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Original Research

Enzyme replacement therapy for mucopolysaccharidosis VI: Growth and pubertal development in patients treated with recombinant human *N*-acetylgalactosamine 4-sulfatase

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Abstract. *Background and Methods:* Growth failure is characteristic of untreated mucopolysaccharidosis type VI (MPS VI: Maroteaux-Lamy syndrome). Growth was studied in fifty-six MPS VI patients (5 to 29 years old) prior to and for up to 240 weeks of weekly infusions of recombinant human arylsulfatase B (rhASB) at 1 mg/kg during Phase 1/2, Phase 2, Phase 3 or Phase 3 Extension clinical trials. Height, weight, and Tanner stage data were collected. Pooled data were analyzed to determine mean height increase by treatment week, growth impacts of pubertal status, baseline urinary GAG, and age at treatment initiation. Growth rate for approximately 2 years prior to and following treatment initiation was analyzed using longitudinal modeling.
Results: Mean height increased by 2.9 cm after 48 weeks and 4.3 cm after 96 weeks on enzyme replacement therapy (ERT). Growth on ERT was not correlated with baseline urinary GAG. Patients under 16 years of age showed greatest increases in height on treatment. Model results based on pooled data showed significant improvement in growth rate during 96 weeks of ERT when compared to the equivalent pretreatment time period. Delayed pubertal onset or progression was noted in 10 patients entering the clinical trials; all of whom showed progression of at least one Tanner stage during 2 years on ERT, and 6 of whom (60%) completed puberty.
Conclusion: Analysis of mean height by treatment week and longitudinal modeling demonstrate significant increase in height and growth rate in MPS VI patients receiving long-term ERT. This impact was greatest in patients aged below 16 years. Height increase may result from bone growth and/or reduction in joint contractures. Bone growth and resolution of delayed puberty may be related to improvements in general health, bone cell health, nutrition, endocrine gland function and reduced inflammation.

Keywords: Mucopolysaccharidosis VI, N-acetylgalactosamine 4-sulfatase, arylsulfatase B, enzyme replacement therapy, glycosaminoglycans, growth, puberty

1. Introduction

1.1. Drug/disease background

Mucopolysaccharidosis type VI (MPS VI; Maroteaux-Lamy syndrome) is a lysosomal storage disease in which deficient activity of the enzyme N-acetylgalactosamine 4-sulfatase (arylsulfatase B, or ASB; E.C. 3.1.6.12) impairs the stepwise degradation of the glycosaminoglycan (GAG) dermatan sulfate (DS) [22]. Partially degraded GAG accumulates in lysosomes in a wide range of tissues, causing a chronic progressive disorder characterized by significant functional impairment and a shortened lifespan [7].

Clinically, MPS VI is characterized by short stature and dysostosis multiplex, as well as characteristic facial features, corneal clouding, cardiac and pulmonary manifestations [22]. Dysostosis multiplex is the term identifying the characteristic skeletal features revealed by plain film radiological examination in MPS types and certain other storage diseases. Although growth charts are not available for MPS VI, accelerated growth has been reported in the first years of life in MPS VI with advanced bone maturation at birth, followed by slowing growth rate and growth failure after 2 years of age [14]. This pattern of growth is similar to those described for MPS IVA and achondroplasia, two other skeletal dysplasias [15,20].

A Survey Study of 121 untreated MPS VI patients (age range 4 to 56 years), representing an estimated 10% of the developed world population, demonstrated mean height of 115.2 cm $\pm$ 26.1 cm and median height of 103.7 cm with a range of 80.0–169 cm [30]. The

large difference between the mean and median values reflected the contribution of a several taller subjects with slowly advancing disease.

The short stature in MPS disease is believed to be related to a combination of joint contracture, bone growth plate disorganization and failure, and endocrine abnormalities [23]. Growth plate failure in MPS disease may be related to abnormal GAG storage in chondrocytes leading to inflammatory response, cell dysfunction, and death [25–27]. Endocrine gland dysfunction [23] or nutritional deficiencies may also cause growth failure in MPS patients. Animal models of MPS VI have demonstrated improved growth plate structure with early initiation of ERT [3].

Three clinical studies using recombinant human ASB (rhASB) ERT to treat MPS VI patients have been reported (see Table 1). A Phase 1/2 study and a Phase 2 study both demonstrated that weekly infusions of 1 mg/kg rhASB were well tolerated, produced a rapid reduction in urinary GAG levels, and improved endurance [9,11]. A Phase 3 double-blind, placebo-controlled study demonstrated greater improvement in endurance on the 12-minute walk test (12MWT) in patients treated with rhASB for 24 weeks compared with patients receiving placebo, and infusions were well tolerated [10]. In all studies improvement in endurance was maintained during an open-label extension phase with patients followed up to 240 weeks [12]. In addition to endurance measures, all three studies monitored change in weight, height, and stage of puberty.

The present paper evaluated pooled long-term data from the clinical rhASB ERT trials and the Survey Study to determine the impact of ERT on growth and puberty in patients with MPS VI.

2. Methods

2.1. Study design

Detailed study design and evaluation criteria have been reported in previous publications of the MPS VI Survey Study and the Phase 1/2, Phase 2, and Phase 3 clinical studies of rhASB treatment in MPS VI, which reported results of 48 weeks of treatment [9–12]. Collection of efficacy data continued for up to 240 weeks during the extension phase of these studies. These studies are summarized in Table 1. Because most patients participating in the Phase 3 clinical trial had previously participated in the MPS VI Survey Study, pre-ERT data were obtained from the Survey Study. In addition, pre-ERT data were collected in patients randomized to the placebo group during the first 24 weeks of the Phase 3 clinical trial.

