the basal lamina. Molecules can cross the BBB by either passive transport, if they are small enough or are lipid soluble. Alternatively, they may cross through active uptake by receptors, transporters, or carriers that are expressed by the cells of the BBB [3, 4]. Therapeutic approaches that target the CNS can use these transport systems to cross the BBB. Other possible techniques to circumvent the BBB are gene therapy, through various approaches, including but not limited to a) *in vivo* administration and expression of the gene into the CNS, b) *ex vivo* expression of the gene product in hematopoietic cells that subsequently localize to the CNS, or c) overexpression of the gene product in non-CNS tissues leading to limited penetration of the BBB.

Therapeutic agents may also be delivered by direct administration of the treatment in one of the fluid-filled compartments of the CNS (the subarachnoid space, the central canal of the spinal cord, or the ventricles in the brain). Several new therapies for brain disease in MPS disorders are currently being tested in animal models and some promising approaches are already being studied in phase 1/2 clinical trials in patients with MPS.

This review discusses existing and potential future approaches to treat CNS manifestations of MPS, and summarizes outcomes of studies with these treatments in MPS animal models and patients. The content is based on presentations and discussions at an expert meeting on the brain in MPS on April 28-30, 2016 in Stockholm, Sweden, which was attended by an international group of 39 MPS experts. Additional relevant literature was obtained from PubMed searches using search terms listed in Table 1. Searches were performed without date restriction. Publications not available in English were excluded. Additional publications were identified from reference lists within the most relevant MPS-related papers focusing on CNS drug delivery. The literature search was completed in March 2017.

2. Hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HSCT) has been extensively studied as a treatment approach for MPS disorders over the past decades. This procedure involves delivery of donor stem cells that produce the deficient enzyme. Donor cells of the monocyte/macrophage-lineage can cross the BBB and infiltrate the CNS [5], where they can produce the enzyme and influence MPS-related neurological manifestations.

Successful HSCT can improve, but not normalize, some somatic signs and symptoms and prolong survival in MPS patients [6-9]. However, neurological outcomes of studies vary widely.

Several studies have provided evidence for a beneficial effect of HSCT in patients with MPS I Hurler [6, 8, 10], including prevention of intellectual decline [11-13] and CNS damage [14, 15], and reduction or stabilization of hydrocephalus [7, 16, 17] and spinal cord compression [17]. In some studies, development declined immediately post-transplantation but appeared to stabilize after approximately 1 year [8, 14].

The transplant-related morbidity and mortality, as well as neurofunctional outcomes and survival, have improved over the past years [6, 11]. Of the various stem cell sources that have been used (bone marrow, peripheral, and umbilical cord blood of related and unrelated donors) [17-19], there have been reports that umbilical cord blood provides advantages as a graft source [11, 19]. In addition, transplantation at a young age (<2 years) [19, 20], in patients with minimal cognitive impairment (intelligence quotient [IQ] >70) (Figure 1), and omission of total body irradiation from the conditioning regimen resulted in better developmental outcomes [10-12, 17, 21, 22]. Due to its positive effect on clinical manifestations and the ability to act directly on the CNS, HSCT is currently considered the treatment of choice for MPS I Hurler patients younger than 2.5 years (Figure 2) [23]. Several centers combine HSCT with enzyme replacement therapy (ERT) as this improves the patient's condition before transplant [24-27] and there is some evidence that this may enhance the beneficial effect of HSCT [27-29]. This beneficial effect has been suggested to result from increased permeability of the BBB due to the transplant regimen and/or the high dose of ERT in the blood [28]. Therefore, a European modified Delphi expert panel recommended to also start ERT at diagnosis in young MPS I Hurler patients and to perform HSCT as soon as possible thereafter (Figure 2) [23]. As MPS I Hurler patients will increasingly be identified at birth through newborn screening, the optimal preparative regimen and timing of transplantation, and the effect of ERT in the peri-transplant period will prove an important area of study. It is recognized that the toxicity of transplant regimens in very young patients with

metabolic disorders can be significant, and therefore needs to be considered in devising strategies to achieve the optimal outcomes [30].

Experience of HSCT in other MPS disorders associated with neurological decline, i.e. II, III, and VII, is limited. Overall, HSCT appears to have less impact on neurological deterioration in these disorders [6, 10, 31-37]. However, the available information is limited, and in most studies transplantation was performed after the onset of neurological manifestations (>2 years), which hampers assessment of outcomes [6]. There are a few reports of a modest impact of HSCT on neurological disease in MPS II and III when patients were treated before the age of 2 years [31] or in the early stage of the disease [38]. More information on this is clearly required.

Figure 1. Developmental trajectory of cognitive skills in mucopolysaccharisosi (MPS) I Hurler patients post-hematopoeitic stem cell therapy (HSCT) subdivided by cognitive status pre-HSCT (median developmental quotient [DQ]/IQ = 85) and age at HSCT (median age = 16 months). (A) Patients with normal or mild cognitive impairment at baseline (DQ/IQ ≥85) had a better cognitive development post-HSCT than those with moderate or severe cognitive impairment at baseline (DQ/IQ <85). (B) Patients aged ≥16 months at HSCT had inferior neurodevelopmental outcome post-HSCT than patients <16 months. The continuous and dashed black lines in the shaded area represent reference curves (+2 SD, 0 SD, and -2 SD). Reproduced from Aldenhoven et al. 2015 [17] with permission from the Amercian Society Of Hematology.

