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Enzyme replacement therapy for treating mucopolysaccharidosis type IVA (Morquio A syndrome): effect and limitations

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Abstract

Introduction—Following a Phase III, randomized, double-blind, placebo (PBO)-controlled, multinational study in subjects with mucopolysaccharidosis IVA (MPS IVA), enzyme replacement therapy (ERT) of elosulfase alfa has been approved in several countries. The study was designed to evaluate safety and efficacy of elosulfase alfa in patients with MPS IVA aged 5 years and older.

Areas covered—Outcomes of clinical trials for MPS IVA have been described. Subjects received either 2.0 mg/kg/week, 2.0 mg/kg/every other week, or PBO, for 24 weeks. The primary endpoint was the change from baseline 6-min walk test (6MWT) distance compared to PBO. The 6MWT results improved in patients receiving 2 mg/kg weekly compared to PBO. The every other week regimen resulted in walk distances comparable to PBO. There was no change from baseline in the 3 Min Stair Climb Test in both treatment groups. Following completion of the initial study, patients, who continued to receive elosulfase alfa 2 mg/kg weekly (QW) for another 48 weeks (for a total of up to 72-week exposure), did not show additional improvement on 6MWT.

Expert opinion—We suggest that ERT is a therapeutic option for MPS IVA, providing a modest effect and the majority of the effects are seen in the soft tissues.

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Declaration of interest

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Keywords

Chondroitin-6-sulfate; enzyme replacement therapy; keratan sulfate; mucopolysaccharidosis IVA; skeletal dysplasia

1. Introduction

Mucopolysaccharidosis IVA (MPS IVA; Morquio A syndrome, OMIM 253000) is an autosomal recessive lysosomal storage disorder (LSD) caused by deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS). Deficiency of the enzyme leads to a progressive accumulation of the glycosaminoglycans (GAGs): chondroitin-6-sulfate (C6S) and keratan sulfate (KS). C6S and KS are synthesized primarily in cartilage, and sequentially, these GAGs are stored in lysosomes of chondrocytes, their associated ligaments, and the extracellular matrix (ECM) [1-4]. Accumulation of undegraded C6S and KS triggers progressive systemic skeletal dysplasia [5-8].

In general, most patients with MPS IVA have no clinical symptoms or signs at birth; however, main signs and symptoms in patients with a severe form appear by 1 year of age, including kyphosis (gibbus) and petus carinatum (protrusion of the chest). They are usually diagnosed during their second year of life due to their unique skeletal features including genu valgum (knock-knee), growth retardation, kyphoscoliosis, pectus carinatum, hypermobile joints (fingers and hands, cervical instability), and abnormal gait with a tendency to fall. MPS IVA is distinguished from other MPSs by preservation of intelligence and unique skeletal features including hypermobile joints. Odontoid hypoplasia in combination with ligamentous laxity and extradural GAGs deposition causes atlantoaxial subluxation with cervical cord compression, cervical myelopathy, or even death [4,9,10].

Other potential complications comprise: muscle atrophy, valvular heart disease, hearing loss, fine corneal clouding, teeth abnormality, and pulmonary function impairment [4,9,10]. The delivery of drugs to avascular tissue such as cornea, cartilage, and heart valves are limited. These tissues are particularly severely affected in MPS IVA.

The clinical phenotype of patients with MPS IVA is very heterogeneous. First symptoms can appear as late as the second decade of life in less severe (attenuated) patients [9], and patients with mild forms of MPS IVA can attain near normal stature [11-13]. An intermediate form of MPS IVA has also been defined where patients are between severe and mild forms [14].

Patients with the severe form of MPS IVA may not survive beyond the second or third decade of life due to complications such as cervical spinal cord compression, respiratory insufficiency, or cardiac compromise. Most patients with MPS IVA have difficulty with anesthesia and surgical procedures because of a small and obstructive airway [4,9,10,15]. A well-trained medical team for the surgical procedures is indispensable to avoid complications during intubation, surgery, and ex-tubation.

Accumulation of KS in cartilage is considered to be the primary contributing factor to the clinical phenotype of MPS IVA. An autopsied case showed the appearance of foam cells and macrophages in multiple tissues including cartilage, ligaments, heart valves, aorta, lungs, liver, and kidneys, indicating that chronic inflammation involves worsening of clinical features in patients with MPS IVA [16]. C6S also accumulates in aorta and heart valves and is elevated in blood [16,17], which means that both KS and C6S play a significant role in producing the clinical features of MPS IVA.

Therapies for MPS have been developed and applied clinically. These consist of enzyme replacement therapy (ERT), gene therapy, hematopoietic stem cell transplantation (HSCT), and substrate reduction therapy (SRT). Conventional ERT of elosulfase alfa for MPS IVA was approved in Europe and USA, following results of a 24-week clinical trial.

