

# Agalsidase Alfa and Kidney Dysfunction in Fabry Disease

Michael West,\* Kathy Nicholls,<sup>†</sup> Atul Mehta,<sup>‡</sup> Joe T.R. Clarke,<sup>§||</sup> Robert Steiner,<sup>¶</sup>  
Michael Beck,\*\* Bruce A. Barshop,<sup>††</sup> William Rhead,<sup>‡‡</sup> Robert Mensah,<sup>§§</sup> Markus Ries,<sup>\*\*|||</sup>  
and Raphael Schiffmann<sup>|||</sup>

\*Department of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada; <sup>†</sup>Royal Melbourne Hospital, Parkville, Victoria, Australia; <sup>‡</sup>Royal Free Hospital and University College Medical School, London, United Kingdom; <sup>§</sup>Division of Clinical and Metabolic Genetics, Hospital for Sick Children and University of Toronto, Toronto, Canada; <sup>||</sup>Service de génétique médicale, Centre hospitalier universitaire de Sherbrooke, Sherbrooke, Canada; <sup>¶</sup>Oregon Health and Science University, Portland, Oregon; <sup>\*\*</sup>Center for Lysosomal Storage Diseases, University of Mainz, Mainz, Germany; <sup>††</sup>University of California San Diego School of Medicine, La Jolla, California; <sup>‡‡</sup>Medical College of Wisconsin, Milwaukee, Wisconsin; <sup>§§</sup>Biostatistics, Shire HGT, Cambridge, Massachusetts; and <sup>|||</sup>Developmental and Metabolic Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

## ABSTRACT

In male patients with Fabry disease, an X-linked disorder of glycosphingolipid metabolism caused by deficient activity of the lysosomal enzyme  $\alpha$ -galactosidase A, kidney dysfunction becomes apparent by the third decade of life and invariably progresses to ESRD without treatment. Here, we summarize the effects of agalsidase alfa on kidney function from three prospective, randomized, placebo-controlled trials and their open-label extension studies involving 108 adult male patients. The mean baseline GFR among 54 nonhyperfiltrating patients (measured GFR <135 ml/min per 1.73 m<sup>2</sup>) treated with placebo was 85.4  $\pm$  29.6 ml/min per 1.73 m<sup>2</sup>; during 6 mo of placebo, the mean annualized rate of change in GFR was  $-7.0 \pm 32.9$  ml/min per 1.73 m<sup>2</sup>. Among 85 nonhyperfiltrating patients treated with agalsidase alfa, the annualized rate of change was  $-2.9 \pm 8.7$  ml/min per 1.73 m<sup>2</sup>. Treatment with agalsidase alfa did not affect proteinuria. Multivariate analysis revealed that GFR and proteinuria category (<1 or  $\geq 1$  g/d) at baseline significantly predicted the rate of decline of GFR during treatment. This summary represents the largest group of male patients who had Fabry disease and for whom the effects of enzyme replacement therapy on kidney function have been studied. These data suggest that agalsidase alfa may stabilize kidney function in these patients.

*J Am Soc Nephrol* 20: –, 2009. doi: 10.1681/ASN.2008080870

Fabry disease is an X-linked disorder of glycosphingolipid metabolism caused by deficiency of the activity of the lysosomal enzyme  $\alpha$ -galactosidase A ( $\alpha$ -Gal A),<sup>1</sup> resulting from one of many mutations of the gene *GLA* located at the Xq22.1.<sup>2</sup> The disease occurs with an incidence of approximately 1 in 117,000 live male births,<sup>3</sup> although recent surveys suggest that the incidence may be much higher.<sup>4</sup> Fabry disease primarily affects male individuals; female heterozygotes are reported to experience all of the signs and symptoms of Fabry disease but with a later onset and a more variable phenotype than is seen in men.<sup>5,6</sup> The signs and symptoms of Fabry disease are thought to be due to progressive accu-

mulation of globotriaosylceramide (Gb<sub>3</sub>) within tissues and organs. Among other signs and symp-

Received August 19, 2008. Accepted January 26, 2009.

Published online ahead of print. Publication date available at www.jasn.org.

M.R.'s current affiliation is Clinical Research, Shire HGT, Cambridge, Massachusetts; R.S.'s current affiliation is Baylor Institute of Metabolic Disease, Dallas, Texas.

**Correspondence:** Dr. Michael L. West, Division of Nephrology, Department of Medicine, Dalhousie University, 5090 ACC QE II Health Sciences Centre, 5820 University Avenue, Halifax, NS, Canada B3H 1V8. Phone: 902-473-4023; Fax: 902-473-2675; E-mail: mlwest@dal.ca

Copyright © 2009 by the American Society of Nephrology

toms, progressive kidney dysfunction is nearly universal in male individuals with Fabry disease. The initial sign of decline in kidney function is proteinuria or microalbuminuria, which has been reported in affected male individuals as young as 16 yr<sup>7</sup> and is present in half of male individuals by age 35 yr.<sup>8</sup> Gb<sub>3</sub> accumulation within the glomeruli results in mesangial widening and glomerular sclerosis, with a resultant loss of filtering capacity.<sup>9</sup> Chronic renal insufficiency (defined as serum creatinine levels  $\geq 1.5$  mg/dl) has an onset in the third decade of life and progresses rapidly to ESRD, with a reported average rate of decline in filtering capacity of 12.2 ml/min per yr (range 3.3 to 33.7 ml/min per yr) once chronic renal insufficiency has been reached.<sup>8</sup> The average age at initiation of dialysis for ESRD in male patients with Fabry disease ranged between 36.7 and 42.0 yr.<sup>5,10</sup> Kidney dysfunction in female patients with Fabry disease is less prevalent than and usually not as severe as that in male patients but does progress to ESRD in some cases.<sup>6,10</sup>

