Enzyme Replacement Therapy for Lysosomal Storage Diseases

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Abstract

Therapies for lysosomal storage disorders (LSDs) have been developed clinically and experimentally (Table 1). These include hematopoietic stem cell therapy (HSCT).

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enzyme replacement therapy (ERT), and gene therapy, all of which lead to the partial restoration of enzyme activity. Substrate reduction therapy (SRT) and the use of pharmacological chaperones have also been attempted. Although HSCT is not entirely effective, impairment of cognitive function has been prevented if the patients are treated at an early stage. However, HSCT still brings concerns because of the high morbidity and mortality rates. Gene therapy is still experimental at this moment. SRT is orally administered and clinical assessment is under investigation.

Treating LSDs with ERT relies on the cellular uptake of exogenous enzyme by receptor-mediated endocytosis. ERT using macrophage-targeted recombinant β-glucocerebrosidase is successful in treating the nonneuronopathic form of Gaucher disease, which opened the door to the development of this treatment for further LSDs. ERT was also approved for use in patients with Fabry and Pompe diseases, mucopolysaccharidosis I (MPS I), MPS II, and MPS VI. Patients treated with ERT had clinical improvement of somatic manifestations and improved quality of life. However, there are several limitations with current regular ERT: 1) immunological issues (raising the antibody level leads to reduced efficacy), 2) rapid clearance from blood circulation, 3) limited effect on neurological and skeletal symptoms, 4) high cost, and 5) lifelong dependence on weekly infusions.

Thus, in spite of substantial success of ERT, no curative therapies exist for LSDs, especially with bone dysplasia and central nervous system (CNS) involvement. Supportive measures are also used to treat the clinical manifestations of LSDs. Medications are used for palliative care, such as non-steroidal anti-inflammatory drugs for joint pain, antibiotics for pulmonary infections and oxygen supplementation for pulmonary compromise. Surgical interventions such as cervical fusion, spinal cord decompression, osteotomy, and hip replacement are often required.

In this chapter, we describe the efficacy of ERT and its limitations in comparison with other therapeutic options available for a particular group of LSDs, The Mucopolysaccharidoses (MPS).

I. Introduction

Enzyme replacement therapy (ERT) is an established strategy of treating lysosomal storage diseases (LSDs) including mucopolysaccharidoses (MPS). Tremendous progress in research toward development of ERT has been made in the last three decades. ERT using genetically engineered human enzyme manufactured by recombinant DNA technology in high-output cell lines is an established treatment for several LSDs based on the unique ability of human cells to bind and transport exogenous enzyme into the lysosomal compartment. In Gaucher disease, delivery of enzyme to affected cells was achieved by modifying the N-linked carbohydrate on the enzyme to expose core mannose residues [1,2], enabling the enzyme to bind to the mannose receptor (MR), which is highly abundant on cells of the reticuloendothelial system [3,4]. These findings led to achievement of clinical management of Gaucher disease by ERT with dramatic clinical results [5-7]. The MPS are a group of LSDs caused by deficiency of the lysosomal enzymes needed to degrade glycosaminoglycans (GAGs), which are long unbranched polysaccharides consisting of repeating disaccharide units [8]. GAGs include: chondroitin sulfate (CS), dermatan sulfate (DS), heparan sulfate (HS), keratan sulfate (KS), and hyaluronan. Their catabolism may be blocked singly or in combination depending on the specific enzyme deficiency. Lysosomal accumulation of GAG molecules results in cell, tissue, and organ dysfunction. In MPS, the undegraded or partially
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degraded GAGs are stored in lysosomes and/or secreted into the blood stream and subsequently excreted in urine. There are 11 known enzyme deficiencies that give rise to seven distinct MPS.

### Table 1. Therapies available to reduce storage materials

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal models</th>
<th>Clinical trial (investigational)</th>
<th>Approved (standard care)</th>
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<tbody>
<tr>
<td><strong>Degradation</strong></td>
<td></td>
<td></td>
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<tr>
<td>ERT (intravenous)</td>
<td>I, II, IIA, IIB, IV, VI, VII</td>
<td>I, II, IIV, VI, VII</td>
<td>I, II</td>
</tr>
<tr>
<td>ERT (Intrathecal)</td>
<td>I, II, IIA, IIB, IV, VI, VII</td>
<td>I, II</td>
<td>IIA (planned in 2010)</td>
</tr>
<tr>
<td>Gene therapy</td>
<td>I, II, IIA, IIB, IV, VI, VII</td>
<td>I, II (unsuccessful)</td>
<td>IIA (planned in 2010)</td>
</tr>
<tr>
<td>HSCT</td>
<td>I, VI, VII</td>
<td>I, II, IIA, IIB, IV, VI, VII</td>
<td>IIA (under 2 years)</td>
</tr>
<tr>
<td>Chemical Chaperon</td>
<td>NA</td>
<td>NA</td>
<td></td>
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<tr>
<td><strong>SYNTHESIS</strong></td>
<td></td>
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<tr>
<td>SRT</td>
<td>I, II, IIA, IIB, IV, VII</td>
<td>I, II, IIA</td>
<td></td>
</tr>
<tr>
<td>SOT</td>
<td>IIA</td>
<td></td>
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</tr>
<tr>
<td>RNAI</td>
<td>IIA, IIB</td>
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Figure 1. A. Clinical picture of three-year-old female patient with the severe form of MPS I (Hurler syndrome) (kindly provided by Dr. Roberto Giugliani). At that time she was 3 year-old (weight 15kg, height 83cm). She had a marked lumbar kyphosis, coarse hair, abnormal facies, delay on speech, corneal clouding, mild articular restriction, hepatosplenomegaly, and upper airway infections. Somatic involvement was improved after starting of ERT. The genotype was p.W402X/p.W402X defining a severe form and the most popular mutation among Caucasian MPS I patients. B. Clinical pictures of MPS II patients (spectrum of disease severity) (kindly provided by Dr. T. Orii). Patients with Hunter syndrome were called MPS II following Hurler syndrome (MPS I) since Hunter and Hurler syndromes were caused by the deficiency of a different enzyme. The historical classification, an arbitrary categorization of symptom clusters, was subsequently proven to be inadequate. Biochemically, patients with the attenuated disease forms cannot be reliably separated from the most severely affected patient either, in all enzyme activity is low, certainly below 10 % of normal, and usually below 1 %. Therefore it is better to speak of MPS II as a spectrum of disease ranging from most severe to patients with an attenuated phenotype. The important clinical characteristics of MPS II are: 1) It affects most tissues and organs, 2) There is a spectrum of disease, both as regards severity as well as symptom heterogeneity, 3) It is a progressive disease, and eventually leads to the patient being left debilitated, 4) The morbidity is severe, with patients losing dexterity, mobility, vision, hearing, having severe
cardiac and respiratory conditions, and so on. In most patients life span is reduced, at least if the disease is left untreated (long term data on HSCT or ERT is under investigation).