An improvement in a 6-min walk test (6MWT) and a reduction of urinary KS were observed [18,19].

However, several limitations with conventional ERT for MPS IVA have been pointed out: i) it has a limited effect on skeletal, corneal, and heart valvular issues [20,21]; ii) the enzyme has a short half-life (40 min), being rapidly cleared from the circulation [22]; iii) there are immunological issues and iv) the cost is high. These limitations have also been seen for ERT in other forms of MPS [7,23-25].

To resolve the above issues, an improved ERT with a long circulating enzyme and a bone-targeting enzyme have been proposed [26,27]. Alternative sources for production of the recombinant GALNSs for MPS IVA have been developed that may eventually reduce the cost of ERT [28,29]. HSCT for MPS has shown that benefits on physical activity and bone mineral density (BMD) in treated mice, and early intervention provides a better outcome [30-32]. ERT and HSCT have a comparable impact on growth of patients with MPS II [33], but HSCT is better than ERT in improvement of activities of daily living (ADL). However, the therapeutic effect of either treatment on bone growth is limited [34]. For MPS IVA, several patients have been treated with HSCT, resulting in improvement of ADL, BMD, and (in one patient) lung function has improved [35-38]. However, hypermobile joints and established skeletal dysplasia has not shown improvement with HSCT or ERT.

Overall, therapies to resolve bone lesions remain an unmet challenge although several promising results with targeting or long-circulating drugs to bone have been demonstrated to be effective experimentally and clinically [27,39-47].

In this review, we describe the current knowledge of ERT for MPS IVA: the expectations, limitations, and future prospects. While conventional ERT has limited effects on the bone pathology of MPS IVA, new approaches on ERT are emerging and discussed in this review.

2. Onset of pathological features

Skeletal development of MPS IVA patients is typically indistinguishable from healthy babies at birth, although some patients show mild signs of skeletal dysplasia such as gibbus or pectus carinatum [35-38]. It is noteworthy that some patients show excessive growth at

birth [2,35]. Most skeletal features are progressive and irreversible with age. The first several months of life provide the best window of possibility for ameliorating bone deformities in patients with MPS IVA. Development of newborn screening programs will help to identify patients with MPS IVA so that they can receive an appropriate therapy in the first weeks of their lives [48,49]. Excessive storage of GAGs in cartilage is already present in the fetus [35]. An affected fetus at 18 – 20 weeks of gestation showed storage vacuoles in chondrocytes [50]. Similarly, lysosomal GAGs accumulate in chondrocytes of newborn MPS IVA mice [51]. Initial clinical signs and symptoms in newborn patients with MPS IVA are observed as sacral dimple, gibbus, abnormal shape of vertebrae, and/or pectus carinatum [52]. Thus, substantial storage materials have already accumulated in chondrocytes before birth in both mice and patients affected with MPS IVA.

3. Enzyme replacement therapy

3.1 Conventional ERT

3.1.1 Background—Enzyme Replacement Therapy (ERT) is one of the most important therapies for patients with LSD for the last 30 years. ERT has been developed for MPS I [7], MPS II [8,53], and MPS VI [54-57]. Recombinant human GALNS (elosulfase alfa, Vimizim®) has also been approved as ERT for patients with MPS IVA by the US FDA and the European Medicines Agency (EMA).

The enzyme contains oligosaccharide chains with mannose-6-phosphate (M6P) residues that promote uptake of the infused enzyme into the lysosome of cells via the M6P receptor [58]. Then, the enzyme can catabolize the GAGs that have accumulated in the lysosome [59]. The majority of the infused enzyme is delivered to liver, kidneys, spleen, and lungs. The infused enzyme has a very short half-life (40 min) [22] in the circulation due to rapid binding to M6P or mannose receptors and delivery into the visceral organs. The enzyme is not delivered directly to avascular cartilage. Therefore, conventional ERT is expected to have a limited impact on growth plate pathology in patients with MPS IVA, even after long-term treatment.

3.1.2 Clinical trials for ERT—A Phase I/II clinical trials of ERT (elosulfase alfa) for MPS IVA included 20 patients in an open-label, dose escalation trial. A Phase III 24-week, randomized, double-blind, placebo (PBO)-controlled trial in 176 patients evaluated two dosing regimens: 2 mg/kg/dose weekly (2 mg/kg QW; $n = 58$) and 2 mg/kg/dose every other week (2 mg/kg QOW; $n = 59$), compared with PBO ($n = 59$) [60,61]. The Phase III trial included patients aged between 5 and 57 years old.

