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Minireview

# Early initiation of enzyme replacement therapy for the mucopolysaccharidoses☆☆

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ABSTRACT

The mucopolysaccharidoses (MPS), a group of rare genetic disorders caused by defects in glycosaminoglycan (GAG) catabolism, are progressive, multi-systemic diseases with a high burden of morbidity. Enzyme replacement therapy (ERT) is available for MPS I, II, and VI, and may improve walking ability, endurance, and pulmonary function as evidenced by data from pivotal trials and extension studies. Despite these demonstrable benefits, cardiac valve disease, joint disease, and skeletal disease, all of which cause significant morbidity, do not generally improve with ERT if pathological changes are already established. Airway disease improves, but usually does not normalize. These limitations can be well understood by considering the varied functions of GAG in the body. Disruption of GAG catabolism has far-reaching effects due to the triggering of secondary pathogenic cascades. It appears that many of the consequences of these secondary pathogenic events, while they may improve on treatment, cannot be fully corrected even with long-term exposure to enzyme, thereby supporting the treatment of patients with MPS before the onset of clinical disease. This review examines the data from clinical trials and other studies in human patients to explore the limits of ERT as currently used, then discusses the pathophysiology, fetal tissue studies, animal studies, and sibling reports to explore the question of how early to treat an MPS patient with a firm diagnosis. The review is followed by an expert opinion on the rationale for and the benefits of early treatment.


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*Abbreviations:* %FVC, percent predicted forced vital capacity; MMP9, matrix metalloproteinase 9; 6MWT, 6-minute walk test; 12MWT, 12-minute walk test; AHI, apnea/hypopnea index; EM, electron microscopy; ERT, enzyme replacement therapy; FVC, forced vital capacity; GAG, glycosaminoglycans; HSCT, hematopoietic stem cell transplantation; IRR, infusion-related reaction; IV, intravenous; JROM, joint range of motion; MPS, mucopolysaccharidosis/mucopolysaccharidoses; uGAG, urinary glycosaminoglycans.

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## 1. Introduction

The mucopolysaccharidoses (MPS) are a group of rare progressive genetic disorders characterized by defects in glycosaminoglycan (GAG) catabolism, leading to the accumulation of partially degraded GAG within lysosomes [1]. Each of the eleven MPS is caused by a deficiency in the activity of a single, specific lysosomal enzyme required for GAG degradation. The progressive accumulation of GAG results in cellular and organ damage. Clinical manifestations are evident in nearly every system, including respiratory, cardiopulmonary, musculoskeletal, gastrointestinal, and neurological, causing significant morbidity and early mortality in the severe forms of these disorders. All of the MPS are inherited in an autosomal recessive pattern with the exception of MPS II (OMIM 309900). MPS II is an X-linked recessive disorder that generally affects only males, although rare, female patients with MPS II are reported [2,3].

The MPS are heterogeneous disorders, and patients present with a wide spectrum of clinical severity [1]. Most MPS patients appear normal at birth, but patients with severe phenotypes typically develop clinical signs and symptoms before the age of 2 to 4 years and can die before the age of 10 years. Patients with the severe forms of MPS I, II, and VII, and all MPS III patients, will also develop cognitive impairment. Patients with an attenuated phenotype can develop all of the somatic signs and symptoms seen in the severe phenotype, but never develop cognitive impairment. The onset of somatic symptoms is generally later and the disease progression slower. Such patients may live well into adulthood. While the cognitive involvement is the hallmark of the severe phenotype in MPS I, II, III, and VII, it is often difficult to determine whether or not a patient is experiencing cognitive decline until an age at which somatic disease is well established. Young patients with MPS may thus have an indeterminate phenotype.

Enzyme replacement therapy (ERT) via intravenous (IV) infusion of recombinant human enzyme is available for MPS I, II and VI in the United States, Europe, and over 40 countries worldwide. The benefits of ERT may include improvements in joint mobility, walking ability, and pulmonary and respiratory functions; reduction in liver and spleen volumes; and reduction in urinary GAG excretion [4–12]. No evidence exists to suggest that intravenously administered recombinant enzyme crosses the blood–brain barrier at the labeled doses, and clinical observation has not supported neurocognitive benefit for patients with severe MPS I or severe MPS II [13,14]. Although ERT has been approved for MPS I, the current recommended treatment for the severe form of MPS I, Hurler syndrome, is hematopoietic stem cell transplantation (HSCT), which has been associated with neurocognitive stabilization [15,16]. ERT has been used for patients with Hurler syndrome prior to HSCT and until engraftment occurs; ERT is well tolerated and may significantly improve the pre-transplant condition in selected patients [17–19]. A recent expert consensus panel noted that Hurler syndrome patients who are referred for HSCT may benefit from ERT before HSCT, as this can improve their clinical condition, but recommended that initiating ERT should not delay the transplant [16]. Surprisingly, ERT given with HSCT has been associated with better cognitive outcomes in Hurler syndrome [20]. HSCT is not recommended for patients with the severe form of MPS II or MPS III, since previous reports have found no evidence of neurocognitive stabilization [21,22].

In the pivotal ERT trials for MPS I, II, and VI, only patients 5 years of age and older were enrolled [6,7,12]. Younger patients were excluded because of their inability to reliably perform the primary efficacy endpoints, such as the 6-minute walk test (6MWT), 12-minute walk test (12MWT), 3-minute stair climb, and forced vital capacity (FVC) testing [17,18,23]. Only one clinical study of ERT in patients under the age of 5 years with MPS I has been published [5]. Reduction in hepatomegaly, improvements in sleep apnea, and a reduction in the percentage of patients displaying left ventricular hypertrophy were observed. No new safety concerns were seen [5]. Clinical trial data for patients under 5 years of age are lacking for MPS II and VI, although a safety study including patients under the age of 5 years has been completed for MPS II [23]. In that study (NCT00607386), idursulfase (Elaprase®, Shire Human Genetic Therapies, Inc., Lexington, MA, USA) treatment was associated with decreases in urinary GAG (uGAG) levels, liver size, and spleen volume. The safety profile was similar to that observed in previous clinical studies with patients over the age of 5 years, with almost all adverse reactions being infusion-related reactions (IRRs). No new safety concerns were seen in this young cohort. Traditional measures of efficacy, such as 6MWT or FVC testing, were not conducted as the enrolled patients were too young to comply.