Clinical Features of MPS: The MPS share many clinical features, although in variable degrees. Most MPS patients are asymptomatic at birth, with subsequent onset of clinical signs and symptoms. These include a chronic and progressive course, multisystem involvement, organomegaly, dysostosis multiplex, and abnormal facies. Hearing, vision, airway, cardiovascular function, and musculoskeletal and connective tissue are also affected. Profound neurological impairment is a characteristic of MPS I (Hurler syndrome) (Figure 1A), the severe form of MPS II (Hunter syndrome) (Figure 1B, Figure 2), all subtypes of the MPS III (Sanfilippo syndrome) (Figure 3), and MPS VII (Sly syndrome) (Figure 4A), while MPS IV (Morquio syndrome) (Figures 4B,5,6) and MPS VI (Maroteaux-Lamy syndrome) (Figure 7) have characteristic bone lesions without CNS involvement. There is clinical similarity between different enzyme deficiencies and, conversely, a wide spectrum of clinical severity within any one enzyme [8]. In nearly all cases, except for the milder ones, the disease is fatal with an average life expectancy of one to two decades without treatment.

Figure 2. Skeletal/joint problem in an MPS II patient with the attenuated form (20 years old; 120 cm tall) (copyright belongs to Gifu and Saint Louis Universities). A. Hip and Knee restriction and contractures. B. Metatarsal abnormalities and contractures of toes. C. Shoulder and elbow restriction and contracture. D. Claw hands. Although this patient had visceral and skeletal/joint involvement, no mental retardation has been recognized. Hepatosplenomegaly and the range of motion for the joints were improved after ERT at the age of 20 years.
Incidence: The MPS can be found worldwide. Although the incidence of MPS is estimated as 1:25,000 live births, the incidence of a particular type of MPS varies [9]. There are no studies about the incidence of MPS in the USA, but we estimate that approximately 200 newborns affected with MPS are born annually.

Figure 3. A. Progression of the disease in an MPS III male patient with the severe form (kindly provided by Japanese Society of the Parents and the Families with MPS). There are four types of MPS III based on enzyme deficiency (MPS IIIA, B, C and D). Patients make up biochemically diverse, but clinically similar. MPS III is a progressive disorder with multiple organ and tissue involvement. Patients have variable onset but usually obvious between 2 and 8 years of age. Progressive and severe central nervous system degeneration is characteristic with mild somatic and skeletal disease. The patients survive into the 2nd decade. Airway obstruction and cardiac failure are the most common direct causes of death. This patient had no sign and symptom at 6 months old with normal appearance. However, at three to five years old, he had started with delayed development, restlessness, and hyperactivity. By eight year old, he revealed very difficult behavior (aggressively, temper tantrums, destructive behavior, and physical aggression) and insomnia. Language and understanding was gradually lost: speech development delayed and poor articulation. By 14 years old, he developed severe neurologic degeneration and rapid deterioration of social and adaptive skills. He slowed down and lost the ability to walk. B. MRI in a five year-old MPS IIIA patient. Brain cortex is markedly atrophic. High density signal is observed in cortex by T2-weighted axial image.
Figure 4. A. Clinical pictures of three patients with an attenuated form of MPS VII (kindly provided by Dr. T. Orii). Case 1: An 8-year-old girl homozygous for the p.A619V mutation, had a successful allogeneic BMT at 12 years old, donored by an HLA-identical unrelated female to replace the deficient enzyme (see the text). She revealed the clinical features of hepatosplenomegaly, umbilical herniation, slight bone deformities, moderate mental retardation and short stature. Case 2: 24 year-old boy homozygous for the p.A619V mutation, showed similar clinical features as Case 1 expressed. Case 3: A 7 year-old girl homozygous for p.R382C mutation, revealed unique clinical features of umbilical herniation, Morquio-like severe bone deformities, short stature, normal intelligence, hepatosplenomegaly, normal facies and no abnormal granules.

B. Clinical pictures of MPS IVA patients (Copyright permission from Gifu University). Case 1: This 31 year old female patient with a severe form of MPS VII shows skeletal deformities typical of Morquio A syndrome. She is 90 cm tall, showing pectus carinatum, bilateral genu valgum, diffuse corneal clouding, atlantoaxial subluxation, and hyper laxity of joints. The genotype is c.1288_1289delCA/c.1288_1289delCA. Case 2: This 18 year old male patient with an intermediate form is 135 cm tall with the milder skeletal deformities of pectus carinatum, bilateral genu valgum, and hyper laxity of joints and corneal clouding. The genotype is p.R94G/p.N204K. Case 3: This 25 year old male patient with a mild form is 157 cm tall and has milder skeletal deformities of thoracolumbar gibbus, mild platyspondyly and anterior wedging of the bodies in X-rays but no genu valgum. The genotype is p.N204K/p.N204K. All three patients have normal intelligence.
Figure 5. Top panel: Skeletal/joint problem in an MPS IVA patient (10 years old; 123 cm tall) (Copyright permission from International Morquio Organization). The genotype is p.M41L/ p.M41L defining the attenuated phenotype. He had characteristic features of MPS IVA and underwent several surgical operations such as osteotomy, hip surgery and cervical fusion. A. Knock-knee; B. Metatarsal abnormalities and flat foot with sandal gap; C. Floppy wrist; D. Protrusion of the chest and deformity of the ribs; E. Laxity of joint. Bottom panel: Skeletal problems in an MPS IVA patient (3 years old; 85 cm tall) (Copyright permission from International Morquio Organization). This three year-old male patient showed a severe bone deformity: protrusion of the chest, kyphosis, and genu valgum are characteristic with waddling gait.
Figure 6. A. Lateral view of progressive changes of the spine with age in the patient (Copyright permission from International Morquio Organization). 1 day: a sacral dimple was noted at delivery and suspected anterior beaking at the level of L2 was seen. 2 months: the anterior beaking was more prominent at the level of L2 with kyphosis. 15 months: Flaring of the anterior lateral ribs was observed. Kyphosis centered at L2 and flamed-shaped anterior beaking of the vertebral bodies were marked. 32 months: Accentuated dorsal thoracolumbar kypholordosis with gibbous deformity was remarkable. Advanced platyspondyly, irregularity, and anterior beaking of the vertebral bodies characteristic of Morquio syndrome were prominent. Mutation analysis of the GALNS gene by PCR of all exons and subsequent direct sequencing indicated that the patient had a novel missense mutation with c.278T>A in exon 3. The mutation present in the other allele was not identified. At the current age of 5 years and 8 months, the patient has nearly stopped growing at height of 97.7 cm and at weight of 15.7 kg. The patient has a majority of the common skeletal findings of MPS IVA including prominent chest and forehead, hypermobile joints, wrist weakness, diffuse platyspondyly, hypoplasia of the superior aspect of the odontoid process of C2, narrowing of cranio cervical junction, mild scoliosis, thoracolumbar kyphosis and mild organomegaly. B. MRI of a 4 year-old Morquio A patient (Copyright permission from International Morquio Organization). A baseline study of the upper cervical anatomy is recommended no later than 2 years or at diagnosis using flexion/extension X-ray films. If severe pain or pain associated with weakness of strength or tremors (or clonus) in the arms or legs occur, the patient should have studies of the neck to evaluate for the slippage (subluxation) of the neck vertebrae and compression of the spinal cord. MRI will be taken with the head bent forward (flexion) and with the neck back (extension) and will be monitored annually to check the situation of cervical spine in a patient aged. This MRI shows substantial spinal cord compression in C1-C2 and L1-L2 portion.
Figure 7. Progression of the disease in a female patient with the severe form of MPS VI (kindly provided by Dr. R. Giugliani). This patient was diagnosed at 1 year old with mild visceral and skeletal disease. She is 10 year-old with the progression of the disease. She sleeps with continuous positive airway pressure (CPAP) and has mitral valve thickening, MRI with hydrocephalus (mild - conservative management), corneal clouding and neuro-sensorial deafness. Her intelligence is normal. The patient attends normal school but has difficult in walking because of the skeletal disease. Her height and weight are 97 cm and 18 kg.