Most patients received rhASB at a dose of 1 mg/kg/week infused over a 4-hour period; three patients in the Phase 1 study received 0.2 mg/kg/wk for the initial part of that study and 19 patients completing the Phase 3 extension study received placebo during the initial 24 weeks of treatment in the Phase 3 study. Patients receiving placebo during the initial 24 weeks of the Phase 3 study were evaluated 24 and 72 weeks after initiation of active therapy.

2.2. Anthropometric, Tanner, and thyroid assessments

Standing height and weight were measured according to standardized techniques using calibrated stadiometer and scale [29]; BMI was calculated as weight/(height)^2. Study physicians determined stage of puberty as a composite of the genital and pubic hair Tanner development scores [13,18,19]. Stage of puberty was defined at baseline as either pre-puberty without delay, pre-puberty with delayed onset, puberty in progress with delayed progression, puberty in progress with no delay, or puberty completed, using longitudinal standards established by Tanner and Davies [31]. Puberty was considered delayed if subject's age was $>$ 2 standard deviations (SD) from mean expected age for the patient's Tanner stage. Thyroid stimulating hormone (TSH) and free thyroxine (T4) were determined at baseline at either local hospital or central laboratory depending on the specific study.

2.3. Analysis and statistical methods

Plots display mean height by treatment week for all patients combined (overall) and by pubertal status (Tanner 1, 2–3, 4–5), baseline GAG level ($<$ 200, 200–299, 300–399, or $\geqslant$ 400 μg/mg creatinine), and age grouping (5–7 years, 8–11 years, 12–15 years, or $\geqslant$ 16). The age groupings were selected to approximate very young (5–7 years of age), pre- pubertal (8–11 years of age), pubertal (12–15 years of age), and post-pubertal ($\geqslant$ 16 years of age) developmental stages. Tables summarize baseline anthropometric, Tanner, and thyroid function characteristics with corresponding summary statistics for each treatment week, including changes from baseline; pubertal development over the course of follow-up; and model results for the effects of ERT on growth. Summaries and analyses of data combined from all phases use the first 96 weeks of treatment; summaries display statistics for all available weeks of treatment for which at least 40 patients had data.

Table 1

Summary of study populations

Study	Study Time Period (Yrs)	Study Design	Weeks of study: Efficacy	Number of Patients Enrolled/ Completed	Dose of rhASB	Age (yrs) Mean ± SD (range yrs)	Sex (M/F)	Height (cm) Mean ± SD	Number of Patients Withdrawn: Time of Withdrawal	Baseline Urinary GAG (μg/mg creatinine) Mean ± SD
Phase 1/2	2001–05	Double-Blind, Randomized, Dose Comparison/Open-Label Extension	240	7/5	0.2 mg/kg 1.0 mg/kg	12.0 ± 3.8 (7 to 16)	4/3	107.5 ± 21.5	1 (0.2 mg/kg) after Week 3; 1 (0.2 mg/kg) after Week 32	365 ± 148
Survey Study (patients not on ERT)	2002–03	Cross sectional study of patients not on ERT		123/121	None	13.9 ± 10 (4 to 56)	58/63	115 ± 26		321 ± 200
Phase 2	2002–06	Open-Label, Non-randomized	144	10/10	1.0 mg/kg	12.1 ± 5.3 (6 to 21)	7/3	103.7 ± 14.4	0	336 ± 116
Phase 3	2003–06	Double-Blind, Placebo-Controlled, Randomized/Open-Label Extension	96	39/38	1.0 mg/kg	13.7 ± 6.5 (rhASB) 10.7 ± 4.4 (placebo) (5 to 29)	13/26	104.4 ± 2.9 (rhASB) 100.3 ± 13.5 (placebo)	1 (placebo group) after Week 5	346 ± 128 (rhASB) 330 ± 114 (placebo)

Table 2
Baseline characteristics

	Overall		5–7 years		8–11 years		12–15 years		⩾ 16 years	
	n	Observed Mean ± SD	n	Observed Mean ± SD	n	Observed Mean ± SD	n	Observed Mean ± SD	n	Observed Mean ± SD
Weight (kg)	54	22.1 ± 7.1	7	16.3 ± 1.9	25	20.4 ± 6.8	8	23.5 ± 4.8	14	27.2 ± 7.0
Height (cm)	54	101.9 ± 12.1	7	96.1 ± 6.9	25	100.4 ± 12.0	8	99.3 ± 11.1	14	108.9 ± 12.7
Z-score[1]		−6.6		−4.4		−5.7		−8.3		−8.3
BMI (kg/m^2)	54	21.0 ± 3.7	7	17.6 ± 1.5	25	20.0 ± 3.1	8	23.9 ± 3.5	14	22.8 ± 4.0
Z-score[1]		1.1		1.2		1.3		1.4		0.4
Urine GAG (μg/mg creatinine)	54	338 ± 111	7	373 ± 121	25	329 ± 119	8	364 ± 59	14	319 ± 117
Albumin (g/dL)*[2]	50	4.3 ± 0.4	7	4.3 ± 0.4	24	4.3 ± 0.4	8	4.2 ± 0.6	11	4.5 ± 0.4
Range		(3.2, 5.3)		(3.7, 4.8)		(3.2, 5.3)		(3.4, 5.1)		(3.5, 5.0)
Tanner score										
1	34		7		23		1		3	
2	11		0		2		6		3	
3	2		0		0		1		1	
4	4		0		0		0		4	
5	3		0		0		0		3	

Summary statistics calculated using measures at study entry, rather than treatment baseline;[1] Z-scores based on World Health Organization 2007 tables; [2] Normal Range: Albumin (3.9–5.4 for 5 yr to adult); (Harriet-Lane 2002).

To determine the mean rate of height increase prior to and following initiation of ERT, a longitudinal linear mixed-effects (LME) model [28] was constructed using pooled data. The model incorporated both pre-ERT and post-ERT data by including a linear spline for time with a knot at treatment week 0. This formulation allows different slopes of the mean trend before and after start of ERT. A random intercept and slope give all individuals their own regression lines with separate intercepts and slopes that deviate from the population line. The longitudinal model includes repeated measures over time and allows correlations of observations within a patient. The model uses empirical estimates for the standard error, which in large samples "corrects" for misspecifying the correlation structure.