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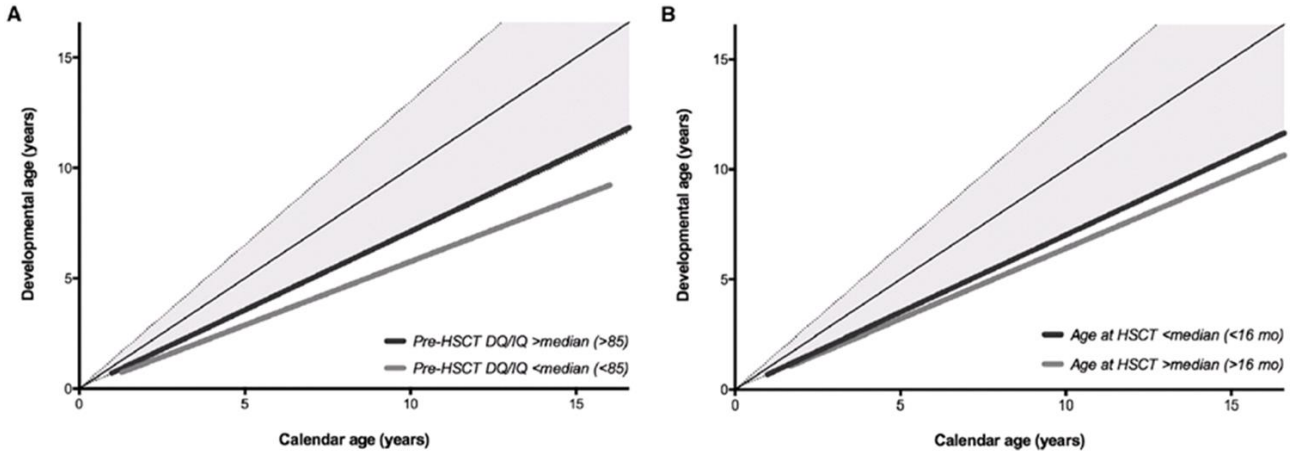

Figure 2. Treatment algorithm for diagnosed mucopolysaccharidosis (MPS) I patients (Reproduced from de Ru et al. 2011 [23] with permission from BioMed Central). *hematopoietic stem cell therapy (HSCT) might be considered under special circumstances. **In patients with presumed MPS I Hurler.

ERT: enzyme replacement therapy

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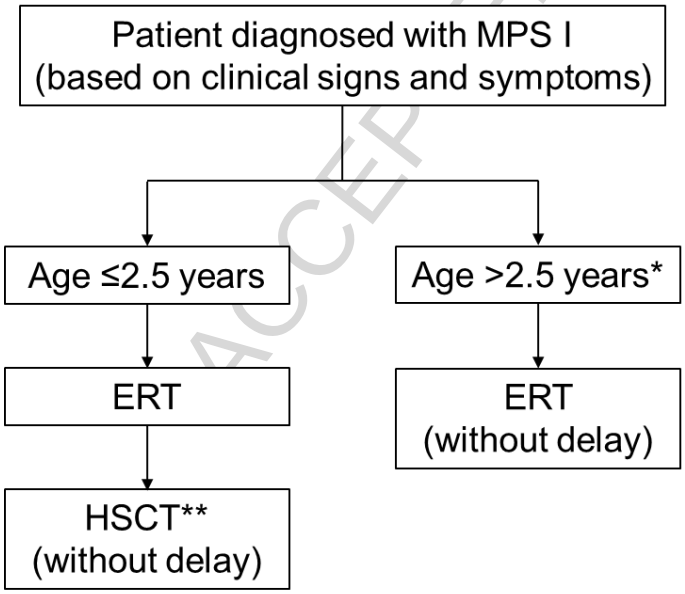

3. Direct delivery of enzyme replacement therapy in the cerebrospinal fluid

Currently, ERT is considered an effective treatment for non-neurological manifestations of MPS I, II, IVA, and VI [39]. The concept involves systemic delivery of exogenously produced enzyme, which is internalized by cells through the mannose(-6-phosphate) receptor pathway [40-42]. However, intravenously delivered enzyme has not been shown to cross the BBB in an adequate amount to prevent progression of neurological manifestations [4]. A possible way to circumvent the BBB is direct delivery of the enzyme in the cerebrospinal fluid (CSF) through either intracerebroventricular (ICV) injection into the lateral ventricle (via a catheter/reservoir), or intrathecal (IT) injection into the lumbar spine or subarachnoid space at the cisterna magna (via lumbar puncture or an IT drug delivery device [IDDD]).

Lumbar IT is already applied clinically, for example for analgesics, and is less invasive than intraparenchymal injection as this requires piercing of the skull to deliver the treatment directly in the CNS.

Studies in small (mice and rats) and large (cats, dogs, and monkeys) MPS animal models suggest that IT and ICV-administered recombinant enzyme is safe, distributes within the brain parenchyma, throughout the neuraxis (including the spinal cord) and deeper brain structures [43-49], and is taken up by neuronal and glial cells [43, 44, 50, 51]. In addition, it appears to reduce GAG levels in the CSF [52] and storage material in the brain (Figure 3) [44, 48-50, 53-60], diminish inflammation [45], reduce neurological damage (i.e. ventricular enlargement and cortical atrophy) as assessed by magnetic resonance imaging [58], and normalize behavior [51, 53]. These results triggered clinical studies with IT ERT in patients with MPS.