**Therapeutic options:** Therapies for MPS have been developed experimentally and clinically. These include hematopoietic stem cell therapy (HSCT), ERT, gene therapy, and substrate reduction therapy (SRT), all of which lead to the partial restoration of the enzyme activity or inhibition of synthesis of GAGs. HSCT is not entirely effective and has a relatively high mortality rate mainly due to the development of graft versus host disease [10-11]. Treating MPS with ERT relies on the cellular uptake of the enzyme by receptor-mediated endocytosis.

ERT tested in animal models has been quite successful leading to application in human patients [12-16]. ERT was FDA approved for use in patients with MPS I [8,17], MPS II [18,19] and MPS VI [20-23]. Patients treated with ERT had clinical improvement of somatic manifestations and improved quality of life. Additional trials for MPS IVA and other type of MPS are under way. ERT holds much promise for the treatment of MPS. However, there are several limitations for ERT. 1) Current experiences with MPS animal models and human clinical trials indicate that it is unlikely that therapeutic amounts of enzyme: i) cross the blood brain barrier to provide correction of the CNS pathology and ii) reach the bone cells to correct the skeletal pathology. Thus, only a small fraction of enzyme is delivered to bone and brain, resulting in an unmet challenge to restore such lesions. 2) Over 50% of the patients treated with regular ERT produce antibodies against the enzyme and have immune reactions [24-26]. 3) *In vivo*, proteins are unstable and rapidly eliminated from circulation because of clearance...
via glomerular filtration, endocytosis, phagocytosis, enzyme degradation, inhibitors, and immune system processing [27-28]. Thus, ERT is not a complete curative strategy for MPS disorders. As an alternative option, experimental gene therapies, HSCT and SRT for MPS are under way in vivo [29-31] (Figure 8). Other supportive therapies such as surgical operations, rehabilitation, and anti-inflammatory drugs are considered in combination.

![Diagram of potential therapeutic approaches for MPS](image)

Figure 8. Potential therapeutic approaches for MPS.

Overall, medical care is directed at treating systemic conditions and improving the person's quality of life. Supportive or symptomatic management can improve the quality of life for affected individuals and their families. Follow-up of affected individuals to anticipate possible complications and to provide early intervention maximizes outcome. We review the optional therapies for MPS especially ERT and summarize advantages and disadvantages of this therapy in comparison with other options (Table 2).

<table>
<thead>
<tr>
<th>Table 2. Comparison among therapies</th>
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ERT  
Gene Therapy  
HSCT  
Chemical Chaperon  

GAGs  
Monosaccharides  

Substrate reduction therapy  
Substrate optimization therapy  
RNAi therapy  

DEGRADATION  
SYNTHESIS
Although HSCT is generally supposed to have a high incidence of mortality rate, most cases were performed in a progressive stage and above 2 years. Therefore, there is no statistically significant clinical outcome when the patients are treated at the presymptomatic stage. NA: not available.

II. Enzyme Replacement Therapy for MPS

ERTs are currently in use or are being tested for use in MPS. ERT has proven useful in reducing non-neurological symptoms and pain. ERT for MPS type I, II and VI are available and ERT for MPS IVA is under clinical trial. Current ERT does not cross the blood-brain barrier (BBB), therefore no effect on CNS disease is anticipated. With the success of ERT for MPS demonstrated by clinical trials, an increased effort is underway to improve responsiveness to ERT and to develop other forms of therapy directed at areas/organs that may not be responsive to ERT.

Clinical Trials

Mucopolysaccharidosis I (MPS I):

1. Phase I/II clinical trial: The Phase I/II open label study included ten individuals with attenuated MPS I aged from five to 22 years, treated with doses of 0.58 mg/kg of human α-L-iduronidase once a week for 52 weeks. This study showed improvement in liver size, growth, joint mobility, breathing, sleep apnea and urinary GAG excretion. Increased ability to perform daily functions was reported [17]. A six-year follow-up of five of the treated individuals showed sustained improvements in joint range of motion and sleep apnea and no progression of heart disease, but evidence of progression of valvular involvement [32].

2. Phase II/III clinical trial: The Phase III double-blind placebo-controlled study included 45 individuals with MPS I (most classified as Hurler-Scheie) treated for 52 weeks with a 26-week placebo phase [33]. This study showed statistically significant improvements in pulmonary function and a six-minute walk test (6MWT) and clear biologic effect with reduction in urinary GAG excretion and liver volume. Patients who had significant sleep apnea and difficulties in shoulder flexion at the beginning of the study improved significantly. Laronidase was well tolerated and nearly all patients developed IgG antibodies without apparent clinical effect. In 2009, Clarke et al. published the results of a prospective, open-label study, with 40 patients, aged from six to 43 years, who received 0.58 mg/kg of laronidase weekly for 182 weeks.
This study confirmed a favorable safety profile and the improvements which had been seen in phase III study [34]. Other case reports representing smaller numbers of treated patients show variable responsiveness to treatment. The heterogeneity of treated patients published to date complicates any conclusions that can be drawn. It appears that the ability of ERT to reverse disease symptoms in individuals with attenuated disease relates closely to the burden of disease prior to commencement of treatment.

3. Phase IV studies: An open label prospective study evaluated 20 children with MPS I under five years of age (16 with Hurler syndrome and four with Hurler-Scheie syndrome) receiving laronidase 0.58 mg/kg or 1.16 mg/kg, weekly for 52 weeks. Out of the 20 patients, four received twice the dose in the second half of the study, as they showed GAG excretion greater than 200 mg/g creatinine at week 22. The main findings included good laronidase tolerance in patients under five years of age at both doses, reduction of GAG levels by approximately 50% in the 13th week of treatment and 61.3% at week 52. The liver edge decreased 69.5% at palpation. There was a decrease in the number of patients with left ventricular hypertrophy and apnea/hypopnea [35].

4. Other trial: An open label, multinational, randomized, 26 weeks long dose optimization study was reported in 2009 [36]. In this study four different regimens of laronidase (0.58 mg/kg every week, 1.2 mg/kg every two weeks, 1.2 mg/kg every week or 1.8 mg/kg every two weeks) were administered to 33 patients with MPS I. The four treatment regimens showed no significant differences in the reduction of urinary GAG excretion and liver size, and regimens were considered safe. Out of the four groups of patients, those who used the approved dose (0.58 mg/kg once a week) showed the least adverse effects. Although the long-term effects of the use of alternative regimes are unknown, the regimen of 1.2 mg/kg every two weeks may be, according to the authors, a convenient and acceptable alternative to patients, particularly those who have difficulties in performing weekly infusions.

