

Innovative Treatments for Mucopolysaccharidoses

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Abstract

Mucopolysaccharidoses (MPSs) are caused by deficiency of specific lysosomal enzymes that affect the degradation of mucopolysaccharides or glycosaminoglycans. Since more than 15 years enzyme replacement therapies are available for an increasing number of MPSs. These therapies together with hematopoietic stem cell transplantation today are the gold standard of causal treatment in MPS. Despite confirmed efficacy, both do not cure these severe conditions. In this article, we discuss the limitations of established and promises of emerging therapies. The limitations of intravenous enzyme replacement and cell therapy can be summarized as immune reactions against the therapeutic molecules/cells and the failure to restore enduring and sufficient enzyme concentration in all relevant tissues. Accordingly, innovative approaches comprise small molecules and encapsulated cells that do not activate antitherapeutic immune reactions, several gene therapy approaches that aim for sustained enzyme expression, and new enzymes that penetrate blood–brain and other barriers for drug distribution. This article provides an update on the state of development of these new therapies and highlights enduring challenges.

Keywords

- ▶ mucopolysaccharidoses
- ▶ gene therapy
- ▶ innovative therapies
- ▶ cell therapy
- ▶ pharmacology

Introduction

Mucopolysaccharidoses (MPSs) are a group of inborn errors of metabolism (IEM) caused by deficiency of specific lysosomal enzymes that affect the degradation of mucopolysaccharides or glycosaminoglycans (GAGs). The accumulation of GAGs in various organs and tissues of patients affected by MPS results in a series of signs and symptoms that lead to a multisystemic clinical picture.¹ The clinical features have been described in the first (MPS I–H,^{2,3} and IV⁴) and second (MPS I–S,⁵ MPS III,⁶ VI,⁷ and VII⁸) half of the last century. The identification of the underlying specific enzyme deficiencies allowed not only a more definitive classification of MPS, but it also inspired Christian de Duve and Roscoe Brady in the late 1960s^{9,10} to propose the concept of treating these and other lysosomal storage diseases (LSDs) by replacing the defective enzyme. First preclinical proof of principle was provided by the mutual

cross correction of cultured fibroblasts from patients with MPS I and MPS II in 1968 by the group of Elizabeth Neufeld.¹¹ Brady's clinical success in treating Fabry and Gaucher patients with ceramidetrihexosidase and cerebrosidase isolated from human placenta was a paramount milestone in establishing enzyme replacement therapy (ERT) as a treatment concept. For technical reasons (low protein abundance, proteolytic degradation, etc.), this protein source could not be used in MPSs.¹² The cloning of the genes coding for the defective enzymes, however, paved the way to develop recombinant therapeutic enzymes. So far, enzyme replacement products have been marketed for the following mucopolysaccharidoses: MPS I (laronidase; FDA 2003/EMA 2003), MPS II (idursulfase; 2006/2007), MPS IV A (elosulfase alfa; 2014/2014), and MPS VI (galsulfase; 2005/2006), and recently MPS VII (vestronidase alfa, 2017). These therapies together with hematopoietic stem cell transplantation (HSCT) (only in early detected cases of MPS

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I) today are the gold standard of causal treatment in MPS. Despite bringing a significant, positive change to the natural history of these conditions with a corresponding improvement and/or stabilization of several disease manifestations, intravenous ERT does not represent a cure for these severe conditions. In this article, we want to highlight the limitations of currently established therapies and give an overview on emerging strategies to overcome these.

Limitations of ERT and BMT/HSCT

Enzyme replacement and cell therapy are causal therapies. As such, ideally they should persistently restore sufficient enzyme activity to normalize lysosomal function, stop GAG accumulation, and clear stored material without causing relevant inadvertent effects. In reality, this is only partly the case. Intravenous ERT and bone marrow transplant (BMT)/HSCT can normalize GAG excretion and liver size; however, splenomegaly, cardiac function, walking ability, endurance, airway obstruction, and pulmonary function can only be improved to some extent. Cardiac valve disease, joint range of motion, skeletal disease, and central nervous system (CNS) manifestation seem not to benefit generally. This can be explained by the following limitations of the two approaches:

Immunoreactivity

For cell therapy, immune suppression is a prerequisite. Although this is not the case for ERT, it is associated with a relevant potential for immune reaction. The spectrum of

immune reactions that have been observed upon the administration of recombinant enzymes reaches from silent antibody production to anaphylactic shocks. Practically, all cases of infusion reactions can be controlled with anti-allergic drugs, yet there is some evidence that in some cases neutralizing antibodies can mitigate therapeutic efficacy.^{13,14}

Low Bioavailability in Certain Tissues

The low vascularization of tissues like bone, cartilage, and cardiac valves as well as physiological barriers that protect the brain or the eye seem to prevent sufficient concentrations of infused recombinant enzyme.^{15,16} Also, cell therapy does not reach all relevant tissues and cells.

Short-Term Exposure

In contrast to the natural continuous enzyme production, the infusion of recombinant enzymes acts as a bolus as it is distributed and eliminated immediately after the infusion stops.^{17–21} On the one hand, this implies the need for weekly (MPS) or biweekly (other LSDs) infusions, on the other hand, recently it has been shown that continuous slow release can be more efficient than boluses.^{22,23}

This article provides an overview on emerging therapies that aim to overcome these limitations and/or mitigate the negative effects of enzyme deficiency (► **Table 1**)

Late Initiation of Treatment

Besides the inherent limitations of the above-mentioned approaches, late initiation of treatment has a major effect on