"Time" refers to time from start of ERT. Because data were also obtained before the start of treatment, some times are negative. The length of follow-up for each trial phase differs; most patients have approximately two years of follow-up height data (96 weeks) after start of ERT. The LME method is flexible in that subjects need not have all measurements at all time-points. Because historical data are limited, the analysis restricts the length of time to a two-year window on either side of ERT initiation.

3. Results

3.1. Baseline assessments

The age of patients at the time of enrollment in a clinical therapy trial ranged from 5 to 29 years; the mean age was approximately 12 years. Baseline anthropometric, albumin, urine GAG, and Tanner assessments are presented in Table 2 for all patients and by age category.

Heights in all age groups were much lower than the corresponding age-related heights in a normal U.S. population [8]. BMI was in the normal range with the mean Z scores above zero for all age groups at baseline (Table 2). This is consistent with other skeletal dysplasias, MPS IVA [20] and achondroplasia [15], which demonstrate BMI patterns that are shifted up in relation to the CDC reference curves. As evidenced by a preponderance of Tanner scores of 1 and 2, MPS VI study patients were primarily pre-pubertal or in an early stage of puberty at baseline (45/54, 83%; Table 2), although 19% of the overall group was classified as having delayed onset or progression of puberty at baseline. The urinary GAG levels at baseline were far above those seen in a healthy population [30] and were similar in all age groups. No patient entering a clinical therapy trial was hypothyroid at baseline, but no other endocrine functions were tested.

3.2. Observed increases over time on treatment

Over the two years of therapy, mean height increased over time (Table 3): overall (Fig. 1); for each pubertal stage (Tanner 1, 2–3, 4–5) (Fig. 1); for each urinary GAG category ($<$ 200, 200–299, 300–399, $>$ 399) (Fig. 2) and for each age category (5–7 yr, 8–11, 12–15, $\geqslant$ 16 yr) (Fig. 2).

Those aged below 16 years of age were shorter at baseline but showed greater increase in height over the

Table 3
Mean height (cm) over time, overall and by age

	Overall					
Treatment week	n	Observed Mean (SD)	Change from BL Mean (SD)			
0	54	101.9 (12.1)	–			
12	54	102.4 (11.9)	0.6 (.09)			
24	54	103.1 (11.9)	1.3 (1.2)			
48	50	105.4 (11.9)	2.9 (1.8)			
72	46	105.8 (12.0)	4.0 (2.1)			
96	46	107.6 (12.1)	4.3 (2.9)			
	5–7 years			8–11 years		
Treatment week	n	Observed Mean (SD)	Change from BL Mean (SD)	n	Observed Mean (SD)	Change from BL Mean (SD)
0	7	96.1 (6.9)	–	25	100.4 (12.0)	–
12	7	96.8 (6.9)	0.7 (0.6)	25	100.9 (11.9)	0.5 (1.0)
24	7	98.0 (7.0)	1.9 (1.3)	25	101.8 (12.1)	1.4 (1.0)
48	5	100.6 (7.4)	3.8 (1.2)	24	103.5 (12.3)	3.5 (1.5)
72	5	99.3 (4.9)	5.2 (1.8)	24	104.3 (12.0)	4.7 (1.6)
96	6	101.9 (7.5)	5.1 (2.3)	21	106.8 (12.7)	5.6 (2.1)
	12 – 15 years			$\geqslant$ 16years		
Treatment week	n	Observed Mean (SD)	Change from BL Mean (SD)	n	Observed Mean (SD)	Change from BL Mean (SD)
0	8	99.3 (11.1)	–	14	108.9 (12.7)	–
12	8	100.0 (10.8)	0.7 (0.8)	14	109.4 (12.5)	0.5 (0.9)
24	8	100.3 (10.9)	1.1 (1.1)	14	109.7 (12.4)	0.7 (1.6)
48	6	105.5 (10.6)	2.2 (1.6)	14	110.7 (12.3)	1.8 (1.9)
72	6	105.1 (12.8)	3.6 (1.9)	11	112.6 (12.2)	2.3 (2.3)
96	5	108.2 (14.0)	4.2 (4.0)	14	110.9 (12.3)	2.0 (2.7)