The paucity of clinical trial data in children under 5 years old, and particularly the lack of efficacy data, has resulted in some physicians and reimbursement agencies failing to support treatment for MPS I, II and VI patients in this age group. This review will examine the benefits and limitations of ERT when used in older patients with established disease as reported in published studies, then correlate those findings with what is known about the pathophysiology of the MPS. Finally, we offer an expert opinion about the use of ERT in MPS patients under 5 years of age.

## 2. Enzyme replacement therapy for mucopolysaccharidosis I, II and VI

### 2.1. Pivotal trial and extension data

The efficacy and safety of ERT with laronidase (Aldurazyme®, BioMarin/Genzyme LLC, Cambridge, MA, USA), idursulfase, and galsulfase (Naglazyme®, BioMarin Pharmaceutical Inc., Novato, CA, USA) for MPS I, II, and VI, respectively, have been established in phase III or II/III clinical trials and open-label extension studies [4,6,7,10,12]. Liver and spleen volumes, when assessed, and uGAG levels were statistically significantly decreased in the treated groups compared with placebo across all three compounds in the double-blind phases, and these gains were maintained or increased throughout the open label extensions [4,6,7,10,12]. Statistically significant improvements in mobility as assessed by the 6MWT (laronidase, idursulfase) and 12MWT (galsulfase) were seen in the pivotal trials [6,7,12]. In the laronidase open-label extension study, 78% (31/40) of patients showed either further improvement or stabilization in the 6MWT distance at the final assessment, while the remaining 22% (9/40) of patients showed declines [4]. In the idursulfase open-label extension study, the mean increase in 6MWT distance seen in weekly treated patients was maintained [10]. The galsulfase open-label study demonstrated continued increases in overall 12MWT distance through the end of the open-label extension study [7]. Pulmonary function as assessed by percent predicted FVC (%FVC) was significantly increased in the double-blind trial of laronidase, but declined at a very slow rate during the open-label study (0.78 percentage points per year) [4,6]. For MPS II, weekly infusions of idursulfase did not result in significant improvement in %FVC in the double-blind trial [10,12]. In the trial of galsulfase for MPS VI, FVC did not significantly improve for treated patients during either the double-blind or extension portions [9]. A sleep study evaluation was performed only in the laronidase trial, and the mean apnea/hypopnea index (AHI) did not decrease significantly with treatment in the intent-to-treat analysis of the double-blind trial. However, the AHI significantly decreased by 6.0 events per hour of sleep among

those treated patients whose baseline AHI suggested sleep apnea, and these gains were slightly furthered during the extension period [6]. Statistically significant improvements in elbow joint mobility were seen in the idursulfase double-blind trial but not in the extension study [4,10,12]. Significant improvements in shoulder mobility were seen for patients with the greatest baseline impairment in the laronidase double-blind trial and extension study, as well as in the idursulfase extension study [6,7,12].

ERT was generally well tolerated [4,6,7,10,12]. The most common adverse events in the clinical trials and extension studies were infusion-related reactions (IRRs) and were successfully managed by slowing or stopping the infusion and/or premedication with antihistamines or steroids. Serious or potentially life-threatening IRRs were uncommon but were seen in three patients treated with idursulfase [12] and two patients treated with laronidase [6]. The prescribing information for laronidase and idursulfase carries a black-box warning that life-threatening anaphylactic reactions have been observed in some patients during infusions; therefore, appropriate medical support should be readily available during treatment [24,25]. For MPS II, the risk of IRRs appears to be greatest in the first 6 months of treatment, while the onset of a first IRR has been reported to be much more variable for MPS I and VI, occurring as late as week 134 of treatment for MPS I and week 84 for MPS VI [26]. However, in postmarketing reports, patients treated with idursulfase have experienced anaphylactic reactions up to several years after initiating treatment [25]. The prescribing information for all three products contains warnings about the risks of anaphylaxis and allergic reactions and recommendations against administering ERT to patients with acute respiratory complications [24,25,27]. Patients with compromised respiratory function or acute respiratory disease may be at risk of serious acute exacerbation of their respiratory compromise due to IRRs, and these patients require additional monitoring.

### 2.2. Limitations of enzyme replacement therapy

#### 2.2.1. Cardiac valve disease

ERT does not appear to improve cardiac valve disease for the majority of patients with valve disease present at the start of ERT (Table 1). This was first observed in a small retrospective case series of five children (one male, four females) with MPS I who ranged in age from 2.3 to 15.3 years at ERT initiation and who continued on weekly ERT for an average duration of 3.6 years [28]. Before ERT, mitral valve regurgitation and aortic valve regurgitation were present in four patients and one patient, respectively. On treatment, mitral valve regurgitation progressed in three patients and improved in one patient. The remaining patient was stable. Aortic regurgitation increased in four patients and stayed stable in one patient. Similar results were reported in a 6-year follow-up evaluation of five of the ten MPS I patients originally enrolled in the phase I/II study of laronidase [29]. Three patients showed an increase in aortic insufficiency over baseline after 6 years of ERT, while the other two patients maintained stable aortic insufficiency. Mitral valve regurgitation was stable in four patients and worse in one patient. A larger MPS cardiac natural history study included nine patients who were receiving ERT (five patients with MPS I Hurler–Scheie, two with MPS I Scheie, and two with attenuated MPS II) [30]. Eight of these patients had available follow-up cardiac data. Of these, four were determined to have stable cardiac valve disease and four had progressive valve disease. In a study of idursulfase in treatment-naïve, adult, male Japanese patients with attenuated disease, all nine patients had abnormal, but stable valve disease after 12 months of weekly treatment with idursulfase [31]. No improvements were noted.