Enzyme replacement therapy (ERT) with human  $\alpha$ -Gal A was approved for treatment of Fabry disease in 2001 and has been reported to alleviate neuropathic pain,<sup>11</sup> result in regression of hypertrophic cardiomyopathy,<sup>12</sup> improve sweat function,<sup>13</sup> reduce plasma and urine sediment Gb<sub>3</sub> levels,<sup>11,14</sup> and reduce microvascular endothelial Gb<sub>3</sub> deposits.<sup>11,14</sup> In one long-term (up to 4.5 yr) study of 25 male individuals with Fabry disease, agalsidase alfa,  $\alpha$ -Gal A produced by gene activation in a human cell line, was reported to stabilize kidney function in patients with stage 1 or 2 chronic kidney disease<sup>15</sup> at baseline and to slow the progression of renal dysfunction in adult male patients with stage 3 chronic kidney disease compared with historical control subjects.<sup>16</sup> Observational studies of the patients enrolled in the Fabry Outcome Survey (FOS) suggested a similar renoprotective effect of agalsidase alfa.<sup>17–19</sup> The results of a recent, double-blind, placebo-controlled trial suggested that agalsidase beta, a recombinant form of  $\alpha$ -Gal A produced in Chinese hamster ovary cells, slowed the progression of major clinical events in patients with Fabry disease and mild to moderate kidney disease, with the benefit being greater in patients with estimated GFR (eGFR)  $>55$  ml/min per 1.73 m<sup>2</sup> at baseline than in those with baseline eGFR  $\leq 55$  ml/min per 1.73 m<sup>2</sup>.<sup>20</sup> In this report, we present a summary of the effects of agalsidase alfa on kidney function in all of the adult male patients who were enrolled in prospective, randomized, placebo-controlled clinical studies of agalsidase alfa and their open-label extension studies and who were treated for at least 12 mo.

## RESULTS

### Patients, Demographics, Concomitant Renoprotective Treatment, and Treatment Time

Of the 121 adult male patients who had Fabry disease and were enrolled in these three studies, 108 had sufficient GFR measurements for inclusion in this analysis. The number of patients who had GFR measured before and after placebo, before and during agalsidase alfa, or before and during both placebo

and agalsidase alfa is illustrated in Figure 1. The average age of these 108 patients at baseline was  $34.2 \pm 9.3$  yr (range 18 to 54 yr). Average systolic BP was  $126.5 \pm 14.5$ , and diastolic BP was  $71.6 \pm 10.9$  mmHg. At baseline, 18 (16.7%) patients were reported as having hypertension.<sup>21</sup> During the 6-mo placebo period, angiotensin-converting enzyme inhibitors (ACEIs) and/or selective angiotensin II receptor blockers (ARBs) were administered to nine (16.1%) of 56 patients. During the active treatment phase of the study, ACEIs and/or ARBs were administered to 26 (30.0%) of the 93 patients. The average mean agalsidase alfa treatment duration for patients in this pooled analysis was  $2.0 \pm 1.0$  yr (median 1.6 yr). Six patients had their final GFR measurement recorded after a total of 4.5 yr of agalsidase alfa therapy; and 5, 8, 11, 10, 26, and 27 patients had their final GFR measurement made after 4.0, 3.0, 2.5, 2.0, 1.5, and 1.0 yr of therapy, respectively.

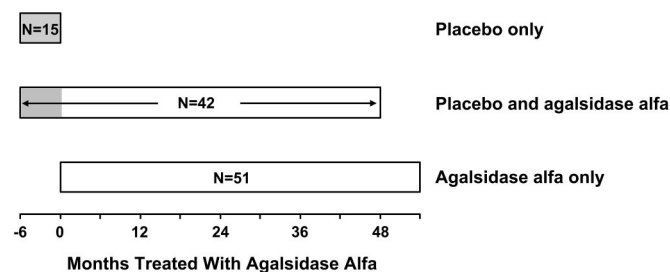
### Renal Function

#### Progression of Renal Function during the Placebo Period.

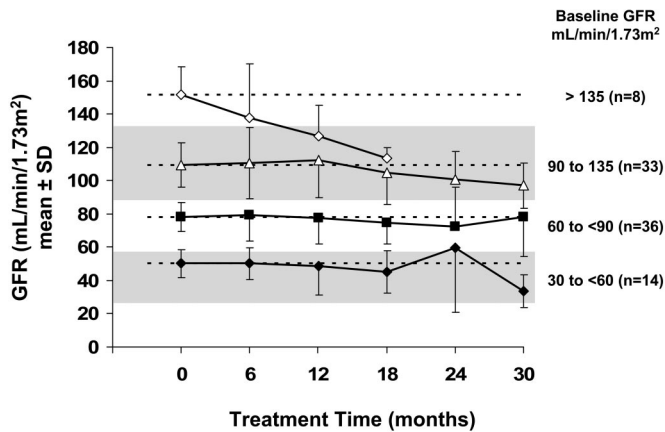
The mean baseline GFR measured before the placebo period was  $88.9 \pm 32.5$  ml/min per 1.73 m<sup>2</sup> (median 92.3 ml/min per 1.73 m<sup>2</sup>; range 12.7 to 160 ml/min per 1.73 m<sup>2</sup>;  $n = 57$ ). After 6 mo of placebo, mean GFR had declined to  $85.0 \pm 37.6$  ml/min per 1.73 m<sup>2</sup> (median 83.3 ml/min per 1.73 m<sup>2</sup>; range 12.5 to 184.0 ml/min per 1.73 m<sup>2</sup>), representing an average annualized rate of change of  $-7.7 \pm 38.0$  ml/min per 1.73 m<sup>2</sup>/yr ( $P = 0.14$ , paired  $t$  test). Three patients had renal hyperfiltration (*i.e.*, baseline GFR  $>135$  ml/min per 1.73 m<sup>2</sup>). When these three patients were removed from the analysis, the baseline GFR was  $85.4 \pm 29.6$  and the GFR after 6 mo was  $81.9 \pm 34.8$ , representing an average annualized rate of change of  $-7.0 \pm 32.9$  ml/min per 1.73 m<sup>2</sup> in this subgroup ( $P = 0.12$ , paired  $t$  test).

