

## A phase I/II clinical trial of enzyme replacement therapy in mucopolysaccharidosis II (Hunter syndrome)

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### Abstract

**Objective:** To evaluate the safety and explore the efficacy of idursulfase (recombinant human iduronate-2-sulfatase) treatment for mucopolysaccharidosis II (MPS II).

**Study design:** Twelve patients were enrolled into a randomized, double-blind, placebo-controlled trial for 24 weeks followed by an open-label extension study. Three groups of 4 patients were enrolled sequentially, with 3 patients in each group receiving idursulfase and 1 patient receiving placebo. The first group received idursulfase at 0.15 mg/kg infused every other week with the 2nd and 3rd groups receiving 0.5 and 1.5 mg/kg, respectively. After 24 weeks the placebo-treated patients were changed to idursulfase at the dose of their group. The primary endpoint was a change from baseline in urinary excretion of glycosaminoglycans. Results were pooled for analysis by ANOVA and compared to baseline.

**Results:** Urinary glycosaminoglycans were reduced within 2 weeks of initiating idursulfase and were decreased 49% after 48 weeks of treatment ( $P < 0.0001$ ). Both liver and spleen volume were decreased at 24 weeks ( $P < 0.01$ ) and 48 weeks ( $P < 0.001$ ). The 6-minute walk test distance increased an average of 48 meters after 48 weeks ( $P = 0.013$ ). Six patients in the higher dose groups developed IgG antibodies that did not influence the clinical effects of idursulfase.

**Conclusions:** This study describes the first experience with enzyme replacement therapy for the treatment of patients with MPS II. Idursulfase was generally well tolerated and was associated with reductions in urine glycosaminoglycans levels and organ size, as well as an increased 6-minute walk test distance.

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### Introduction

The mucopolysaccharidoses (MPS) are a group of rare, inheritable disorders that are each caused by a single deficiency in a lysosomal enzyme that catalyzes a step in the catabolism of glycosaminoglycans (GAG). MPS II (Hunter syndrome) is due to the deficiency of the enzyme iduronate-2-sulfatase (I2S), which cleaves an O-linked sulfate from

dermatan sulfate and heparan sulfate [1]. MPS II is an X-linked recessive disorder with an incidence of between 1 in 100,000 to 160,000 [2–4] and occurs primarily in males, although females with MPS II have been reported [1]. The mutations responsible for the I2S enzyme deficiency include missense and nonsense mutations, and insertions or deletions of the gene located at Xq28 [5–10].

The progressive accumulation of the GAG within tissues and organs is responsible for the clinical disease seen in MPS II [1]. The common clinical signs and symptoms include developmental delay, short stature, skeletal

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deformities, hearing loss, enlarged tongue, abnormal dentition, upper airway obstruction, hepatosplenomegaly, valvular heart disease, and decreased joint range of motion. Historically, MPS II was divided into two distinct forms, severe and mild, but it is now recognized that MPS II is a spectrum of clinical severity ranging from severe to attenuated. In patients with the severe form, the onset of somatic involvement is usually between 2 and 4 years of age, with variable progressive neurologic involvement leading to mental impairment [1]. Death occurs in the first or second decade, usually due to obstructive airway disease and/or cardiac failure associated with a progressive loss of neurologic function [1]. In the attenuated form, neurological involvement is minimal, and patients exhibit normal intelligence and survive into adulthood, but can have severe joint, airway and cardiac disease.

The treatment of MPS II has historically been palliative and focused on the management of clinical problems. Hemapoietic stem cell transplantation (HSCT) has been suggested as a means of providing donor cells capable of expressing I2S, but long-term results are limited, and HSCT is not currently recommended for individuals with MPS II [11,12]. Successful HSCT has resulted in stabilization of somatic soft tissue disease, but has not had any proven benefit for the central nervous system or skeleton [13–15]. Enzyme replacement therapy with recombinant human proteins has been used successfully in the treatment of other lysosomal storage diseases, such as Gaucher disease [16], Fabry disease [17,18], MPS I [19,20], and MPS VI [21,22]. Experiments using a mouse model of MPS II suggested that enzyme replacement therapy with idursulfase is effective in reducing GAG stores [23]. Idursulfase has recently been approved for the treatment of MPS II by the US Food and Drug Administration and the results of the phase II/II 1 year double-blind placebo-controlled clinical trial have been published [24]. In this report, we describe the initial clinical experience of enzyme replacement therapy with idursulfase in 12 patients with MPS II.