A recent prospective study of 24 patients with MPS I, II, or VI confirms these earlier reports that ERT does not appear to improve valve disease in most patients [32]. Patients began ERT at a median age of 5.3 years (1–18 years) and received therapy for a median duration of 2.0 (0.9–6.0) years. At baseline, all of the patients had abnormal valves. Mitral valve regurgitation was seen in seven patients (three MPS I and four MPS VI), aortic valve regurgitation in seven patients (two MPS I, three MPS II and two MPS VI), and tricuspid valve and pulmonary valve regurgitation in two patients (both MPS VI). Complete follow-up data were available for 20 out of the 24 patients, and of these, increased valve regurgitation was seen in 12 (60%), stabilization was seen in five (25%), and improvement was seen in three (15%).

Although overall cardiac function did not improve with ERT in the above studies, some evidence indicates that younger patients may be more likely to improve. Braunlin and colleagues conducted a post-hoc analysis of prospective data from 54 MPS VI patients who underwent echocardiograms at baseline, at 24–48 weeks, and at 72–96 weeks after initiation of weekly galsulfase treatment during the phase I/II, phase II, and phase III trials [33]. The mean age at baseline was 11.8 ± 5.4 years (range 6–29 years). At baseline, the mean mitral valve gradient was 7.28 ± 5.68 mm Hg (normal: <5 mm Hg), indicating mitral stenosis, and the mean mitral valve regurgitation score was "mild" at baseline (1.35 ± 1.05). The mean peak systolic gradient across the aortic valve was 13.72 ± 10.78 mm Hg (normal: 11–13 mm Hg) at baseline, and the mean aortic regurgitation score was "trace" (0.53 ± 0.87). After 96 weeks of ERT, the mean mitral valve gradient, the mean aortic valve gradient, and the mean mitral valve regurgitation score did not change significantly. Aortic regurgitation, however, did increase significantly, although still within the "trace to mild" category. A subgroup analysis of patients less than 12 years old versus those 12 years of age or older found that mitral and aortic valve stenosis and mitral regurgitation did not change significantly from baseline after 96 weeks of therapy for either group. However, the aortic valve regurgitation score did increase significantly from baseline in those patients 12 years of age or older ($p = 0.015$). The authors speculated that, although the increase in aortic valve regurgitation score among the older patients was not large enough to have a physiologic consequence for those patients, it may imply that cardiac valve pathology, once begun, may not be reversible.

**Table 1**
Studies of aortic valve disease outcomes after enzyme replacement therapy in MPS I, II, and VI.

| Study | N | MPS | Time on treatment at study end | Regurgitation at the end of follow up | | |
|---|---|---|---|---|---|---|
| | | | Median (range) years | Increased | Stable | Decreased |
| Braunlin (2006) [28] | 5 | I | 3.6 (0.3 to 7.3) | Mitral: 3/5 (60%)<br>Aortic: 4/5 (80%) | Mitral: 1/5 (20%)<br>Aortic: 1/5 (20%) | Mitral: 1/5 (20%)<br>Aortic: 0/5 |
| Sifuentes (2007) [29] | 5 | I | 6 | Mitral: 4/5 (80%)<br>Aortic: 3/5 (60%) | Mitral: 0/5<br>Aortic: 2/5 (40%) | Mitral: 1/5 (20%)<br>Aortic: 0/5 |
| Fesslova (2009) [30] | 8 | I and II | 2.7 (2.0–5.0) | 4/8 (50%) | 4/8 (50%) | 0/8 |
| Okuyama (2010) [31] | 9 | II | 1 | 0/9 | 9/9 (100%) | 0/9 |
| Brands (2012) [32] | 20 | I, II, VI | 2.0 (0.9–6.0) | 12/20 (60%) | 5/20 (25%) | 3/20 (15%) |
| Braunlin (2012) [33] | 54 | VI | 1.8 (96 weeks) | Mean aortic regurgitation score increased from 0.53 ± 0.87 at baseline to 0.88 ± 0.99 at the end of the study ($p = 0.004$) | Mean mitral regurgitation score was 1.35 ± 1.05 at baseline and 1.51 ± 0.73 at the end of the study ($p = 0.459$) | |

MPS, mucopolysaccharidosis.

*2.2.2. Skeletal disease (dysostosis multiplex) and joint disease*

There is limited information available about the impact of ERT on bone disease in the MPS. There is some evidence to suggest that established dysostosis multiplex typically remains stable and does not improve appreciably with ERT. In the long-term follow-up of patients from the phase I/II trial of laronidase, comprehensive skeletal surveys from baseline through 6 years of treatment did not reveal any distinct changes in dysostosis multiplex over time, except for one patient with increased spine and hip disease [29]. An Italian study of 36 patients with MPS II (mean age 12.6 years, range 2.8–32.2 years) used magnetic resonance imaging to evaluate brain and spine features, including cranial bone abnormalities and spinal anomalies, over time [34]. A subset of 15 patients were treated with idursulfase, and after a median treatment duration of 1.6 years (range 1–2.9 years), cranial bone abnormalities and spinal anomalies remained stable for most patients. Two idursulfase-treated patients experienced a worsening of intervertebral disc abnormalities. The authors concluded that GAG-mediated damages to cranial bone and spine might be difficult to revert once they have occurred.