Table 1 Overview: Innovative treatments for mucopolysaccharidoses

MPS	Preclinical studies	Clinical trials
Intrathecal enzyme replacement		
MPS I		Case study ³⁰ and phase I study ³⁴
MPS II		Phase I/II study ³⁶ and interim results of Phase II/III study ³⁷
MPS III A		Phase I/II study ³⁵
MPS III B		Interim results phase 1/2 study ³⁸
“Trojan Horse” approach with fusion proteins		
MPS I	AGT-181 (HIRMAB-IDUA) in non-human primates ^{42,43}	AGT-181 Phase I (NCT02371226) AGT-181 Phase 1/2 (NCT03053089) interim results ^{48,49}
MPS II	AGT-182 (HIRMAB-IDS) in nonhuman primates ⁴⁴	AGT-182 Phase I (NCT02262338)
MPS III A	HIRMAB-SGSH in nonhuman primates ⁴⁵ cTFRMAB-SGSH in nonhuman primates ⁴⁶	
MPS III B	HIRMAB-LL-NAGLU in nonhuman primates ⁴⁷	
Nanotechnology		
MPS I	Nano capsules with Aldurazyme in MPS I cells ⁵⁰	
MPS VI	Nano capsules with Naglazyme in MPS VI cells ⁵¹	
Gene therapy		
MPS I	Intrathecal AAV-mediated in-vivo gene therapy in MPS I dogs ^{94,95}	SB-318 AAV6/zinc finger nuclease mediated genome editing NCT02702115

Table 1 (Continued)

MPS	Preclinical studies	Clinical trials
Gene therapy		
MPS II	Ex-vivo gene therapy with lentiviral corrected HSC in MPS II-mice ⁵⁴	Retroviral in-vivo gene therapy ⁹⁶ SB-913 AAV6/zinc finger nuclease mediated genome editing NCT03041324
MPS IIIA	Ex-vivo gene therapy with lentiviral corrected HSC in MPS IIIA-mice ⁵⁵	Phase I/II intracerebral AAV mediated in-vivo gene therapy ^{58,60}
MPS IIIB	Ex-vivo gene therapy with lentiviral corrected HSC in MPS IIIB-mice ⁹⁷	Phase I/II intracerebral AAV mediated in-vivo gene therapy ⁵⁹
MPS VI		Phase I/II systemic AAV mediated in-vivo gene therapy NCT03173521
Microencapsulated cells		
MPS I	Intraperitoneally implanted α -L-iduronidase overexpressing cells in MPS I mice ^{63,64}	
MPS II	Intraperitoneally implanted iduronate-2-sulfatase overexpressing myoblasts in MPS II mice ⁶²	
MPS VII	Intraventricularly injected β glucuronidase overexpressing fibroblasts in MPS VII mice ⁶¹	
Stop Codon read-through approach		
MPS I	Chloramphenicol, ⁶⁶ gentamicin, ^{67–69} amikacin, lividomycin, paromomycin ⁶⁷ in MPS I cells	Phase II Ataluren EudraCT Number 2015–003105–41
MPS VI	Ataluren, NB30 and NB54 in MPS VI cells ⁷⁰	
Pharmacological chaperones		
MPS II	Heparin derivative D250 in MPS II-cells ⁷²	
MPS III	Iminosugars in MPS IIIC mice ⁷⁷	
MPS IV	Iminosugars DLHex-DGJ β -Gal-inhibitors and isofagomine derivatives in MPS IV cells ^{73–75}	
Genistein (4,5,7-trihydroxyisoflavone)		
MPS III		Case series to randomized controlled studies ^{79–81}
Pentosan polysulfate (PPS)		
MPS I		Randomized open phase 2 study ⁸⁷
Rhodamine B ([9-(2-carboxyphenyl)-6-diethylamino-3-xanthenylidene]-diethylammonium chloride)		
MPS I	Rhodamine in MPS I mice ⁹²	
MPS IIIA	Rhodamine in MPS IIIA mice ^{90,91}	
MPS VI	Rhodamine in MPS VI cells ⁹¹	

Abbreviations: AAV, adeno associated virus; cTFRMAb-SGSH, chimeric monoclonal antibodies against the mouse transferrin receptor fused to *N*-sulfoglucosamine sulfohydrolase; EudraCT, European clinical trials registry identifier-number; HIRMAb-IDS, monoclonal antibodies against human insulin receptor fused to iduronate sulfatase A; HIRMAb-IDUA, monoclonal antibodies against human insulin receptor fused to iduronidase A; HIRMAb-LL-NAGLU, monoclonal antibodies against human insulin receptor fused to α -*N*-acetylglucosaminidase; MPS, mucopolysaccharidosis.

► **Table 1** Preclinical and clinical development status of innovative therapies.
NCT clinicaltrials.gov-identifier number.

its success. This is because irreversible tissue destructions seem to occur very early in life. Studies in aborted affected fetuses as well as animal studies indicate that GAG accumulation is prevalent even before the 30th week of gestation.^{24,25} If started at birth, however, ERT can normalize GAG storage and reduce pathology even in otherwise hard-to-reach tissues such as cardiac valves, bone, and brain.²⁶ Newborn screening for MPS, as a key measure against late treatment initiation, is currently investigated in several pilot programs,^{27–29} but this is beyond the focus of this publication.

Intrathecal Enzyme Replacement in MPS I, MPS II, and MPS III A and B

A straightforward method to overcome blood–brain barrier (BBB) is the direct injection into cerebrospinal fluid (CSF). It is well established in other indications such as drug treatment of cerebral tumors. The intrathecal space can be accessed by lumbar puncture or subcutaneously implanted drug delivery devices. Several preclinical and clinical studies have been conducted in small and large animal models of MPS I,³⁰ II, IIIA, and IIIB and patients, respectively. Obviously,

the ultimate goal is to treat CNS manifestation of these diseases. Clinical trials that evaluate if cognitive decline can be stopped or decelerated are currently underway in MPS I, MPS II, MPS IIIA, and MPS IIIB but conclusive results so far are not yet published. Before these pivotal studies could be conducted several pharmacokinetic and pharmacodynamic characteristics of the drugs had to be clarified upfront. In the following section, this knowledge is summarized.

What Doses and Intervals Are Needed to Restore Normal Enzyme Activity in Relevant Tissues by Intrathecal Injection?

In different murine and canine³¹ MPS models as well as in nonhuman primates doses have been identified that lead to a normal enzyme activity in brain parenchyma down to deep layers, spinal cord, and spinal meninges.^{31,32} The injected recombinant enzyme was detectable up to 1 to 3 months after injection³⁰ and had a brain half-life of 10 days.³³ These results suggested biweekly or even monthly infusion intervals consecutive clinical trials.

Does the Restored Enzyme Activity Reduce Storage Material and Brain Pathology?