## Materials and methods

### Patients

Patients who were at least 5 years old, cooperative, and who met both clinical and biochemical criteria for a diagnosis of MPS II were eligible for enrollment. Clinical criteria included having clinical disease consistent with MPS II, such as hepatosplenomegaly, radiographic evidence of dysostosis multiplex, cardiomyopathy, or evidence of upper airway obstruction. The biochemical criterion for a diagnosis of MPS II was defined as either an I2S activity in plasma or in leukocytes  $\leq 5\%$  of normal. All patients had to be able to actively cooperate in study measurements, thus excluding severe MPS II patients who have significant CNS disease.

### Study design

This study was a 6-month randomized double-blind trial followed by an open-label extension designed to investigate the safety and to explore the efficacy of enzyme replacement therapy with idursulfase in the treatment of MPS II. The study was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill, NC. All adult

patients or the parents of patients under 18 years old gave written informed consent prior to enrollment.

Three groups of four patients were enrolled sequentially to receive idursulfase across a 10-fold dose range: 0.15, 0.5, and 1.5 mg/kg every other week. Patients were selected by the principal investigator (JM) for each group based on availability, and within each dose group patients were randomized in a double-blind fashion to idursulfase or placebo in a 3:1 ratio. The study started with the lowest dose, initiating treatment in a single patient each week. Progression to the next dose level was based on a comprehensive assessment of safety, tolerability, and adverse events and was not begun until all patients in the lower dose group had received at least 3 doses and been monitored for 7 days after the third dose. During the double-blind phase, all patients were treated for 23 weeks. All patients elected to continue in the open-label extension study and continued to receive the same dose of idursulfase for at least an additional 6 months. Patients originally treated with placebo received the idursulfase dose of their treatment group, and measurements made at the time of their final placebo visit were considered the baseline values. Data are presented for 48 weeks of treatment with idursulfase for all patients. For the 3 placebo assigned patients this represents the first 72 weeks of their experience in the trial, 24 weeks of placebo and 48 weeks of idursulfase treatment.

### Safety assessments

Safety evaluations were performed at every visit and included a physical examination, serum chemistry, complete blood count, urinalysis, measurement of vital signs, height, weight, and an electrocardiogram. Adverse events were monitored and recorded throughout the study, either at study visits or by telephone contact by a study coordinator during the weeks without a scheduled visit. Anti-idursulfase antibodies in plasma were detected by an enzyme-linked immunosorbent assay (ELISA) and positive results were confirmed by radioimmunoprecipitation (RIP).

### Urinary GAG analysis

Urine samples for GAG analysis were collected prior to each infusion during the first 6 months of the study (double-blinded phase). In the open-label extension trial, urine samples for GAG analysis were collected prior to idursulfase infusion at weeks 5, 13, and 25. Urine samples were analyzed for GAG content colorimetrically by the method of de Jong [25,26]. GAG concentrations were quantified by comparison to a standard curve prepared from serial dilutions of a dermatan sulfate stock solution. Final GAG concentrations were normalized to urinary creatinine concentration and reported as  $\mu\text{g}$  GAG per mg creatinine.

### Clinical assessments

The primary endpoint was the extent of reduction in urinary GAG excretion. Secondary endpoints included liver and spleen size, 6-minute walk test (6MWT), pulmonary function, joint mobility, heart size and function, and a sleep study. All clinical assessments were performed at the UNC Hospitals. The baseline clinical evaluations (abdominal magnetic resonance imaging, echocardiography, 6MWT, pulmonary function testing, joint range of motion measurements, and sleep study) were conducted during the week prior to receiving the first infusion of idursulfase. Repeat evaluations were performed at weeks 13 and 24 during the double-blinded phase and at weeks 25 and 51 of the open-label phase. Evaluations performed during the study were conducted at study visits just before administration of idursulfase. Liver and spleen size were measured by a single investigator using abdominal magnetic resonance imaging with the volumes calculated using the manual contour-tracing method [27]. Hepatosplenomegaly was defined as a liver volume (L)  $>3.5\%$  of body weight (kg) in patients aged 5–12 years,  $>2.2\%$  of body weight in patients aged 13–17 years, and  $>2.6\%$  of body weight in patients more than 18 years old [20]. Splenomegaly was defined as having a splenic volume greater than the 95th percentile of the normal distribution in children [28]. Heart size and valve function were assessed by echocardiography. Estimates of heart size were made by a single investigator using standard formulas [29]. For

the 6-minute walk test, subjects were instructed to walk back and forth between two marks that were 15 m apart, and an observer recorded the total distance covered in 6 min according to American Thoracic Society guidelines [30]. Two tests, typically one day apart, were performed during each evaluation and the further distance was used as the result for all analyses. Pulmonary function was assessed by spirometry to measure forced expiratory volume in 1 s (FEV<sub>1</sub>) and forced vital capacity (FVC). Three consecutive determinations of FVC and FEV<sub>1</sub> within 5% of each other were required for a successful measurement [31,32]. Two tests, typically one day apart, were performed during each evaluation and the largest values were used as the result for all analyses. Passive joint mobility was defined as the range of motion of neck, shoulder, elbow, wrist, hip, knee, and ankle joints as assessed by a physical therapist using a goniometer [33]. Standard overnight sleep studies were performed in the UNC Hospitals Sleep Disorders Laboratory to measure the frequency of apneas, hypopneas, and oxygen desaturations following the American Academy of Sleep Medicine guidelines and standards [34].

