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# Changes in plasma and urine globotriaosylceramide levels do not predict Fabry disease progression over 1 year of agalsidase alfa

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**Purpose:** Globotriaosylceramide concentrations were assessed as potential predictors of change from baseline after 12 months by estimated glomerular filtration rate and left-ventricular mass index using pooled data from three randomized, placebo-controlled agalsidase alfa trials and open-label extensions of patients with Fabry disease.

**Methods:** Males (aged 18 years or older) with Fabry disease received agalsidase alfa (0.2 mg/kg every other week for 12 months). A backward-elimination approach evaluated potential predictors (baseline estimated glomerular filtration rate and left-ventricular mass index; age at first dose; baseline and change from baseline at 12 months of globotriaosylceramide (urine, plasma); urine protein excretion; and systolic and diastolic blood pressure). Subgroups included patients randomized to placebo or agalsidase alfa (double-blind phase), then to agalsidase alfa (open-label extensions; placebo→agalsidase alfa or agalsidase alfa→agalsidase alfa, respectively) and stage 2/3 chronic kidney disease patients.

**Results:** Baseline estimated glomerular filtration rate, age at first dose, baseline urine globotriaosylceramide excretion, and baseline and change from baseline urine protein excretion significantly predicted change from baseline estimated glomerular filtration rate in the analysis population ($N = 73$; all $P<0.05$), although not in all subgroups. Change from baseline urine and plasma globotriaosylceramide (baseline and change from baseline) concentrations did not predict change from baseline estimated glomerular filtration rate. No predictors of left-ventricular mass index were significant.

**Conclusion:** Changes in globotriaosylceramide concentrations do not appear to be useful biomarkers for prediction of Fabry disease–related changes in estimated glomerular filtration rate or left-ventricular mass index.

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**Key Words:** agalsidase alfa; biomarkers; enzyme replacement therapy; Fabry disease; globotriaosylceramide

Globotriaosylceramide ($Gb_3$) is often elevated in the urine of patients with Fabry disease,[1] and some studies support its use as a diagnostic biomarker.[2–4] Plasma $Gb_3$ concentration has been found to be consistently elevated in hemizygous males with classic Fabry disease but variably elevated in some variant hemizygous males with residual enzyme activity and in heterozygous females.[5,6] No evidence has been published supporting the use of plasma or urine $Gb_3$ concentrations as a biomarker for disease progression or response to treatment. For patients with elevated plasma or urine $Gb_3$ concentrations before treatment, enzyme replacement therapy (ERT) results in an initial drop in $Gb_3$ concentrations.[5,7] The lower $Gb_3$ concentrations do not remain low in all patients and do not always coincide with clinical improvement.[8]

Biomarkers are generally defined as measurements that reflect the activity of a disease process[9] and can be (i) prognostic, (ii) predictive, or (iii) pharmacodynamic.[10]

The goal of this study was to assess the relationship of plasma and urine $Gb_3$ concentrations with renal or cardiac outcome measures. A previous analysis of pooled data from three randomized, placebo-controlled clinical trials and their open-label extensions (sponsored by Shire Human Genetic Therapies) of male patients with Fabry disease suggested a stabilizing effect of agalsidase alfa (agalα) on renal function assessed by measured glomerular filtration rate (GFR).[11] In that analysis, baseline GFR or elevated proteinuria category (≥1 g/24 h) significantly predicted GFR decline during treatment. Using a suitable selection approach of pooled data

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from the same three clinical trials, we asked whether $Gb_3$ concentrations could also be a predictor of changes in kidney function and/or left-ventricular mass index (LVMI) in a large study population of patients with Fabry disease receiving agalα ERT.

## MATERIALS AND METHODS

### Clinical trial designs and treatments

Data were pooled from three 24-week, randomized, double-blind, placebo-controlled trials (RCTs) and their open-label extension studies (EXTs; TKT003/TKT006; TKT005/TKT007; TKT010/TKT013; and TKT015). Two of these trials (TKT003 and extension[12] and TKT005 and extension)[13] were single-center phase II trials, and one was a multicenter phase III trial (TKT010 and extension).

Treatments in these trials included agalα (0.2 mg/kg body weight) infused intravenously over a 40-min period every other week or placebo (infused on the same schedule). For these analyses, patient data were analyzed for 12 months of total agalα treatment (**Figure 1**). Patients were either treated with agalα for 6 months (RCTs), followed by an additional 6 months of agalα (EXTs), or they received placebo for 6 months (RCTs) and then transitioned to 12 months of agalα (EXTs).