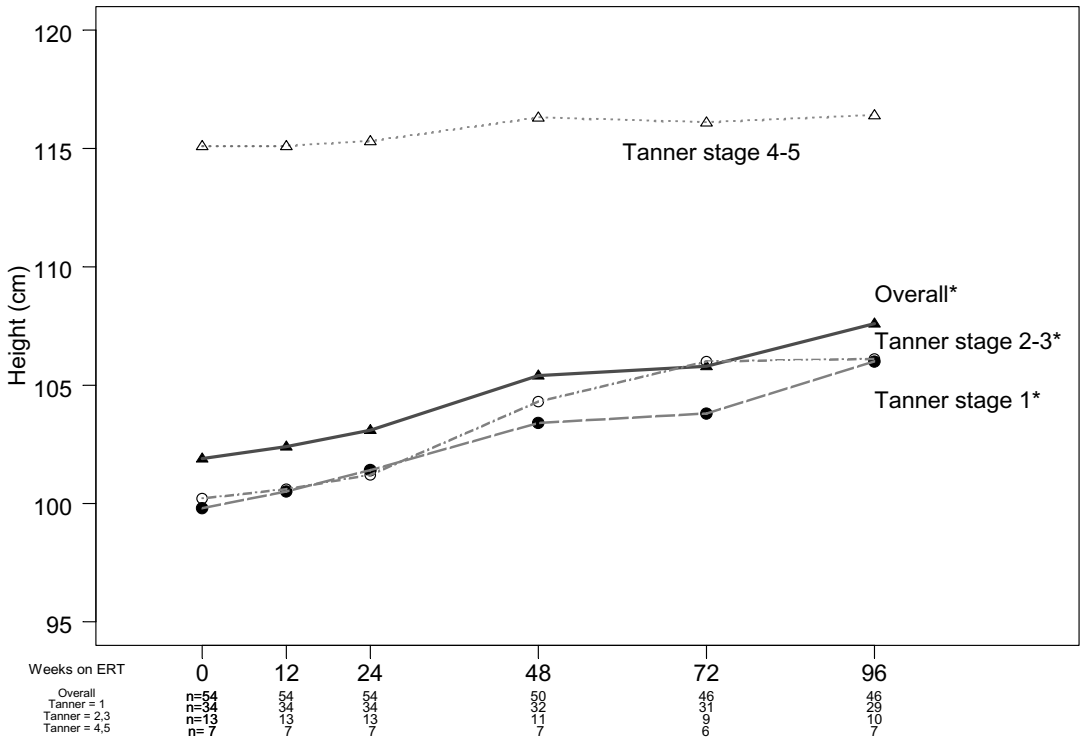

Fig. 1. Height, observed mean overall and by pubertal status at initiation of ERT. Each point represents the mean height (cm) for the group of patients observed at number of weeks on ERT indicated by x-axis. Asterisks (*) denote p-values < 0.05 at week 96 compared to baseline.

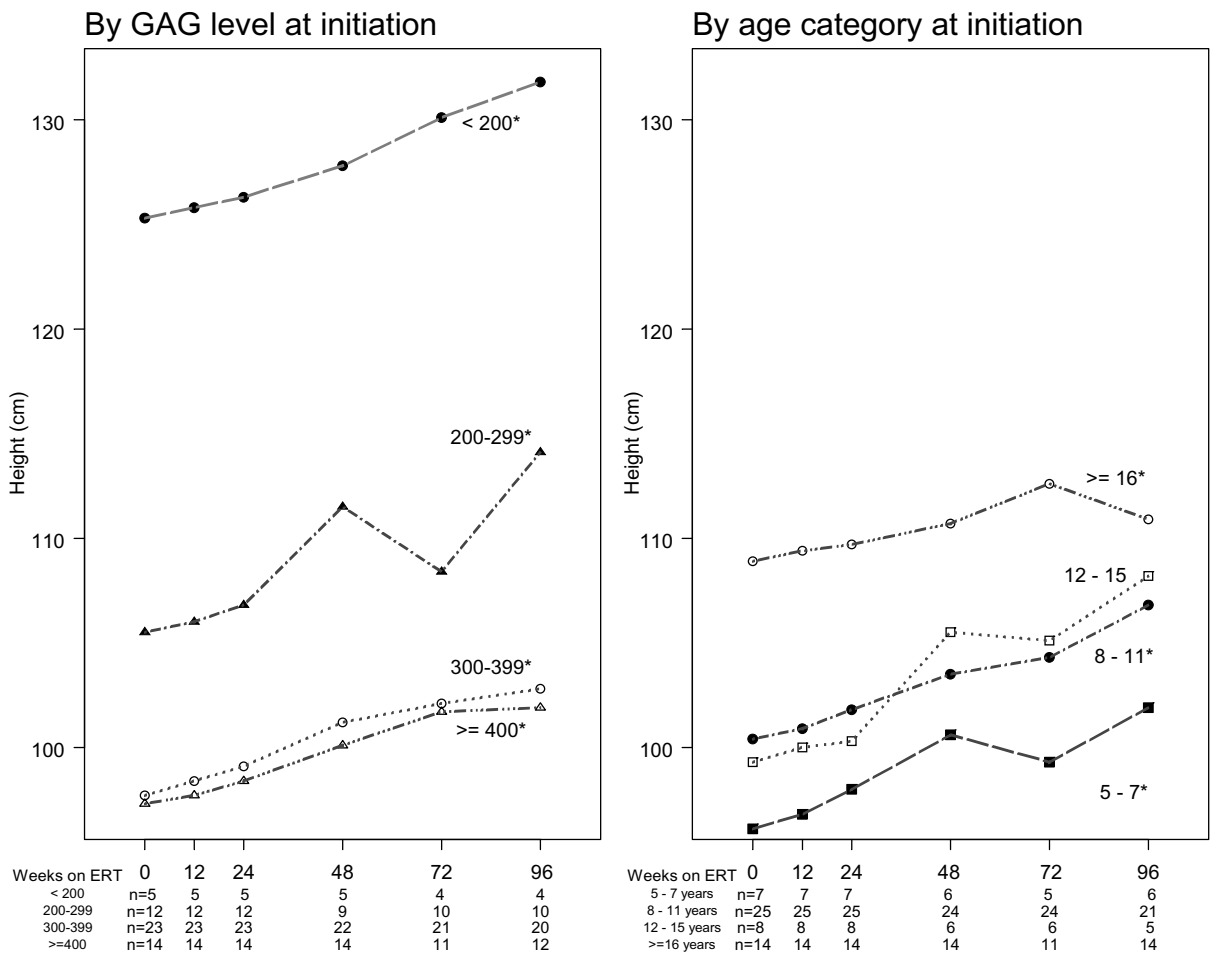

Fig. 2. Height, observed mean by GAG level and by age category at initiation of ERT. Each point represents the mean height (cm) for the group of patients observed at number of weeks on ERT indicated by x-axis. Asterisks (*) denote p-values < 0.05 at week 96 compared to baseline.

treatment period (Table 3; Fig. 2). On average, growth rate was greater in patients who were prepubertal or in puberty than in patients who had completed puberty (Fig. 1). Growth rate did not differ, however, with increasing urinary GAG level at baseline (Fig. 2).