### Idursulfase

Idursulfase (Shire Human Genetic Therapies, Inc., Cambridge, MA) is a recombinant form of human I2S that is produced using genetic engineering in a continuous human cell line that yields a glycosylated protein analogous to the native human enzyme. The idursulfase-producing cell line was generated by transfecting HT-1080 cells with an expression plasmid encoding the 550 amino acids of human iduronate-2-sulfatase, including a 25 amino acid signal sequence, which is cleaved in the secreted protein. The purified protein is >99.9% pure as assessed by a number of chromatographic and electrophoretic assays. The 8 N-linked glycosylation sites are fully occupied and consist of two bis mannose-6-phosphate (M6P) containing glycans that enable high affinity receptor mediated cell uptake and targeting to the lysosomes via M6P receptors. In addition, purified idursulfase contains complex highly sialylated glycans that prolong the circulating half-life of the enzyme. The extent of post-translational modification of cysteine 59 to formylglycine that is required for enzymatic activity is approximately 50%. The placebo contained the same formulation, but without purified idursulfase protein. All study infusions were performed in the University of North Carolina General Clinical Research Center.

The appropriate dose of idursulfase was diluted in normal saline to yield a final volume of 100 mL, which was initially administered over a period of 1 h as a continuous infusion. If a patient developed an infusion reaction, which typically involved chills, fever, headache, and/or flushing, the infusion was terminated, and medical intervention provided as needed. For all moderate to severe infusion reactions, a serum tryptase level and complement studies (C3, C4, and total serum complement) were obtained within 1 h of stopping the infusion. For subsequent infusions, patients were premedicated with antihistamines and/or steroids depending on the severity of the initial reaction. The duration of all subsequent infusions was lengthened to 3 h with an initial stepwise increase every 15 min during the first hour (20% of dose) and then continuous infusion of 80% of the dose over the next 2 h.

### Analysis of results

Changes from baseline of urinary GAG excretion were evaluated using ANOVA. For the purposes of analysis, patients randomized to placebo were considered as a single treatment group (placebo) during the double-blind phase. These patients then became part of their respective dose group for the evaluation of long-term effects of idursulfase. Descriptive statistics were used for the other exploratory efficacy variables. Because of the small number of patients in each dose group ( $n = 4$ ) and the expected heterogeneity in liver and spleen volumes, pulmonary function, and 6MWT distance at baseline, no prospective analysis of the effect of treatment on these measurements was planned. Post-hoc analyses of the effect of idursulfase on these measurements for all patients combined (pooled study population) were performed with a Student's *t*-test. All calculations were performed with SAS, Version 8.0 (Cary, NC). All values are expressed as mean  $\pm$  standard deviation.

## Results

Demographics and baseline characteristics of the study participants are shown in Table 1. All patients were Caucasian males with an attenuated form of MPS II. Each treatment group varied with respect to age and clinical severity

Table 1  
Patient demographics and baseline characteristics

Treatment patient no.	Age (years)	Height (cm)	Weight (kg)	CPAP <sup>a</sup>	Tracheostomy	FVC <sup>c</sup> (% predicted)	Urine GAG ( $\mu$ g/mg creatinine)	Liver size (% of BW)	6MWT <sup>c</sup> (m)
<i>Idursulfase 0.15 mg/kg</i>									
2	9	126	26.9	No	No	68	511	3.8 <sup>d</sup>	372
3	14	130	33.5	No	No	86	291	3.4 <sup>d</sup>	554
4	10	123	43.2	Yes	No	64	475	2.4	420
<i>Idursulfase 0.5 mg/kg</i>									
5	20	130	42.7	Vent <sup>b</sup>	Yes	15	364	4.5 <sup>d</sup>	252
6	20	137	45.7	No	Yes	34	309	3.8 <sup>d</sup>	301
8	20	151	85.2	Yes	No	38	354	2.6	420
<i>Idursulfase 1.5 mg/kg</i>									
10	8	120	25.3	No	No	78	391	4.2 <sup>d</sup>	480
11	10	131	36.9	No	No	64	369	3.9 <sup>d</sup>	498
12	6	119	25.5	No	No	45	591	3.5	341
<i>Placebo</i>									
1	17	132	48.6	No	No	84	237	3.4 <sup>d</sup>	405
7	13	119	29.3	No	No	62	509	5.3 <sup>d</sup>	465
9	20	130	47.4	Yes	No	23	368	3.3 <sup>d</sup>	254

<sup>a</sup> CPAP, continuous positive airway pressure for obstructive sleep apnea.