In animal models, the concentration of GAGs in brain and meninges, brain vacuolization, and signs of neuro inflammation were reduced under biweekly or monthly infusions. Clinical data from case reports and small studies in adult and pediatric patients with MPS I, II, IIIA, and IIIB indicate that GAGs in CSF are reduced by up to 90%.^{34–36}

Do the Drugs Induce Immune Reaction and/or Other Relevant Adverse Reactions?

Currently, safety of intrathecal ERT has been studied and reported in a total of 70 (6 MPS I,^{30,34} 49 MPS II,^{36,37} 12 MPS IIIA,³⁵ and 3 MPS IIIB³⁸) patients with a maximum follow-up of up to 67 months. No major adverse events have been reported. Serum antidrug antibodies were a common finding in animal and clinical studies. Antibodies in CSF were only found in patients with substantial serum antibody titers. Thus, it was concluded that these presumably crossed the BBB rather than being built intrathecally. Clinical significance of antibodies has not been reported.

Does Intrathecal ERT Improve the CNS Disease in MPS?

The major motivation for intrathecal ERT is to treat or prevent myelon compression and neurocognitive deterioration. Subjective improvement of symptoms associated with myelon compression has been observed in a phase I study by Dickson et al with intrathecal laronidase in MPS I patients.³⁴ Increased mobility, improved bowel and bladder control, a reduction in crampy leg pain, and reduced sensation of “pins & needles” were reported by patients. Neurological examination showed small gains in the sensory and motor function. Objective study endpoints, however, such as CSF GAG reduction, MRI signs for myelon compression, somatosensory testing, and a score for activities of daily living were missed. The failure to demonstrate efficacy was attributed to the low number of participant ($n = 5$), the lack of sensitive

outcome measures for the measurement of myelon compression, the presence of long-standing (likely irreversible) disease in the subjects, and spinal ligamentous thickening and other contributors to myelon compression that would be unlikely to respond to intrathecally delivered enzyme.³⁴

In the phase I/II study of Jones et al with MPS IIIA patients, neurodevelopment (Vineland Adaptive Behavior Scales-II [VABS-II], Bayley Scales of Infant Development III [BSID-III], Kaufman Assessment Battery for Children [KABC-II]) and gray matter volume was evaluated 22 weeks after intrathecal heparan-*N*-sulfatase. Of the 12 patients, 4 patients showed a decline in developmental quotient assessed, 6 patients were essentially stable, and 2 patients had only a single data point. All except two patients showed reduction in gray matter volume.³⁵ The above-mentioned studies were primarily designed to proof safety and tolerability; thus, it was not entirely unexpected that efficacy could not be statistically proved. In contrast, the randomized controlled phase II/III trial in children with Hunter syndrome of Muenzer et al primarily aimed for the proof of efficacy. The effects of monthly intrathecal idursulfase ($n = 32$) on cognitive impairment were assessed with General Conceptual Ability (GCA) score (part of DAS-II) and Adaptive Behavior Composite (ABC) score (part of VABS-II) and compared versus no treatment ($n = 16$). The top line results presented in December 2017 showed no significant improvement in these parameters.^{39–41}

So, in conclusion it has been shown that intrathecal ERT can be safely used in MPS I, II, IIIA, and IIIB, but it remains unclear if the CNS pathology can be reversed or reduced in progression once developed. This underlines the need for alternative approaches.

Trojan Horse Approach with Fusion Proteins

Although many therapeutic proteins cannot pass the BBB, it is not a complete barrier for large molecules. Macromolecules such as hormones, neurotransmitters, and xenobiotics enter the brain via receptor-mediated active transport systems. This can be utilized by fusing active compounds to antibodies against these receptors. The antibodies act as Trojan horses that ferry the therapeutic protein across BBB. Namely fusion proteins of antibodies against human insulin receptors (HIRMAb) or transferrin receptors (TfRMAb) have been used to develop treatments for MPS I (HIRMAb-IDUA^{42,43}), MPS II (HIRMAb-IDS⁴⁴), MPS IIIA (HIRMAb-SGSH,⁴⁵ cTfRMAb-SGSH⁴⁶), and MPS IIIB (HIRMAb-LL-NAGLU⁴⁷). These studies indicate that approximately 1% of intravenously infused enzyme is taken up into brain, which is considered sufficient to reduce intracellular GAG accumulation. In MPS IIIA mice GAG accumulation is substantially reduced after treatment with cTfRMAb-SGSH.⁴⁶

Human insulin receptors caused hypoglycemia in high doses by a weak insulin agonist activity. However, this was not observed when dextrose was added to the infusion.⁴³ Above that the preclinical studies indicated a good toxicity profile. Currently, several clinical trials are ongoing including a phase I study (NCT02371226) and a phase ½ (NCT03053089) study with AGT-181 (HIRMAb-IDUA) in 3 and 21 MPS I

patients, respectively; extension studies (NCT02597114; NCT03071341); and a phase I study with AGT-182 (HIR-MAb-IDS) in eight MPS II patients (NCT02262338). Recent reports on preliminary results of a trial with AGT-181 in MPS I patients indicate good effects on GAG levels, spleen, and liver volume as well as on neurocognitive function.^{48,49}

Nanotechnology

Another promising strategy to ferry enzyme across the BBB is to coat it with polymer-based nanoparticles. The particles conjugate the therapeutic enzyme and build nanocapsules that can pass BBB by transcytosis and other mechanisms. *In vitro* studies have been done with arylsulfatase B (Naglazyme for MPS VI; BioMarin Pharmaceutical)⁵⁰ and iduronidase (Aldurazyme for MPS I, Genzyme Corporation, Boston, Massachusetts, United States).⁵¹