A greater amount of data are available about the effects of ERT upon joint range of motion (JROM) in the MPS. In the phase III trial of laronidase for MPS I, the mean change in active shoulder flexion, the only joint studied, did not differ significantly between the treated and untreated groups [6]. Statistically significant improvements in shoulder flexion were seen, however, in the open-label extension study, with a mean change of 17.4° ± 3.6° from baseline [4]. At the end of the extension study, 17 of 37 patients (46%) had an increase of ≥20° in mean shoulder flexion, 18 of 37 patients (49%) remained stable (<20° change in either direction), and 2 of 37 patients (5%) had a decrease of ≥20°. No significant changes were seen for knee flexion. A longer-term, 6-year follow up of five patients from the original phase I/II study reported that shoulder extension increased over 6 years, improving by a mean of 38.7° on the right and by 32.1° on the left at the end of the study [29]. Tylki-Szymańska and colleagues conducted a study of motor movement in 17 treatment-naïve Polish patients with MPS I (10 with MPS I-Hurler, 2 with MPS I-Hurler/Scheie, and 5 with MPS I-Scheie) by assessing upper extremity JROM [35]. After 52–208 weeks of ERT with laronidase, only mean passive shoulder flexion ($p = 0.013$) improved significantly from baseline. When only data from the subset of patients with the Hurler/Scheie or Scheie phenotypes was analyzed, active should flexion also showed significant improvements from baseline ($p = 0.046$). Eight of the patients with the Hurler phenotype in this study were 1 year old at enrollment. Among these young patients with the severe phenotype, the authors noted no change or only a slight increase in JROM over the study, saying that "earlier introduction of ERT led to slower progression of symptoms". Unfortunately, no subgroup analysis for these young patients was published.

In the phase II/III trial of idursulfase for MPS II, passive elbow mobility, a secondary endpoint, improved in the weekly treated group as compared with placebo ($p = 0.0476$), but no other significant changes in passive JROM were found [12]. The extension study found progressive, statistically significant, and clinically important (≥10%) improvements in JROM for the shoulder [10]. No significant differences in JROM were observed for the elbow, wrist, digits, hip, knee, or ankle. A Japanese open-label, 12-month study of idursulfase treatment for 10 adult patients found improved mean shoulder flexion, shoulder abduction, knee flexion, hip flexion, and elbow extension, but only hip flexion reached statistical significance ($p = 0.031$) [31].

The phase III trial galsulfase for MPS VI evaluated JROM as a tertiary endpoint and found that ERT had no statistically significant effects upon any joint [7]. Only shoulder JROM data was analyzed in the extension study, and again, no effect of galsulfase was found.

*2.2.3. Airway disease*

Pulmonary function measured by %FVC or absolute FVC was shown to improve in the pivotal trials of laronidase and idursulfase (but not galsulfase); however, the data suggest that airway disease does not normalize with treatment [6,7,12]. In the phase III study of laronidase, the baseline mean %FVC for the laronidase treatment group was 48.4 ± 14.5% [6], but all enrolled patients had a %FVC <80% at baseline per inclusion criteria. After 26 weeks of treatment, the mean %FVC increased by 5.6% (median, 3.0%). While this improvement is statistically significant and clinically meaningful [36], the final mean %FVC for the treated patients was still well below the normal range [37]. In addition, the gains were not fully maintained, as a small but steady decrease of 0.78 percentage points per year was seen in the 3.5-year extension study [4]. When individual patient %FVC responses were tabulated in the extension study, the authors reported improvement in 18% of patients, a stable value in 55%, and a decline in 28% [4].

The double-blind phase II/III trial of idursulfase found a 3.45 ± 1.77% increase in %FVC among patients treated weekly, which was not statistically significant [12]. However, absolute FVC increased by 0.22 ± 0.05 L from a baseline of 1.19 ± 0.10 L for weekly treated patients in the double-blind trial, compared with an increase of 0.06 ± 0.03 L from a baseline of 1.09 ± 0.09 L in the placebo group ($p = 0.0011$). This improvement was further increased by 0.44 ± 0.10 L over the extension study (D.A.H. Whiteman, personal communication). Given that the mean age of patients at baseline was approximately 14 years, the final values after 3.5 to 4 years of idursulfase treatment remained below the normal range [37]. In a small study of open-label idursulfase treatment of adult Japanese patients with attenuated MPS II, the mean %FVC was 39.9 ± 6.6%, which increased by 3.8 ± 2.8 percentage points after 1 year [31]. The mean absolute FVC increased by 0.1 ± 0.1 L over the baseline of 1.4 ± 0.3 L. Neither value reached statistical significance.

The only reported clinical trial data for sleep apnea in patients receiving ERT comes from the phase III and extension studies of laronidase [4,6]. In the pivotal trial, the AHI significantly decreased by 6.0 events per hour among those treated MPS I patients whose baseline AHI was abnormal ($p = 0.014$). A final mean reduction of 7.6 ± 4.5 events per hour was seen in the extension. The actual baseline value for the subgroup of patients whose AHI suggested sleep apnea was not reported, so it is difficult to determine if the final value is within normal limits (AHI ≥10 for ages ≤15 years, AHI ≥15 for ages >15 years); however, the baseline AHI for all patients was 17.5 ± 15.5 events per hour, making it unlikely [4,6].

Some retrospective data for airway disease have also been reported. A chart review of 17 Australian patients with MPS (four MPS I-Hurler, ten MPS II, one MPS III, one MPS IV, and one MPS VI) who had received a total of 141 anesthetics between 1998 and 2011 found through a logistic regression analysis that ERT did not reduce the incidence of difficult airway management [38]. Five out of the ten enrolled MPS II patients were treated with idursulfase beginning at a mean age of 9.4 years (range 8–13 years). The odds of a difficult intubation were greater for treated patients than for those receiving no treatment, although this was not statistically significant. The authors noted that the use of idursulfase late in the clinical course when significant deposition of GAG in the airway had already occurred likely contributed to the apparent lack of effect.