#### Renal Function during Treatment.

Before the initiation of agalsidase alfa therapy, GFR ranged from 25 to 184 ml/min per 1.73 m<sup>2</sup> and averaged  $90.3 \pm 31.2$  ml/min per 1.73 m<sup>2</sup> (median 87.4 ml/min per 1.73 m<sup>2</sup>;  $n = 93$ ). Two patients had GFR between 15 and  $<30$  ml/min per 1.73 m<sup>2</sup> at baseline. No patient had GFR  $<15$  ml/min per 1.73 m<sup>2</sup>. GFR measurements at baseline and during agalsidase alfa therapy are presented in Figure 2, and the rates of change of GFR



**Figure 1.** Distribution of the 108 patients who had GFR measured before and after placebo (■), agalsidase alfa (□), or both placebo and agalsidase alfa. The number of patients is noted in each treatment box.



**Figure 2.** GFR during treatment with agalsidase alfa in male patients with Fabry disease, divided into subgroups according to baseline GFR.

during treatment are presented in Table 1. The average rate of change in GFR for the entire study population (including the hyperfiltrators) was  $-4.8 \pm 10.6$  ml/min per  $1.73 \text{ m}^2/\text{yr}$  ( $P = 0.0003$  compared with no change,  $t$  test; median  $-4.1$  ml/min per  $1.73 \text{ m}^2/\text{yr}$ ). As shown in Table 1, much of the mean decrease in GFR was due to the large decreases exhibited in the subgroup of patients who had baseline GFR  $>135$  ml/min per  $1.73 \text{ m}^2$  (*i.e.*, with evidence of hyperfiltration). When the eight patients with hyperfiltration were removed from the analysis, the average rate of change of GFR was  $-2.9 \pm 8.7$  ml/min per  $1.73 \text{ m}^2/\text{yr}$  ( $P = 0.002$  compared with no change). In subgroups of patients with normal GFR (90 to  $<135$  ml/min per  $1.73 \text{ m}^2$ ) or with decreased GFR at baseline, the rate of decline in GFR was small and not significantly different from 0 (Figure 2, Table 1). Although all eight patients with baseline hyperfiltration demonstrated large decreases in GFR during treatment, all remained within the normal range during the studies (*i.e.*, GFR  $>90$  ml/min per  $1.73 \text{ m}^2$ ).

A subgroup of 42 patients had GFR measured both before and after a 6-mo placebo period and again after 12 mo of agalsidase alfa therapy (see Figure 1). In the 36 patients who started and ended the placebo period with GFR  $<135$  ml/min per  $1.73 \text{ m}^2$ , average GFR at placebo baseline was  $84.8 \pm 28.7$  ml/min per  $1.73 \text{ m}^2$  and declined by an average of  $-10.5 \pm 27.5$  ml/min per  $1.73 \text{ m}^2/\text{yr}$  during the 6-mo

placebo period. The rate of decline in GFR was  $-0.10 \pm 12.88$  ml/min per  $1.73 \text{ m}^2/\text{yr}$  during the 12 mo of agalsidase alfa ( $P = 0.097$ , paired  $t$  test). A similar statistically not significant (NS) effect was seen when this analysis was confined to the 18 patients with baseline GFR between 30 and  $<90$  ml/min per  $1.73 \text{ m}^2$  at baseline (Figure 3).

The two patients with GFR  $<30$  ml/min per  $1.73 \text{ m}^2$  at baseline progressed to ESRD during treatment after 12 mo of agalsidase alfa therapy. In addition, three other patients who had received  $<12$  mo of agalsidase alfa treatment also progressed to ESRD. Before starting agalsidase alfa therapy, two of these three patients had GFR between 15 and  $<30$  ml/min per  $1.73 \text{ m}^2$  and one had GFR  $<15$  ml/min per  $1.73 \text{ m}^2$ .

### Responder Analyses

Table 2 presents the responder analyses. The response rate was better in patients with preserved kidney function at baseline regardless of whether GFR or the level of proteinuria was used to categorize baseline kidney function. Importantly, only five of 36 patients with GFR between 60 and  $<90$  ml/min per  $1.73 \text{ m}^2$  at baseline progressed to the next, more severe category during the study.

### Proteinuria

No significant change in urinary protein excretion was observed with 1 or 2 yr of agalsidase alfa treatment. At baseline, mean urinary protein excretion was  $1.030 \pm 1.680$  g/d (median 0.40 g/d; range 0.006 to 10.550 g/d;  $n = 80$ ). After 1 yr of agalsidase alfa therapy, mean urinary protein excretion was  $0.970 \pm 1.520$  g/d (median 0.420 g/d; range 0.040 to 8.660 g/d;  $n = 76$ ), and after 2 yr of treatment, mean urinary protein excretion was  $0.970 \pm 1.140$  g/d (median 0.610 g/d; range 0.036 to 4.680 g/d;  $n = 34$ ).

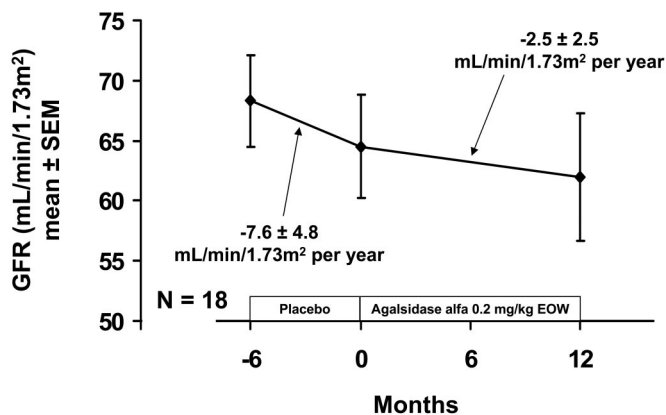
In the 80 patients with quantitative baseline proteinuria measurements and at least 1 yr of therapy with agalsidase alfa, multivariate analysis revealed that baseline GFR ( $P = 0.005$ ) and proteinuria category ( $<1$  or  $\geq 1$  g/d;  $P = 0.002$ ) were significant factors in predicting the rate of decline in GFR. The average rate of decline of GFR in the 58 patients with proteinuria  $<1$  g/d was  $-2.1 \pm 8.8$  ml/min per  $1.73 \text{ m}^2/\text{yr}$ , whereas the rate of decline in the 22 patients with baseline proteinuria  $\geq 1$  g/d was  $-6.4 \pm 5.8$  ml/min per  $1.73 \text{ m}^2/\text{yr}$ .