<sup>b</sup> Patient 5 was ventilated and was receiving supplemental oxygen at night.

<sup>c</sup> Highest values of 2 tests performed at baseline.

<sup>d</sup> Denotes enlarged liver based on liver volume normalized to body weight.

due to the small size of the groups and clinical heterogeneity of MPS II patients. Age ranged from 6 to 20 years with an average age at enrollment of 14 years. All patients had hepatomegaly at baseline based on physical examination, although when normalized to body weight, hepatomegaly was present in 9 of the 12 patients (75%). Baseline FVC ranged from normal (86% of predicted) to severely compromised (15% of predicted). The 0.5 mg/kg group had severe respiratory involvement with 2 of 4 patients having tracheostomies and an average % predicted FVC of 37% compared to a pooled baseline average % predicted FVC of 55%. All patients completed the 6-month double-blinded study and enrolled in the open-label extension study.

### Urinary GAG excretion

All patients had elevated urine GAG levels at baseline, ranging from 237 to 591  $\mu\text{g}/\text{mg}$  urine creatinine (the upper limit of normal is 127  $\mu\text{g}/\text{mg}$  urine creatinine as determined in a separate group of 28 normal subjects aged 5–21 years old). As shown in Fig. 1, marked decreases in urinary GAG were seen as early as the 2 weeks following the initial dose of idursulfase with more rapid declines in the 0.5 and 1.5 mg/kg groups. Repeated measures ANOVA showed

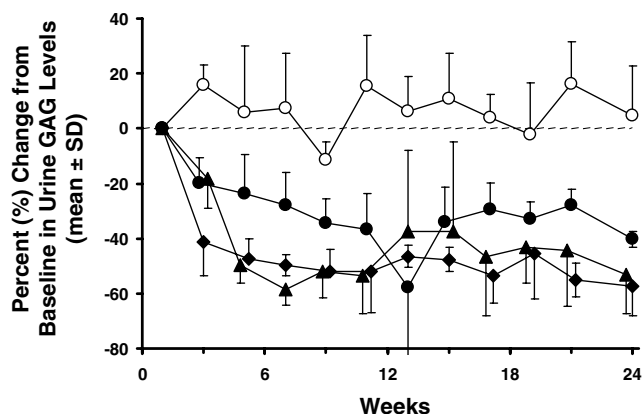


Fig. 1. Percent change in urinary GAG excretion during the double-blind phase of the study. The open circles represent the placebo group and the closed circles, triangles, and diamonds represent the 0.15, 0.5, and 1.5 mg/kg groups, respectively. Some symbols are slightly shifted along the time axis for clarity.  $N = 3$  in each group.  $P = 0.0007$  for % change from baseline for all idursulfase groups at all time points after the initial infusion.

Table 2  
Effect of idursulfase on urinary GAG excretion in male MPS II patients during the first 12 months of treatment

	Urinary GAG ( $\mu\text{g}$ GAG/mg urine creatinine)			
	Dose (mg/kg)			
	0.15 ( $n = 4$ )	0.5 ( $n = 4$ )	1.5 ( $n = 4$ )	Pooled ( $n = 12$ )
Baseline	386 $\pm$ 124	364 $\pm$ 50	445 $\pm$ 101	398 $\pm$ 94
6 months (% change from baseline)	230 $\pm$ 76 (–41 $\pm$ 3)	211 $\pm$ 110 (–44 $\pm$ 22)	168 $\pm$ 61 (–58 $\pm$ 6)	203 $\pm$ 82* (–49 $\pm$ 17)
12 months (% change from baseline)	203 $\pm$ 55 (–47 $\pm$ 3)	209 $\pm$ 98 (–43 $\pm$ 24)	178 $\pm$ 32 <sup>a</sup> (–58 $\pm$ 6)	200 $\pm$ 18 <sup>b,*</sup> (–49 $\pm$ 15)

All values are means  $\pm$  SD.

<sup>a</sup>  $N = 3$ .

<sup>b</sup>  $n = 11$  because patient 9 had been switched to 0.5 mg/kg before reaching 48 weeks treatment with 1.5 mg/kg.