## 3. The pathophysiology of MPS and treatment limitations

Historically it was believed that the primary storage of GAG and their deposition in tissues was solely responsible for the signs and symptoms associated with the MPS [39], but recent research suggests a more complex picture [40]. (Please see the review articles by Clarke [41] and Simonaro et al. [42] for an in-depth discussion of this topic.) Far from inert storage materials, GAG have been described as one of the most biologically active, information-dense biological molecules [43]. Their storage appears to lead to the perturbation of cellular, tissue, and organ homeostasis through secondary pathogenic cascades [40,41,44–47]. Accumulation of GAG and secondary storage metabolites has also been linked with increased excretion of cytokines and inflammatory mediators, activation of the inflammatory response, and

production of reactive free radicals associated with oxidative stress. Indeed, peripheral leukocytes from untreated MPS I and MPS II patients have significantly elevated levels of lipid peroxidation, carbonyl group content, and DNA damage, all indicators of severe oxidative damage [48,49]. Intracellularly, GAG storage can alter the endosomal network, impacting intracellular targeting pathways, endocytosis, and autophagy [50–52]. Once activated, these secondary events may not be easily reversible. In addition, the presence of excess GAG within lysosomes has been shown to inhibit lysosomal enzymes that otherwise would function normally, leading to the secondary storage of a variety of molecules that may influence pathology [53–56].

### 3.1. Pathophysiology of cardiac valve disease

Many of the above-mentioned pathological mechanisms may come into play in a complex clinical feature like cardiac valve disease. First, there is presumed primary GAG storage in cardiac tissue. Proteoglycans such as decorin and biglycan contain dermatan sulfate, which are stored in MPS I, II, and VI; these proteoglycans are normally found in appreciable quantities in cardiac valve tissue [57]. In the MPS, GAG levels are elevated, and histopathological and electron microscopy (EM) studies have revealed the infiltration of GAG-loaded "clear" cells and granular cells within cardiac valves (leaflets, annuli and chordae tendineae), endocardium, myocardial walls, coronary arteries, aorta and the conduction system [58,59]. The "clear" cells appear to be activated valvular interstitial cells [60]. Such cells normally are engaged in maintaining valvular structural integrity and mediating response to injury [61], but in patients with MPS, they appear to be engaged in attempted, but ineffective, valve repair [60]. It has been hypothesized that these cells may reach an activated state due to the accumulation of GAG and a resulting stimulation of the lipopolysaccharide signaling pathway. GAG stimulation of the lipopolysaccharide signaling pathway may result in the release of proinflammatory cytokines and matrix metalloproteinases. This hypothesis is supported by the finding that the activated valvular interstitial cells stain positively for matrix metalloproteinase 9 (MMP9), an enzyme responsible for degradation of proteoglycans in the extracellular matrix. Upregulation of MMP9 in cardiac valve tissue has been associated with inflammation and pathological extracellular matrix remodeling, leading to valve regurgitation and stenosis [62].

Autopsy reports from patients with MPS I confirm that ERT does not fully clear GAG from all cardiac tissue types once cardiac disease is evident [63,64]. Postmortem findings in a 3.5-year-old boy with MPS I who had received laronidase treatment for 1 year revealed hypertrophic cardiomyopathy, severe aortic valve and mitral valve thickening with shortened chordae, and endocardial fibroelastosis [64]. Numerous histiocytes with enlarged lysosomes were seen in the thickened endocardium tissue on EM analysis. By contrast, EM examination of the liver and the cardiac muscle revealed no accumulation of GAG. An autopsy of a 20-year-old male with MPS I who had been receiving laronidase treatment for 104 weeks revealed a thickened tricuspid valve, thickened and rigid aortic, mitral, and pulmonary valves, and GAG deposition in the heart valves and endocardium.

### 3.2. Pathophysiology of skeletal and connective tissue disease

As with cardiac valve disease, established dysostosis multiplex and abnormal joint range of motion seem to stabilize or progress on long-term ERT, but generally do not improve. Many features of dysostosis multiplex, such as thoracolumbar kyphosis/scoliosis, odontoid hypoplasia, wide oar-shaped ribs, shortened long bones, coxa valga, dysplastic femoral heads, genu valgum, and bullet-shaped phalanges, are in part due to abnormal endochondral ossification. Chondrocytes in the resting zone of the growth plates of patients with MPS I, II and IV have been reported to be overly large and to contain undegraded GAG. These chondrocytes appear to interrupt the architecture of the growth plate such that the resting zone is abnormally large [65]. GAG have been postulated to play a role in this process through the binding of mitotic growth factors and interruption of cellular signaling [66]. The ossification zone of the growth plate also seems to be affected. In MPS I mice, ossification starts before the GAG have been removed from the cartilage extracellular matrix; thus, remnants of cartilage remain in the newly formed bone, leading to growth retardation and bone malformation [67]. It follows that even if the levels of stored GAG are reduced by ERT, disruptions in the growth plate and remnants of cartilage within bone are not likely to be corrected once they have been established.

Progressive decreased joint range of motion is a common feature in MPS I, II and VI. Inflammation has been shown to play a critical role in the joint and articular cartilage disease in MPS animals [68]. Simonaro and colleagues first reported in MPS VI animal that articular chondrocytes undergo a high rate of apoptosis and release nitric oxide and inflammatory cytokines [69]. The accumulation of GAG results in an activation of a specific toll-like receptor (TLR4) which leads to an inflammatory cascade due to the release of TNF-alpha and other inflammatory cytokines from chondrocytes [70]. The subsequent increased chondrocyte apoptosis and the release of matrix metalloproteinase contribute to the joint destruction and abnormal function [71].

### 3.3. Pathophysiology of airway disease

Airway involvement in the MPS includes upper airway disease, lower airway disease, and restrictive lung disease [72,73]. In the upper airway, narrowed or blocked nasal passages, macroglossia, decreased jaw range of motion, enlarged adenoids and tonsils and increased redundant (folds) supraglottic tissues can lead to obstructive sleep apnea [73,74]. Lower airway disease is primarily caused by tracheomalacia and the narrowing of the tracheal caliber that develop over time due to infiltration of the submucosa and tracheal cartilage with GAG [75,76]. Restrictive lung disease is caused by inefficient mechanical properties of the chest secondary to skeletal abnormalities. The antero-posterior diameter of the chest may be increased placing the ribs in a more horizontal position. Widened "oar shaped" ribs, a short thorax, kyphosis, scoliosis, or kyphoscoliosis are common. Further limitations may be caused by elevation of the diaphragm due to hepatosplenomegaly [77].