Figure 3.

Impact of three intrathecal (IT)-lumbar injections of iduronate-2-sulfatase in MPS II mice [44].

A. **Hematoxylin and eosin-staining of brain tissues of IT-uninjected and injected mice: the number of cellular storage vacuoles (arrows) were markedly reduced in injected mice in the cerebral cortex (a,b), caudate nucleus (c,d), thalamus (e,f), white matter (g,h), and cerebellum (i,j).**
B. **Immunohistochemical staining of lysosomal-associated membrane-1 (LAMP-1): marked reduction of LAMP-1 immunoreactivity in IT-injected mice in the number of LAMP-1 positive cells and lighter staining intensity in the cerebral cortex (a,b), caudate nucleus (c,d), thalamus (e,f), white matter (g,h), and cerebellum (i,j).**

Scale bar: 25 mm. Reproduced from Calias et al. 2012 [44] with permission from the Public Library of Science.

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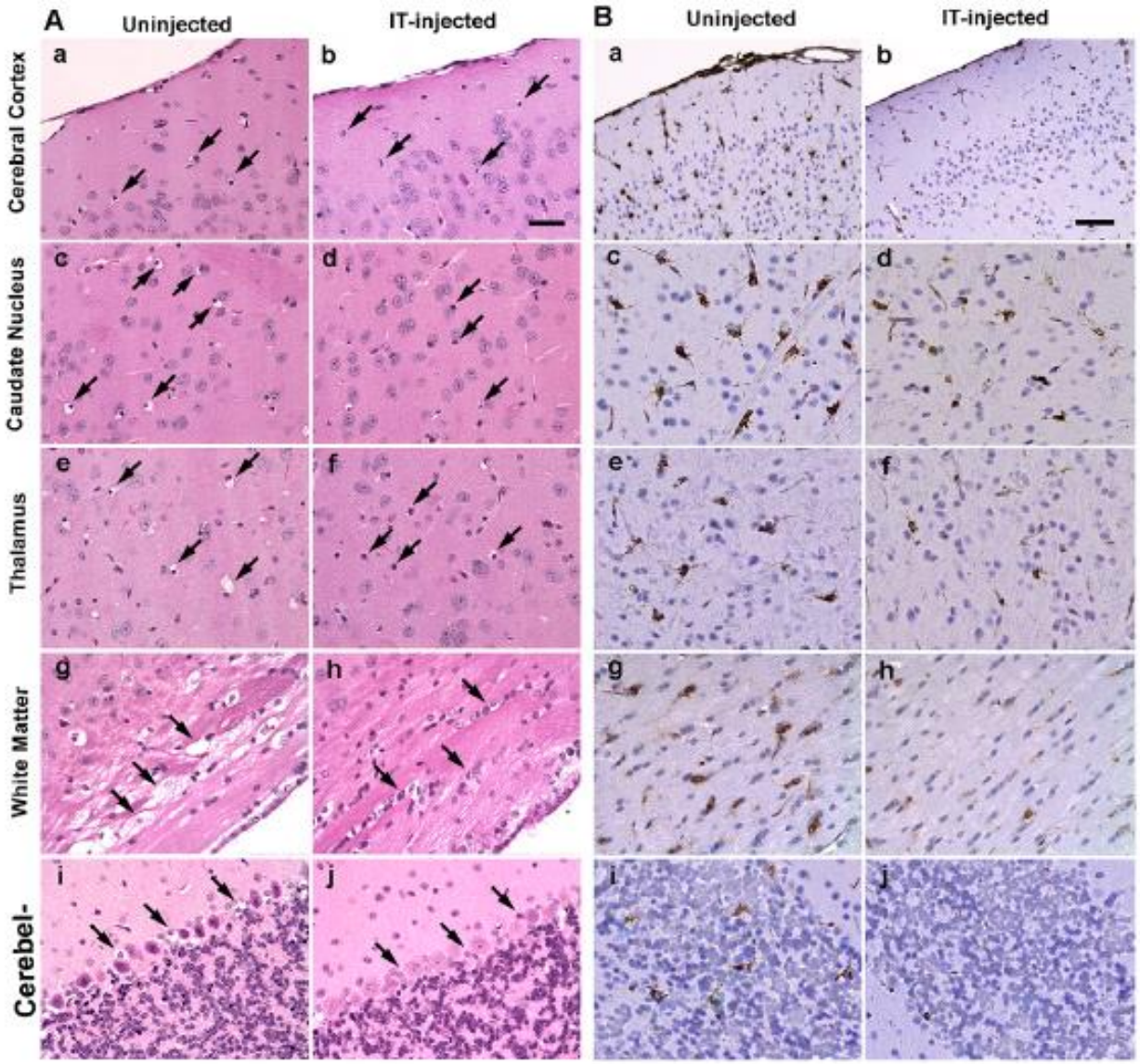