\*  $P < 0.0001$  compared to baseline.

that during the double-blind phase, the mean decrease in urinary GAG for all dose groups as well as the percent changes from baseline at each visit were statistically significant (mean change,  $P = 0.0092$ ; percent change,  $P = 0.0007$ ). Urinary GAG level did not change in patients treated with placebo during the double-blind phase (mean baseline urine GAG, 371  $\pm$  136  $\mu\text{g}/\text{mg}$  creatinine; mean 6-month urine GAG, 375  $\pm$  93  $\mu\text{g}/\text{mg}$  creatinine). However, a rapid decrease in urinary GAG was seen at each dose when these patients were changed to idursulfase during the open-label extension (data not shown). The reduction in urinary GAG levels was maintained through 48 weeks as shown in Table 2. Analysis of the pooled results from all three groups showed that idursulfase significantly reduced urinary GAG excretion at 6 and 12 months (Table 2,  $P < 0.0001$  for both time points). The urine GAG level was reduced to near normal in the majority of patients with 2 patients achieving the normal range at 6 months.

### Liver and spleen size

Baseline mean liver volume expressed as a percentage of body weight was 3.6%  $\pm$  0.6% in the pooled study population. During the 6-month double-blind phase of the study, 8 of the 9 idursulfase-treated patients demonstrated a decrease in liver volume, but no consistent dose-related decrease was observed (data not shown). In the open-label analysis, liver volume was reduced only in the 0.5 and 1.5 mg/kg groups (Fig. 2A). After 6 and 12 months of treatment, liver volumes were significantly decreased in the pooled study population ( $P \leq 0.0001$ ), with 11 of 12 patients having a reduction in liver volume. Liver volume was reduced to within normal limits in 6 of the 9 patients with hepatomegaly (normalized to body weight) at baseline and remained within normal limits in the other 3 patients.

At baseline, mean spleen volume expressed as a % of body weight was 0.93%  $\pm$  0.25% in the pooled study population, and 7 of 12 patients had splenomegaly. Idursulfase-treated patients demonstrated a decrease in spleen volume during the double-blind phase of the study, but no consistent dose-related changes in spleen volume were seen (data not shown). In the open-label analysis, spleen volumes were significantly decreased in the pooled study population after 6 ( $P = 0.0043$ ) and 12 months ( $P < 0.0001$ ) of treatment

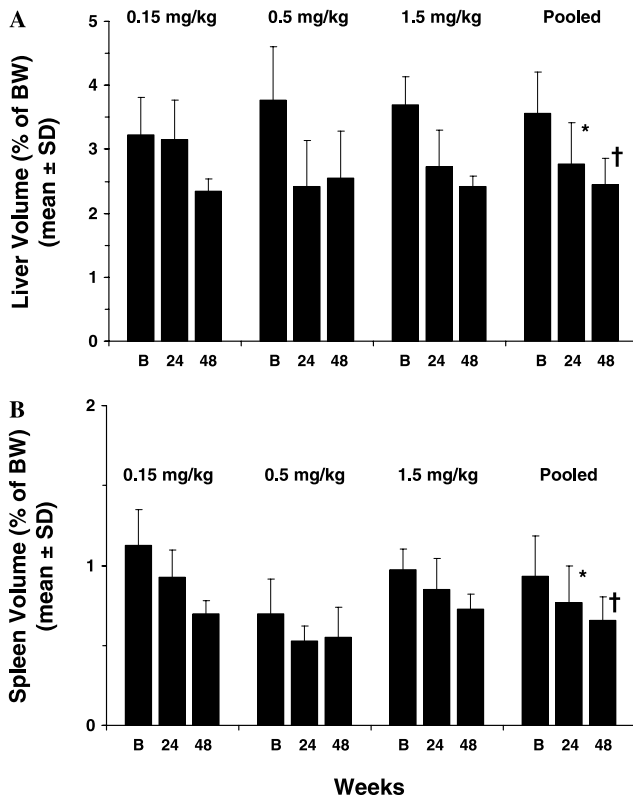


Fig. 2. The effect of idursulfase on liver (A) and spleen (B) volume in MPS II patients. The dose groups are indicated above the bars. \* $P < 0.01$ , † $P < 0.0001$  compared to baseline.  $N = 4$  in each dose group except for the 12 month value in the 1.5 mg/kg group where  $n = 3$ .  $N = 12$  for the pooled data except for the 12 month value where  $n = 11$ .

(Fig. 2B). After 12 months of idursulfase treatment, all patients had a spleen volume within normal limits.