Although the clinical features associated with upper airway disease in MPS, such as narrowed or blocked nasal passages, macroglossia, increased redundant supraglottic tissues and enlarged adenoids and tonsils, may improve with ERT, tracheal abnormalities appear to persist. MPS VI rat studies have confirmed that ERT treatment is associated with improvements in mean tracheal cross-section area in treated animals as compared with controls, but normalization does not occur [71]. The skeletal abnormalities contributing to restrictive lung disease generally do not improve with ERT.

## 4. Summary: the benefits and limitations of ERT for the MPS

ERT for MPS I, II, and VI may produce improvements in walking ability, endurance, and pulmonary function as evidenced by the pivotal trials and extension studies. Cardiac valve disease, JROM, and skeletal disease, on the other hand, do not generally seem to improve with ERT if pathological changes are already present when ERT is started. Airway disease improves, but usually does not normalize. An appreciation of the pathophysiology of the MPS and the multi-faceted role of GAG in the body makes it clear that primary GAG storage is not fully responsible for all of the disease features. Disruption of GAG catabolism has far-reaching consequences both intracellularly and extracellularly due to the triggering of secondary pathogenic cascades. It appears that many of the consequences of these secondary pathogenic cascades, while they may improve, cannot be fully corrected even with long-term ERT. Further studies of ERT in younger patients or in patients without clinically significant somatic disease at baseline are warranted to determine if ERT can prevent the onset of disease features, thereby dramatically altering the natural history of the disease.

## 5. Rationale for early initiation of ERT: an expert opinion

The MPS are progressive heterogeneous lysosomal storage disorders with early morbidity and premature mortality. The cardiac, pulmonary, and musculoskeletal systems are the major contributors to the somatic clinical burden in patients with MPS. The development and availability of IV ERT has resulted in clinical improvement, but current usage has not realized the full potential of this therapy. The long-term outcomes of MPS patients receiving IV ERT have demonstrated that many disease manifestations are not reversible and that prevention should be the expectation. The concept that improved clinical outcomes can be achieved by starting ERT early is supported by the observation that lysosomal GAG storage occurs prenatally, animal studies showing improved outcomes with very early treatment, clinical studies in young patients demonstrating an acceptable safety profile, case reports showing the benefit of early versus later treatment in siblings, and personal clinical experience.

Enzyme replacement therapies for MPS I, II and VI were approved based on double-blinded clinical trials in which clinically meaningful somatic improvements occurred. The patients enrolled were 5 years and older, were intellectually intact, and had significant somatic disease at the onset of treatment. From these trials and other data described above, it is clear that ERT in established disease does not result in normalization/correction of somatic disease, but at best results in some improvements in certain disease manifestations, with stabilization to slow deterioration in others.

The progressive nature of the MPS disorders suggests that ERT should be initiated as soon as possible after diagnosis—perhaps even *before* obvious clinical features are apparent. Indeed, studies of tissues from affected aborted fetuses have demonstrated that GAG storage begins prenatally. Using EM, GAG storage was seen in the mesenchymal cells of the liver and spleen and neurons of the spinal ganglia, spinal cord, and brain from a 22-week-old fetus with MPS II [78]. Another group examined tissues from four fetuses aged 18–30 weeks gestation