### Patient selection

Patients were adult males (aged 18 years or older) with Fabry disease (OMIM 301500) confirmed by clinical characteristics and alpha-galactosidase A deficiency, who were otherwise considered to have adequate general health. Each individual RCT also had specific inclusion criteria, including symptoms of neuropathic pain (TKT003, TKT010), medication for neuropathic pain at screening (TKT010), and left-ventricular hypertrophy (TKT005). None of these trials had specific renal inclusion criteria.

For inclusion in these *post hoc* analyses, patients in the renal analysis population were required to have available data on estimated GFR (eGFR),[14] plasma $Gb_3$ concentration, urine $Gb_3$ excretion, urine protein excretion, systolic and diastolic blood pressure, and age at baseline and 12 months of agalα treatment. The cardiac analysis population must have had cardiac magnetic resonance imaging measurements for LVMI. All patients should have received at least one dose of agalα during the treatment period assessed. In addition, subpopulations were evaluated comprising patients who were initially randomized to the placebo group in the 6-month RCTs and transitioned to 12 months of agalα in the EXTs (placebo→agalα; **Figure 1**), who were initially randomized to agalα (6-month RCTs) and continued on to 6 months of agalα during the EXTs (agalα→agalα; **Figure 1**), or who had stage 2/3 chronic kidney disease (CKD 2/3) at baseline (defined as patients with baseline eGFR of 30–90 ml/min/1.73 $m^2$).

All studies included in these analyses were approved by the appropriate institutional review boards of the investigators' institutions, and all patients provided written informed consent.

### Measurement of eGFR and LVMI

These analyses use eGFR instead of measured GFR because eGFR allows for the inclusion of more patients in the analysis population. eGFR (ml/min/1.73 $m^2$) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation,[14] which incorporates serum creatinine (SCr [mg/dl]), the patient's age, sex, and race. κ Corresponds to a value of 0.7 (females) or 0.9 (males), and α is –0.329 (females) or –0.411 (males). The "min" value indicates the minimum of SCr/κ or 1,

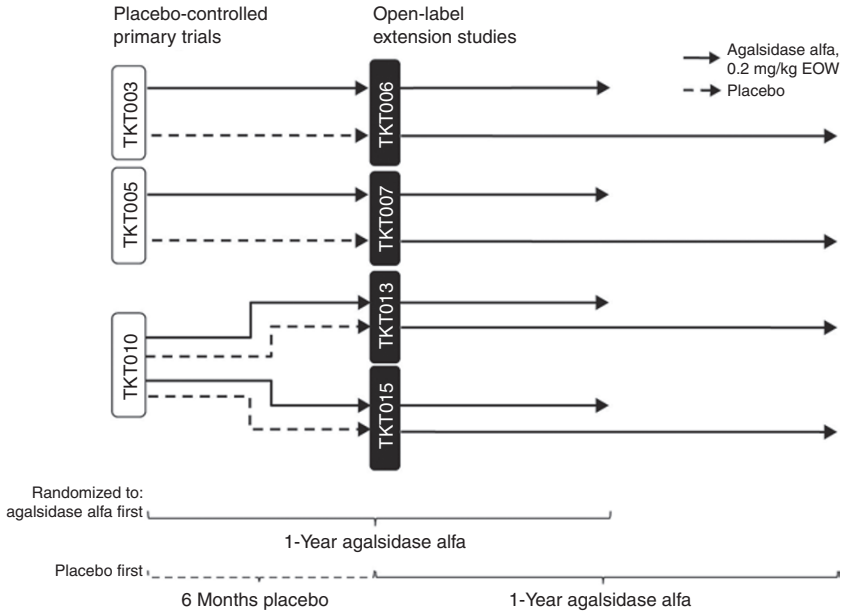


**Figure 1 Clinical trial designs and treatment durations for these analyses.** Each patient in these analyses received 12 total months of agalsidase alfa treatment (solid lines): either 6 months agalsidase alfa in the double-blind primary trial plus 6 more months in the open-label extension study or placebo in the primary trial (dashed line) plus 12 months of agalsidase alfa in the extension. EOW, every other week.

and "max" is the maximum of SCr/κ or 1. The Chronic Kidney Disease Epidemiology Collaboration formula can be expressed as a single equation as follows:

$$\text{eGFR} = 14 \times \min(\text{SCr}/\kappa, 1)^{\alpha} \times \max(\text{SCr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}} \times 1.018\ (\text{if female}) \times 1.159\ (\text{if black})$$