Gene Therapy

Gene therapy aims for the correction of genetic sequences in patient cells. In the *ex vivo* approach, patient cells (e.g., stem cells and fibroblasts) are gathered, cultured *in vitro*, corrected genetically, and consecutively reinjected into the patient. In contrast for *in vivo* gene therapy, the corrected DNA is injected directly into the patient. Most *in vivo* efforts utilize viral vectors to deliver the corrective genetic material into the target cells. In principle, MPSs as well as other LSDs are good candidates for gene therapy approaches. This is because even a relatively small number of corrected cells may be sufficient to produce therapeutic enzyme concentrations in the circulating blood. As in ERT, this will lead to internalization of enzyme into deficient cells, even if the DNA of these cells was not corrected.⁵² Like in ERT, a major challenge also of systemically administered gene therapy is to reach CNS, bones, and eyes sufficiently.^{52,53} Among many efforts, two approaches seem promising for brain-targeted gene therapy. First lentiviral vectors can be used to augment the efficacy of HSCT by inducing overexpression of the therapeutic enzyme. In this sense, mouse models of Hunters and Sanfilippo A disease have been successfully treated with autologous HSC transduced with a lentivirus encoding for iduronate-2-sulfatase and *N*-sulfoglucosamine sulfohydrolase, respectively.^{54,55} Interestingly, in contrast to regular HSCT these modified HSC improved neuropathology significantly. In metachromatic leukodystrophy, another LSD, this strategy has been successfully used in clinical trials.^{56,57}

Second, adeno-associated viral (AAV) vectors have been directly injected into the brain parenchyma or CSF in many preclinical and some clinical studies. Tardieu et al^{58,59} conducted two phase ½ studies in 1.5 to 6 years old children with MPS IIIA and IIIB, respectively. The recombinant AAV vector serotype 2/5 (rAAV2/5) encoding human *N*-sulfoglucosamine sulfohydrolase (SGSH) or α -*N*-acetylglucosaminidase (NAGLU) was injected in cerebral and cerebellar white matter with silica glass capillaries. This was well tolerated and induced sustained enzyme production in the brain. After initial specific anti-NAGLU immune response immunological

tolerance was developed. Some cognitive improvement was observed in all patients with best results in the youngest patient (20 months of age). Another phase ½ study in MPS IIIA was recently reported by Flanigan et al using a scAAV9 vector. GAGs in urine and CSF and liver volume were decreased upon gene therapy. Stabilization or improvement in adaptive behavior and cognitive function was observed.⁶⁰

Although larger studies and longer follow-up are needed, these results indicate a window of therapeutic opportunity in early life for this approach. Clinical trials are also underway for MPS II (NCT00004454), IIIB (NCT03300453, NCT03315182), and VI (NCT03173521).

Cell Microencapsulation

Cell microencapsulation of allogenic cells aims to allow their implantation without the need for immune suppression. By enclosing the cells into a semipermeable membrane immune reactions can be prevented, while exchange of metabolites and nutrients is still possible. Several kinds of microencapsulated cells that have been genetically modified to overexpress the therapeutic enzymes have been studied successfully in MPS types I, II, and VII. In MPS VII mice implantation of microencapsulated β -glucuronidase overexpressing fibroblasts into the lateral ventricles resulted in distribution of the enzyme in most brain areas and the CNS pathology was improved.⁶¹ Peritoneal application of iduronate-2-sulfatase overexpressing myoblasts reduced GAGs in urine and visceral organs in MPS II mice.⁶² In MPS I mice encapsulated baby hamster kidney [BHK] cells were successfully applied,⁶³ but prednisone was needed to control immune response.^{64,65}

Stop-Codon Read Through

Stop-codon read through therapy (SCRT) aims for genetic correction at the RNA level. Nonsense mutations can induce stop codons that lead to premature termination of the RNA translation and consecutive messenger RNA (mRNA) degradation by nonsense-mediated mRNA decay resulting in truncated dysfunctional peptides. This pathomechanism can be disrupted by inserting amino acids into the sequence, so the stop codon is resolved and full length protein can be generated. Several molecules have been shown to apply for SCRT including marketed drugs. Enzyme activity could be increased in MPS fibroblasts and cell lines with chloramphenicol,⁶⁶ gentamicin,⁶⁷⁻⁶⁹ amikacin, lividomycin, and paromomycin.⁶⁷ Furthermore novel less-toxic molecules like PTC124 (Ataluren), NB30, and NB54 (paromomycin derivatives) were successfully tried in MPS VI fibroblasts⁷⁰ and MPS I cells.⁶⁷ Ataluren (Translarna, PTC Therapeutics, South Plainfield, New Jersey, United States) is market approved for SCRT of nonsense mutations caused Duchenne muscular dystrophy. A phase II trial with MPS I patients (EudraCT Number 2015-003105-41) is currently conducted in the United Kingdom. All of these are small molecules that can cross the BBB. However, SCRT is limited to the use in patients with missense mutation.

Pharmacological Chaperones

Some genetic variants in MPS and many other diseases cause misfolding of the respective enzyme or other protein, respectively. Misfolding leads to an aberrant three-dimensional conformation and consecutively to a reduced function and stability as well as aberrant trafficking of the enzyme. Pharmacological chaperones (PCs) counteract this misfolding pathology by acting as scaffolding for the misfolded proteins. PCs are small molecules that can have advantages over therapeutic proteins in their ability to reach target cells and cell compartments. On the other hand, this approach is limited to patients with amenable mutations that lead to potentially reversible misfolding. In Fabry disease, a PC (migalastat) has reached market approval. In MPS, interesting molecules have been described for MPS II, III, and IV^{71–76} and recently first *in vivo* experiments in a murine MPS model have been conducted.⁷⁷

Glycosaminoglycan-Reducing Small Molecules

Substrate reduction is an established therapeutic concept in other LSDs like Gaucher disease and Niemann Pick Type C. Partly motivated by the restrictions of ERT to reach the brain, the bones and the eyes several small molecules that reduce GAG concentration in urine and tissue have been studied in MPS.