Munoz-Rojas et al. were the first to report (in 2008) on IT ERT in an MPS I Scheie patient and an MPS VI patient, both with spinal cord compression (SCC) and refusing surgery. In both cases, IT infusions were considered safe and some improvement in symptoms of SCC was observed [61, 62]. Recently, results of three phase 1/2 studies investigating the safety (as primary objective) and efficacy of IT ERT in patients with MPS I, II, and III were published (Table 2). Overall, IT delivery of ERT was safe and well tolerated [63-65]. Two studies used an IDDD and reported

many adverse events related to device malfunctioning (breakage or migration of the catheter) [64, 65]. These problems might have been caused by the restlessness and hyperactive behavior often observed in MPS patients with neurological involvement ([1] and personal communication Dr. J. Muenzer). Therefore, IDDD devices used in these patients should be robust enough to sustain high activity levels ([64, 65] and personal communication Dr. J. Muenzer). A recent study on an attenuated MPS I patient suggests that IT ERT has neurological benefits, improving brain structure and reversing cognitive decline [66]. Preliminary results of a phase 1/2 study for ICV treatment in MPS IIIB (NCT02754076) showed that ICV ERT is well tolerated and able to reduce heparan sulfate in the CSF [67]. In addition, this technique is currently also being investigated in a phase 1/2 study and phase 2 studies (NCT01907087, NCT02485899, NCT02678689) in children with ceroid lipofuscinosis neuronal type 2 (CLN2) disease, also a lysosomal storage disorder. Preliminary results indicate that ERT infusion via an ICV delivery reservoir is well tolerated and slows down the progression of functional decline ([68] and personal communication Dr. A. Schulz).

The animal studies showed that IT or ICV ERT could provoke a (dose-dependent) immune response [48, 55, 57]. Antibodies against the recombinant enzyme were found in the CSF and serum. Although these may interfere with the effectiveness of ERT [52], they were not associated with adverse events [48, 50, 55]. Induction of immune tolerance with prior intravenous ERT could prevent this antibody response [52], as could transplantation. Most patients in the clinical studies, that received intravenous ERT at least 6 months before IT administration, did not develop an immune response in the CSF [64, 65, 69]. Those patients with anti-enzyme antibodies in the CSF, either present at baseline or *de novo*, did not report any safety issues and did not show reduced enzyme uptake or activity [65, 69]. More information on the impact and safety of IT and ICV ERT in MPS patients is expected from clinical trials that are currently recruiting (including

MPS I: NCT02232477; MPS IIIB: NCT02754076) or ongoing (including MPS I Hurler: NCT00638547; MPS II: NCT01506141 and NCT02055118; MPS IIIA: NCT01299727).

4. Gene therapy

Much attention has been given to gene therapy as a treatment for MPS, as it has the potential to provide a stable and continuous source of enzyme. cDNA of the recombinant enzyme can be delivered either *in vivo* or *ex vivo*. The *in vivo* approach involves systemic or localized injection of a vector containing the cDNA, resulting in enzyme expression by the transduced cells in the target tissue. This approach has been investigated in several MPS animal models, with most studies using viral vectors [70]. Administration designed to target the brain can be intracerebral, IT, ICV, or intravenously [71-73]. The viral vectors used for gene therapy in MPS animal models were mostly derived from adeno-associated viruses (AAV), lentiviruses, or retroviruses [70]. Lenti- and retroviruses have the capacity to integrate into the genome of the cell, which increases the risk of insertional mutagenesis. This risk is not present with AAV as they are generally non-integrating [70, 74]. Intracerebral, IT, or ICV injection of AAV and lentiviral vectors successfully treated brain disease in MPS I, IIIA, IIIB, and VII animal models, inducing stable expression of the vector (as shown for example by *in situ* hybridization) [75] and enzyme [71, 73, 76-89] and clearance of pathological storage [71, 73, 76-79, 82-90] (Table 3), especially when administered in the neonatal period [77, 84, 87, 91, 92]. Some studies report positive effects on brain disease in MPS I, IIIA, and IIIB animal models after intravenous vector administration [91, 93-95]. Retroviral vectors have also been used for gene therapy in MPS animal models [70, 74, 92, 96, 97]. However, these vectors can only transduce dividing cells, and, therefore, are not useful to

treat brain disease. These results suggest that optimal combination therapy may include intravenous and direct CNS administration to treat MPS-related somatic and CNS manifestations. Importantly, gene therapy can provoke an immune response against the vector and protein [82, 98], reducing enzyme activity and eliminating transduced cells. This immune response could be avoided by immunosuppression or immunomodulation [78, 83, 85, 95, 99, 100]. A phase 1/2 study evaluating intracerebral injection of an AAVrh10-hMPS3A vector, an AVV vector encoding both the enzyme *N*-sulfoglucosamine sulfohydrolase (SGSH) and the sulfatase modifying factor SUMF-1 (which activates the catalytic site of the enzyme), in combination with immunosuppressive treatment in four children with MPS IIIA showed good safety and tolerability and suggested moderate improvements in behavior, attention, and sleep in the first year after surgery [101]. In addition, preliminary results of an ongoing phase 1/2 trial, in which six MPS IIIA patients received a single, intravenous injection of the AAV-SGSH vector ABO-102, recently demonstrated good safety, reduction in CSF HS, and preliminary evidence of decreased neurocognitive decline (http://www.raredr.com/news/abeona-data-for-gene-therapy-trial).