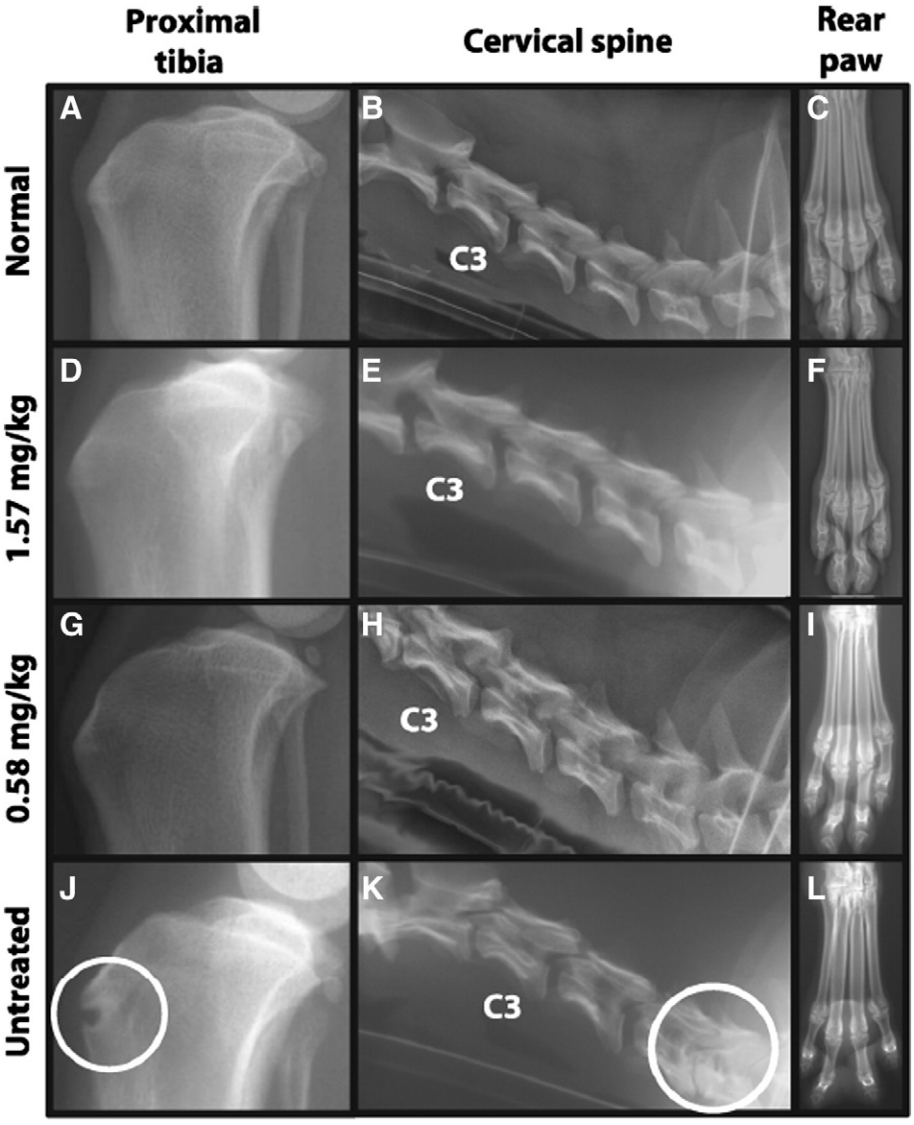


**Fig. 1.** (A to I) Effects of intravenous laronidase from birth on skeletal abnormalities in MPS I dogs. Radiographs of normal (A to C), treated (1.57 mg/kg) MPS I-affected canines (D to F), treated (0.58 mg/kg) MPS I-affected canines (G to I), and untreated MPS I-affected canines (J to L). C3, third cervical vertebra (B, E, H, and K). A physeal remnant/open physis (circled) is seen in an untreated 17-month-old animal (J). (K) Severe narrowing of the intervertebral disc space (circled) in an untreated, affected dog and moderate intervertebral space narrowing in a weekly treated dog (0.58 mg/kg) (H), not seen in (B), a normal dog, or (E) a treated dog (1.57 mg/kg per week). Also evident in (K) is C2–C3 narrowing of the intervertebral space. (L) Toe splaying seen in untreated animal, but not seen in normal or treated dogs (C, F, and I).
Reproduced from 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.

(two MPS I-Hurler, one MPS II, and one MPS III) and found storage vacuoles in hepatocytes, Kupffer cells, splenic reticulo-histiocytic cells, parietal cells of Bowman's capsule, heart valves, fibroblasts, endothelial and smooth muscle cells, chondrocytes, and cells of the peripheral and central nervous system [79].

Several studies have investigated whether outcomes improve when ERT is begun from birth in animal models of MPS I and VI. For MPS I, intravenous (IV) laronidase treatment (either 0.58 mg/kg or 1.57 mg/kg weekly) was initiated in affected model dogs between 3 and 23 days of age and was continued for 56 to 81 weeks [80]. At the end of the study, the GAG levels in the liver, spleen, lung, myocardium, renal cortex, and renal medulla were at or below the normal range of values in all treated dogs, and liver size was normal. Of note, cardiac valvular GAG content and mean anterior mitral leaflet thickness were normal in all treated animals. Skeletal abnormalities were reduced in the low-dose group of treated dogs and were nearly completely prevented in the high-dose group of treated dogs (Fig. 1). Similar results have been reported for MPS VI cats who initiated ERT between 14 and 58 h after birth and continued on weekly treatment for 5, 6 or 11 months [81]. Treated cats had improved bone quality, density, and dimensions, and GAG storage in heart valves was nearly normal. Recent studies in the MPS I mouse have demonstrated that IV ERT started at birth compared to two months of age improves outcome in difficult-to-treat organs, such as aorta and heart valves [82].

Sibling case studies from human patients report similar findings documenting the benefits of early treatment in patients with MPS. In MPS I, a 5-month-old boy who was pre-symptomatic began laronidase after diagnosis at 3 days of age. His older sister was diagnosed with MPS I Hurler–Scheie at 4.5 years of age. After 5 years of ERT, the boy displayed no signs of coarse facies, joint disease, organomegaly, cardiac