**Table 1.** The rate of change of GFR in male patients with Fabry disease during 12 to 54 mo of ERT with agalsidase alfa

Baseline GFR Range (ml/min per $1.73 \text{ m}^2$ )	Age (yr; Mean $\pm$ SD)	$n$	Treatment Time (yr; Mean $\pm$ SD)	Baseline Proteinuria (mg/24 h; Median [Min, Max]) <sup>a</sup>	Baseline GFR (ml/min per $1.73 \text{ m}^2$ ; Mean $\pm$ SD)	Rate of Change in GFR (ml/min per $1.73 \text{ m}^2/\text{yr}$ ; Mean $\pm$ SD)
$\geq 135$	$35.0 \pm 8.8$	8	$1.2 \pm 0.2$	1236 ( $n = 1$ )	$151.9 \pm 16.4$	$-24.5 \pm 9.0$
90 to $<135$	$28.5 \pm 7.8$	33	$2.0 \pm 1.0$	207 (6, 912; $n = 29$ )	$109.6 \pm 13.4$	$-2.2 \pm 9.3$
60 to $<90$	$36.5 \pm 7.8$	36	$2.2 \pm 1.2$	500 (611, 5382; $n = 36$ )	$78.1 \pm 8.4$	$-3.2 \pm 6.7$
30 to $<60$	$41.3 \pm 7.8$	14	$2.1 \pm 0.9$	1192 (228, 7980; $n = 13$ )	$50.1 \pm 8.2$	$-2.9 \pm 11.8$
15 to $<30$	$41.5 \pm 6.4$	2	1.0	10,545 ( $n = 1$ )	26.7	$-9.5$
Total <sup>b</sup>	$34.4 \pm 9.0$	85	$2.1 \pm 1.1$	388 (6, 10,545; $n = 79$ )	$84.5 \pm 25.5$	$-2.9 \pm 8.7$

<sup>a</sup> $n$  = the number of patients who had quantitative assessment of baseline proteinuria.

<sup>b</sup>Excluding patients with a baseline GFR  $>135$  ml/min per  $1.73 \text{ m}^2$ .



**Figure 3.** Effect of agalsidase alfa on GFR in male patients with Fabry disease and with baseline GFR between 30 and 90 ml/min per 1.73 m<sup>2</sup>. EOW, every other week.

**Table 2.** Responder analysis of the effect of agalsidase alfa on kidney function in male patients with Fabry disease<sup>a</sup>

Baseline Category	n	Responder Definition (%)		
		I	II	III
90 to 135 ml/min per 1.73 m <sup>2</sup>	33	76	88	85
60 to 89 ml/min per 1.73 m <sup>2</sup>	36	53	75	78
30 to 59 ml/min per 1.73 m <sup>2</sup>	14	43	43	71
Proteinuria <300 mg/24 h	31	74	87	87
Proteinuria ≥300 and <1000 mg/24 h	27	59	74	74
Proteinuria ≥1000 mg/24 h	22	27	50	64

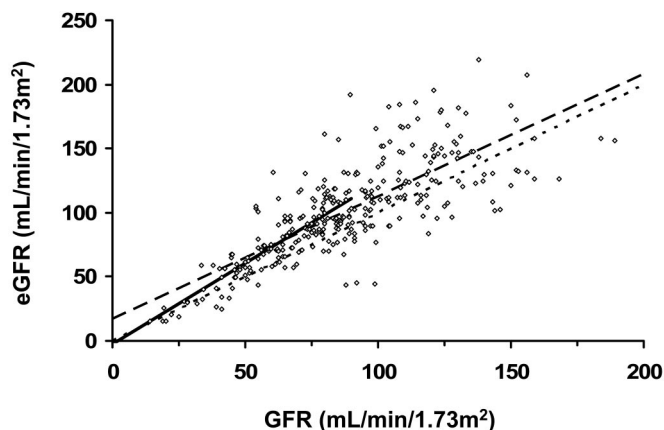
<sup>a</sup>I, rate of loss of GFR ≤5 ml/min per 1.73 m<sup>2</sup>/yr<sup>30</sup>; II, <20% decrease in GFR from baseline<sup>25</sup>; III, no shift to more severe GFR category.

**Measured GFR versus eGFR**

A total of 314 simultaneously measured GFR and eGFR values were available for analysis. GFR was significantly correlated with eGFR over the entire range of GFR (eGFR [ml/min per 1.73 m<sup>2</sup>] = 17.3 ml/min per 1.73 m<sup>2</sup> + 0.95 · GFR [ml/min per 1.73 m<sup>2</sup>]; r<sup>2</sup> = 0.623, P < 0.001; Figure 4). For GFR values <90 ml/min per 1.73 m<sup>2</sup>, eGFR tended to overestimate measured GFR in the higher range (eGFR [ml/min per 1.73 m<sup>2</sup>] = -2.79 ml/min per 1.73 m<sup>2</sup> + 1.26 · GFR [ml/min per 1.73 m<sup>2</sup>]); however, the slope of the regression line was not significantly different from 1 (slope = 1.26 ± 0.074; P = 0.18; Figure 4).