3.1.2.1 Primary endpoint: 6 min walk test (6MWT): The primary clinical endpoint was defined as the change in distance walked in 6MWT from baseline to Week 24. At baseline, all enrolled patients walked more than 30 meters but less than 325 meters in 6 min. Patients in the 2 mg/kg QW treatment group showed a significant change in the 6MWT, compared to PBO at Week 24, based on the pre-specified analysis of covariance (ANCOVA) model (mean difference of 22.5 meters; 95% CI [4.0, 40.9]; $p = 0.0174$); however, patients in 2 mg/kg QOW treatment group performed similarly to those in the PBO group (mean difference of 0.5 meters; 95% CI [-17.8, 18.9]; $p = 0.9542$). The numerical difference of

22.5 meters between 2 mg/kg QW and PBO groups is classified as modest (an average city block is approximately 80 to 100 meters long) [19]. Several exploratory analyses were conducted by the FDA Advisory Committee to better assess the clinical meaningfulness of this result [19]. The Committee evaluated the mean change in 6MWT by baseline walking distance (6MWT $\leq$ 200 meters vs $>$ 200 meters at baseline). Patients who walked shorter distances at baseline (6MWT $\leq$ 200 meters) had a greater improvement in walking distance than those who walked $>$ 200 meters at baseline. Among patients who walked $\leq$ 200 meters at baseline, the mean change in 6MWT from baseline to Week 24 was 53 ± 67 meters on 2 mg/kg QW treatment vs 13 ± 39 meters on PBO. Among patients who walked $>$ 200 meters at baseline, the mean change in 6MWT from baseline to Week 24 was 25 ± 49 meters on 2 mg/kg QW treatment vs 14 ± 57 meters on PBO (**Figure 1**). These exploratory results suggest that ERT might be more effective in patients with a more severe phenotype at baseline [19].

In an extension of this clinical study, patients who continued to receive 2 mg/kg QW for another 48 weeks (a total of 72-week treatment) did not show further improvement on 6MWT beyond what had been demonstrated during the first 24-week PBO-controlled trial (Table 1); however, their walking ability remained stable. Since disease progression is expected in these patients, the demonstrated stability suggests that ERT continues to have a positive impact. It is critical to interpret these data carefully since there was no comparable PBO group in the extension trial and in the first part of the trial the PBO group showed a small improvement in the walk test. Patients who received PBO during the 24-week-controlled trial and were subsequently randomized to receive 2 mg/kg QW in the extension study did not show any improvement in the 6MWT compared to baseline but their walking ability remained stable throughout the extension trial.

The 6MWT was originally developed to measure the sub-maximal level of functional capacity in adult patients with moderate to severe heart or lung diseases, as a predictor of morbidity and mortality in these patients [62-64]. Since its introduction, it has also been applied to evaluate functional outcome in other patient groups including Cystic fibrosis, Duchenne muscular dystrophy, and obesity [64-66]. Thus, the 6MWT has been used to achieve US marketing approval, including ERTs for Pompe disease, MPS I, MPS II, and MPS VI; however, clinical features and prognoses vary between different diseases. Patients with Pompe disease and MPS I, II, and VI have more extensive cardiopulmonary involvement that influence their endurance while patients with MPS IVA suffer from difficulty of ambulation mainly due to skeletal dysplasia, such as joint deformities (knee and hip) and contractures. In this sense, since the 6MWT is influenced by at least three organ systems affected in MPS IVA (musculoskeletal, respiratory, and cardiovascular), its application to measure the ambulation in MPS IVA patients could be limited.

The 6MWT is affected by multiple factors. Implementation of the 6MWT varies widely, despite availability of specific guidelines. The test is effort-dependent, which can be particularly problematic in pediatric patients. Performance in the 6MWT is often influenced by training, participants' stage of developmental, ability to understand instructions, willingness to cooperate, and the site of the clinical test. The 6MWT results also can be affected by the type of disease and, therefore, should be interpreted with caution [62].

Experts have advised the use of additional outcome measures to interpret the clinical meaningfulness of the 6MWT results in each chronic disease population [19].

3.1.2.2 Secondary endpoint: 3-min stair climb test (3MSCT): The secondary clinical endpoint, the change in 3MSCT, did not show treatment-related improvement. Neither 2 mg/kg QW nor 2 mg/kg QOW treatment group demonstrated a significant improvement in the 3MSCT, compared to PBO at Week 24. The 2 mg/kg QW treatment group showed a greater difference from PBO on the 3MSCT, but the mean difference in stair climb rate between these two treatment groups was only 1.1 stairs per min. Patients who walked < 200 meters on baseline 6MWT climbed fewer stairs (15 – 20 stairs/min on 3MSCT) than those who walked > 200 meters on baseline 6MWT (35 – 43 stairs/min on 3MSCT); however, there were no differences in the mean change from baseline in 3MSCT among treatment groups. In this group of patients, the baseline 6MWT did not correlate with either the 3MSCT at baseline or the change from baseline in the 3MSCT. The extension trial did not show any improvement in either the PBO-QW or the QW-QW groups.