Age specific BMI SDS scores were determined at baseline and 96 weeks using WHO BMI data. Among those patients with measurements at both baseline and week 96, mean BMI at week 96 changed significantly from baseline for the the 8–11 group, $\geqslant 16$ group and overall (data not shown).

3.3. Regression analyses

Model results show that for all patients, the estimated mean height at baseline is approximately 102 cm (Table 4). For the year prior to ERT, mean height increased less than 1 cm on average. After a year on ERT, mean height increased about 2.6 cm on average ($p < 0.001$). Growth velocity within this time frame differs significantly pre-ERT and post-ERT initiation ($p < 0.001$). Individual observations for each patient over time, as well as the modeled population regression lines pre-ERT and post-ERT initiation, are displayed in Fig. 3. The plot shows the scatter of observations including fewer historical measurements prior to ERT, the completeness of measurements after ERT initiation, as well as the variability both across and within individuals.

The model's flexibility also allows estimation of each individual's growth over the two years pre-ERT and post-ERT initiation. Figure 4 displays plots of the modeled growth, in centimeters, as a function of age at baseline for each individual. Height measurement of patients with skeletal dysplasia is challenging; observations below the reference line representing "no growth" may result from variability in measurement or standing

Table 4
Predicted change in height per year (cm) based on model results

Model	Average height at baseline (cm)	Time period	N	Change in height per year (SE)	Difference (Post-Pre) p-value
All patients	102	Pre-ERT	47	0.65 (0.25)	< 0.001
		Post-ERT	54	2.6 (0.22)	
5–7	96	Pre-ERT	7	1.9 (0.47)	< 0.001
		Post-ERT	7	3.1 (0.43)	
8–11	100	Pre-ERT	23	0.93 (0.28)	< 0.001
		Post-ERT	25	3.3 (0.22)	
12–15	99	Pre-ERT	6	0.93 (0.66)	< 0.001
		Post-ERT-	8	2.1 (0.66)	
$\geqslant 16$	109	Pre-ERT	11	−0.79 (0.37)	0.32
		Post-ERT	14	1.2 (0.39)	

N is the number of patients with observations during the given time period. Heights are measured in centimeters.

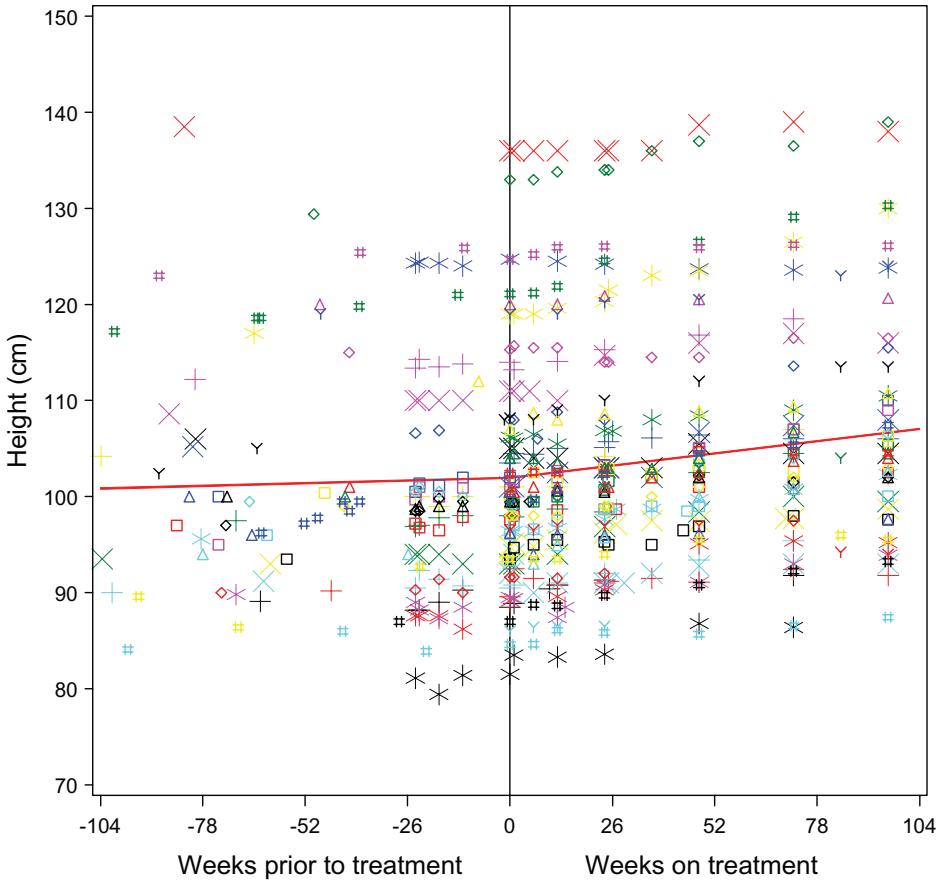

Fig. 3. Observed height over two years, with fitted regression line. Each symbol/color combination represents a single patient's height measurement at the time indicated on the x-axis. In this figure, each dot represents an actual measurement. Time 0 is the time of initiation of ERT.

posture. The two plots show that individual growth increases more in the year on ERT than in the year prior to ERT initiation.

The growth (Table 4) occurs primarily in younger patients: those 5–7 years old grew approximately 2 cm per year before ERT and 3 cm per year after ERT initiation and those 8–11 grew approximately 1 cm per year prior to ERT and approximately 3 cm per year after ERT initiation. Patients in the 12–15 year range grew approximately 1 cm per year prior to ERT and approxi-

mately 2 cm per year after ERT. The oldest patients ($\geqslant$ 16 years) did not grow in the year prior to ERT, but grew approximately 1.2 cm per year after ERT initiation.