SCr was measured in local laboratories as part of the standard assessments. A local clinical laboratory quantitatively determined 24-h urine protein excretion. Renal disease was staged into CKD subgroups.[15] LVMI was calculated using cardiac magnetic resonance imaging measurements and using techniques described previously.[13]

### Measurement of $Gb_3$ concentration

Plasma samples were collected for assessment of $Gb_3$ after 8 h of fasting before drawing the blood for analyses. Urine $Gb_3$ excretion was evaluated in 24-h urine sediment. Both plasma and urine samples were analyzed with high-performance liquid chromatography using a validated assay, as described previously.[16]

### Potential predictors

In the renal analysis population, the primary outcome measure was the change from baseline (CFB) value in eGFR at 12 months. The potential predictors considered for renal outcomes included baseline (before agalα treatment) eGFR and age at start of agalα, as well as baseline and CFB (at month 12) plasma $Gb_3$ concentrations, urine $Gb_3$ excretion, natural log (ln) of urine protein excretion, and systolic and diastolic blood pressure. In the cardiac analysis population, the same potential predictors (except baseline eGFR) as well as baseline LVMI were evaluated for CFB in LVMI ($g/m^{2.7}$) at month 12. Interaction effects were also evaluated as described below.

### Statistical analyses

The significance of potential predictors of renal outcomes was assessed via a backward-elimination linear regression model, which started with the full model, including interaction terms, and systematically and sequentially factored out all nonsignificant potential explanatory variables one by one, starting with the least significant variable. Nonsignificant ($P > 0.05$) interaction terms were removed initially, followed by the removal of nonsignificant ($P > 0.05$) main effects that were not a component of a statistically significant interaction term. Because the starting model contains interaction terms, the elimination process did not permit the removal of a main effect term before all interactions related to that main effect were removed. The elimination model was terminated when all remaining main effect terms were either significant or part of a significant interaction term ($P_{\text{elimination}} > 0.05$). The backward-elimination approach was chosen because it selects models that are more explanatory. A similar approach was used for the cardiac outcomes, with the exception of inclusion of interaction terms due to small sample size. The robustness of the model was also assessed using a linear regression analysis in the three patient subpopulations: placebo→agalα, agalα→agalα, and CKD 2/3 subgroups. A Student's *t*-test was used to calculate *P* values, assessing whether parameters (intercepts and slopes) were significantly different from zero. Statistical significance levels were set at $\alpha = 0.05$ (two-sided).

## RESULTS

Baseline demographic and clinical characteristics are shown in **Table 1**. These characteristics were similar among the entire renal analysis population and the placebo→agalα and agalα→agalα subgroups. Patients in the CKD 2/3 subgroup had lower baseline eGFR than the whole analysis population and other subpopulations, as expected from the criteria for defining CKD 2/3 (baseline eGFR of 30–90 ml/min/1.73 $m^2$).

Baseline and CFB values at month 12 are shown for each of the parameters evaluated as a potential predictor of renal function (**Table 2**). The backward-elimination approach identified baseline eGFR, age at start of agalα, baseline urine $Gb_3$ excretion, log-transformed baseline, and CFB of urine protein excretion at month 12 as significant predictors of renal function in the renal analysis population (all $P < 0.05$; **Table 3**). Furthermore, significant interactions were found between baseline eGFR and log-transformed baseline and CFB in urine protein excretion at month 12 (both $P < 0.005$; **Table 3**). No

**Table 1** Demographic and clinical characteristics: analysis population and subgroups

| Characteristic | Analysis population (all subjects), *N* = 73 | Pbo→agalα subgroup[a] (*n* = 36) | Agalα→agalα subgroup[b] (*n* = 37) | CKD 2/3 subgroup[c] (*n* = 25) |
|---|---|---|---|---|
| Age at first dose, years, median (range) | 36 (20–53) | 36 (20–52) | 35 (20–53) | 40 (26–53) |
| Race, *n* (%) | | | | |
| White | 66 (90) | 33 (92) | 33 (89) | 21 (84) |
| Other | 7 (10) | 3 (8) | 4 (11) | 4 (16) |
| Sex, male, *n* (%) | 73 (100) | 36 (100) | 37 (100) | 25 (100) |
| eGFR, ml/min/1.73 $m^2$, mean ± SD | 92.6 ± 32.4 | 88.9 ± 37.7 | 96.2 ± 26.3 | 66.6 ± 16.0 |

Agalα, agalsidase alfa; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; EXT, extension study; Pbo, placebo; RCT, randomized placebo-controlled trial.