Overall, the mean change in 3MSCT was small for both 2 mg/kg QW and 2 mg/kg QOW treatment groups but through 72 weeks performance did not drop below baseline (Table 1). It should be noted that after 48 weeks, data are from approximately half of the enrolled patients and the extension trial has no PBO control group for comparison. Given the short stature and lower extremity alignment issues common to Morquio patients, difficulties in demonstrating efficacy with the 3MSCT may be more a function of the skeletal dysplasia of the patients than ERTs' overall effectiveness.

3.1.2.3 Other clinical effects: Patients treated with ERT showed positive changes in maximal voluntary ventilation, MPS-Health Assessment Questionnaire (MPS-HAQ), and height/growth rate, although these changes were not statistically significant [19,61].

Patients, who received elosulfase alfa 2 mg/kg weekly, indicated a small numerical improvement in the Caregiver Assistance and Mobility Domains, but not in the Self-Care Domain, in the Mucopolysaccharidosis Health Assessment Questionnaire (MPS HAQ). No apparent treatment effects were observed in hearing, echocardiogram, corneal clouding, or lower extremity long bone length [19].

3.1.2.4 Limitations of clinical endpoints: To date there is no widely accepted and appropriate method of assessing therapeutic efficacy in patients with MPS IVA. As discussed above, the 6MWT is primarily a measure of endurance that is mostly affected by cardiovascular performance and not skeletal dysplasia, the most critical feature of MPS IVA. The 3MSCT may be a better measure of efficacy for patients with MPS IVA because it requires coordination of multiple joints including those in the hip, knee, and foot. However, the clinical trial was not sufficiently powered to show an improvement in this test, and longer-term studies are required to conclude whether ERT will improve this endpoint. The physical assessments in the Phase III clinical trial excluded patients younger than 5 years old, those wheel-chair bound, and post-operative patients with severe muscle weakness since these patients cannot perform such effort-dependent physical assessments. Effort-independent noninvasive tests are needed to better determine efficacy of ERT for these

patients. Bone issues such as hyperlaxity of joints, BMD, and bone pathology are of particular importance to MPS IVA and need to be studied in more detail.

3.1.2.5 Limitations of conventional ERT: We have analyzed cartilage tissues from 12 patients with MPS IVA (4 – 17 years old), who underwent 6 – 30 months of ERT, and did not detect any reduction of vacuoles in chondrocytes or abnormal ECM in any patient (**Figure 2**). These early data suggest poor penetration of enzyme into avascular tissue in keeping with animal studies [67]. Thus, lack of impact on established bone lesions during a trial which lasted for 24 weeks is not unexpected since the cartilage is avascular. Longer-term studies are needed to determine efficacy for bone pathology.

Another issue is that ERT is very expensive. The estimated cost in USA is \$1,068 per vial, which for a typical 22.5 kg patient requires nine vials for each weekly infusion, equating to approximately \$380,000 per patient annually. Increasing the half-life of the enzyme and developing a more efficient method of production could reduce costs significantly.

The GAGs that accumulate in MPS IVA, KS and C6S, are synthesized mainly in chondrocytes, and blood and urinary KS and C6S fall to normal levels with age after the growth plate is closed or destroyed. ERT may remove storage materials from airway and lungs and thereby ease breathing problems of patients with airway obstruction in soft tissues, leading to recovery of stamina and improved ADL. Patient death is usually caused by complications related to airway or spinal cord compromise. In patients where these complications are absent or resolved, therapy may have limited impact. ERT is more likely to have a significant effect on storage in soft tissues with good vascularization, than in hard or avascular tissues. Thus, dosing of ERT for patients with MPS IVA could potentially be modified to control accumulation of storage materials in soft tissues such as lung and airway as opposed to trying to reverse bone pathology.

3.1.2.6 Adverse effects: Of the 235 patients in the clinical trials, 175 (74%) patients had at least one adverse reaction. The most common adverse reactions occurring in 10% of patients were pyrexia (26%), vomiting (22%), headache (20%), nausea (18%), abdominal pain (14%), and fatigue (12%). In the Phase III trial, the most common adverse reactions occurring in 10% of patients treated with elosulfase alfa and with a higher incidence than in the PBO-treated patients were pyrexia (33%), vomiting (31%), headache (26%), nausea (24%), abdominal pain (21%), chills (10%), and fatigue (10%). There was no mortality. Twenty-five (10.6%) patients presented a serious adverse effect. The most serious adverse reactions were acute reactions associated with infusion, such as anaphylaxis or hypersensitivity reactions. The FDA review team identified a total of 18 (7.7%) cases of anaphylaxis from 235 patients treated with ERT. Signs and symptoms of anaphylactic events observed in treated patients included dyspnea, bronchospasm, cough, hypoxia, hypotension, flushing, angioedema of the throat, urticaria, and gastrointestinal symptoms in conjunction with urticaria. Anaphylaxis occurred as early as 30 min from the time of infusion and up to 3 h after infusion. Anaphylaxis has also occurred as late into treatment as the 47th infusion. It should be noted that all patients in Phase III received premedication with anti-histamines. Hypersensitivity reactions were reported in 64 (27%) patients. Most commonly reported hypersensitivity reactions included angioedema, urticaria, peripheral edema, facial edema,

wheezing, flushing, cough, dyspnea, and rash. One patient discontinued treatment due to anaphylaxis after the tenth infusion of elosulfase alfa, and another patient discontinued treatment after the 45th infusion due to recurrent severe hypersensitivity reactions and anaphylaxis.