Ex vivo gene therapy involves transplantation of (autologous) cells that were modified *ex vivo* to express the normal cDNA of the deficient enzyme. It has shown positive effects on neuronal deficits in some MPS I, II, and IIIA animal models [70, 102-104]. Recently, El-Amouri et al. used *ex vivo* HSCT gene therapy in MPS I Hurler mice with a vector encoding an apolipoprotein E-coupled alpha-*L*-iduronidase fusion protein (see below *5.1 Trojan horse strategy*), resulting in reduced brain GAG accumulation and improved exploratory behavior [105]. Despite these promising results, many questions regarding dosing, toxicity, and safety of gene therapy in MPS remain to be addressed, warranting more research.

5. Interventions that facilitate penetration across the blood-brain barrier

An alternative approach to provide ERT to the CNS is facilitation of BBB penetration. This can be achieved by techniques that use the transport mechanisms present on the lining cells, without affecting the integrity of the BBB.

5.1 Trojan horse strategy

One technique to facilitate penetration of recombinant enzyme through the BBB is to fuse it to a genetically engineered molecule, usually an antibody, that can bind to a receptor present on the BBB that allows entry in the CNS through receptor-mediated transcytosis (i.e. endocytosis followed by exocytosis across brain capillary endothelial cells). This is also referred to as the 'Trojan horse' strategy. The fusion protein can be administered directly, or through expression mediated by a vector that contains the fusion gene. Candidate receptors include the human insulin receptor, the low density lipoprotein receptor, and the transferrin receptor [3, 106]. Treatment with recombinant alpha-*L*-iduronidase fused to transferrin or a monoclonal antibody to the transferrin receptor resulted in increased enzyme activity levels in the brain of MPS I mice (Figure 4A), and decreased vacuolation/GAG storage (Figure 4B and C) [107, 108] and GAG levels [107]. Similar results were obtained in MPS I and IIIA mice with apolipoprotein-fused alpha-*L*-iduronidase [109] and sulphamidase [72], respectively. Studies on primate models treated with the respective human enzyme fused to a monoclonal antibody against the human insulin receptor showed good safety and rapid uptake of the enzyme in the brain [110-113]. The results in primates allowed dose estimation for humans. Phase 1 clinical trials in MPS I and MPS II patients with human insulin receptor monoclonal antibody-enzyme fusion proteins are currently ongoing or enrolling patients (MPS I: NCT03053089, NCT02371226, NCT03071341, NCT02597114; MPS II: NCT02262338).

Figure 4. Treatment of mucopolysaccharidosis (MPS) I mice with alpha-*L*-iduronidase fused with a monoclonal antibody of the transferrin receptor resulted in uptake of the enzyme in the brain 60 minutes after treatment (1 mg/kg) (A). IDUA enzyme activity in the normal mouse brain is 3.2 µmol/h/mg protein. The central nervous system (CNS) of MPS I mice treated with saline contained large perivascular lysosomal inclusion bodies (B, C), which were significantly reduced after 8 weeks of twice-weekly treatment with 1 mg/kg of the fusion protein (B, C).

Scale bars: 2 µm in left panel C and 5 µm in right panel C.

*** *P* < 0.005 (A and B based on and C reprinted with permission from Boado et al. 2011 [108] Copyright 2011 American Chemical Society).**

SE: standard error; TfR mAb-IDUA: transferrin receptor monoclonal antibody-alpha L iduronidase fusion protein