**DISCUSSION**

The study presented here represents the largest group of male patients who have Fabry disease and who have had repeated measurements of GFR either before or during treatment with ERT. While treated with placebo, GFR declined at a mean rate of 7.7 ml/min per 1.73 m<sup>2</sup>/yr. Although this placebo observation period was short, the observed rate of change in GFR was similar to that reported by others.<sup>8,19</sup> During treatment with agalsidase alfa in this study, the rate of loss of GFR in patients with baseline GFR from 30 to 135 ml/min per 1.73 m<sup>2</sup> was



**Figure 4.** Relationship between eGFR and simultaneously measured GFR in men with Fabry disease (n = 314 pairs of measurements). The dotted line is the line of identity. The dashed line represents the results of linear regression across the entire range of GFR. This correlation is described by the following equation: eGFR (ml/min per 1.73 m<sup>2</sup>) = 17.3 ml/min per 1.73 m<sup>2</sup> + 0.95 · GFR (ml/min per 1.73 m<sup>2</sup>) (r<sup>2</sup> = 0.623, P < 0.001). The bold line represents the results of linear regression for GFR values <90 ml/min per 1.73 m<sup>2</sup> and is described by the following equation: eGFR (ml/min per 1.73 m<sup>2</sup>) = -2.79 ml/min per 1.73 m<sup>2</sup> + 1.26 · GFR (ml/min per 1.73 m<sup>2</sup>).

numerically less than that seen during the placebo period, suggesting that renal function was stabilized in male patients with Fabry disease for periods between 1.0 and 4.5 yr (Table 1); however, these observed differences were not statistically significantly different, likely as a result of the large variation in the rate of change of GFR during either the placebo or the agalsidase alfa treatment period. An important observation was that the magnitude of proteinuria at baseline was a strong predictor of the rate of loss of GFR during ERT.

The results from the 6-mo placebo period of this study provides some insight into the natural history of kidney function in untreated Fabry disease, particularly because GFR was measured rather than estimated. Hyperfiltration (GFR ≥135 ml/min per 1.73 m<sup>2</sup>) was common; it was observed in three placebo patients at enrollment and developed in three additional patients during the placebo period. That it developed during these studies suggests that it may be an initial indication of kidney dysfunction in Fabry disease, as has been postulated by Barbey *et al.*<sup>22</sup> In patients with hyperfiltration, a fall in GFR toward normal could represent either a positive result of a treatment or continued disease progression. The wide range of the rate of change in GFR during the placebo period suggests that 6 mo may be too short an observation period to describe accurately the progression of kidney disease in non-ERT-treated patients with Fabry disease.

The advent of ERT has raised the possibility of positively affecting the progression of kidney disease in patients with Fabry disease. Two different forms of α-Gal A are available: Agalsidase alfa<sup>11</sup> and agalsidase beta.<sup>14</sup> Agalsidase beta has been approved in >40 countries, including the United States, for

dosing at 1.0 mg/kg every other week.<sup>23</sup> Both proteins reduce plasma and urine sediment Gb<sub>3</sub> levels,<sup>11,14</sup> and both are reported to stabilize kidney function in long-term, open-label studies.<sup>16,24,25</sup> The stabilization of kidney function by agalsidase alfa in the present study (Figures 2 and 3, Table 1) is nearly identical to that reported by Germain *et al.*<sup>25</sup> with long-term use of agalsidase beta; however, neither of these studies included a long-term, concurrently followed placebo group, and thus the conclusion that ERT stabilized kidney function must remain somewhat speculative.

Proteinuria has emerged as an important risk factor for the decline in kidney function while receiving ERT. In this study, baseline proteinuria ≥1 g/d was strongly and statistically significantly associated with a greater rate of decline in GFR during ERT. This observation is similar to those reported for two long-term studies of agalsidase beta.<sup>20,25</sup> In both of those studies, renal events were significantly more frequent in patients with baseline proteinuria >1 g/d. The influence of proteinuria at baseline on the average rate of decline of GFR during ERT is illustrated in Figure 5, which shows that ERT with either agalsidase alfa or agalsidase beta seems to slow the rate of decline of kidney function to an equivalent degree when compared with the expected loss of filtering capacity in untreated or placebo-treated patients but that this benefit is experienced only by patients with proteinuria <1 g/24 h. Because of the progressive nature of kidney dysfunction in Fabry disease, it may be unreasonable to think that ERT will quickly stop or reverse renal damage. Even in those without Fabry disease, GFR declines by approximately 1 ml/min per 1.73 m<sup>2</sup>/yr after the age of 30 yr.<sup>26</sup>

Further evidence supporting the importance of proteinuria in influencing the response to ERT is provided by Tahir *et al.*,<sup>27</sup> who reported that aggressive antiproteinuric therapy with ACEIs or ARBs combined with agalsidase beta (1 mg/kg every other week) stabilized kidney function in male and female patients with Fabry disease during a median 30 mo of treatment. This study was started before the antiproteinuric effect of ACEIs or ARBs in Fabry disease was appreciated (*e.g.*, Brenner

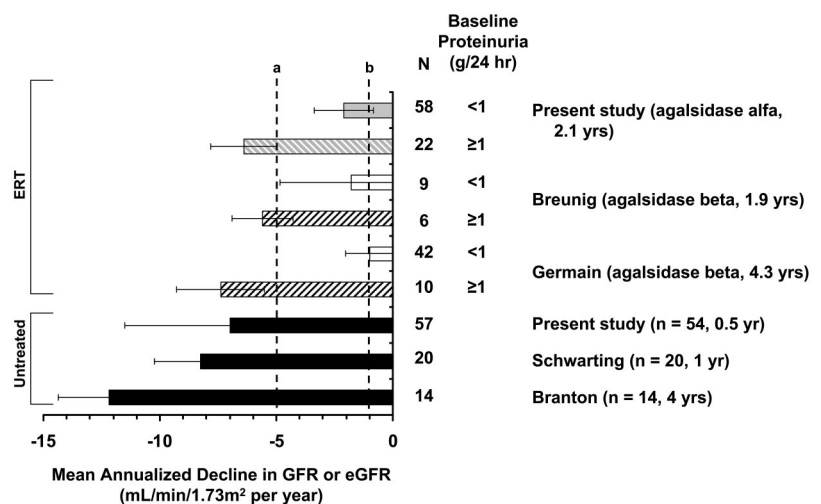
*et al.*<sup>28</sup>), and the fraction of patients treated with these agents increased during the study period. For example, 16.1% of the patients reported use of an ACEI or ARB during the placebo period, and 29.2% reported their use during the treatment period. The study was not designed to evaluate the effect of ACEIs or ARBs in a controlled setting; therefore, the failure of the multivariate analysis to identify a significant benefit associated with these agents may reflect their uncontrolled use and less than optimal dosing and should not be considered as evidence against their importance in treating kidney disease in patients with Fabry disease.