3.1.3 Conventional ERT in MPS IVA mouse models—A pre-clinical trial was performed using GALNS enzyme in 3-month-old MPS IVA mice tolerant to human enzyme [67]. Twelve weeks of intravenous treatment with GALNS showed that lysosomal storage vacuoles are eliminated from visceral organs such as liver, spleen, and heart while the storage materials in articular and epiphyseal cartilage did not reveal improvement even with high doses of GALNS (2.5 mg/kg). The column structure of the growth plate region remained disorganized [67]. Thus, ERT for MPS IVA mice provided a limited effect in chondrocytes in the femur, ligaments, and synovium.

When ERT was started in newborn MPS IVA mice, clearance of storage materials in bone was better than seen in adult MPS IVA mice treated at 3 months old. The chondrocytes remained vacuolated while the column structure was organized [68]. Thus, conventional ERT alone, even if started at birth, is unlikely to ameliorate bone pathology completely since most patients may already have progressive vacuolated chondrocytes prior to treatment and the avascular nature of cartilage will prevent enzyme delivery.

Overall, the clinical and mouse data indicate that conventional ERT may not function efficiently to improve established growth plate lesions.

3.2 Targeting ERT

Combination of ERT with a bone-targeting strategy has been developed. Hydroxyapatite (HA) $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ is a positively charged, major inorganic component of bone that is absent in soft tissues. Bone matrix proteins (osteopontin, bone sialoprotein, etc.) have a repetitive sequence of negatively charged acidic amino acids (aspartic acid - Asp and glutamic acid - Glu), which are proposed as possible HA-binding sites [69,70]. In osteoblastic cell culture, osteopontin and bone sialoprotein rapidly bind to HA after their secretion by osteoblasts [71]. A drug attached to bisphosphonate is targeted to HA and released during the bone resorption process, indicating that targeting a drug to HA is a potential approach for a selective drug delivery to bone [39,72]. Targeting to bone has been achieved by attaching the drug to six Glu (E6) or more residues [40-42]. This bone-targeting system has been applied to a large molecule, an enzyme (tissue nonspecific alkaline phosphatase), and the tagged enzymes are delivered efficiently to bone [42-44]. Clinical and pathological improvements in the systemic bone disease hypophosphatasia were observed more by using the tagged enzyme, compared with native enzyme [43].

Human GALNS has been bioengineered to add an aspartic acid hexamer E6 tag. The tagged GALNS enzyme had a markedly prolonged clearance from the circulation, leading to 10 – 20 times higher concentration of the enzyme activity in blood than those of the native enzyme in an MPS IVA mouse model [47]. The bone-targeting enzyme was kept longer in bone, with substantial residual enzyme activity 48 h after intravenous infusion. Pathological examination of MPS IVA mice after ERT with the targeting enzyme demonstrated more

clearance of storage materials in bone than mice treated with unmodified enzyme. Therefore, the tagged enzyme enhances delivery and improvement of the pathological effects in bone.

Overall, these results indicate that targeting of the enzyme to bone and/or treating at the newborn stage presents more improvements in the clinical manifestations and pathological bone lesions in mouse models. Despite these improvements in therapeutic efficacy, no therapy that completely removes completely storage vacuoles from chondrocytes has been established. It is critical to evaluate the therapeutic effect to bone and joint lesions by using molecules with increased tropism towards those tissues.

4. Biomarkers for MPS IVA

4.1 Useful biomarker

Biomarkers are used for diagnosing, staging, assessing disease severity, and monitoring disease progression and the response to therapy. Generally, biomarkers should be cheaper and easier to measure than “true” clinical endpoints and can be measured in a shorter period [73,74]. In clinical trials, an ideal biomarker is a measure of therapeutic efficacy that correlates with a *real* clinical endpoint. The first step in defining appropriate biomarkers is to clarify the pathophysiology of the disease and to identify its determinant factors. The second step is to identify biomarkers based on the mechanism and pathway of action of the intervention associated with the pathophysiology of the disease. The last step is to determine the extent to which the putative biomarker correlates with the process and how useful it is in predicting the clinical outcome [74,75].