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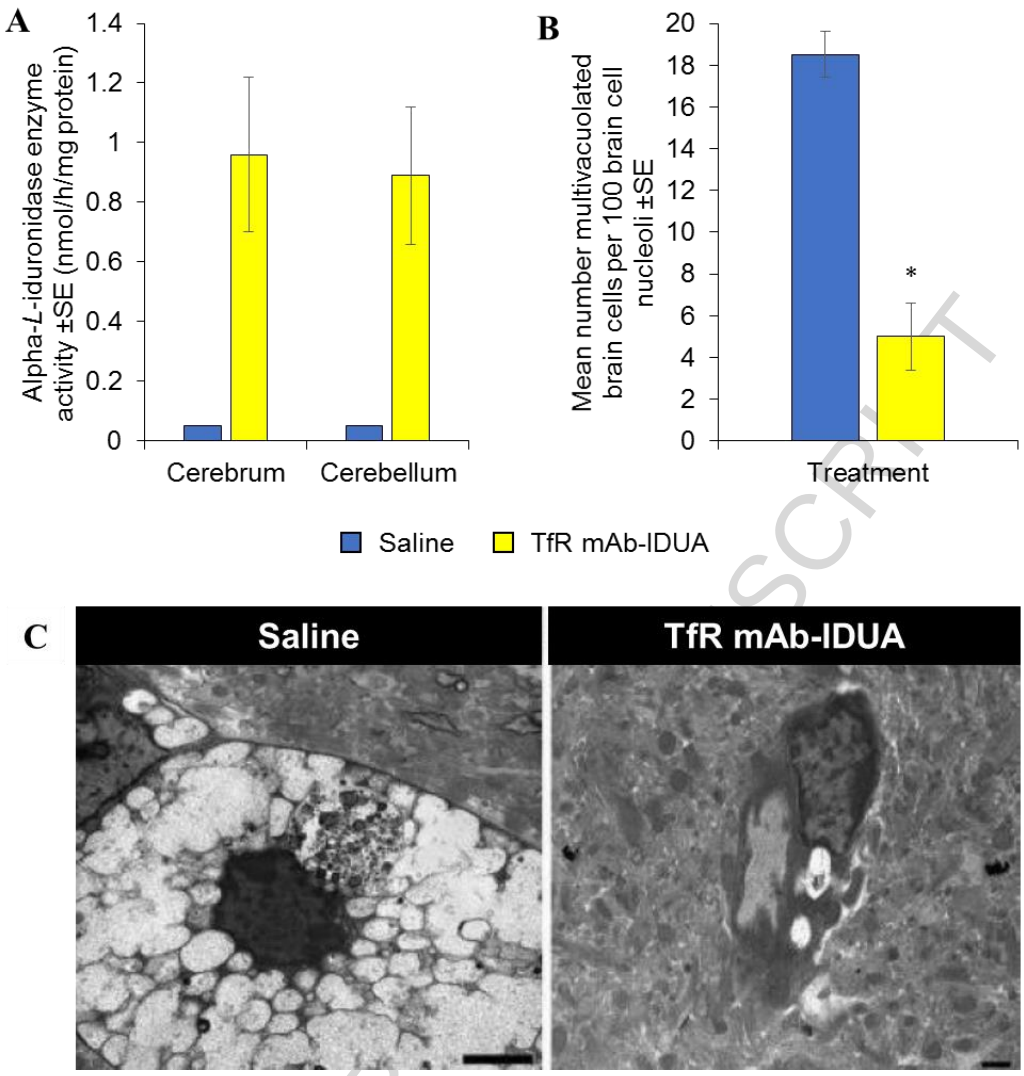

5.2 Nano-enabled therapy

A second strategy to facilitate CNS access is the use of coated nanoparticles as a carrier to transport recombinant enzyme across the BBB [3]. Several studies in patients with Alzheimer's and Parkinson's disease showed promising results with nano-enabled therapies for the treatment of CNS disease [114]. Uptake of intravenously injected apolipoprotein-E-coated particles into the murine brain has been shown to occur within 30 minutes [115]. More recently, it was shown that high molecular weight molecules, such as the recombinant enzymes for MPS disorders, can be

efficiently encapsulated by or linked to nanoparticles [116, 117]. Arylsulfatase B-loaded poly(butyl cyanoacrylate) nanoparticles demonstrated stable absorption for at least 1 h in human blood serum [117]. In addition, a study in MPS I and MPS II mice showed the ability of biodegradable and biocompatible poly(lactide-co-glycolide) nanoparticles modified with a 7-aminoacid glycopeptide (g7) to transfer a model drug with a high molecular weight through the BBB after intravenous injection [118]. An important advantage of g7- modified polymeric nanoparticles is that they tend to accumulate within lysosomes, where GAG storage occurs in MPS patients. Recently, Mayer et al. synthesized laronidase surface-functionalized multiple-wall lipid-core nanocapsules, which were able to improve biodistribution of laronidase in MPS I mice [116]. The findings of these studies open up the opportunity for further development of nano-enabled therapies for the MPS disorders.

6. Small molecules that can cross the blood-brain barrier

Several substances have the potential to alter GAG synthesis or enzyme function and are sufficiently small to cross the BBB.

6.1 Substrate reduction therapies

Substrate reduction therapy (SRT) aims to inhibit the early stage of the lysosomal degradation pathway, reducing GAG synthesis [3]. Molecules used for SRT, such as genistein and rhodamine B, are small and believed to cross the BBB after oral delivery. Genistein is a plant isoflavone tyrosine kinase inhibitor that inhibits the epidermal growth factor-mediated signal transduction responsible for the expression of GAG-synthesizing genes. In addition, genistein has anti-oxidant and anti-inflammatory capacity, which may contribute to a decrease in proinflammatory cytokines that have been suggested to play an important role in the pathogenesis of MPS [119].

Rhodamine B is a fluorescent dye that acts as a non-specific inhibitor of GAG synthesis. Both molecules reduced lysosomal GAG storage in the brain of MPS II (limited effect) and III mice [120-122] and normalized behavior in MPS IIIB [120] and MPS IIIA mice [123]. Genistein also reduced neuroinflammation in the cerebral cortex and hippocampus of MPS IIIB mice [120]. In a clinical study in 10 MPS IIIA and B patients, genistein was safe, reduced urine GAG concentration, and prevented or slowed the development of behavioral and cognitive deficits in some patients [124, 125]. Miglustat, a licensed inhibitor of glucosylceramide synthase, reduces ganglioside levels and is used to treat Gaucher disease. It has also been suggested to improve neurological disease in MPS. However, results of a 6-month, double-blind, randomized, placebo-controlled, clinical trial with miglustat in MPS III patients showed no improvement of cognition or behavior [126].