#### Six-minute walk test

The distance walked in 6 min at baseline was  $375 \pm 109$ ,  $449 \pm 94$ ,  $324 \pm 86$ , and  $440 \pm 86$  m in the placebo, 0.15, 0.5, and 1.5 mg/kg groups, respectively. No substantial changes in the mean 6MWT distance were seen in any treatment group during the 6-month double-blind phase of the study. In the open-label analysis, after 12 months of treatment with idursulfase, 8 of 12 patients experienced an increase of at least 30 m in walking distance, and no patients had a decrease in walking distance. Although there was no change in the 0.15 mg/kg group, walking distance increased by  $10.9\% \pm 7.15\%$  in the 0.5 mg/kg group and by  $27.9\% \pm 15.1\%$  in the 1.5 mg/kg group. When the results from all 3 groups were pooled, average walking distance significantly improved from  $398 \pm 117$  to  $445 \pm 124$  m after 12 months on idursulfase ( $P = 0.013$ ,  $t$ -test).

#### Pulmonary function tests

At baseline, 10 of the 12 patients had primarily a restrictive ventilatory disorder as evidenced by a FVC of less than 80% of predicted and a normal FEV<sub>1</sub>/FVC ratio

of greater than 70%. Of the remaining 2 patients, one had normal FVC and FEV<sub>1</sub>/FVC ratio and the other had low FVC and FEV<sub>1</sub>/FVC ratio. In the open-label analysis of the pooled study population, 9 of 12 patients had an increase in FVC after 12 months of idursulfase, but the average increase was small and not statistically significant (FVC =  $1.03 \pm 0.34$  L at baseline and  $1.10 \pm 0.13$  L at 12 months,  $P = 0.08$ ). FEV<sub>1</sub> was not improved after 12 months ( $0.82 \pm 0.28$  L at baseline and  $0.84 \pm 0.30$  at 12 months,  $P = 0.61$ ). The older patients tended to have more severely compromised pulmonary function and might not be expected to improve with treatment. When these four patients were removed from the analysis, a trend towards an improvement in FVC was observed ( $1.11 \pm 0.14$  L at baseline to  $1.22 \pm 0.36$  at 12 months,  $P = 0.065$ ). In this MPS II study population, the total lung capacity, residual lung volume, and pulmonary diffusion capacity were difficult to perform for many subjects, resulting in measurements that were highly variable.

#### Joint mobility

The broad heterogeneity of joint disease, the variability of measurements, and the small sample size complicated interpretation of joint mobility assessment. Although some joint motion measurements indicated improvement in one or more dose groups, overall no consistent significant improvements were noted during 12 months of therapy. Despite the lack of objective improvement in joint motion, many patients noted that they felt that joint movement had improved during the study.

#### Overnight sleep study

Five patients were excluded from the sleep study analysis because of a pre-existing tracheostomy ( $n = 2$ ) or the use of CPAP at night for treatment of sleep apnea ( $n = 3$ ). Four of the five patients excluded were the oldest patients in the study, which reflects the progressive nature of pulmonary disease in MPS II. Five of the remaining 7 patients had abnormal apnea hypopnea index (AHI, apneas plus hypopneas per hour of sleep) at baseline. An AHI  $> 5$  was considered to be abnormal and consistent with obstructive sleep apnea [35,36]. One of the patients (10 years old) with a normal AHI at baseline had an increase from 3.7 to 13.5 after 12 months of idursulfase. In contrast, four of five patients with an abnormal AHI at baseline had at least a 50% reduction after 12 months of treatment with an average reduction of 73.4%. In one patient (13 years old) the AHI dropped from 63.1 at baseline to 12.2 after 12 months of idursulfase. One patient (17 years old) with an abnormal AHI at baseline had no significant change in his AHI (8.9 at baseline to 9.6 at 12 months) after idursulfase treatment. The average number of O<sub>2</sub> desaturation events per hour decreased from 19.2 at baseline to 2.4 after 12 months of idursulfase, with 6 of 7 patients experiencing a decrease of  $> 78\%$ .

### Echocardiography

At baseline 6 patients had left ventricular hypertrophy, defined as a left ventricular mass indexed to body surface area (LVMI) > 103 g/m<sup>2</sup>. After 12 months of idursulfase, LVMI of these patients declined from 151 ± 41 to 120 ± 25 g/m<sup>2</sup>, with 2 of the 6 patients reaching the normal limits of LVMI. Four of the 6 patients with normal LVMI at baseline remained normal after 12 months, whereas 2 increased into the hypertrophic category (final LVMI = 105 and 122 g/m<sup>2</sup>). Improvements in other cardiac parameters, including aortic morphology, mitral and tricuspid valve function, were variable and no clear treatment effect was observed.