Deficiency of GALNS in MPS IVA was identified [76] by use of oligosaccharide substrates that had been prepared from C6S containing N-acetylgalactosamine (GalNAc) 6-sulfate. Therefore, the enzyme was originally named GalNAc 6-sulfate sulfatase, namely GALNS [76]. Successively, it was demonstrated that the enzyme also removes the 6-sulfated galactose residues of KS [77].

Thus, GALNS is required for the degradation of both C6S and KS by removing the sulfate. The degradation of C6S and KS is impaired in MPS IVA, which causes the accumulation of undegraded C6S and KS. Accumulated C6S and KS are released into the circulation where they can be measured as an important biomarker for characterizing MPS IVA.

4.2 KS

Measurement of blood and urinary KS in patients with MPS IVA have been established using ELISA and liquid chromatography tandem mass spectrometry (LC-MS/MS) [78,79]. Levels of blood and urinary KS in MPS IVA [80,81] decline with age and become normal or subnormal by the age of 20 years.

4.2.1 Tertiary endpoint: urinary KS—Urinary KS levels correlate with clinical severity in patients with MPS IVA [78,81-84]. In the Phase III trial, urinary KS levels in patients treated with 2.0 mg/kg/week decreased by 41% from baseline [18]. In the extension clinical trial, urinary KS levels continued to decline through Week 72 in patients receiving

elosulfase alfa 2 mg/kg QW. However, changes in urinary KS levels did not correlate with changes in 6MWT [18,19,84].

Thus, reduction of urinary KS levels did not correlate with the clinical effects measured by 6MWT or other clinical endpoints. Patients treated with 2.0 mg/kg/every other week also had a 30% reduction in urinary KS level from the baseline; however, no improvement was observed in any clinical endpoint. Since most clinical features of MPS IVA [short stature, hypermobile joints, (cervical instability, genu valgum, floppy hands, and double fingers)] are due to skeletal dysplasia caused by malfunction of vacuolated chondrocytes and successive abnormal ECM formation, it is critical to determine whether a measured biomarker correlates with improvement of these clinical outcomes. To date urinary KS appears to be useful to differentiate MPS IVA from other forms of MPS and to demonstrate pharmacodynamic effects of ERT treatment, but it does not appear to be valuable for both therapeutic efficacy and prediction of short-term outcomes of ERT for MPS IVA. The recent study by Donida *et al.* showed that MPS IVA patients who received weekly ERT for 8 months presented still significant higher levels of urinary GAGs ($2 \pm 0.02 \mu\text{g}/\text{mg Cr}$) compared to controls ($1.57 \pm 0.07 \mu\text{g}/\text{mg Cr}$) [85].

4.2.2 Blood KS—Blood KS levels are associated with clinical severity of MPS IVA [78,80-83]. Blood KS peaks between 5 and 15 years while urinary KS peaks in newborns [78,80-82]. With the start of ERT, most patients demonstrate a decrease of urinary KS levels, while the changes in blood KS levels have not yet been reported. The discrepancy between blood and urinary KS levels is justified as follows. Urinary KS results from small KS fragments that filter through the kidneys from the circulation, and therefore, urinary KS does not reflect total blood KS that includes both small and large fragments of KS. Thus, blood KS may be a better indicator of improvement in true clinical endpoints including bone pathology or any other skeletal signs and symptoms.

A limitation of both blood and urinary KS analysis is that these biomarkers are only of value in younger patients before closure or destruction of growth plates since synthesis of KS decreases markedly and levels of KS in MPS IVA patients are normalized or subnormal.

4.3 Ratio of mono-sulfated and di-sulfated KS

GALNS plays a role as galactose-6-sulfatase (G6S) since the enzyme hydrolyses the sulfated galactose of KS and converts di-sulfated KS to mono-sulfated KS. Therefore, deficiency of GALNS activity leads to the accumulation of di-sulfated KS, and consequently, the ratio of di-sulfated KS in total KS of patients with MPS IVA increases, compared with normal controls.

Levels of both mono-sulfated and di-sulfated KS measured by LC-MS/MS in blood from patients with MPS IVA were elevated compared with age-matched controls [86]. The elevation of di-sulfated KS in MPS IVA patients was more significant than the elevation of mono-sulfated KS. The proportion of di-sulfated KS in total KS in blood rose with age in control subjects while it was age-independent in patients with MPS IVA. The proportion of di-sulfated KS is better at distinguishing younger MPS IVA patients than older patients from

age-matched controls. Levels of mono- and di-sulfated KS in urine of MPS IVA patients were also higher than age-matched controls for all studied ages.

Overall, a significant difference in sulfation levels of KS between control subjects and patients with MPS IVA indicates that the di-sulfated KS is another potential biomarker for MPS IVA.

4.4 C6S

GALNS also plays a role as N-acetylgalactosamine-6-sulfate sulfatase since the enzyme hydrolyses the sulfated acetylgalactosamine of C6S, converting it into CS. C6S is distributed mainly in growth plates, aorta, and cornea. Disaccharides of C6S have been measured by LC-MS/MS after digestion of polymeric C6S with chondroitinase ABC (or C) [17]. Levels of C6S in blood and urine were significantly elevated in patients compared with age-matched controls and declined with age in both MPS IVA patients and control subjects.