6.2 Stop-codon read-through

A small molecule treatment strategy for MPS caused by premature stop-codon mutations is to facilitate stop-codon read-through. This approach aims to suppress the effect of the premature stop-codon, inducing synthesis of a functional protein. Aminoglycosides, such as gentamicin, and chloramphenicol are candidate drugs using this approach. *In vitro* studies on MPS patient cell lines showed that aminoglycosides, chloramphenicol, and ataluren induced read-through and synthesis of the deficient enzyme [127-131]. In addition, reductions in urine GAG concentration and GAG storage in the brain of MPS I Hurler mice have been described after treatment with a designer aminoglycoside (NB84) [132]. As the effect of stop-codon read through depends on the specific mutation causing the premature stop codon, it is important to determine the patient's genotype before treatment [128-132]. An example of such a therapy is ataluren, a licensed compound in the European Union for the treatment of Duchenne muscular dystrophy [133].

Ataluren is currently being investigated in a phase 2 study in patients with MPS I (COMPASS: EudraCT 2014-002596-28 and 2015-003105-41).

6.3 Pharmacological chaperone therapy

Chaperones are small proteins that help to restore the natural folding of enzymes containing amenable mutations, leading to an increase of residual enzyme activity [3]. Their small size suggests that they are able to cross the BBB and can be used to treat CNS disease in MPS. *In vitro* experiments on several MPS IIIB- and IIIC-causing mutations indicated that pharmaceutical chaperones can bind and stabilize mutant enzymes and improve their enzymatic activity (up to approximately 7%) [134-136]. As an enzyme activity level of 10% is considered to be sufficient to prevent lysosomal GAG storage in MPS patients [137, 138], chaperone therapy is a promising treatment approach for MPS that needs further investigation.

7. Conclusions

Currently, HSCT (for MPS I Hurler) and, in particular, intravenous ERT are considered to be effective treatments for non-neurological manifestations of MPS I, II, IVA, and VI, as they can improve the patient's clinical status and quality of life. HSCT is primarily used to treat CNS manifestations in MPS I Hurler, while this effect is less clear for the other MPS disorders [39]. However, there is still a large, unmet need for the prevention and treatment of the neurocognitive decline, neurobehavioral problems, and other neurological manifestations that can occur in patients with MPS I (Hurler), II, III, and VII. With the current evidence, early diagnosis is essential to start treatment early to limit the neurological complications [1], but for most disorders the optimal treatment period and/or preparative regimens still need to be determined. In the past decade, much progress has been made in the development of therapeutic approaches for

neurological disease in these MPS disorders, partly due to the availability of small and large MPS animal models. It is currently accepted that early HSCT can limit or stabilize brain disease in MPS I Hurler patients and IT and ICV delivery of ERT has proven to be a feasible and safe option to provide enzyme directly to the CNS. In addition, promising new therapeutic strategies, including gene therapy, the Trojan-horse approach, SRT, stop-codon read-through, and nanotechnology, have been developed and are being tested. Clinical trials are required to ascertain the safety and efficacy of these strategies in MPS patients. Essential in this is the evaluation of neurocognitive function with standardized assessment tools that measure changes in cognition in MPS patients. Due to the variety of somatic and neurological manifestations present in MPS patients, combination therapy will likely provide the most optimal results. A better understanding of additional pathological changes and abnormalities will allow the development of synergistic therapies.

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Tables

Table 1. Overview of the Pubmed literature searches for each topic and number of items retrieved.

BBB: blood-brain barrier; CSF: cerebrospinal fluid; ERT: enzyme replacement therapy; HSCT: hematopoietic stem cell transplantation

Topic	Search terms	Items
HSCT	("Mucopolysaccharidoses"[Mesh]) AND "Hematopoietic Stem Cell Transplantation"[Mesh]	154
CSF delivery of ERT	("Mucopolysaccharidoses"[Mesh]) AND "Enzyme Replacement Therapy"[Mesh] AND (intrathecal OR intracerebroventricular) (("Enzyme Replacement Therapy"[Mesh]) AND "Mucopolysaccharidoses"[Mesh]) AND "Injections, Spinal"[Mesh]	20 11
Gene therapy	("Mucopolysaccharidoses/therapy"[Mesh]) AND "gene therapy" AND ("brain" OR "spinal cord" OR "Central Nervous System")	98
Therapies crossing the BBB	("Mucopolysaccharidoses"[Mesh]) AND "Blood-Brain Barrier"[Mesh] ("Mucopolysaccharidoses"[Mesh]) AND (cross blood-brain barrier) ("Mucopolysaccharidoses"[Mesh]) AND (fusion protein) ("Mucopolysaccharidoses"[Mesh]) AND "Nanoparticles"[Mesh] "Mucopolysaccharidoses"[Mesh] AND "substrate reduction therapy" "Mucopolysaccharidoses"[Mesh] AND "stop-codon read through" ("Mucopolysaccharidoses"[Mesh]) AND "Molecular Chaperones"[Mesh]	35 29 36 3 27 4 9

Table 2. Overview of published clinical trials on intrathecal (IT) enzyme replacement therapy (ERT) in mucopolysaccharidosis (MPS) patients.