Genistein (4,5,7-trihydroxyisoflavone) is a plant isoflavone, which blocks the epidermal growth factor-mediated signal transduction. This pathway regulates the expression of GAG synthesizing enzymes. Thus, the reduction in GAG levels in brains and other organs of Genistein-treated MPS III B mice^{78,79} was attributed to substrate inhibition. Despite promising preclinical data, clinical studies with 5 to 10 mg/kg Genistein per day including one placebo-controlled study so far failed to conclusively confirm effects on neurocognition whereas safety seems to be good even in high doses.^{79–82}

Pentosan polysulfate (PPS) is an anti-inflammatory drug approved for the treatment of interstitial cystitis and osteoarthritis.⁸³ The rationale to use it in MPS is based on its effects on inflammation processes that contribute to bone and joint disease in MPS.⁸⁴ PPS improved systemic and joint inflammation, motility, grooming behavior, skull and tracheal malformations,⁸⁵ and reduced GAG concentration in urine and tissue of MPS VI rats.⁸³ Comparable results were found in MPS I dogs.⁸⁶ Yet so far it remains unclear how PPS reduces GAG levels and substrate reduction, increased degradation, direct effects on lysosomal function, and chaperone function are discussed as mechanisms.⁸³ In a monocentric phase II study with four MPS I patients, Hennermann et al found a 24-week treatment with PPS well tolerated. Urinary GAG concentrations and pain were reduced; range of motion was improved.⁸⁷

Rhodamine B ([9-(2-carboxyphenyl)-6-diethylamino-3-xanthenylidene]-diethylammonium chloride) reduced GAG concentration in MPS VI and MPS IIIA skin fibroblasts.^{88,89}

Rhodamine B-treated MPS IIIA mice showed reduced liver size and GAG levels in urine, liver, and brain tissue. Additionally, an improvement of the neurological function was proved by water maze experiments.^{90,91} MPS I mice improved in learning and skeletal disease upon rhodamine B treatment.⁹² Although long-term administration of low-dose rhodamine B was well tolerated in mice,⁹² safety and efficacy in patients as well as the active mechanism are unknown so far.⁹³

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References

- Neufeld EMJ. The mucopolysaccharidosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Basis of Inherited Disease*. 8th ed. New York, NY: McGraw-Hill; 2001: 3421–3452
- Hurler G. Über einen Typ multipler Abartungen, vorwiegend am Skelettsystem. *Z Kinderheilkd* 1920;24:220–234
- Hunter C. A rare disease in two brothers. *Proc R Soc Med* 1917;10 (Sect Study Dis Child):104–116
- Morquio L. Sur une forme de dystrophie osseuse familiale. *Archives de medecine des infants*. 1929;32:129–135
- Scheie HG, Hambrick GW Jr, Barness LA. A newly recognized forme fruste of Hurler's disease (gargoylism). *Am J Ophthalmol* 1962;53:753–769
- Sanfilippo S, Podosin R, Langer LJ, et al. Mental retardation associated with acid mucopolysacchariduria (heparin sulfate type). *J Pediatr* 1963;63:837–838
- Maroteaux P, Leveque B, Marie J, Lamy M. A new dysostosis with urinary elimination of chondroitin sulfate B [Article in French]. *Presse Med* 1963;71:1849–1852
- Sly WS, Quinton BA, McAlister WH, Rimoin DL. Beta glucuronidase deficiency: report of clinical, radiologic, and biochemical features of a new mucopolysaccharidosis. *J Pediatr* 1973;82(02):249–257
- De Duve C. From cytochromes to lysosomes. *Fed Proc* 1964:1045–1049
- Brady RO. The sphingolipidoses. *N Engl J Med* 1966;275:312–318
- Fratantoni JC, Hall CW, Neufeld EF. Hurler and Hunter syndromes: mutual correction of the defect in cultured fibroblasts. *Science* 1968;162(3853):570–572
- Heartlein MWKA. Discovery and clinical development of idursulfase (Elaprase®) for the treatment of mucopolysaccharidosis II (Hunter syndrome). In: David CPM, ed. *Orphan Drugs and Rare Diseases*. London: Royal Society of Chemistry; 2014: 164–182
- Lenders M, Schmitz B, Brand SM, Foell D, Brand E. Characterization of drug-neutralizing antibodies in patients with Fabry disease during infusion. *J Allergy Clin Immunol* 2018;141(06): 2289–2292.e7
- Kim S, Whitley CB, Jarnes Utz JR. Correlation between urinary GAG and anti-idursulfase ERT neutralizing antibodies during treatment with NICIT immune tolerance regimen: a case report. *Mol Genet Metab* 2017;122(1–2):92–99
- Lin HY, Lin SP, Chuang CK, Chen MR, Chen BF, Wraith JE. Mucopolysaccharidosis I under enzyme replacement therapy with laronidase—a mortality case with autopsy report. *J Inher Metab Dis* 2005;28(06):1146–1148
- Yano S, Moseley K, Pavlova Z. Postmortem studies on a patient with mucopolysaccharidosis type I: histopathological findings after one year of enzyme replacement therapy. *J Inher Metab Dis* 2009;32(Suppl 1):S53–S57
- Hendriksz CJ. Elosulfase alfa (BMN 110) for the treatment of mucopolysaccharidosis IVA (Morquio A syndrome). *Expert Rev Clin Pharmacol* 2016;9(12):1521–1532