Overall, it would be of great significance to clarify i) whether blood KS levels decline over time during treatment, ii) the extent to which blood and urinary KS levels are correlated, iii) how reduction of blood/urinary KS and/or C6S correlates with a specific clinical manifestation, iv) the extent to which the ratio of mono-sulfated to di-sulfated KS change during treatment, and v) whether blood and urinary C6S or combination with urinary or blood KS is a better biomarker than KS alone (**Figure 3**).

The US Institute of Medicine recommends evaluating bio-markers with the following steps. i) Analytical validation to ensure that biomarker tests are reliable, reproducible, and adequately sensitive and specific. ii) Qualification to ensure the biomarker is associated with the clinical outcome of concern. iii) Utilization analysis to determine that the biomarker is appropriate for the proposed use [73,74].

C6S and KS measurement by LC-MS/MS covers the first step. C6S and KS levels closely correlate with the clinical outcome and severity of skeletal dysplasia, covering the second step. Further study is needed to determine whether KS and/or C6S levels in blood and urine correlate with its clinical response to therapy for step 3.

5. Clinical pharmacology

Following a single intravenous dose of elosulfase alfa 2 mg/kg at Week 0 in the Phase III clinical trial, circulating enzyme concentrations rapidly declined with a mean half-life of 7 min. After repeated dosing at Week 22, the mean clearance of the enzyme decreased, while the maximal plasma concentration (C_{max}) and the area under the plasma concentration–time curve (AUC) increased for both 2 mg/kg QOW and 2 mg/kg QW groups. The mean t_{1/2} increased to 19 and 35 min for 2 mg/kg QOW and 2 mg/kg QW groups, respectively. These increases in exposure at Week 22 may be attributed to the formation of anti-GALNS neutralizing or non-neutralizing antibodies, which are capable of binding to and interfering with the cellular uptake of the enzyme to the target tissues [19].

6. Expert opinion

In MPS IVA, we have two therapeutic options: one is ERT and the other one is HSCT. ERT provides an immediate therapeutic option without the donor and complicated process to patients with MPS IVA. ERT of elosulfase alfa is a therapeutic option for MPS IVA patients, providing at least a modest effect. Following 24 weeks of treatment, patients receiving 2 mg/kg weekly showed an improvement in a 6MWT compared to PBO. The major beneficial effects appear to be in the soft tissues. The patient's skeletal dysplasia did not appear to be affected by ERT. More definitive research is needed, but elosulfase alfa has the potential to provide clinical improvement and stability in the soft tissue effects of MPS IVA. Improvement of skeletal dysplasia is an unmet challenge.

To achieve a better activity of daily living, clinical management utilizing a multidisciplinary approach to care for the patient is needed. When used together with appropriate medical care, surgical interventions, physiotherapy, and an appreciation of all the possible complications, elosulfase α appears to improve the quality of life of patients with MPS IVA. ERT does not cure the disease, although it provides the potential to rescue some patients from consequences of the disease and to improve their quality of life. Therapy for established systemic bone dysplasia remains unsolved, and robust, innovative approaches such as bone-targeting ERT and gene therapy as well as HSCT should be considered.

In the clinical trials, no significant improvements were detected in hearing, echocardiogram, corneal clouding, or lower extremity long bone length. However, the maximum voluntary ventilation (maximum amount of air that can be inhaled and exhaled within 1 min) improved 10.3% from baseline compared with PBO, with a trend toward statistical significance (CI95, -1.8, 22.4; $p = 0.0943$). The clinical trial did not include patients younger than 5 years of age who might benefit more than older patients.

In any progressive disorder, a treatment that prevents worsening or leads to stability is an efficacious treatment, so ERT is certainly a new tool for treating MPS IVA. A new approach is still required to enhance therapeutic efficacy in bone lesions of MPS IVA patients.

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- or of considerable interest

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Article highlights

- Description of effect and limitations of ERT
- Biomarkers for MPS IVA
- Limitations of current clinical endpoints
- Improvement of ERT

This box summarizes key points contained in the article.

Figure 1. Change in 6MWT from baseline to week 24

Patients who walked $\leq$ 200 meters at baseline: the mean change in the 6MWT from baseline to Week 24 is 40 meters greater than placebo.

Patients who walked $>$ 200-meter at baseline: the mean change in the 6MWT from baseline to Week 24 is 11 meters greater than placebo (revised from FDA Advisory Committee Briefing Document Elosulfase alfa for Mucopolysaccharidosis Type IVA) [19].

3MSCT: 3-minute stair climb test; 6MWT: 6-min walk test.