We and others have chosen to use proteinuria of 1 g/d as a threshold for analysis of the influence of urinary protein excretion on the response to ERT<sup>20,25</sup>; however, on the basis of the informal responder analysis presented in Table 2, it seems likely that the negative influence of proteinuria extends to as low as 0.3 g/d. This observation, coupled with the fact that the response rate to agalsidase alfa was higher in patients with preserved baseline GFR (Table 2), emphasizes the importance of early treatment.

**Study Limitations**

This report has summarized the results of three separate clinical trials that were conducted at different times and at different study sites. Although the studies specifically excluded patients who had reached ESRD, no other renal-related entry criteria were used. Thus, the inclusion of a large fraction of patients with relatively normal baseline kidney function (GFR ≥90 ml/min per 1.73 m<sup>2</sup>), who would not be expected to demonstrate substantial loss of GFR, may have reduced the power to detect statistically significant effects. Too few patients participated in both the placebo and the active treatment periods for a robust statistical analysis to be performed. On the basis of the results of the 42 patients who participated in both the placebo period and the subsequent open-label treatment period, a prospective study would require approximately 186 patients to have an 80% chance of detecting an improvement of slope of GFR of 5.1 ml/min per 1.73 m<sup>2</sup>/yr at the 5% significance level.

**Figure 5.** A summary of rates of change of GFR in Fabry disease with or without ERT. The dotted line designated “a” represents the entry criterion for inclusion into a study of aggressive ERT.<sup>30</sup> The dotted line designated “b” shows the decline in GFR in the population without Fabry disease after age 30 yr.<sup>26</sup> The rate of change for the Breunig study<sup>31</sup> was calculated from data presented in the article for patients with baseline eGFR between 30 and <135 ml/min per 1.73 m<sup>2</sup>. Similarly, the results presented for this study exclude patients with baseline GFR ≥135 ml/min per 1.73 m<sup>2</sup>. The number of patients in each study and the mean duration of follow-up are indicated in parentheses. The differences between the rates of change of GFR or eGFR in ERT-treated patients who had baseline proteinuria values <1 g/d in the three studies shown above were not statistically significant by t test (this study versus Germain, *P* = 0.53; this study versus Breunig, *P* = 0.93; Germain versus Breunig, *P* = 0.77).



Finally, because the prevalence of uncontrolled hypertension was low in this study, no meaningful analysis of the influence of BP conclusions on the renal response to ERT could be performed.

This summary represents the largest group of male patients who have Fabry and in whom the effects of ERT on renal function have been determined by monitoring changes in measured GFR, the gold standard for evaluating kidney function. In patients with renal dysfunction (*i.e.*, GFR <90 ml/min per 1.73 m<sup>2</sup>), eGFR overestimated renal function in the higher range. The results of this analysis suggest that agalsidase alfa may stabilize kidney function in male patients with Fabry disease and are nearly identical to results reported for agalsidase beta in a similar long-term, open-label study. Elevated proteinuria at baseline was a significant predictor of loss of GFR during this study, with an apparent threshold of 1 g/d. Formal confirmation of these preliminary observations will be challenging, because, ideally, it would require a large, long-term, placebo-controlled study. Further observational data from the Fabry Outcome Survey may be useful in evaluating the effect of agalsidase alfa on kidney function in Fabry disease.

## CONCISE METHODS

### Study Design

Three separate prospective studies of agalsidase alfa (Replagal; Shire Human Genetic Therapies, Cambridge, MA) for the treatment of Fabry disease in male hemizygotes have been conducted. Male adults ( $\geq 18$  yr old) with laboratory and clinical evidence of Fabry disease were eligible for enrollment in these studies. Each study was supported by Shire HGT and began as a 6-mo, randomized, double-blind, placebo-controlled trial, and each was continued as an open-label extension during which all enrolled patients were treated with agalsidase alfa for an additional 12 to 48 mo. Agalsidase alfa was administered at a dosage of 0.2 mg/kg infused over 40 min every other week in each study. In the first study, 26 adult male patients with chronic neuropathic pain were enrolled, and the effect of treatment on pain was assessed using the Brief Pain Index.<sup>11</sup> A second study of 15 adult men with left ventricular hypertrophy was conducted in which the effect of agalsidase alfa on left ventricular mass was evaluated with magnetic resonance imaging.<sup>12</sup> In the third study, 80 adult male patients with chronic neuropathic pain were enrolled, and the effect of treatment on pain was followed with the Brief Pain Index. No specific guidelines were predefined per protocol with regard to potential confounders (*e.g.*, BP management and concomitant renoprotective medication such as ACEIs and ARB). All patients who participated in these studies gave their written informed consent before enrolling in the double-blind and open-label extension phases of each study. The institutional review board or ethics committee reviewed and approved each individual protocol at each study site.

Although none of the three studies had a specific requirement for the presence of renal dysfunction at baseline, the effect of agalsidase alfa on GFR was assessed by measuring GFR at baseline and after 6 mo during the double-blind studies and every 6 or 12 mo in the open-

label extension studies. This report describes a secondary, pooled analysis of the effects of long-term treatment with agalsidase alfa on renal function in adult male patients who had Fabry disease and were enrolled in these studies. Results from 108 of 121 enrolled patients who had GFR measured by inulin, technetium-DTPA, or chromium-EDTA before and after the placebo period or before and after  $\geq 12$  mo of agalsidase alfa therapy were included in this study. Patients were excluded from analysis when they had fewer than two GFR measurements per study period (placebo, active treatment).

### Agalsidase Alfa

Agalsidase alfa is a form of human  $\alpha$ -Gal A, manufactured in a genetically modified continuous human cell line by methods previously described.<sup>11</sup> The enzyme has the same amino acid sequence as the native human enzyme and has a similar glycosylation pattern. Agalsidase alfa is approved in >40 countries at a dosage of 0.2 mg/kg infused intravenously every other week, but has not yet been approved in the United States. Each patient received agalsidase alfa (0.2 mg/kg) every other week administered intravenously in 100 ml of physiologic saline infused over approximately 40 min. Premedications were not administered prophylactically unless a patient had experienced a previous infusion reaction. In those cases, patients were premedicated with oral H1 and H2 histamine antagonists, nonsteroidal anti-inflammatory drugs, and/or oral corticosteroids before subsequent infusions. These premedications were subsequently withdrawn without sequelae in the majority of patients.