Mucopolysaccharidosis II (MPS II):

1. Phase I/II clinical trial: A phase I/II study enrolled 12 patients into a randomized, double-blind, placebo controlled trial for 24 weeks followed by an open label extension study. The dose of idursulfase was 0.15, 0.5 and 1.5 mg/kg every other week for each group. Urinary GAG level were markedly decreased within 2 weeks following the initial dose. This reduction was maintained through 48 weeks. Both spleen and liver volumes were decreased at 24 and 48 weeks. The average walking distance in the 6MWT significantly improved after 12 months of idursulfase. The results of this trial indicated that idursulfase is generally well tolerated and it has effects on several aspects of MPS II that may confer clinical benefit with long-term therapy [37].

2. Phase III clinical trial: Clinical efficacy of ERT was shown in a randomized, double-blinded, placebo-controlled, multicenter and multinational study of 96 patients [19] in phase III clinical trial. Patients were randomized into three treatments arms: 0.5 mg/kg of idursulfase weekly, 0.5 mg/kg idursulfase every other week (EOW) and placebo. After one year of treatment, patients in the weekly idursulfase group
compared to the placebo group demonstrated statistically significant improvement of the primary endpoint, a composite score consisting of distance walked in 6MWT and the percent of predicted forced vital capacity based on the sum of ranks of change from baseline. A smaller difference was found for idursulfase EOW compared to placebo group. Liver and spleen volumes decreased by over 20% after 18 weeks of treatment in both idursulfase groups. After 53 weeks, about 80% of patients with hepatomegaly who were receiving idursulfase weekly or EOW had normal liver volume and spleen volume remained significantly reduced in these groups. The urinary GAG levels in the idursulfase groups were significantly different than that of the placebo group after one year of treatment and the response in the weekly group was significantly greater than that seen in the EOW group. An improvement in elbow mobility between the weekly idursulfase group compared to placebo was observed. Based on the larger clinical response in the weekly compared to the every other week group, idursulfase was approved for the treatment of MPS II in both the US and European Union at a dose of 0.5 mg/kg weekly.

3. Others: Patients with the severe form of MPS II treated with idursulfase would be expected to have somatic stabilization, but the overall benefit will have to be evaluated depending of the somatic disease burden and rate of progression of the CNS disease. According to Wraith et al (2006) [38], the patients with severe CNS involvement may be offered the possibility of treatment for a ‘trial’ period of 12-18 months, after which time a decision should be made as to whether to continue. Experience in treating children under age of 5 years with idursulfase is limited.

Mucopolysaccharidosis IVA (MPS IVA):

ERT by using recombinant human native GALNS is now under Phase I/II clinical trial for treatment of patients with MPS IVA. GALNS is produced in a continuous CHO cell line and is a purified form of the natural lysosomal enzyme GALNS. It will be anticipated that efficacy of therapy will be limited in a short term observation compared to other types of MPS since MPS IVA is a systemic bone disease and that careful long term observation is required to evaluate treatment.

Mucopolysaccharidosis VI (MPS VI):

Extensive preclinical studies were completed in a feline model of MPS VI [39] that established the dose, safety, as well as efficacy in reversing storage, and limiting bone disease when started very early in life. These preclinical studies were followed by Phase I/II, Phase II and Phase III human clinical trials [40]. Significant improvements in 12-minute-walk test, nearly significant improvement in 3-minute stair climb and significant reduction in urinary GAG levels were demonstrated in the 24-week, randomized, double-blind Phase III trial [41].

On the long-term follow-up, improvement in endurance on the walk test and stair climb, reduction in urinary GAG positive effects on puberty and growth, and pulmonary function were maintained in clinical study subjects from all trials, with observation times up to a maximum of five years with regular ERT [40,41].

A case study has shown improvement following ERT of pulmonary function leading to a reversal of tracheostomy intubation in an MPS VI patient [42]. Another case study has shown
reversal of papilledema and improved vision in an 11-year old MPS VI patient, followed on ERT maintained during follow-up of 130 weeks [43]. A case series of six MPS VI patients showed stabilization of visual acuity after treatment with ERT for 144 weeks [43].

Early introduction of recombinant human arylsulfatase B (rhASB) produced a state of immune tolerance and improved enzyme effectiveness in the cat model; hopefully, similar findings will be demonstrated in humans after early introduction of ERT [44]. An open label, single center case control study compared a pair of siblings, one of whom was diagnosed in utero [45]. Both were treated with rhASB, and the primary end-point was clinical status of the younger sibling at age 3.6 years after 182 weeks of therapy compared with sibling 1, who had no ERT at 3.6 years. No adverse effect occurred and the major differences were that sibling 2 had no scoliosis, minimal joint involvement, normal facial appearance, and normal cardiac valves. Both had decreased growth rate between birth and 3.6 years. This sibling control study provides the first evidence in patients with MPS VI that early initiation of ERT may provide an improved clinical outcome compared with reported studies of affected older children, adolescents and adults.

Unmet Challenges for ERT

1. Delivery of the enzyme to bone: Most of the enzyme-based drugs are delivered to major visceral organs like liver and spleen and only a small amount of enzyme is delivered to bone and brain. Many lysosomal enzymes have a short half-life because of rapid clearance in liver by carbohydrate-recognizing receptors, particularly the mannose receptor (MR) that is highly abundant on Kupffer cells [3] or the mannose-6-phosphate receptor (M6PR). Although a fraction of enzyme reaches bone marrow, a slight amount reaches bone, especially the cartilage cells in the avascular growth plate. Therefore, it is still a challenge to achieve clinical efficacy for the skeleton, especially for diseases with multi-bone involvement like MPS IVA. Thus, improvement of bone lesions in Gaucher patients by ERT is quite limited even after a long term treatment [44]. Similarly, very little improvement in bone was seen in clinical trials on MPS I. The current conventional ERT targeting both MR and M6PR may not work efficiently on bone and cartilage lesions. To solve the intrinsic issue, an alternative approach of ERT is devised in combination with bone targeting system. Hydroxyapatite (HA) is a positively-charged, major inorganic component in a hard tissue (bone) and does not exist in soft tissues. A drug attached to HA may be released in bone resorption process and targeting a drug on HA could be a potential strategy for a selective drug delivery to bone and cartilage cells. Estradiol uptake by bone was enhanced and osteoporosis was prevented by using six stretch of Glu (E6) targeted hormone [46-47]. This bone-targeting system was applied to a large molecule, an enzyme (tissue nonspecific alkaline phosphatase), showing that the tagged enzymes were delivered more efficiently to bone and that the clinical and pathological improvement in systemic bone disease, hypophosphatasia, was observed [48-49]. Human N-acetylgalactosamine-6-sulfate sulfatase (GALNS) which is deficient in MPS IVA patients was also bioengineered with the N-terminus extended by the hexa-glutamate sequence (E6) to improve targeting to bone (E6-GALNS). The E6-GALNS tagged enzyme
had markedly prolonged clearance from circulation, giving over 20 times exposure time in blood, compared to untagged enzyme. The tagged enzyme was retained longer in bone, with residual enzyme activity. The pathological findings in adult mice treated with tagged enzyme showed substantial clearance of the storage materials in bone, bone marrow and heart valves, especially after 24 weekly infusions. These findings indicate the feasibility of using tagged enzyme to enhance delivery and pathological effectiveness in MPS IVA mice [50].