We examined the sensitivity of the estimated effect of ERT to baseline urinary GAG level by modifying the model to include the baseline GAG level. Whether we used GAG as a continuous variable or GAG as a categorical variable with categories defined as in Fig. 2, the resulting estimate of the effect of time on ERT remained unchanged (data not shown). Since the sample size was small we did not perform analysis by gender. We also reanalyzed the model excluding the three patients with baseline Tanner score of 5 the results remained essentially the same.

Patients' pre-ERT data were considered the best estimate of expected growth in the absence of treatment. In an effort to simulate a potential "control group" for these data, a supportive analysis considered hypothetical multiple "trials" by calculating the observed rates of growth, randomly selecting half of the patients pre-treatment, and comparing these rates to those of the remaining patients post-treatment. After computing the t-tests for 1000 such samples, the average p-value is 0.025.

3.4. Change in pubertal status with ERT treatment

Most (56%) of patients were pre-pubertal and not delayed at the initiation of therapy, reflecting the relatively young age of recruited patients related to the rapid progression of the disease and death of patients in late second or early third decades, and the requirement that patients be able to walk in the Phase 2 and 3 studies. Sixty-seven percent (20/30) of these pre-pubertal patients without delay at baseline remained pre-pubertal by the 96 week observation. Excluding the pre-pubertal patients, without delay, a large percentage (42%, 10/24) of patients who should be pubertal or post-pubertal had delayed onset or delayed progression at baseline. All of these patients showed progression during a minimum of 72 weeks follow-up; with 60% completing puberty.

4. Discussion

Increased growth rate was demonstrated in the 2-year period after initiating Naglazyme®(galsulfase; rhASB) ERT as compared to the 2-year period prior to initiating ERT; this increase was greatest in patients younger than 16 years. The increased growth velocity in the pre-pubertal age groups suggests a positive impact of ERT on growth, since growth velocity in normal children is relatively constant or decreasing between age 5 years and the onset of puberty [1,16,17]. Although we cannot rule out an effect of puberty on growth rate, previous studies in patients with achondroplasia or MPS IVA, two other skeletal dysplasias [15,20], showed that a pubertal growth spurt did not occur in these populations and may not therefore be expected in the present study. Baseline urinary GAG levels did not influence this response in growth to ERT. Although gender is an important variable when considering growth rate [1,31], the sample sizes, when broken down by both age and gender, do not have sufficient power to allow meaningful conclusions. Improved growth in response to long-term ERT (Aldurazyme® [laronidase]) has also been noted for MPS I patients in 2 studies. Sifuentes et al. [24] described a "substantial increase in growth" in patients started on therapy before puberty. Clarke et al. [4] noted that median height velocity normalized in pediatric patients with MPS I and the shortest patients showed the greatest catch-up growth. Although the MPS VI patients in the present report did not achieve normal median growth velocity for pre-pubertal children of 6–8 cm/year [1], they had more severe skeletal disease than the MPS I patients included in the Clarke study, with mean height at baseline of 101.9 cm (mean age 12–13 years) versus mean height 136.3 cm (mean age 15.7 years) of patients in the Clarke study.

The nature of the growth data presented in this paper presented challenges that affected the feasible analyses and results. Relatively small sample size, differing study lengths, collection of data at different study time points and the limited amount of pre-treatment or natural history data all impacted the presented analyses. Some individuals have only one or two observations within the two-year period prior to ERT initiation, which limits the ability to model an individual's growth pattern (Fig. 3). At times, either variability in measurement or standing posture related to worsening muscle strength or joint contractures may have resulted in 1 to 2 centimeters of apparent "shrinkage" in growth; particularly evident in the pre-ERT measurements. A supportive analysis simulated potential control group data by considering multiple "randomizations" of the existing study data and comparing hypothetically independent groups pre- and post-ERT. While it was not possible to control for all the covariates of interest (i.e., Tanner score, exact age, or gender in a small study population), these simulation results offer some reassurance that the conclusions from the model are sound.

Growth comprises three phases: infancy, childhood, and puberty [16,17]. Each phase is under unique en-