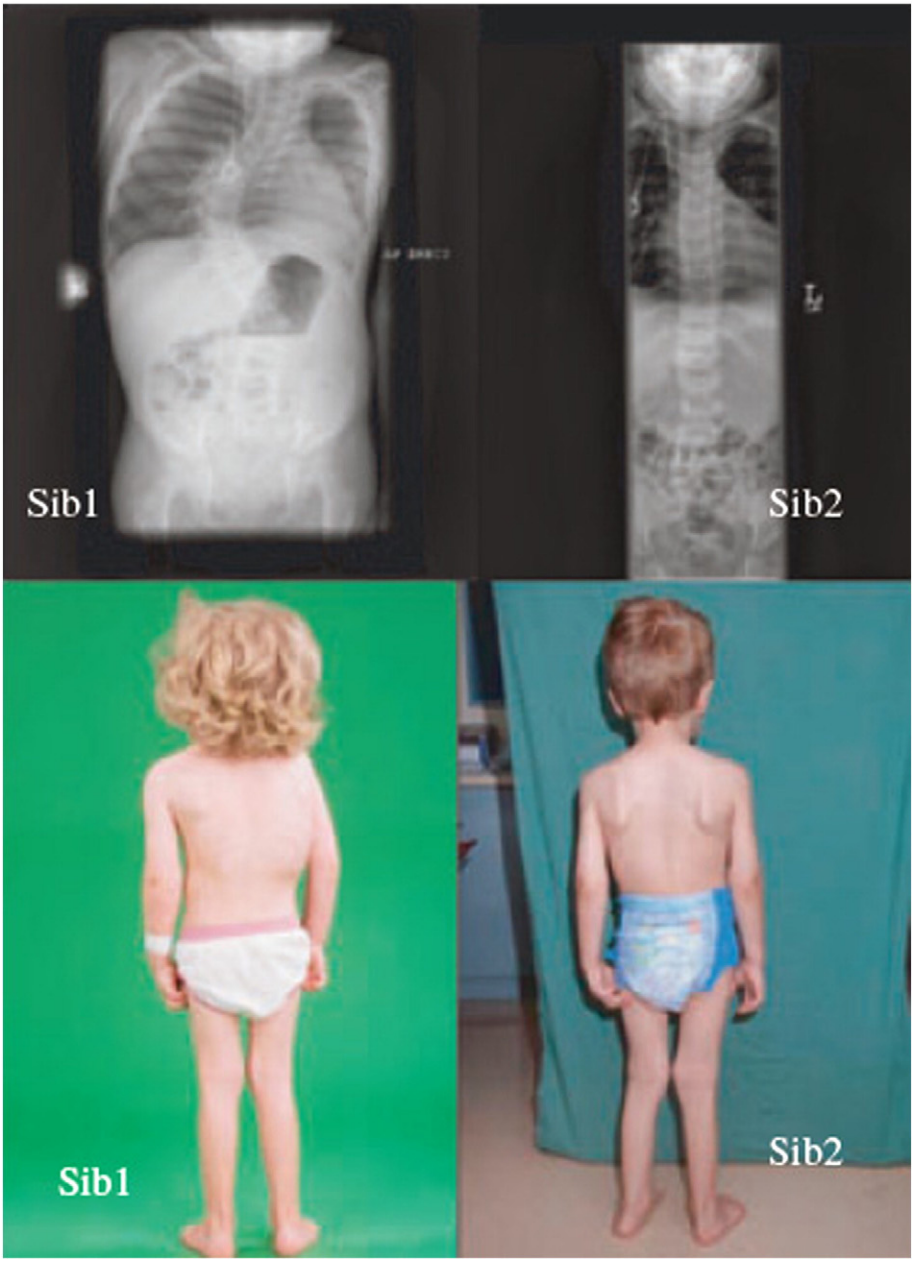


**Fig. 2.** Scoliosis in the older affected sister (left) of the sibling pair with MPS I at 3.6 years of age. Sibling 1 and Sibling 2 are both affected with MPS I and are both pictured at 3.6 years of age. Sibling 1 had not received laronidase at this time, while Sibling 2 had received 182 weeks of laronidase after being diagnosed prenatally.
Reproduced from McGill JJ, Inwood AC, Coman DJ, Lipke ML, de Lore D, Swiedler SJ, et al. Enzyme replacement therapy for mucopolysaccharidosis VI from 8 weeks of age—a sibling control study. Clin Genet 2010;77:492–8.

valve disease, or dysostosis multiplex. The only sign of MPS I was mild, stable corneal clouding. The older sister at 4 years of age experienced joint stiffness, thick skin, corneal clouding, hepatosplenomegaly, moderate mitral insufficiency with thickened valve leaflets and anterior edge prolapse, mild left atrium thickening, dysostosis multiplex, and obstructive hydrocephalus which required shunting [83]. A similar case study has been published for MPS VI in which two affected siblings were compared at the age of 3.6 years. The older sister had not received ERT at that age; the younger brother was diagnosed prenatally and was started on ERT at 8 weeks of age. At the age of 3.6 years, the older sister presented with scoliosis (32° and fixed; Fig. 2), short stature, moderate joint restriction in most joints, claw hands, mild mitral valve incompetence, hepatomegaly, and coarse facies. At the same age, but after 182 weeks of ERT, the younger brother presented with no scoliosis (Fig. 2), normal stature, mild joint restriction in shoulder only, normal hands, normal cardiac features, normal liver and spleen size, and normal facial appearance [84]. For MPS II, a sibling case report was published in which an affected boy was treated with ERT from 3 months of age [85]. After 3 years of treatment, the only somatic sign of the disease was a mild deformity of one vertebra. No other disease features were found. This boy was diagnosed very early because of an older, affected sister who began ERT at 7.5 years of age. Unlike her younger brother, her disease continued on a severe progressive course despite 3 years of ERT. A more traditional sibling case study was recently published for MPS II, with similar findings. Two Japanese brothers with MPS II caused by a complex rearrangement between the *IDS* gene and the *IDS-2* psuedogene were followed [86]. The older brother began treatment with idursulfase at 3.0 years of age, while the younger sibling started treatment at 4 months of age. At the start of treatment, the older brother showed typical somatic features of MPS II, including mitral valve regurgitation, gibbus deformity, joint stiffness, umbilical hernia, coarse facies, short stature, hepatomegaly, and cognitive impairment. After 2 years of treatment, the older brother's somatic disease was stable or improved, while the cognitive decline continued. After 32 months of ERT (age 3.0 years), the younger brother remained free from most of the somatic features that had already appeared in his brother at the same age. His only apparent disease manifestations were otitis media with effusion, mild dysostosis multiplex, and mild cognitive impairment.

Although safety has been a concern with very young patients, the clinical trial in MPS I patients under the age of 5 years has demonstrated that laronidase produces somatic improvements, with no new safety concerns. A similar study has been conducted for MPS II on the use of idursulfase in children under 7 years old (NCT00607386). The authors reported similar effects of ERT upon uGAG levels and liver and spleen size as have been previously reported for older patients, with no new safety concerns found [23]. A review of the clinical experience of MPS II patients under the age of 5 years in the Hunter Outcome Survey [87] also reported reductions in uGAG concentration and decreased liver and spleen size with ERT, with no new safety concerns noted in this younger population [11,88].

In summary, many lines of evidence, albeit with limited data, support my opinion that treatment of patients with MPS should occur early, at least at the onset of clinical disease and most likely presymptomatically (before onset of significant clinical disease) to obtain better long-term outcomes. Lysosomal storage has been demonstrated to occur in affected MPS fetuses prior to 30 weeks gestation, supporting early initiation of ERT. The animal data are more compelling and suggest that early treatment can prevent the progression of some of the clinical disease features that occur in MPS. The handful of sibling case study reports demonstrating rather remarkable outcomes in the younger siblings treated from shortly after birth, with no new safety concerns seen, further support the animal study data. Certainly there are no data whatsoever to suggest that waiting to treat results in a better outcome for this population, while there are some data and clinical experience to support commencing ERT as soon as possible. With this in mind, I support the use of ERT in pre-symptomatic infants with a confirmed diagnosis of MPS as a treatment option, given that, in my opinion, IV ERT is much better at preventing than correcting clinical disease in MPS. A strong need for further clinical trials in this area remains.

## Conflict of interest

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