2. Delivery of enzyme to the CNS: IV infusion of recombinant proteins does not lead to transfer of proteins across the BBB. Various means to provide enzyme to the CNS are currently being researched. These approaches include cerebrospinal fluid (CSF) instillation of enzyme via direct injection, continuous pumps, microcapsule implants, and production of chimeric recombinant proteins, enabling passage across the BBB. A clinical trial of intrathecal (IT) ERT for MPS I, II and VI is now underway in patients who have evidence of spinal cord involvement.

The IT ERT could hold promise as a treatment for the CNS manifestations of LSDs. Treatment via the CSF represents a potential method of delivering recombinant enzyme across the BBB. Experiments in animal models of MPS I, MPS II, MPS IIIA and MPS IIIB have shown that ERT delivered via the IT route distributes throughout the CNS and penetrates brain tissue, where it promotes clearance of lysosomal storage material. Studies are underway to investigate the safety and efficacy of IT ERT in patients with MPS I, II and VI [51-55]. The clinical trial of IT ERT for MPS IIIA is expected to start in 2010.

MPS VI patients often develop spinal cord compression during the course of the disease due to GAG storage within the cervical meninges, requiring neurosurgical intervention, as intravenous ERT is not expected to cross the BBB. In recent reports, the use of IT ERT was performed for a MPS VI child with spinal cord compression whose parents initially refused the surgical treatment. Assessments were performed at baseline, with clinical, neurological and biochemical evaluations, urodynamic studies and MRI of the CNS. Despite significant urodynamic improvement and some neurological amelioration, the patient developed worsening of walking capacity. After IT ERT was started, the patient presented with a generalized hypotonia and a life-saving surgical fixation of the neck was then performed. The results observed on this MPS VI patient suggest that instability of the cervical vertebrae could be unmasked by IV ERT as joint storage is reduced, and the decrease in neck stiffness and stability could confound the expected improvement of spinal cord compression manifestations following IT ERT. Thus, IT ERT for MPS should be evaluated carefully with assessment of its adverse effect [56].

Another approach for CNS involvement is to utilize alternative uptake mechanisms that rely on protein-based, rather than oligosaccharide-based, targeting determinants. Some studies have focused on the use of small basic peptides, like human immunodeficiency virus TAT, to enhance the delivery to specific tissues [57-58]. Others have demonstrated the feasibility of this approach using insulin-like growth factor-2, a peptide ligand for the M6PR or the low-density lipoprotein receptor [59-61].

The critical issue for ERT is that proteins have complex secondary and tertiary structures that make them susceptible to conformational changing factors which lead to instability [62]. In vivo, many proteins are unstable and/or rapidly eliminated from circulation because of clearance via glomerular filtration, endocytosis, phagocytosis, enzymatic degradation, inhibitors, and antibodies [27-28]. The previous clearance studies on phosphorylated
lysosomal enzymes showed a rapid clearance (half-life time): α-iduronidase, 0.9 min [62]; α-galactosidase A, 2 - 5 min [63]; glycosylasparaginase, 4 min [64]; β-glucuronidase, 5.2 min [65-66]; and GALNS, 3 min [67]. Recent studies have shown that longer treatment with higher doses of enzyme or enzyme modified to effect slower circulation resulted in improved therapeutic responses in brain in mouse model [66,58-71]. These results indicate that therapeutic enzyme can be delivered across the BBB if administered in higher doses than used in conventional ERT trials or if the enzyme is modified to attain a longer circulation during a certain period.

3. Side effects:

A. Infusion reactions: Infusion-related reactions that may occur with use of ERT are seen in treatment of LSDs. The etiology of the more severe forms of these non-allergic reactions, referred to as anaphylactoid, is unknown. Current evidence suggests that anaphylactoid (as opposed to anaphylactic) reactions are not immune mediated [72]. Anaphylactoid reactions refer to an identical clinical pattern with anaphylaxis, however it is non-IgE mediated. Certain allergens including drugs can trigger the mast cell cascade directly without involving IgE as the initial mediator. Anaphylactoid reactions therefore do not require prior sensitization as they are direct mass cell releasers and may produce anaphylaxis-like reactions in a dose-dependent manner. By contrast, classic anaphylaxis is not dose-dependent as the immune system is primed to recognize even minute amounts of the allergen and able to amplify the reaction via IgE mediation. For practical purposes, we can consider the clinical effects and management of anaphylaxis and anaphylactoid reactions to be identical.

Most infusion-related reactions are mild, manifest as brief, insignificant decreases or increases in heart rate, blood pressure, or respiratory rate. Other mild reactions are itching, rash, flushing, and headache. Mild reactions can usually be managed by slowing the infusion rate for several treatments and then slowly returning to the prior rate.

Pretreatment with anti-inflammatory drugs or anti-histamines is often done for ERT. If mild or moderate infusion reactions (e.g., dyspnea, urticaria, or systolic blood pressure changes) cannot be ameliorated by slowing the infusion rate, the addition of treatment one hour before infusion with diphenhydramine and acetaminophen (or ibuprofen) to the regimen usually resolves the problem. Pretreatment can typically be discontinued after six to ten weeks.

Severe non-allergic anaphylactoid reactions such as major changes in blood pressure, wheezing, stridor, rigors, or drop in oxygen saturations should be immediately addressed by stopping the infusion and giving appropriate doses of subcutaneous epinephrine, IV diphenhydramine, and hydrocortisone or methylprednisolone. Subsequent infusions should then be given at a significantly reduced rate with prednisone pretreatment 24 hours and eight hours before the infusion, diphenhydramine and acetaminophen or ibuprofen orally one hour before the infusion, and IV methylprednisolone just before beginning the infusion. It is unknown at this time whether the incidence or severity of infusion-related reactions is different for patients younger than age five years with severe respiratory compromise or with severe CNS disease.

B. Immune response: One intrinsic limitation of current ERT is the induction of anti-enzyme antibodies, leading to the neutralization of the infused protein by the immune system. ERT commonly produces a cellular or humoral immune response to the therapeutic enzyme, with reported rates of antibody formation in treated patients ranging from 13% for Gaucher
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**disease to 90% and 100% for MPS I, MPS VI and Pompe disease, respectively [26,73,74].** IgG anti-idursulfase antibodies were detected in 46.9% of MPS II patients in each idursulfase-treated group. The reduction in urinary GAG levels in antibody-positive patients was about two-thirds of that seen in antibody-negative patients. In contrast, there was no association with the presence of antibodies and adverse events or with clinical assessments.

Immune response may depend on the nature of the replaced protein, the genetic background of the patient, the dose of the infused enzyme, the frequency of treatment, the structural differences between the infused and defective protein, the presence or absence of residual mutant protein in the individual and other environmental factors that could increase the susceptibility to develop an immune response. The source of the protein in terms of processing, production, and post-manufacture storage and handling are additional factors that affect the immunogenicity of the enzyme [74,75]. There is a correlation between genetic mutations and immune response. Especially, patients with null type of mutation and subsequent severe clinical phenotypes could show a more vigorous immune response [74]. There are potentially two main adverse effects of an immune response to ERT. The first immune-mediated complication is the development of hypersensitivity (anaphylactic) reactions during or immediately after administration of the enzyme. The second potential complication involves the effect of circulating reactive antibodies to the replaced protein resulting in the reduction of the efficacy of ERT [73,74,76].