AE: adverse event; CSF: cerebrospinal fluid; GAG: glycosaminoglycans; IDDD: IT drug delivery device; MRI: magnetic resonance imaging; SCC: spinal cord compression

MPS type	Patients	Treatment regimen	Outcomes
MPS I [63]	N=5, ≥8 years old with symptomatic (cervical) SCC	Monthly laronidase injections (1.74 mg), 4 months in pilot phase, then every 30-90 days in extension phase (for up to 1 year)	- 9 serious AEs (including 2 deaths not related to ERT), 3 possibly related to ERT (pneumonia, headache, facial flushing) which resolved - Subjective improvement in SCC symptoms (including mobility, bowel/bladder control, crampy leg pain) and small gains in neurological examination (sensory and motor) - No change in SCC on MRI
MPS II [64]	N=16, 3-18 years old with cognitive impairment; 4 treatment-naïve	Monthly idursulfase-IT administrations (1, 10 or 30 mg) for 6 months with IDDD implant	- 14 serious AEs in 7 of 12 patients, 12 related to IDDD malfunctioning (and related hospitalization); none related to ERT - Decline (≥79%) in mean CSF GAG concentration
MPS IIIA [65]	N=12, ≥3 years old with developmental age ≥1 year	Monthly recombinant human heparan-*N*-sulfatase administrations (10, 45 or 90 mg) for 6 months with IDDD implant	- 10 serious AEs in 7 patients, 9 related to IDDD malfunctioning (and related hospitalization); none related to ERT - Consistent decline in CSF heparan sulfate - Decline in cortical gray matter volume - Developmental quotient: stable in 50% and decline in 33%

Table 3. Overview of published studies with *in vivo* gene therapy in mucopolysaccharidosis (MPS) animal models.

AAV: adeno-associated viruses; CNS: central nervous system; CSF: cerebrospinal fluid; GAG: glycosaminoglycan; ICV: intracerebroventricular; IT: intrathecal; ND: not determined

Vector	**Injection**	**MPS model**	**Outcomes CNS**				
			↑enzyme activity	**↓GAG levels**	**↓vacuolation/ GAG storage**	**↓ inflammation/ neurodegeneration**	**Improvement behavioral deficits**
AAV2	IT	Mouse MPS IIIB (adult) [76]	Yes	Yes	Yes	ND	Yes
	Intracerebral	Mouse MPS IIIB (adult) [89]	Yes	ND	Yes	ND	Yes
AAV2/5	Intracerebral	Mouse MPS IIIB (neonate) [77]	Yes	ND	Yes	ND	ND
		Canine MPS I and IIIB (young adult) [78]	Yes[a]	Yes	Yes	ND	ND
	ICV	Mouse MPS IIIA (neonate) [84]	Yes	ND	Yes	Yes	Yes
AAV2/8	ICV (fusion	Mouse MPS IIIA (adult)	Yes	Yes	Yes	Yes	Yes

Vector	Injection	MPS model	Outcomes CNS				
			↑enzyme activity	↓GAG levels	↓vacuolation/ GAG storage	↓ inflammation/ neurodegeneration	Improvement behavioral deficits
	gene)	[72]					
AAV4	ICV	Mouse MPS VII (adult) [79]	Yes	ND	Yes	ND	Yes
AAV5	Intracerebral and ICV	Mouse MPS I Hurler (adult) [80, 81]	Yes	Yes	Yes	ND	ND
	Intracerebral	Mouse MPS IIIB (adult) [89]	Yes	ND	Yes	ND	Yes
AAV9	IT	Feline MPS I (adolescent) [82] and canine MPS I (pediatric) [83]	Yes	Yes[b]	Yes	ND	ND
	Intracisternal	Mouse MPS IIIA (adult) [85], IIIB (adult) [86], and MPS IIID (adult)	Yes	Yes	Yes	Yes	Yes

Vector	Injection	MPS model	Outcomes CNS				
			↑enzyme activity	↓GAG levels	↓vacuolation/ GAG storage	↓ inflammation/ neurodegeneration	Improvement behavioral deficits
		[139]					
	Intravenous	Mouse MPS IIIA (adult) [93]	Yes	Yes	Yes	Yes	ND
		Mouse MPS IIIB (adult) [94]	Yes	Yes	Yes	Yes	Yes
		Primate MPS IIIB (sexually immature and adult) [95]	Yes	ND	ND	ND	ND
	Intranasal	Mouse MPS I (adult) [140]	Yes	ND	Yes	ND	Yes
AAV9/rh10	IT (+intravenous)	Canine MPS VII (neonate) [87]	Yes	Yes	Yes	Yes	ND
AAVrh10	Intracerebral	Mouse MPS IIIA (adult) [90]	Yes	Yes	Yes	Yes	ND

Vector	Injection	MPS model	Outcomes CNS				
			↑enzyme activity	↓GAG levels	↓vacuolation/ GAG storage	↓ inflammation/ neurodegeneration	Improvement behavioral deficits
Lentiviral (HIV-1-based)	ICV	Mouse MPS IIIA (adult) [71]	Yes	ND	Yes	ND	Yes
	ICV	Mouse MPS IIIA (adult) [88]	Yes	ND	Yes	ND	Yes
	Intravenous	Mouse MPS I (neonate and adult) [91]	Yes[c]	Yes[c]	Yes[c]	ND	ND
	ICV	Mouse MPS VII (adult) [73]	Yes	ND	Yes	ND	Yes

a: only in those receiving immunosuppressant

b: only measured in CSF of the canine model

c: only in those treated as neonate

The author instructions do not include guidelines for the preparation of highlights.