### Renal Function

GFR was measured using inulin, <sup>99m</sup>technetium DTPA, or <sup>51</sup>Cr-EDTA (non-US sites) and was expressed as ml/min per 1.73 m<sup>2</sup> body surface area. In addition, GFR was estimated using the modified Modification of Diet in Renal Disease (MDRD) equation.<sup>26</sup> Patients were categorized as to their baseline GFR as follows: GFR  $\geq 135$  ml/min per 1.73 m<sup>2</sup> (defined as hyperfiltration<sup>29</sup>), GFR  $\geq 90$  to <135 ml/min per 1.73 m<sup>2</sup>, GFR 60 to <90 ml/min per 1.73 m<sup>2</sup>, GFR 30 to <60 ml/min per 1.73 m<sup>2</sup>, GFR 15 to <30 ml/min per 1.73 m<sup>2</sup>, and GFR <15 ml/min per 1.73 m<sup>2</sup> or on dialysis. Twenty-four-hour urinary protein excretion was quantitatively determined by the local clinical laboratory.

### Statistical Analysis

Baseline GFR was defined as the last value measured before beginning therapy with agalsidase alfa or placebo. The rate of change in GFR during the initial 12 to 54 mo of ERT was calculated for each patient individually by subtracting the baseline value from the final value and dividing by treatment duration. A responder analysis was performed using three different definitions of a responder: (1) Rate of loss of GFR no greater than 5 ml/min per 1.73 m<sup>2</sup>/yr, (2) less than a 20% decrease in GFR from baseline to final measurement,<sup>25</sup> and (3) no shift to a more severe GFR category. To investigate the influence of baseline factors on the rate of change of GFR during treatment with agalsidase alfa, we fitted a multivariate model. In this model, rate of change in GFR was the outcome measure, and baseline age, baseline GFR, baseline proteinuria category (<1 or  $\geq 1$  g/d), and ACEI/ARB status were explanatory variables. Only patients who had quantitative baseline proteinuria measurements were included in this analysis. A patient who reported the use of an ACEI and/or ARB at any time during the

active treatment period was considered positive for ACEI/ARB status. The correlation between simultaneously measured GFR and eGFR was analyzed by linear regression for the entire range of GFR measurements as well as for the subgroup of GFR measurements <90 ml/min per 1.73 m<sup>2</sup>. All analyses were two-tailed, and statistical significance was defined as  $P < 0.05$ . All values are expressed as means  $\pm$  SD.

## ACKNOWLEDGMENTS

Shire HGT provided editorial assistance to the authors through a third party. Statistical analysis was performed by Shire HGT at the direction of the authors, and Shire HGT reviewed the final manuscript to ensure the analysis was accurately presented.

## DISCLOSURES

Michael West has received research funding, consultancy fees, and/or speaker's fees from Shire Human Genetic Therapies (formerly TKT), Genzyme, and Amicus Therapeutics.

Kathy Nicholls has received research funding, travel support, consultancy, and/or speaker honoraria from Shire Human Genetic Therapies, Genzyme, and Amicus Therapeutics, and is a member of the Fabry Outcome Study International Advisory Board.

Atul Mehta has received research funding, consultancy fees, and/or speaker's fees from Shire Human Genetic Therapies, Genzyme, and Amicus Therapeutics.

Joe T.R. Clarke has received research funding, consultancy fees, and/or speaker's fees from Shire Human Genetic Therapies, Genzyme, and Actelion.

Robert Steiner has received research funding, consultancy fees, honoraria, and/or speaker's fees from Shire Human Genetic Therapies, Genzyme, Actelion, Biomarin, and Amicus Therapeutics.

Michael Beck has received travel support, honoraria, and unrestricted grants from Genzyme, Shire Human Genetic Therapies, and Biomarin.

Bruce Barshop has received research funding, consultation fees, and/or speaker's fees from Genzyme and Biomarin.

William Rhead has received research funding, consultancy fees, and/or speaker's fees from Shire Human Genetic Therapies, Genzyme, Hyperion, Ucylyd, and Actelion.

Robert Mensah is an employee of Shire Human Genetic Therapies.

Markus Ries reports no conflict during the conduct of these studies and is currently an employee of Shire Human Genetic Therapies.

Raphael Schiffmann has received research funding, consultancy fees, and/or speaker's fees from Shire Human Genetic Therapies, Genzyme, and Amicus Therapeutics.