[a]Pbo→agalα subgroup: 6 months placebo (RCT), followed by 12 months agalα (EXT). [b]Agalα→agalα subgroup: 6 months agalα (RCT), followed by 6 months agalα (EXT). [c]CKD 2/3 subgroup: stage 2/3 CKD before beginning agalα treatment.

**Table 2** Potential predictors (baseline and CFB at month 12) of renal function: possible covariates in 73 patients in the renal analysis population

| Variable | Baseline | Month 12 | CFB at month 12 |
|---|---|---|---|
| eGFR, ml/min/1.73 $m^2$, mean ± SD | 92.6 ± 32.4 | 92.1 ± 32.4 | −0.5 ± 14.1 |
| Plasma $Gb_3$ concentration, nmol/ml, mean ± SD | 11.7 ± 3.4 | 5.2 ± 2.6 | −6.5 ± 3.9 |
| Urine $Gb_3$ excretion, nmol/24 h, mean ± SD | 3,622.4 ± 2,374.8 | 1,271.3 ± 1,320.8 | −2,351.1 ± 2,372.6 |
| ln (urine protein excretion), mg/24 h, mean ± SD | 6.2 ± 1.3 | 6.2 ± 1.2 | −0.1 ± 0.7 |
| Systolic blood pressure, mm Hg, mean ± SD | 125.5 ± 13.4 | 123.4 ± 13.9 | −2.1 ± 15.6 |
| Diastolic blood pressure, mm Hg, mean ± SD | 70.6 ± 9.8 | 68.2 ± 9.8 | −2.4 ± 11.1 |
| Age at first dose of active therapy, years, median (range) | 35.7 (19.5–52.9) | — | — |

CFB, change from baseline; eGFR, estimated glomerular filtration rate; $Gb_3$, globotriaosylceramide.

**Table 3** Potential predictors of renal function: backward-elimination approach in 73 patients in the renal analysis population

| Variable | DF | Estimate ± SEM | *t* Value | *P* value |
|---|---|---|---|---|
| Intercept | 1 | 115.05 ± 30.18 | 3.81 | 0.0003 |
| Baseline eGFR, ml/min/1.73 $m^2$ | 1 | −0.93 ± 0.26 | −3.64 | 0.0005 |
| Age at start of agalα, years | 1 | −0.39 ± 0.17 | −2.28 | 0.0257 |
| Baseline urine $Gb_3$ excretion, nmol/mg | 1 | $1.18 \times 10^{-3} \pm 5.52 \times 10^{-4}$ | 2.14 | 0.0362 |
| ln (baseline urine protein excretion) | 1 | −14.25 ± 3.72 | −3.83 | 0.0003 |
| CFB at month 12 ln (urine protein excretion) | 1 | −37.24 ± 4.75 | −7.85 | <0.0001 |
| Baseline eGFR × ln (baseline urine protein excretion) | 1 | 0.12 ± 0.04 | 3.37 | 0.0013 |
| Baseline eGFR × CFB at month 12 ln (urine protein excretion) | 1 | 0.34 ± 0.05 | 6.90 | <0.0001 |

Agalα, agalsidase alfa; CFB, change from baseline; DF, degrees of freedom; eGFR, estimated glomerular filtration rate; $Gb_3$, globotriaosylceramide.

other variables assessed were significant predictors of renal function. Most of these potential parameters and interactions were found to be statistically significant predictors of renal function in the placebo→agalα subgroup (all $P < 0.05$), with the exception of age at start of agalα ($P = 0.0571$; **Table 4**). Patients in the agalα→agalα subgroup retained only baseline eGFR and log-transformed baseline urine protein excretion as potential predictors (both $P < 0.05$), with a significant interaction found between these two parameters ($P = 0.0153$; **Table 4**). For CKD 2/3 patients, only urine $Gb_3$ was determined to be a significant predictor of CFB eGFR at month 12 ($P = 0.0105$; **Table 4**).

The backward-elimination approach did not identify any predictors of CFB LVMI at month 12 (**Supplementary Table S1** online).

## DISCUSSION

A large unmet need exists for finding and validating accurate biomarkers in lysosomal storage disorders and, in particular, for Fabry disease.[17,18] Individual lysosomal diseases exhibit great variability in clinical expression that contributes to the current inability to predict their rate of progression, severity, and response to therapy, which underscores the need for biomarkers.[19] A biomarker is defined as a laboratory measurement that reflects the activity of a disease process. Urine or plasma $Gb_3$ concentrations have been used as biomarkers for diagnostic purposes and to follow the effect of specific therapies such as ERT.[1,2] In addition, the measurement of plasma $Gb_3$ concentration was used as a pharmacodynamic marker to demonstrate the biological activity of agalα *in vivo*.[20] For patients with elevated plasma or urine $Gb_3$ concentrations before treatment, ERT resulted in an initial drop in $Gb_3$ concentrations.[7,8] Lower $Gb_3$ concentrations did not always coincide with clinical improvement.