Antibody levels have not been precisely predictive of hypersensitivity or other adverse reactions to therapy, although the presence of antibodies is likely a factor in infusion-associated adverse events [26]. Thus, IgG antibodies against infused enzymes are present in most patients receiving ERT, although titers may wane with time in some patients [26,69]. Recent report shows that during ERT, urinary GAG excretion is higher in MPS I and MPS VI patients with very high antibody titers to enzyme, resulting in reduction in the efficacy of infused enzyme [26,77]. There is evidence in animal models that antibodies may partially neutralize the effect of ERT by reducing the efficiency of uptake and redirecting the enzyme to other target tissues [26,78]. The antibody probably binds to an epitope at or near the M6P modification and sterically inhibits the protein from binding to the M6PR and being taken up by the cell [79].

**C. Current treatments to immune response:** Protein therapeutics can be used as a drug for the treatment of diverse diseases and in some cases represents the only available therapeutic option. Therefore it is a particular challenge to avoid undesired side effects derived from the unexpected immunogenicity of these drugs, which may decrease their efficacy and safety [80]. In order to manage the immune response in patients, several immunosuppressive protocols have been tested, including methotrexate, mycophenolate mofetil and cyclosporin A with azathioprine [74,81]. A tolerization regimen was developed in a canine model for MPS I that prevents a strong antibody response to the enzyme during ERT by using cyclosporine A and azathioprine. In this study a decrease of immune response was obtained due to a profound T cell suppression based on the maintenance of high plasma levels of cyclosporine A and azathioprine [82]. A type 3 Gaucher patient, treated with ERT, developed a progressively increasing titer of IgG antibodies that blocked the catalytic activity of the enzyme. For a tolerant regimen, the patient was treated with a combination of plasma exchange (to reduce the concentration of circulating antibodies), cyclophosphamide (as an immune suppressor of B cells), intravenous immune globulins (to block Fc receptors and control immune reactivity) and large doses of enzyme. A methotrexate regimen was used as
an immunosuppressive protocol to reduce recombinant human acid α-glucosidase-specific antibody response in a mouse model [81]. The use of high amounts of protein in long-term treatments or the induction and maintenance of immunosuppression protocols to the patients in order to induce immune tolerance could be a disadvantage that raises adverse effect and increases the cost of the therapy. The tolerance to the infused enzyme was obtained after one to two years since the beginning of ERT. Those findings in preclinical and clinical trials indicate that the induction of immune tolerance to the enzyme has a direct relevance to the clinical management of the patient in terms of the efficacy and safety of treatments. Therefore, an alternative novel tolerance protocol that minimizes the immune response avoiding side-effects will be required.

III. Other therapeutic options

1. **HSCT**: HSCT using umbilical cord blood or bone marrow is a potential way of providing sufficient enzyme activity to slow or stop the progression of the disease. The beneficial effect of HSCT is thought to result from the replacement of deficient macrophages by marrow-derived donor macrophages (Kupffer cells; pulmonary, splenic, nodal, tonsilar, and peritoneal macrophages; and microglial cells) that constitute an ongoing source of normal enzyme capable of gaining access to the various sites of storage [84]. Although HSCT may modify disease progression and improve survival in some children, it is not curative. HSCT should be used only in carefully selected children with extensive pretransplantation clinical assessment and counseling in whom systematic long-term monitoring of the results will be possible.

In general, the clinical outcome of children undergoing HSCT is varied and depends on the degree of clinical involvement and the age of the child at the time of transplantation. Adults have not undergone HSCT. Failure to achieve stable engraftment and graft-versus-host disease represent significant barriers to successful HSCT for many children [85-87]; thus, the procedure carries a high risk of morbidity and mortality.

HSCT has been successful in reducing the progression of some findings in children with severe MPS I [88-90]. Although the heterogeneity of the disease makes interpretation of the outcomes of HSCT somewhat difficult, available data show that successful HSCT increases survival, reduces facial coarseness, as well as hepatosplenomegaly, improves hearing, and maintains normal heart function. In a series of individuals predicted to have severe MPS I based on the presence of known severe mutations, use of HSCT resulted in stabilization and improvement of cardiac function with regression of hypertrophy and normalization of chamber dimensions. In that cohort, HSCT did not appear to show significant effects on the presence and progression of valvular involvement [91]. In contrast, the skeletal manifestations and corneal clouding continued to progress at the same rate in children treated with HSCT as in untreated individuals [92].

Neuropsychological responses to HSCT vary and are related to the age and intellectual capacity of the child at the time of the engraftment. In children undergoing HSCT before evidence of significant developmental delay (i.e., usually between ages 12 and 18 months), HSCT appears to slow the course of cognitive decline. Children showing significant cognitive impairment prior to undergoing HSCT do not appear to benefit developmentally. Recent
reports showed positive cognitive effects on MPS II and III patients after HSCT if the patients are at an early stage [93].

In part because of increased longevity after HSCT, treated individuals develop increasing pain and stiffness of the hips and knees, carpal tunnel syndrome, spinal cord compression, and progressive thoracolumbar kyphosis. As a result, various orthopedic procedures intended to maintain function and gait have been performed post-HSCT.

We had experienced a successful MPS VII BMT case (Figure 4A: case 1). Within 5 months after BMT at 12 years old, the enzyme activity of the recipient's lymphocytes increased to normal range without acute or chronic GVHD. For the successive 31 months post-BMT, the enzyme activity in her lymphocytes was maintained at almost normal levels and excretion of urinary GAGs was greatly diminished. Ultrastructural findings demonstrated no abnormal vacuoles and inclusion bodies in the cytoplasm of her rectal mucosal cells. Especially notable were improvements in motor function. The patient was able to walk alone for a long time without aid, and she even became able to ride a bicycle and take a bath. In addition, recurrent infections of the upper respiratory tract and the middle ears decreased in frequency and severity, and dyspnea on exertion, severe snoring and vertigo have substantially improved. Thus, allogeneic BMT in this patient produced a better quality of life and provided a more promising outlook [94].

2. Combined ERT and HSCT: The use of ERT in the peri-HSCT period should be considered. In 26 patients [95] ERT used during this time period neither increased nor decreased the frequency of engraftment or survival. Whether ERT begun prior to transplantation and continued in the peri-transplantation period reduces the frequency of these complications is yet to be systematically reviewed. It is reasonable to suggest that the use of ERT prior to HSCT may alleviate disease manifestations, thus reducing complications during HSCT. Whether long-term combined ERT and HSCT may improve the outcome of a severely affected individual is of interest.