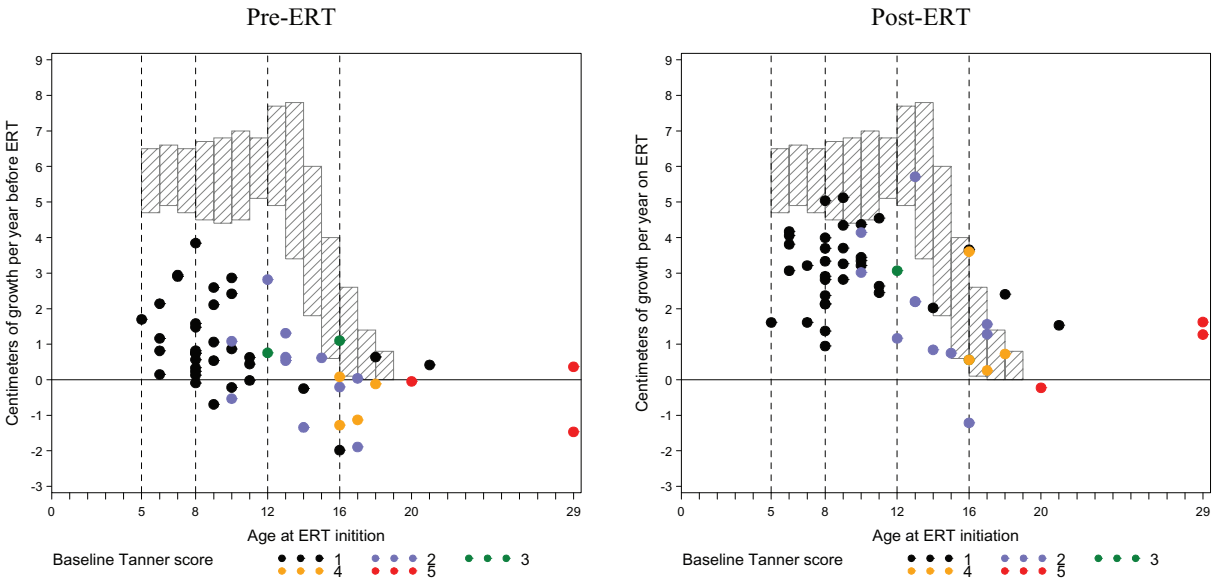

Fig. 4. Modeled rates of growth over one year compared to WHO growth charts. Each hatched bar represents the normal range of rates of growth by year of age. The bottom of the box represents the 1st percentile; the top represents the 99th percentile. Each dot shows an individual patient's Tanner score at time of initiation of ERT and the rate of growth pre- and post- initiation of ERT. For patients without a pre-ERT measurement, the baseline values were imputed by a longitudinal model (see text). (Reference for normal rates: http://www.who.int/childgrowth/standards/en/).

docrine control with childhood growth being controlled by growth hormone (GH)/ insulin-like growth factor (IGF) axis, and puberty under the additional influence of sex steroids. An estimated 17% to 18% of final height is accounted for by pubertal growth in healthy individuals [1]. After completion of puberty, bone growth centers are fused and growth ceases. In the present evaluation, puberty makes the effect of ERT on linear growth more difficult to assess because of puberty's variable time of onset, the potential failure of puberty to occur, and the large number of pre-pubertal age subjects in the study population. Age at onset of puberty is highly variable and gender-dependent [6]; both age and gender could affect statistical analysis in the small population. The population studied here had more females than males. Further, the sample size was too small to allow analysis of each year of age. Therefore, we chose age groupings: 5–7 years (the very young), 8–11 years (pre-puberty), 12–15 years (puberty), and $\geqslant$ 16 years (the typical post-pubertal age where growth would stop).

Finally, ERT may also have exerted a direct effect on endocrine gland function, thus improving pubertal growth or even muscle strength or joint contractures, thus increasing height even after the bone growth plates had fused.

To see if the patterns of growth shift toward those typically seen in a healthy pediatric population, we used growth charts from the World Health Organization (WHO) to estimate expected growth in a year for ages ranging from 5 to 18 years. To calculate an expected range of growth for a given year, we took the expected growth within a year for a child in the first and 99th percentiles of height for a given age, pooled over gender. These ranges produce the shaded regions of expected yearly growth as seen in Fig. 4. Within each age range, we first plotted the modeled growth in the year prior to ERT initiation. The plot shows that most patients grow within each age range. It also shows some variability in historical measurements as represented by the number of "no growth" points lying below the horizontal reference line. In contrast, in the corresponding post-ERT plot most of the points shifted up towards the lower ranges of the shaded regions of expected growth for a healthy population. This shift is seen in all age groups, including patients at least 16 years, demonstrating that ERT may still increase the height of older patients. If those who typically do not grow after puberty do increase in height on ERT, then one can speculate that this increase is not due to bone growth but to better posture and decreased contractures.

Because the data in the analyses are limited to two years post-ERT, one should not extrapolate beyond the range of the data presented. Longer-term trends will probably not be linear over time. Longer-term da-

ta could potentially address whether growth velocity changes over time in the MPS VI population.

Endocrine abnormalities in the hypothalamic-pituitary-growth hormone (GH)/insulin-like growth (IGF) factor axis have been reported in rare cases of MPS disease [2,32], although GH has also been documented as normal in isolated cases [21]. Precocious puberty has been noted in several MPS III patients [5], but pubertal delay or abnormality of the hypothalamic-pituitary-gonadal axis has not been reported in an MPS disease. The present study excluded thyroid hormone deficiency in all patients, but did not evaluate growth hormone or sex hormone status. After excluding patients who were pre-pubertal without delay, we found that 10 of 24 patients expected to be pubertal or post-pubertal had delay onset or progression of puberty. This is far above the expected rate in a normal population and suggests that some aspect of MPS VI disease leads to this endocrine failure. Further studies will be necessary to determine whether this increased rate reflects a primary failure of hypothalamic-pituitary function or secondary failure related to gonadal gland failure. It is possible but unlikely that end tissue response (breast/penis or pubic hair) is blocked. It is unlikely that malnutrition is responsible for growth failure in MPS VI with BMI in the normal range. Notably, all 10 patients showed progression through puberty after starting ERT with six patients reaching Tanner score 4 or 5.

4.1. Overall conclusions

In conclusion, analysis of combined data from the MPS VI clinical studies showed that overall patients experienced an increased rate of growth after ERT initiation. Although the analysis is based on a limited number of observations and cannot separate out the growth impacts of gender and puberty, it suggests a positive impact of ERT on growth. The increase in height was largest in patients aged under16 years suggesting the advantage of initiating ERT as early as possible to maximize potential benefits in final height. Patients with pubertal delay showed pubertal progression after treatment, and several completed puberty. ERT appears to effectively treat delayed puberty which is common in MPS VI patients. The mechanisms for growth or pubertal failure are unknown, but future studies can examine possible relationship to endocrine deficiencies.

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Conflict of interest

Drs. Harmatz, Beck, Giugliani, Berger, Steiner and Yu have provided consulting support to BioMarin Pharmaceutical Inc., Novato, CA. Dr. Hopwood has received commercial research project funding to assist the development of enzyme replacement therapy for MPS VI patients. Drs. Harmatz, Arash and Beck each report receiving speaker's honorarium and travel support from BioMarin. BioMarin is a supporter of the Lysosomal Disease Network's WORLD Symposium organized by Dr. Whitley. Drs. Swiedler and Decker are former and current employees of BioMarin Pharmaceutical Inc., respectively; both are stockholders.

References

[1] V. Abbassi, Growth and normal puberty, *Pediatrics* **102**(2 Pt 3) (1998), 507–511.