Figure 2. Bone pathology of a patient with MPS IVA after clinical trial and 6 months extension study of ERT

Left panel (patient with MPS IVA): The surgical remnant was collected from the iliac crest in a 17-year-old patient with MPS IVA when he underwent cervical spinal fusion.

Chondrocytes are vacuolated and nucleus disappears. Right panel (control): Surgical remnant from an 8-year-old male shows normal chondrocyte without any vacuoles (toluidine blue-stained 0.5- μ m-thick sections: X 100).

MPS IVA: Mucopolysaccharidosis IVA.

Figure 3. Levels of urinary C6S (Di-6S) in patients with MPS IVA and control subjects
Levels of urinary C6S in patients with MPS IVA were distinguished from those in age-matched control subjects. Values of urinary C6S from patients and control subjects were plotted on a semilogarithmic scale.
C6S: Chondroitin-6-sulfate; MPS IVA: Mucopolysaccharidosis IVA.

Table 1

Summary of clinical endpoints of MPS IVA.

Procedures	Treatment (Elosulfase alfa)	24 weeks	72 weeks	Remarks
<i>I. Physical test</i>				
6 MWT	2 mg/kg QW 2 mg/kg QOW 2 mg/kg PBO-QW 2 mg/kg PBO-QOW 2 mg/kg QW-QW 2 mg/kg QOW-QOW	Significant change compared to placebo (PBO) (Mean difference of 22.5 m, $p = 0.0174$) Similar to PBO (Mean difference of 0.5 m, $p = 0.9542$) NA NA NA NA	No further improvement No improvement: Kept similar to 24 weeks similar to the level of QW-QW No improvement: Kept similar to 24 weeks similar to the level of QW-QW	FDA Advisory Committee Briefing Document [19] Use additional outcome measures to interpret the clinical meaningfulness of the 6MWT results [19]. Develop other potential measures of short-and long-term treatment benefits. No consistent data that QW-QW group shows significant improvement.
Change in 6MWT from Baseline	2 mg/kg QW: Patients who walked < 200 meters at baseline 2 mg/kg QW: Patients who walked > 200-meter at baseline	40 meters greater than PBO. 11 meters greater than PBO	NA NA	FDA Advisory Committee Briefing Document [19] QW-QW group with the lowest level does not have any improvement.
3MSCT	2 mg/kg QW 2 mg/kg QOW 2 mg/kg PBO-QW 2 mg/kg PBO-QOW 2 mg/kg QW-QW 2 mg/kg QOW-QOW	No significant improvement than PBO (Mean difference of 1.1 stairs/min, $p = 0.494$) No significant improvement than PBO (Mean difference of -0.5 stairs/min, $p = 0.778$) NA NA NA NA	Kept similar to 24 weeks Kept similar to 24 weeks No improvement: Kept similar to 24 weeks No improvement No improvement: Kept similar to 24 weeks No improvement: Kept similar to 24 weeks	FDA Advisory Committee Briefing Document [19]
PIT	2 mg/kg QW 2 mg/kg QOW	Trend toward improvement. No significant improvement No significant improvement	NA NA	FDA Advisory Committee Briefing Document [19]
Growth	2 mg/kg QW	No significant improvement	NA	FDA Advisory Committee Briefing Document [19]
Other tests (ECG, hearing, corneal clouding, lower extremity bone length)	2 mg/kg QW	No significant improvement	NA	FDA Advisory Committee Briefing Document [19]
Questionnaire (ADL)	2 mg/kg QW	No significant improvement	NA	FDA Advisory Committee Briefing Document [19]
<i>II. Biomarker</i>				
Procedures	Treatment	24 weeks	72 weeks	Remarks
Urinary KS	Elosulfase alfa 2 mg/kg QW Elosulfase alfa 2 mg/kg QOW	Decreased by 41% from the baseline Decreased by 30% from the baseline	Further decreases NA	FDA Advisory Committee Briefing Document [19] No correlation with changes in 6MWT. Not a good biomarker

Procedures	Treatment (Elosulfase alfa)	24 weeks	72 weeks	Remarks
Blood KS	No treatment	NA	NA	for therapeutic efficacy [19,81] Could be better biomarker only in younger patients [77-79,82]
Mono-sulfated and di-sulfated KS in blood	No treatment	NA	NA	Di sulfated KS significantly higher than mono sulfated KS. Better biomarker [82]
Mono-sulfated and di-sulfated KS in urine	No treatment	NA	NA	Both mono and di-sulfated KS higher than age-matched controls Better biomarker [82]
C6S	No treatment	NA	NA	Higher levels in both blood and urine compared with control but goes down with age in both controls and MPS groups [17]

3MSCT: 3-minute stair climb test; 6MWT: 6-min walk test; C6S: Chondroitin-6-sulfate; KS: Keratan sulfate; MPS IVA: Mucopolysaccharidosis IVA.