3. Substrate reduction therapy (SRT): Decreasing the quantity of stored substrate in LSDs is currently being investigated for the treatment of Gaucher disease [96]. Potential use of similar molecules (genistein) that may decrease the production of GAGs or other substances that are stored in MPS disease may have a future role in treatment. A gene expression-targeted isoflavone therapy (GET-IT) is another, though still experimental, option for treatment of MPS patients [97-98]. Nevertheless, currently, some individual MPS I, II, IIIA and IIIB patients are treated worldwide by SRT as an experimental therapy. This therapy is based on the use of genistein, a naturally occurring isoflavone (4’, 5, 7-trihydroxyisoflavone or 5, 7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one), which indirectly impairs the efficiency of GAG synthesis through inhibiting phosphorylation of epidermal growth factor receptor, which results in impairment of the specific signal transduction process and less efficient activation of expression of genes coding for some enzymes involved in GAG production [99]. Slow GAG synthesis appears to prevent further accumulation of these substances in patient’s cells, and residual activity of the deficient enzyme may cause a reduction in the level of already accumulated compounds and achievement of a new balance between GAG synthesis and degradation [100]. As such, GET-IT is a specific case of SRT, which groups the treatment procedures leading to impairment of production of compounds (substrates) that cannot be
efficiently degraded. The current dose of genistein used in human is 5 mg/kg daily. In this dose, there is a limited effect in patients. Therefore, the optimal dose of genistein must be explored carefully by monitoring genistein level in blood and clinical effects. In MPS IIIB mouse model, to achieve the clear clinical effect, the dose was increased to 160 mg/kg/day leading to a reversal of brain pathology and complete correction of mouse cognitive behavior [101-102]. Importantly, no adverse effects were observed in these mice even at doses as high as 160 mg/kg/day [101-102]. Positive effects of genistein on brain were also observed in MPS II mice [103]. Moreover, a recent promising report on optimized reduction therapy (Substrate Optimization Therapy) for HS could be applied to MPS I, II and III as well as MPS VII [104-106].

4. **RNA interference (RNAi):** Another way to reduce GAG synthesis is the use of RNAi phenomenon. This method appeared to be effective in cell culture experiments when siRNA or shRNA were employed [107,108]. However, an efficient delivery of specific RNA molecules to affected cells is still a problem in developing real therapies for humans.

5. **Non-steroid anti-inflammatory drug (NSAID) therapy:** NSAID therapy by aspirin has been experimentally performed on an MPS IIIA mouse model since pathways such as inflammation or oxidative stress have been highlighted in many neurodegenerative disorders, including LSDs as major contributors to the neuropathology (Figure 9). Treatment with aspirin led to the normalization of inflammation and oxidative stress-related mRNA levels, suggesting long term NSAID administration may be of potential benefit in MPS III and other types of MPS in combination with ERT, gene therapy and cell therapy [109].
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Figure 9. Potential mechanism of pathogenesis for MPS and future therapeutic pathway involving autophagy, inflammation, and ER stress. Primary storage material (GAG) triggers accumulation of the secondary products (GM2 ganglioside, GM3 ganglioside, disialoganglioside, subunit c of mitochondrial ATP synthase, ubiquitin, and/or cholesterol), which affects the process of autophagy, inflammation, alteration of Ca++ homeostasis. 1) Lysosomal storage leads to a reduced ability of lysosomes to fuse with autophagosomes. 2) This results in a block of autophagy maturation and defective degradation. 3) Consequently autophagy substrates such as polyubiquitinated protein aggregates and dysfunctional mitochondria accumulate and promote cell death. 4) The inflammatory response to cell damage further contributes to cell death [112,113]. By administering therapeutic agent which blocks the pathway or promote maturation of autophagosome, the secondary accumulation or response will be reduced.

ERT has little effect in bone tissue, so treating the skeletal changes is one of the objectives of new treatments. It was hypothesized that lysosomal and/or extracellular GAG storage in MPS disorders induces inflammation and altered growth of connective tissue and other cells through activation of Toll-like receptor 4 (TLR4) signaling pathway [110]. Hyaluran fragments and oligosaccharides released from the breakdown of the extracellular matrices also have been shown to signal through TLR4 providing further support for the concept that GAG storage in MPS disorders activates this pathway. The drug infliximab (Remicade®, Centocor Ortho Biotech, Inc) is an FDA-approved anti-TNF-α drug which showed to attenuate the inflammatory response in MPS VI animals, leading to improve joint pathology. The anti-inflammatory treatments should be evaluated in MPS patients alone or associated with ERT due to the possibility of reducing
inflammation, increase the accessibility of synovial tissues to recombinant proteins and improve the efficacy of ERT.

6. Others: Moreover, lysosomes are organelles central to degradation and recycling processes in animal cells. Most lysosomal genes exhibit coordinated transcriptional behavior and are regulated by the transcription factor EB (TFEB). Under aberrant lysosomal storage conditions, TFEB translocated from the cytoplasm to the nucleus, resulting in the activation of its target genes. TFEB overexpression in cultured cells induced lysosomal biogenesis and increased the degradation of complex molecules, such as GAGs in MPS IIIA mouse fibroblasts and neuronal stem cells. Thus, a genetic program controls lysosomal biogenesis and function, providing a potential therapeutic target to enhance cellular clearing in lysosomal storage disorders and neurodegenerative diseases [111].

Figure 10. Scheme of therapy for MPS. Currently, ERT, HSCT and SRT are available in clinical practice for MPS disorders. The decision about the appropriate modality of treatment depends on various factors, including prognosis of the disease, the age of presentation, genotype/phenotype correlations when known, the degree of organ involvement, the performance status of the patient at diagnosis, donor availability for HSCT, and the availability and feasibility of ERT. Generally, in the case of the untreated symptomatic patients, ERT and/or SRT should be the first option if available. It is reasonable to suggest that the use of ERT and/or SRT prior to HSCT may alleviate disease manifestations, thus reducing complications during HSCT. Certainly for HSCT to have any significant effect on CNS development, it should be done prior to age two years, preferably earlier. If neurological and cognitive benefits are not expected, it is not indicative for HSCT. When a newborn screening is established, prognosis should be carefully assessed prior to commencement of therapy.
IV. Conclusion

In the near future, we will have more therapeutic options in clinical practice in parallel with establishment of newborn screening for MPS. It is hoped that physicians will take the challenge to familiarize themselves with the most common symptom complexes, in order to suspect the disease and to refer the patients to an expert center. Physicians also should know therapies available on each type of MPS. It may be required to have tailored-made therapy for each individual patient. Hopefully this will lead to more patients being diagnosed earlier on, so adequate combination therapy can be provided and some irreparable damage can be prevented. Not only ERT, gene therapy, HSCT and SRT but other supportive treatments should be considered in combination (Figure 10).

It is unclear at present whether early diagnosis by newborn screening is beneficial from the aspect of early initiation of ERT for CNS involved MPS since no effect of regular ERT on children with the CNS form of the disease is anticipated. Consequently, SRT and/or ERT combination therapy and HSCT as a permanent therapy will be considered when the asymptomatic newborn is diagnosed.

References


[20] Harmatz, P; Whitley, CB; Waber, L; Pais, R; Steiner, R; Plecko, B; Kaplan, P; Simon, J; Butensky, E; Hopwood, JJ. Enzyme replacement therapy in mucopolysaccharidosis VI (Maroteaux-Lamy syndrome). J. Pediatr., 2004, 144, 574-580.

[21] Harmatz, P; Ketteridge, D; Giugliani, R; Guffon, N; Teles, EL; Miranda, MC; Yu, ZF; Swiedler, SJ; Hopwood, JJ. Direct comparison of measures of endurance, mobility, and joint function during enzyme-replacement therapy of mucopolysaccharidosis VI (Maroteaux-Lamy syndrome): results after 48 weeks in a phase 2 open-label clinical study of recombinant human N-acetylgalactosamine 4-sulfatase. Pediatrics, 2005, 115, e681-e689.