## REFERENCES

- Brady RO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L: Enzymatic defect in Fabry's disease: Ceramidetrihexosidase deficiency. *N Engl J Med* 276: 1163–1167, 1967
- Eng CM, Niehaus DJ, Enriquez AL, Burgert TS, Ludman MD, Desnick RJ: Fabry disease: Twenty-three mutations including sense and anti-sense CpG alterations and identification of a deletion hot-spot in the alpha-galactosidase A gene. *Hum Mol Genet* 3: 1795–1799, 1994
- Meikle PJ, Hopwood JJ, Clague AE, Carey WF: Prevalence of lysosomal storage disorders. *JAMA* 281: 249–254, 1999
- Spada M, Pagliardini S, Yasuda M, Tukul T, Thiagarajan G, Sakuraba H, Ponzzone A, Desnick RJ: High incidence of later-onset Fabry disease revealed by newborn screening. *Am J Hum Genet* 79: 31–40, 2006
- MacDermot KD, Holmes A, Miners AH: Anderson-Fabry disease: Clinical manifestations and impact of disease in a cohort of 98 hemizygous males. *J Med Genet* 38: 750–760, 2001
- MacDermot KD, Holmes A, Miners AH: Anderson-Fabry disease: Clinical manifestations and impact of disease in a cohort of 60 obligate carrier females. *J Med Genet* 38: 769–775, 2001
- Ries M, Gupta S, Moore DF, Sachdev V, Quirk JM, Murray GJ, Rosing DR, Robinson C, Schaefer E, Gal A, Dambrosia JM, Garman SC, Brady RO, Schiffmann R: Pediatric Fabry disease. *Pediatrics* 115: e344–e355, 2005
- Branton MH, Schiffmann R, Sabnis SG, Murray GJ, Quirk JM, Al-tarescu G, Goldfarb L, Brady RO, Balow JE, Austin HA, Kopp JB: Natural history of Fabry renal disease: Influence of  $\alpha$ -galactosidase A activity and genetic mutations on clinical course. *Medicine* 81: 122–138, 2002
- Alroy J, Sabnis S, Kopp JB: Renal pathology in Fabry disease. *J Am Soc Nephrol* 13[Suppl 2]: S134–S138, 2002
- Thadhani R, Wolf M, West ML, Tonelli M, Ruthazer R, Pastores GM, Obrador GT: Patients with Fabry disease on dialysis in the United States. *Kidney Int* 61: 249–255, 2002
- Schiffmann R, Kopp JB, Austin HA, Sabnis S, Moore DF, Weibel T, Balow JE, Brady RO: Enzyme replacement therapy in Fabry disease: A randomized controlled trial. *JAMA* 285: 2743–2749, 2001
- Hughes DA, Elliott PM, Shah J, Zuckerman J, Coughlan G, Brookes J, Mehta AB: Effects of enzyme replacement therapy on the cardiomyopathy of Anderson-Fabry disease: A randomized, double-blind, placebo-controlled clinical trial of agalsidase-alfa. *Heart* 94: 153–158, 2008
- Schiffmann R, Floeter MK, Dambrosia JM, Gupta S, Moore DF, Sharabi Y, Khurana RK, Brady RO: Enzyme replacement therapy improves peripheral nerve and sweat function in Fabry disease. *Muscle Nerve* 28: 703–710, 2003
- Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, Caplan L, Linthorst GE, Desnick RJ: Safety and efficacy of recombinant human  $\alpha$ -galactosidase A replacement therapy in Fabry's disease. *N Engl J Med* 345: 9–16, 2001
- National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (K/DOQI) Advisory Board: K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Kidney Disease Outcomes Quality Initiative. *Am J Kidney Dis* 39: S1–S266, 2002
- Schiffmann R, Ries M, Timmons M, Flaherty JT, Brady RO: Long-term therapy with agalsidase alfa for Fabry disease: Safety and effects on renal function in a home infusion setting. *Nephrol Dial Transplant* 21: 345–354, 2006
- Dehout F, Schwarting A, Beck M, Mehta A, Ricci R, Widmer U: Effects of enzyme replacement therapy with agalsidase alfa on glomerular filtration rate in patients with Fabry disease: Preliminary data. *Acta Paediatr Suppl* 92: 14–15, 2003
- Beck M, Ricci R, Widmer U, Dehout F, de Lorenzo AG, Kampmann C, Linhart A, Sunder-Plassmann G, Houge G, Ramaswami U, Gal A, Mehta A: Fabry disease: Overall effects of agalsidase alfa treatment. *Eur J Clin Invest* 34: 838–844, 2004
- Schwarting A, Dehout F, Feriozzi S, Beck M, Mehta A, Sunder-Plassmann G: Enzyme replacement therapy and renal function in 201 patients with Fabry disease. *Clin Nephrol* 66: 77–84, 2006
- Banikazemi M, Bultas J, Waldek S, Wilcox WR, Whitley CB, McDonald M, Finkel R, Packman S, Bichet DG, Warnock DG, Desnick RJ: Agalsidase-beta therapy for advanced Fabry disease: A randomized trial. *Ann Intern Med* 146: 77–86, 2007
- Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ: The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 Report. *JAMA* 289: 2560–2571, 2003
- Barbey F, Lidove O, Schwarting A: Fabry nephropathy: 5 years of

- enzyme replacement therapy—A short review. *Nephrol Dial Transplant* 1: 11–19, 2008
23. Fabrazyme® (agalsidase beta) for Intravenous Infusion [Prescribing Information]. Cambridge, MA: Genzyme; 2006
  24. Wilcox WR, Banikazemi M, Guffon N, Waldek S, Lee P, Linthorst GE, Desnick RJ, Germain DP: Long-term safety and efficacy of enzyme replacement therapy for Fabry disease. *Am J Hum Genet* 75: 65–74, 2004
  25. Germain DP, Waldek S, Banikazemi M, Bushinsky DA, Charrow J, Desnick RJ, Lee P, Loew T, Vedder AC, Abichandani R, Wilcox WR, Guffon N: Sustained, long-term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease. *J Am Soc Nephrol* 18: 1547–1557, 2007
  26. Levey AS: Clinical practice: Nondiabetic kidney disease. *N Engl J Med* 347: 1505–1511, 2002
  27. Tahir H, Jackson LL, Warnock DG: Antiproteinuric therapy and Fabry nephropathy: Sustained reduction of proteinuria in patients receiving enzyme replacement therapy with agalsidase-beta. *J Am Soc Nephrol* 18: 2609–2617, 2007
  28. Brenner BM, Cooper ME, De Zeeuw D, Keane WF, Mitch WE, Parving H-H, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S: Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 345: 861–869, 2001
  29. Cotroneo P, Manto A, Todaro L, Manto A Jr, Pitocco D, Saponara C, Vellante C, Maussier ML, D'Errico G, Magnani P, Ghirlanda G: Hyperfiltration in patients with type I diabetes mellitus: A prevalence study. *Clin Nephrol* 50: 214–217, 1998
  30. Schiffmann R, Askari H, Timmons M, Robinson C, Benko W, Brady RO, Ries M: Weekly enzyme replacement therapy may slow decline of renal function in patients with Fabry disease who are on long-term biweekly dosing. *J Am Soc Nephrol* 18: 1576–1583, 2007
  31. Breunig F, Weidemann F, Strotmann J, Knoll A, Wanner C: Clinical benefit of enzyme replacement therapy in Fabry disease. *Kidney Int* 69: 1216–1221, 2006