### Safety

One year of treatment with idursulfase was well tolerated with the majority of adverse events (AEs) being consistent with those expected to be seen in an MPS II population. Patients in the 0.15 mg/kg dose group did not experience an infusion reaction, whereas 6 of the 8 patients in the higher dose groups had infusion reaction. Elevations of tryptase or complement were not found in these patients after any infusion reaction. All patients who had an infusion reaction were able to receive subsequent infusions delivered over 3 h, but some required premedication with antihistamines and/or corticosteroids.

One patient (20 year old) experienced three serious and potentially life-threatening episodes of respiratory distress during his infusions of idursulfase at 0.5 mg/kg. This patient had a history of severe upper airway obstruction which required a tracheostomy and needed nighttime ventilation and supplemental oxygen at baseline. During his 5th and 6th infusions of idursulfase, he experienced respiratory distress with a decrease in oxygen saturation to a low of 47%. Treatment consisted of stopping the enzyme infusion and starting supplemental oxygen which resulted in rapid improvement in his oxygen saturation. Subsequent infusions were successfully managed with premedication and a 3-h infusion time. After stopping the premedication prior to his 21st infusion, the patient had a life-threatening episode of respiratory distress during his 23rd infusion of idursulfase. One hour into that 3-h infusion, the patient was shivering, cyanotic and required bagging. The patient sub-

sequently experienced a seizure lasting about 30 s and lost consciousness. He was treated with diphenhydramine, epinephrine, methylprednisolone, and albuterol by nebulizer. His respiratory distress resolved within 60 min and he recovered without any sequelae. Subsequently, he has continued to receive idursulfase infusions with the use of premedications (steroids and antihistamine) and a slower infusion rate.

No significant changes in serum chemistries, urinalysis or complete blood count were noted during the 12 months of idursulfase infusions. One study subject who presented with a history of thrombocytopenia requiring IVIG infusions prior to starting the trial, had a slow rise in his platelet counts and did not need any additional IVIG infusions during the first 12 months of idursulfase infusions.

### Antibodies

IgG antibodies to idursulfase were detected at one or more time points in 6 of 12 patients: 3 in the 0.5 mg/kg group, and 3 in the 1.5 mg/kg group. Anti-idursulfase IgG antibodies were first detected after 3 infusions in 1 patient, after 5 infusions in 1 patients, and after 6 infusions in 4 patients. One of the 6 antibody-positive patients reverted to an antibody-negative status at 1 year of idursulfase treatment. The development of antibodies did not impact the biological or clinical activity of idursulfase as evidenced by the similar reduction in urinary GAG levels and liver and spleen volumes and the changes in 6MWT and %FVC observed in antibody negative compared to antibody positive patients (Table 3). No anti-idursulfase IgE antibodies were detected at any time.

### Discussion

This study represents the first use of enzyme replacement therapy with idursulfase in patients with MPS II. These results indicate that idursulfase administered every other week is generally well tolerated and that it has effects on several aspects of MPS II that may confer clinical benefit with long-term therapy. The sustained reduction in urinary GAG excretion supports that idursulfase maintained its biological activity with repeated dosing. Further evidence of its biological activity was seen in the reduction in size of liver and spleen. Clinical benefit is suggested by the

Table 3  
The effect of idursulfase in patients who remained IgG anti-idursulfase negative during the study compared with patients who were IgG anti-idursulfase positive during the study

IgG antibody status	N	Percent change from baseline				
		Urine GAG	Liver volume <sup>a</sup>	Spleen volume <sup>a</sup>	6MWT distance	FVC (% predicted)
Negative	6	-54 ± 12	-27 ± 16	-33 ± 10	9 ± 20	-0.7 ± 7.6
Positive	6	-45 ± 17	-34 ± 7	-23 ± 7	19 ± 15	3.6 ± 11.2
		P = 0.35	P = 0.33	P = 0.078	P = 0.34	P = 0.45

Values expressed as means ± SD.

P values determined by unpaired *t* test.

<sup>a</sup> Liver and spleen volume expressed as a percent of body weight.

improvement in 6MWT distance, a test which integrates the function of the respiratory, cardiovascular, and musculo-skeletal systems, which are all adversely affected in MPS II. Further clinical benefit is suggested by the reduction in heart size in patients with left ventricular hypertrophy at baseline and the improvement in sleep apnea.