[22] Harmatz, P; Giugliani, R; Schwartz, I; Guffon, N; Teles, EL; Miranda, MC; Wraith, JE; Beck, M; Arash, L; Scarpa, M; et al. Enzyme replacement therapy for mucopolysaccharidosis VI: a phase 3, randomized, double-blind, placebo-controlled, multinational study of recombinant human N-acetylgalactosamine 4-sulfatase (recombinant human arylsulfatase B or rhASB) and follow-on, open-label extension study. J. Pediatr., 2006, 148, 533-539.

[23] Harmatz, P; Giugliani, R; Schwartz, IV; Guffon, N; Teles, EL; Miranda, MC; Wraith, JE; Beck, M; Arash, L; Scarpa, M; et al. Long-term follow-up of endurance and safety outcomes during enzyme replacement therapy for mucopolysaccharidosis VI: Final


[26] Dickson, P; Peinovich, M; McEntee, M; Lester, T; Le, S; Krieger, A; Manuel, H; Jabagat, C; Passage, M; Kakkis, ED. Immune tolerance improves the efficacy of enzyme replacement therapy in canine mucopolysaccharidosis I. J. Clin. Invest, 2008, 118, 2868-2876.


[29] Ellinwood, NM; Vite, CH; Haskins, ME. Gene therapy for lysosomal storage diseases: the lessons and promise of animal models. J. Gene Med., 2004, 6, 481-506


[32] Sifuentes, M; Doroshow, R; Hoft, R; Mason, G; Walot, I; Diamant, M; Okazaki, S; Huff, K; Cox, GF; Swiedler, SJ; Kakkis, ED. A follow-up study of MPS I patients treated with laronidase enzyme replacement therapy for 6 years. Mol Genet Metab, 2007, 90, 171–80.


[42] Valayannopoulos, V; Nicely, H; Harmatz, P; Turbeville, S; Mucopolysaccharidosis VI. Orphanet J. Rare Di, 2010, 5, 5.


[44] Millán, JL; Narisawa, S; Lemire, I; Lois, TP; Boileau, G; Leonard, P; Gramatikova, S; Terkeltaub, R; Camacho, NP; McKee, MD; et al. Enhancement of drug delivery to bone: characterization of human tissue-nonspecific alkaline phosphatase tagged with an acidic oligopeptide. Mol. Therapy, 2010, 18(6), 1094-1102.


Kakkis, E; McEntee, M; Vogler, C; Le, S; Levy, B; Belichenko, P; Mobley, W; Dickson, P; Hanson, S; Passage, M. 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.

Xia, H; Mao, Q; Davidson, BL. The HIV Tat protein transduction domain improves the biodistribution of beta-glucuronidase expressed from recombinant viral vectors. *Nat. Biotechnol.*, 2001, 19, 640-644.

Green, I; Christison, R; Voyce, CJ; Bundell, KR; Lindsay, MA. Protein transduction domains: are they delivering? *Trends Pharmacol Sci.*, 2003, 24, 213-215.


Prince, WS; McCormick, LM; Wendt, DJ; Fitzpatrick, PA; Schwartz, KL; Aguilera, AI; et al. Lipoprotein receptor binding, cellular uptake, and lysosomal delivery of fusions between the receptor-associated protein (RAP) and alpha-L-iduronidase or acid alpha-glucosidase. *J. Biol. Chem.*, 2004, 279, 35037-35046.


Sands, MS; Vogler, CA; Ohlemiller, KK; Roberts, MS; Grubb, JH; Levy, B; Sly, WS. Biodistribution, kinetics, and efficacy of highly phosphorylated and non-phosphorylated beta-glucuronidase in the murine model of mucopolysaccharidosis VII. *J. Biol. Chem.*, 2001, 276, 43160-43165.

Vogler, C; Levy, B; Grubb, JH; Galvin, N; Tan, Y; Kakkis, E; Pavloff, N; Sly, WS. Overcoming the blood-brain barrier with high-dose enzyme replacement therapy in murine mucopolysaccharidosis VII. *Proc. Natl. Acad. Sci.*, 2005, 102, 14777-14782.


[69] Matzner, U; Herbst, E; Hedayati, KK; Lüllmann-Rauch, R; Wessig, C; Schröder, S; Eistrup, C; Möller, C; Fogh, J; Gieselmann, V. Enzyme replacement improves nervous system pathology and function in a mouse model for metachromatic leukodystrophy. *Hum. Mol. Genet.*, 2005, 14, 1139–1152.


[81] Joseph, A; Munro, K; Housman, M; Garman, R; Richards, S. Immune tolerance induction to enzyme-replacement therapy by co-administration of short-term, low-dose


[87] Peters, C; Balthazor, M; Shapiro, EG; King, RJ; Kollman, C; Hegland, JD; et al. Outcome of unrelated donor bone marrow transplantation in 40 children with Hurler syndrome. *Blood*, 1996, 87, 4894–4902.


[89] Souillet, G; Guffon, N; Maire, I., Pujol, M; Taylor, P; Sevin, F; et al. Outcome of 27 patients with Hurler’s syndrome transplanted from either related or unrelated haematopoietic stem cell sources. *Bone Marrow Transplant.*, 2003, 31, 1105–1117.


[94] Yamada, Y; Kato, K; Sukegawa, K; Tomatsu, S; Fukuda, S; Emura, S; Kojima, S; Matsuyma, T; Sly, WS; Kondo, N; Orii, T. Treatment of MPS VII (Sly disease) by allogeneic BMT in a female with homozygous A619V mutation. *Bone Marrow Transplant.* 1998, 21, 629-634.

[95] Cox-Brinkman, J; Boelens, JJ; Wraith, JE; O’Meara, A; Veys, P; Wijburg, FA; et al. Haematopoietic cell transplantation (HCT) in combination with enzyme replacement therapy (ERT) in patients with Hurler syndrome. *Bone Marrow Transplant.*, 2006, 38, 17–21.


[104] Schuksz, M; Fuster, MM; Brown, JR; Crawford, BE; Ditto, DP; Lawrence, R; Glass, CA; Wang, L; Tor, Y; Esko, JD. Surf en, a small molecule antagonist of heparan sulfate. *Proc. Natl. Acad. Sci.*, 2008, 105, 13075-13080.

[105] Brown, JR; Yang, F; Sinha, A; Ramakrishnan, B; Tor, Y; Qasba, PK; Esko, JD. Deoxygenated disaccharide analogs as specific inhibitors of beta1-4-galactosyltransferase 1 and selectin-mediated tumor metastasis. *J. Biol. Chem.*, 2009, 284, 4952-4959.


[110] Simonaro, C; Ge, Y.; Eliyahu, E; He, K; Jepsen, K; Schuchman, E Involvement of the toll-like receptor 4 pathway and use of TNFα antagonists for treatment of the mucopolysaccharidoses PNAS 2010, 107(1), 222-227.

[111] Sardiello, M; Palmieri, M; di Ronza, A; Medina, DL; Valenza, M; Gennarino, VA; Di Malta, C; Donaudy, F; Embrione, V; Polishchuk, RS; Banfi, S; Parenti, G; Cattaneo, E; Ballabio, A. A gene network regulating lysosomal biogenesis and function. Science, 2009, 325(5939), 473-477.