- 18 Ruane T, Haskins M, Cheng A, et al. Pharmacodynamics, pharmacokinetics and biodistribution of recombinant human N-acetylgalactosamine 4-sulfatase after 6 months of therapy in cats using different IV infusion durations. *Mol Genet Metab* 2016;117(02):157–163
- 19 Qi Y, Musson DG, Schweighardt B, et al. Pharmacokinetic and pharmacodynamic evaluation of elosulfase alfa, an enzyme replacement therapy in patients with Morquio A syndrome. *Clin Pharmacokinet* 2014;53(12):1137–1147
- 20 Xie H, Chung JK, Mascelli MA, McCauley TG. Pharmacokinetics and bioavailability of a therapeutic enzyme (idursulfase) in cynomolgus monkeys after intrathecal and intravenous administration. *PLoS One* 2015;10(04):e0122453
- 21 Burton BK, Berger KI, Lewis GD, et al. Safety and physiological effects of two different doses of elosulfase alfa in patients with Morquio A syndrome: a randomized, double-blind, pilot study. *Am J Med Genet A* 2015;167A(10):2272–2281
- 22 King B, Hassiotis S, Rozaklis T, et al. Low-dose, continuous enzyme replacement therapy ameliorates brain pathology in the neurodegenerative lysosomal disorder mucopolysaccharidosis type IIIA. *J Neurochem* 2016;137(03):409–422
- 23 King B, Marshall NR, Hassiotis S, et al. Slow, continuous enzyme replacement via spinal CSF in dogs with the paediatric-onset neurodegenerative disease, MPS IIIA. *J Inher Metab Dis* 2017;40(03):443–453
- 24 Martin JJ, Ceuterick C. Prenatal pathology in mucopolysaccharidoses: a comparison with postnatal cases. *Clin Neuropathol* 1983;2(03):122–127
- 25 Wiesmann UN, Spycher MA, Meier C, Liebaers I, Herschkowitz N. Prenatal mucopolysaccharidosis II (Hunter): a pathogenetic study. *Pediatr Res* 1980;14(05):749–756
- 26 Dierenfeld AD, McEntee MF, Vogler CA, et al. Replacing the enzyme alpha-L-iduronidase at birth ameliorates symptoms in the brain and periphery of dogs with mucopolysaccharidosis type I. *Sci Transl Med* 2010;2(60):60ra89
- 27 Scott CR, Elliott S, Buroker N, et al. Identification of infants at risk for developing Fabry, Pompe, or mucopolysaccharidosis-I from newborn blood spots by tandem mass spectrometry. *J Pediatr* 2013;163(02):498–503
- 28 Hopkins PV, Campbell C, Klug T, Rogers S, Raburn-Miller J, Kiesling J. Lysosomal storage disorder screening implementation: findings from the first six months of full population pilot testing in Missouri. *J Pediatr* 2015;166(01):172–177
- 29 Mechtler TP, Stary S, Metz TF, et al. Neonatal screening for lysosomal storage disorders: feasibility and incidence from a nationwide study in Austria. *Lancet* 2012;379(9813):335–341
- 30 Munoz-Rojas MV, Vieira T, Costa R, et al. Intrathecal enzyme replacement therapy in a patient with mucopolysaccharidosis type I and symptomatic spinal cord compression. *Am J Med Genet A* 2008;146A(19):2538–2544
- 31 Dickson P, McEntee M, Vogler C, et al. Intrathecal enzyme replacement therapy: successful treatment of brain disease via the cerebrospinal fluid. *Mol Genet Metab* 2007;91(01):61–68
- 32 Kakkis E, McEntee M, Vogler C, et al. Intrathecal enzyme replacement therapy reduces lysosomal storage in the brain and meninges of the canine model of MPS I. *Mol Genet Metab* 2004;83(1–2):163–174
- 33 Kan SH, Aoyagi-Scharber M, Le SQ, et al. Delivery of an enzyme-IGFII fusion protein to the mouse brain is therapeutic for mucopolysaccharidosis type IIIB. *Proc Natl Acad Sci U S A* 2014;111(41):14870–14875
- 34 Dickson PI, Kaitila I, Harmatz P, et al; Mucopolysaccharidosis I Intrathecal Research Collaborative. Safety of laronidase delivered into the spinal canal for treatment of cervical stenosis in mucopolysaccharidosis I. *Mol Genet Metab* 2015;116(1–2):69–74
- 35 Jones SA, Breen C, Heap F, et al. A phase 1/2 study of intrathecal heparan-N-sulfatase in patients with mucopolysaccharidosis IIIA. *Mol Genet Metab* 2016;118(03):198–205
- 36 Muenzer J, Hendriksz CJ, Fan Z, et al. A phase I/II study of intrathecal idursulfase-IT in children with severe mucopolysaccharidosis II. *Genet Med* 2016;18(01):73–81
- 37 Muenzer J, Burton BK, Harmatz P, et al. Efficacy and safety of intrathecal idursulfase in pediatric patients with mucopolysaccharidosis type II and early cognitive impairment: design and methods of a controlled, randomized, phase II/III multicenter study. *Mol Genet Metab* 2018;123:S99–S100
- 38 Muschol N, Cleary M, Couce ML, et al. ICV-administered BMN 250 (NAGLU-IGF2) is well tolerated and reduces heparan sulfate accumulation in the CNS of subjects with Sanfilippo syndrome type B (MPS IIIB). *Mol Genet Metab* 2018;123:S102
- 39 Muenzer J, Hendriksz CJ, Stein MB, Fan Z, Kearney S, Horton J, Vijayaraghavan S, Santra S, Solanki GA, Pan L et al. A long-term extension study evaluating intrathecal idursulfase-IT in children with Hunter syndrome and cognitive impairment. *Mol Genet Metab* 2017;120:S99–S100
- 40 Study of intrathecal idursulfase-IT administered in conjunction with elaprase in pediatric patients with Hunter syndrome and early cognitive impairment available at: <https://clinicaltrials.gov/ct2/show/study/NCT02055118>. Accessed March 5, 2018
- 41 Shire announces top-line results for phase II/III clinical trial in children with Hunter syndrome and cognitive impairment. Available at: <https://www.shire.com/en/newsroom/2017/december/wvdwq3>. Accessed March 5, 2018
- 42 Boado RJ, Pardridge WM. Brain and organ uptake in the Rhesus monkey in vivo of recombinant iduronidase compared to an insulin receptor antibody-iduronidase fusion protein. *Mol Pharm* 2017;14(04):1271–1277
- 43 Boado RJ, Hui EK, Lu JZ, Pardridge WM. Glycemic control and chronic dosing of rhesus monkeys with a fusion protein of iduronidase and a monoclonal antibody against the human insulin receptor. *Drug Metab Dispos* 2012;40(10):2021–2025
- 44 Boado RJ, Ka-Wai Hui E, Zhiqiang Lu J, Pardridge WM. Insulin receptor antibody-iduronate 2-sulfatase fusion protein: pharmacokinetics, anti-drug antibody, and safety pharmacology in Rhesus monkeys. *Biotechnol Bioeng* 2014;111(11):2317–2325
- 45 Boado RJ, Lu JZ, Hui EK, Pardridge WM. Insulin receptor antibody-sulfamidase fusion protein penetrates the primate blood-brain barrier and reduces glycosaminoglycans in Sanfilippo type A cells. *Mol Pharm* 2014;11(08):2928–2934
- 46 Boado RJ, Lu JZ, Hui EK, Pardridge WM. Reduction in brain heparan sulfate with systemic administration of an IgG Trojan Horse-sulfamidase fusion protein in the mucopolysaccharidosis type IIIA mouse. *Mol Pharm* 2018;15(02):602–608
- 47 Boado RJ, Lu JZ, Hui EK, Lin H, Pardridge WM. Insulin receptor antibody- α -N-acetylglucosaminidase fusion protein penetrates the primate blood-brain barrier and reduces glycosaminoglycans in Sanfilippo type B fibroblasts. *Mol Pharm* 2016;13(04):1385–1392
- 48 Giugliani L, Carvalho Doni K, Poswar F, Giugliani R, Boado R. Somatic effects of AGT-181 in patients with mucopolysaccharidosis I enrolled in a phase I/II clinical trial in Brazil. *Mol Genet Metab* 2018;123(02):S53
- 49 Giugliani R, Giugliani L, Dalle Corte A, et al. Safety and clinical efficacy of AGT-181, a brain penetrating human insulin receptor antibody-iduronidase fusion protein, in a 26-week study with pediatric patients with mucopolysaccharidosis type I. *Mol Genet Metab* 2018;123(02):S54
- 50 Mühlstein A, Gelperina S, Kreuter J. Development of nanoparticle-bound arylsulfatase B for enzyme replacement therapy of mucopolysaccharidosis VI. *Pharmazie* 2013;68(07):549–554
- 51 Mayer FQ, Adorne MD, Bender EA, et al. Laronidase-functionalized multiple-wall lipid-core nanocapsules: promising formulation for a more effective treatment of mucopolysaccharidosis type I. *Pharm Res* 2015;32(03):941–954
- 52 Baldo G, Giugliani R, Matte U. Gene delivery strategies for the treatment of mucopolysaccharidoses. *Expert Opin Drug Deliv* 2014;11(03):449–459