Enzyme replacement therapy has been used successfully in other lysosomal storage diseases such as Gaucher disease [37,38], Fabry disease [17,18,39], and most recently MPS I [19,21] and MPS VI [19,21]. MPS I, MPS II, and MPS VI are caused by single, but distinct, enzyme deficiencies in the GAG catabolic pathway. MPS II patients have similar clinical disease compared to MPS I patients, except for the tendency toward an earlier onset and the presence corneal clouding in MPS I. After 6 months of a double-blind, placebo-controlled trial, 22 MPS I patients treated with recombinant human- $\alpha$ -L-iduronidase had an increase in % predicted FVC (based on age and baseline height) of  $4.9 \pm 8.7\%$  (mean  $\pm$  SD), which was significantly different than the  $0.7 \pm 5.9\%$  decrease observed in the placebo-treated group [19]. Similarly, 6MWT distance increased by a median of 27.5 m in the  $\alpha$ -L-iduronidase group compared to a median decrease of 11 m in the placebo group ( $P=0.066$ ). In addition, urinary GAG excretion was reduced by 54%, liver volume was reduced by 18.9%, and the number of AHI events per hour was reduced in patients with sleep apnea at baseline. Patients with MPS VI have a similar phenotype to patients with MPS I, except that neurological involvement is absent [1]. In the MPS VI phase III ERT double-blind, placebo-controlled study, 6 months of treatment with galsulfase (recombinant human *N*-acetylgalactosamine 4-sulfatase) decreased urinary GAG and patients walked 92 meters more in the 12 min walk test ( $P=0.025$ ) and 5.7 stairs per minute more in the 3 min stair climb ( $P=0.053$ ) than patients receiving placebo [21].

The results of the present study suggest that idursulfase has efficacy in patients with MPS II that is similar to the benefits observed by ERT in MPS I and MPS VI. The reduction in urinary GAG excretion and in the size of the liver and spleen clearly indicate that the enzyme is active and taken up into tissues and organs, and the results of the functional tests support a clinical benefit. After 1 year of treatment with idursulfase, 11 of 12 patients improved their 6-minute walking distance, with an average increase of 47.6 m. This increase is greater than observed for ERT in MPS I and is within the range of the minimum improvement considered to be clinically noticeable by patients with chronic obstructive pulmonary disease [40]. Idursulfase also tended to improve pulmonary function in patients who were less than 20 years old, although the increase in FVC was not statistically significant in this small study. The small and inconsistent improvement in FVC suggests that the benefits underlying the increase in 6MWT distance are not limited to improvements in pulmonary function.

The promising results of the present study have been recently confirmed and extended in a 1-year, double-blind study of idursulfase in MPS II patients [24]. Muenzer and

co-workers compared idursulfase (0.5 mg/kg) administered either weekly or every other week to placebo, and reported that both dosing regimens of idursulfase significantly improved the composite clinical endpoint comprising change in percent predicted FVC and change in 6MWT distance compared to placebo. However, the weekly dose appeared to be more effective than the every other week dosing regimen. The weekly dosing group demonstrated a significant 37 m increase in 6MWT distant ( $P=0.013$ ) and a 2.7% increase in percent predicted FVC ( $P=0.065$ ) compared to the placebo group. Thus, these results support the use of weekly idursulfase in the treatment of MPS II.

The safety profile of idursulfase was similar to that seen with other enzyme replacement therapies [17–19,37]. Infusion-related reactions occurred during one or more treatments in 6 of 8 patients in the 0.5 and 1.5 mg/kg groups. However, no patients in the 0.15 mg/kg group experienced an infusion reaction. These infusion-related reactions, which were of moderate to severe intensity, were successfully managed by premedication with an antihistamine and/or corticosteroids and by extending the infusion time from 1 to 3 h, with a stepwise increase in rate during the first hour. All patients successfully completed 12 months of therapy. During the study, IgG anti-idursulfase antibodies were detected in 6 of 12 patients, but the presence of these antibodies did not affect the clinical activity of idursulfase (Table 3). This lack of influence on clinical activity is important because MPS II is a chronic disease and successful treatment with idursulfase is expected to be life-long, and neutralizing antibodies could potentially negate any benefit.

Although this study was small and included patients at various stages of disease progression, the results support the conclusion that enzyme replacement therapy in MPS II patients with idursulfase administered every other week for a period of 12 months is safe and effective. The reductions in urinary GAG excretion and in organ size demonstrate that the idursulfase has biological activity. Further evidence of benefit was seen in the increase in 6-minute walking distance that demonstrated an improvement in mobility and endurance. The present study is being continued as a long-term, open-label study, with all patients treated with a single dose of idursulfase (0.5 mg/kg) given every other week. The results of this extension study will determine if the benefits reported here are maintained and will evaluate whether improvement in other parameters, like pulmonary function and joint mobility, emerge with longer therapy.

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