[2] B. Buyukgebiz et al., Maroteaux-Lamy syndrome associated with growth hormone deficiency, *Journal of Pediatric Endocrinology & Metabolism* **8**(4) (1995), 305–307.

[3] S. Byers et al., Effect of enzyme replacement therapy on bone formation in a feline model of mucopolysaccharidosis type VI, *Bone* **21**(5) (1997), 425–431.
[4] L.A. Clarke et al., Long-term efficacy and safety of laronidase in the treatment of mucopolysaccharidosis I, *Pediatrics* **123**(1) (2009), 229–240.
[5] D. Concolino et al., Precocious puberty in Sanfilippo IIIA disease: diagnosis and follow-up of two new cases, *Eur J Med Genet* **51**(5) (2008), 466–471.
[6] S.Y. Euling et al., Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings, *Pediatrics* **121**(Suppl 3) (2008), S172–S191.
[7] R. Giugliani, P. Harmatz and J.E. Wraith, Management guidelines for mucopolysaccharidosis VI, *Pediatrics* **120**(2) (2007), 405–418.
[8] P. Hamill et al., Physical growth: National Center for Health Statistics percentiles, *Am J Clin Nutr* **32**(3) (1979), 607–629.
[9] P. Harmatz et al., 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* **115**(6) (2005), e681–e689.
[10] P. Harmatz 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* **148**(4) (2006), 533–539.
[11] P. Harmatz et al., Enzyme replacement therapy in mucopolysaccharidosis VI (Maroteaux-Lamy syndrome), *J Pediatr* **144**(5) (2004), 574–580.
[12] P. Harmatz et al., Long-term follow-up of endurance and safety outcomes during enzyme replacement therapy for mucopolysaccharidosis VI: Final results of three clinical studies of recombinant human N-acetylgalactosamine 4-sulfatase, *Mol Genet Metab* **94**(4) (2008), 469–475.
[13] Harriet-Lane, *The Harriet Lane Handbook: A Manual for Pediatric House Officers*. 16 ed, V.L. Gunn and C. Nechyba, eds, 2002, Philadelphia: Mosby.
[14] D. Heron et al., Early diagnosis of Maroteaux-Lamy syndrome in two patients with accelerated growth and advanced bone maturation, *Eur J Pediatr* **163**(6) (2004), 323–326.
[15] J.E. Hoover-Fong et al., Age-appropriate body mass index in children with achondroplasia: interpretation in relation to indexes of height, *Am J Clin Nutr* **88**(2) (2008), 364–371.
[16] J. Karlberg, A biologically-oriented mathematical model (ICP) for human growth, *Acta Paediatr Scand Suppl* **350** (1989), 70–94.
[17] J. Karlberg, On the construction of the infancy-childhood-puberty growth standard, *Acta Paediatr Scand Suppl* **356** (1989), 26–37.
[18] W.A. Marshall and J.M. Tanner, Variations in pattern of pubertal changes in girls, *Arch Dis Child* **44**(235) (1969), 291–303.
[19] W.A. Marshall and J.M. Tanner, Variations in the pattern of pubertal changes in boys, *Arch Dis Child* **45**(239) (1970), 13–23.
[20] A.M. Montano et al., Growth charts for patients affected with Morquio A disease, *Am J Med Genet A* **146A**(10) (2008), 1286–1295.
[21] J. Nelson and D. Carson, Pituitary function studies in a case of mild Hunter's syndrome (MPS IIB), *J Med Genet* **26**(11) (1989), 731–732.
[22] E. Neufeld and J. Muenzer, The Mucopolysaccharidoses Chapter 136, in: *Metabolic and Molecular Bases of Inherited Disease*, C. Scriver, ed., 2001, McGraw-Hill: New York.
[23] L.E. Polgreen et al., Growth and endocrine function in patients with Hurler syndrome after hematopoietic stem cell transplantation, *Bone Marrow Transplant* **41**(12) (2008), 1005–1011.
[24] M. Sifuentes et al., A follow-up study of MPS I patients treated with laronidase enzyme replacement therapy for 6 years, *Mol Genet Metab* **90**(2) (2007), 171–180.
[25] C.M. Simonaro et al., Joint and bone disease in mucopolysaccharidoses VI and VII: identification of new therapeutic targets and biomarkers using animal models, *Pediatr Res* **57**(5 Pt 1) (2005), 701–707.
[26] C.M. Simonaro et al., Mechanism of glycosaminoglycan-mediated bone and joint disease: implications for the mucopolysaccharidoses and other connective tissue diseases, *Am J Pathol* **172**(1) (2008), 112–122.
[27] C.M. Simonaro, M.E. Haskins and E.H. Schuchman, Articular chondrocytes from animals with a dermatan sulfate storage disease undergo a high rate of apoptosis and release nitric oxide and inflammatory cytokines: a possible mechanism underlying degenerative joint disease in the mucopolysaccharidoses, *Lab Invest* **81**(9) (2001), 1319–1328.
[28] J. Singer and J. Willett, *Applied Longitudinal Data Analysis*. 2003, Oxford University Press, Oxford.
[29] R.D. Stevenson, Use of segmental measures to estimate stature in children with cerebral palsy, *Arch Pediatr Adolesc Med* **149**(6) (1995), 658–662.
[30] S.J. Swiedler et al., Threshold effect of urinary glycosaminoglycans and the walk test as indicators of disease progression in a survey of subjects with Mucopolysaccharidosis VI (Maroteaux-Lamy syndrome), *Am J Med Genet A* **134A**(2) (2005), 144–150.
[31] J.M. Tanner and P.S. Davies, Clinical longitudinal standards for height and height velocity for North American children, *J Pediatr* **107**(3) (1985), 317–329.
[32] S.P.A. Toledo et al., SERUM GROWTH HORMONE LEVELS IN HUNTER SYNDROME, *Revista do Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo* **46**(1) (1991), 9–13.