- 53 Ellinwood NM, Vite CH, Haskins ME. Gene therapy for lysosomal storage diseases: the lessons and promise of animal models. *J Gene Med* 2004;6(05):481–506
- 54 Wakabayashi T, Shimada Y, Akiyama K, et al. Hematopoietic stem cell gene therapy corrects neuropathic phenotype in Murine model of mucopolysaccharidosis type II. *Hum Gene Ther* 2015; 26(06):357–366
- 55 Sergijenko A, Langford-Smith A, Liao AY, et al. Myeloid/microglial driven autologous hematopoietic stem cell gene therapy corrects a neuropathic lysosomal disease. *Mol Ther* 2013;21(10): 1938–1949
- 56 Sessa M, Lorioli L, Fumagalli F, et al. Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. *Lancet* 2016;388(10043):476–487
- 57 Biffi A, Montini E, Lorioli L, et al. Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science* 2013;341(6148):1233158
- 58 Tardieu M, Zerah M, Husson B, et al. Intracerebral administration of adeno-associated viral vector serotype rh.10 carrying human SGSH and SUMF1 cDNAs in children with mucopolysaccharidosis type IIIA disease: results of a phase I/II trial. *Hum Gene Ther* 2014; 25(06):506–516
- 59 Tardieu M, Zerah M, Gougeon ML, et al. Intracerebral gene therapy in children with mucopolysaccharidosis type IIIB syndrome: an uncontrolled phase 1/2 clinical trial. *Lancet Neurol* 2017;16(09): 712–720
- 60 Flanigan KM, Truxal KV, McBride KL, et al. A phase 1/2 clinical trial of systemic gene transfer of scAAV9.U1a.HSGSH for MPS IIIA: Safety, tolerability, and preliminary evidence of biopotency. *Mol Genet Metab* 2018;123:S46
- 61 Ross CJ, Bastedo L, Maier SA, Sands MS, Chang PL. *Treatment* of a lysosomal storage disease, mucopolysaccharidosis VII, with microencapsulated recombinant cells. *Hum Gene Ther* 2000;11 (15):2117–2127
- 62 Friso A, Tomanin R, Alba S, et al. Reduction of GAG storage in MPS II mouse model following implantation of encapsulated recombinant myoblasts. *J Gene Med* 2005;7(11): 1482–1491
- 63 Baldo G, Mayer FQ, Martinelli B, et al. Intraperitoneal implant of recombinant encapsulated cells overexpressing alpha-L-iduronidase partially corrects visceral pathology in mucopolysaccharidosis type I mice. *Cytotherapy* 2012;14(07):860–867
- 64 Lagranha VL, de Carvalho TG, Giugliani R, Matte U. Treatment of MPS I mice with microencapsulated cells overexpressing IDUA: effect of the prednisolone administration. *J Microencapsul* 2013; 30(04):383–389
- 65 Lizzi Lagranha V, Zambiasi Martinelli B, Baldo G, et al. Subcutaneous implantation of microencapsulated cells overexpressing α -L-iduronidase for mucopolysaccharidosis type I treatment. *J Mater Sci Mater Med* 2017;28(03):43
- 66 Mayer FQ, Artigalás OA, Lagranha VL, et al. Chloramphenicol enhances IDUA activity on fibroblasts from mucopolysaccharidosis I patients. *Curr Pharm Biotechnol* 2013;14(02): 194–198
- 67 Kamei M, Kasperski K, Fuller M, et al. Aminoglycoside-induced premature stop codon read-through of mucopolysaccharidosis type I patient Q70X and W402X mutations in cultured cells. *JIMD Rep* 2014;13:139–147
- 68 Hein LK, Bawden M, Muller VJ, Sillence D, Hopwood JJ, Brooks DA. α -L-iduronidase premature stop codons and potential read-through in mucopolysaccharidosis type I patients. *J Mol Biol* 2004;338(03):453–462
- 69 Keeling KM, Wang D, Dai Y, et al. Attenuation of nonsense-mediated mRNA decay enhances in vivo nonsense suppression. *PLoS One* 2013;8(04):e60478
- 70 Bartolomeo R, Polishchuk EV, Volpi N, Polishchuk RS, Auricchio A. Pharmacological read-through of nonsense ARSB mutations as a potential therapeutic approach for mucopolysaccharidosis VI. *J Inherit Metab Dis* 2013;36(02):363–371
- 71 Takai T, Higaki K, Aguilar-Moncayo M, et al. A bicyclic 1-deoxygalactonojirimycin derivative as a novel pharmacological chaperone for GM1 gangliosidosis. *Mol Ther* 2013;21(03): 526–532
- 72 Hoshina H, Shimada Y, Higuchi T, Kobayashi H, Ida H, Ohashi T. Chaperone effect of sulfated disaccharide from heparin on mutant iduronate-2-sulfatase in mucopolysaccharidosis type II. *Mol Genet Metab* 2018;123(02):118–122
- 73 Fantur K, Hofer D, Schitter G, et al. DLHex-DGJ, a novel derivative of 1-deoxygalactonojirimycin with pharmacological chaperone activity in human G(M1)-gangliosidosis fibroblasts. *Mol Genet Metab* 2010;100(03):262–268
- 74 Suzuki H, Ohto U, Higaki K, et al. Structural basis of pharmacological chaperoning for human β -galactosidase. *J Biol Chem* 2014; 289(21):14560–14568
- 75 Thonhofer M, Weber P, Gonzalez Santana A, et al. Synthesis of C-5a-substituted derivatives of 4-epi-isofagomine: notable β -galactosidase inhibitors and activity promoters of GM1-gangliosidosis related human lysosomal β -galactosidase mutant R201C. *Carbohydr Res* 2016;429:71–80
- 76 Matos L, Canals I, Dridi L, et al. Therapeutic strategies based on modified U1 snRNAs and chaperones for Sanfilippo C splicing mutations. *Orphanet J Rare Dis* 2014;9:180
- 77 Pshezhetsky AV, Pan X, Héon-Roberts R, et al. Chaperone therapy for mucopolysaccharidosis type IIIC. *Mol Genet Metab* 2018;123: S121–S122
- 78 Malinowska M, Wilkinson FL, Langford-Smith KJ, et al. Genistein improves neuropathology and corrects behaviour in a mouse model of neurodegenerative metabolic disease. *PLoS One* 2010; 5(12):e14192
- 79 Piotrowska E, Jakobkiewicz-Banecka J, Maryniak A, et al. Two-year follow-up of Sanfilippo Disease patients treated with a genistein-rich isoflavone extract: assessment of effects on cognitive functions and general status of patients. *Med Sci Monit* 2011;17(04): CR196–CR202
- 80 Delgadillo V, O'Callaghan MM, Artuch R, Montero R, Pineda M. Genistein supplementation in patients affected by Sanfilippo disease. *J Inherit Metab Dis* 2011;34(05):1039–1044
- 81 de Ruijter J, Valstar MJ, Narajczyk M, et al. Genistein in Sanfilippo disease: a randomized controlled crossover trial. *Ann Neurol* 2012;71(01):110–120
- 82 Kim KH, Dodsworth C, Paras A, Burton BK. High dose genistein aglycone therapy is safe in patients with mucopolysaccharidoses involving the central nervous system. *Mol Genet Metab* 2013;109 (04):382–385
- 83 Frohbergh M, Ge Y, Meng F, et al. Dose responsive effects of subcutaneous pentosan polysulfate injection in mucopolysaccharidosis type VI rats and comparison to oral treatment. *PLoS One* 2014;9(06):e100882
- 84 Simonaro CM, Ge Y, Eliyahu E, He X, Jepsen KJ, Schuchman EH. Involvement of the Toll-like receptor 4 pathway and use of TNF- α antagonists for treatment of the mucopolysaccharidoses. *Proc Natl Acad Sci U S A* 2010;107(01):222–227
- 85 Schuchman EH, Ge Y, Lai A, et al. Pentosan polysulfate: a novel therapy for the mucopolysaccharidoses. *PLoS One* 2013;8(01): e54459
- 86 Simonaro CM, Tomatsu S, Sikora T, et al. Pentosan polysulfate: oral versus subcutaneous injection in mucopolysaccharidosis type I dogs. *PLoS One* 2016;11(04):e0153136
- 87 Hennermann JB, Gökce S, Solyom A, Mengel E, Schuchman EH, Simonaro CM. Treatment with pentosan polysulphate in patients with MPS I: results from an open label, randomized, monocentric phase II study. *J Inherit Metab Dis* 2016;39(06): 831–837
- 88 Banecka-Majkutewicz Z, Jakóbkiewicz-Banecka J, Gabig-Cimińska M, Węgrzyn A, Węgrzyn G. Putative biological mechanisms of

- efficiency of substrate reduction therapies for mucopolysaccharidoses. *Arch Immunol Ther Exp (Warsz)* 2012;60(06):461–468
- 89 Noh H, Lee JI. Current and potential therapeutic strategies for mucopolysaccharidoses. *J Clin Pharm Ther* 2014;39(03):215–224
- 90 Roberts AL, Rees MH, Klebe S, Fletcher JM, Byers S. Improvement in behaviour after substrate deprivation therapy with rhodamine B in a mouse model of MPS IIIA. *Mol Genet Metab* 2007;92(1–2):115–121
- 91 Roberts AL, Thomas BJ, Wilkinson AS, Fletcher JM, Byers S. Inhibition of glycosaminoglycan synthesis using rhodamine B in a mouse model of mucopolysaccharidosis type IIIA. *Pediatr Res* 2006;60(03):309–314
- 92 Derrick-Roberts ALK, Jackson MR, Pyragius CE, Byers S. Substrate deprivation therapy to reduce glycosaminoglycan synthesis improves aspects of neurological and skeletal pathology in MPS I mice. *Diseases* 2017;5(01):5
- 93 Giugliani R, Federhen A, Silva A, et al. Emerging treatment options for the mucopolysaccharidoses. *Res and Rep in Endocrine Disorders* 2012;2:53–64
- 94 Hinderer C, Bell P, Louboutin JP, et al. Neonatal tolerance induction enables accurate evaluation of gene therapy for MPS I in a canine model. *Mol Genet Metab* 2016;119(1–2):124–130
- 95 Ellinwood NM, Ausseil J, Desmaris N, et al. Safe, efficient, and reproducible gene therapy of the brain in the dog models of Sanfilippo and Hurler syndromes. *Mol Ther* 2011;19(02):251–259
- 96 Whitley CB, McIvor RS, Aronovich EL, et al. Retroviral-mediated transfer of the iduronate-2-sulfatase gene into lymphocytes for treatment of mild Hunter syndrome (mucopolysaccharidosis type II). *Hum Gene Ther* 1996;7(04):537–549
- 97 Holley RJ, Ellison SM, Fil D, et al. Macrophage enzyme and reduced inflammation drive brain correction of mucopolysaccharidosis IIIB by stem cell gene therapy. *Brain* 2018;141(01):99–116