In this *post hoc* analysis of prospective RCTs of agalα ERT in Fabry disease, plasma $Gb_3$ concentration was not found to be a significant predictor at month 12 of CFB eGFR (for renal function) or CFB LVMI (for cardiac structure) in the analysis population or any subgroup. Urine $Gb_3$ excretion also failed to predict CFB LVMI at month 12. However, baseline urine $Gb_3$ excretion did appear to be a predictor of change in renal function, but when analyzed by subgroup, urine $Gb_3$ excretion did not consistently correlate with disease stage, progression, or response to ERT (e.g., baseline urine $Gb_3$ excretion predicted renal function in the placebo→agalα and CKD 2/3 subgroups but not in the agalα→agalα subgroup). CFB of urine $Gb_3$ excretion at month 12, however, was not found to be a predictor of renal function, suggesting that change in $Gb_3$ concentrations during the course of therapy would not be a good biomarker for response to therapy or Fabry disease progression. Urine protein excretion (baseline and CFB at month 12) was

**Table 4** Potential predictors of renal function: linear regression analyses in subgroup populations

| Variable | DF | Estimate ± SEM | *t* Value | *P* Value |
|---|---|---|---|---|
| Pbo→agalα subgroup[a] (*n* = 36) | | | | |
| Intercept | 1 | 123.68 ± 41.84 | 2.96 | 0.0063 |
| BL eGFR, ml/min/1.73 $m^2$ | 1 | −0.96 ± 0.35 | −2.79 | 0.0093 |
| Age at start of agalα, years | 1 | −0.48 ± 0.24 | −1.98 | 0.0571 |
| BL urine $Gb_3$ excretion, nmol/mg | 1 | $2.00 \times 10^{-3} \pm 6.44 \times 10^{-4}$ | 3.11 | 0.0043 |
| ln (BL urine excretion) | 1 | −15.13 ± 4.97 | −3.05 | 0.0050 |
| CFB at month 12 ln (UPE) | 1 | −40.27 ± 4.63 | −8.69 | <0.0001 |
| BL eGFR × ln (BL UPE) | 1 | 0.12 ± 0.05 | 2.58 | 0.0155 |
| BL eGFR × CFB at month 12 ln (UPE) | 1 | 0.35 ± 0.05 | 6.44 | <0.0001 |
| Agalα→agalα subgroup[b] (*n* = 37) | | | | |
| Intercept | 1 | 137.91 ± 48.60 | 2.84 | 0.0082 |
| BL eGFR, ml/min/1.73 $m^2$ | 1 | −1.20 ± 0.43 | −2.82 | 0.0085 |
| Age at start of agalα, years | 1 | −0.44 ± 0.26 | −1.69 | 0.1025 |
| BL urine $Gb_3$ excretion, nmol/mg | 1 | $-1.07 \times 10^{-3} \pm 1.04 \times 10^{-3}$ | −1.03 | 0.3112 |
| ln (BL UPE) | 1 | −16.57 ± 6.55 | −2.53 | 0.0171 |
| CFB at month 12 ln (UPE) | 1 | −11.04 ± 17.01 | −0.65 | 0.5215 |
| BL eGFR × ln (BL UPE) | 1 | 0.17 ± 0.06 | 2.58 | 0.0153 |
| BL eGFR × CFB at month 12 ln (UPE) | 1 | 0.12 ± 0.16 | 0.77 | 0.4450 |
| CKD 2/3 subgroup[c] (*n* = 25) | | | | |
| Intercept | 1 | 100.79 ± 71.89 | 1.40 | 0.1789 |
| BL eGFR, ml/min/1.73 $m^2$ | 1 | −0.78 ± 0.92 | −0.85 | 0.4075 |
| Age at start of agalα, years | 1 | −0.19 ± 0.29 | −0.66 | 0.5176 |
| BL urine $Gb_3$ excretion, nmol/mg | 1 | $2.29 \times 10^{-3} \pm 7.96 \times 10^{-4}$ | 2.88 | 0.0105 |
| ln (BL urine protein) | 1 | −13.68 ± 9.03 | −1.52 | 0.1480 |
| CFB at month 12 ln (UPE) | 1 | −12.53 ± 20.72 | −0.60 | 0.5534 |
| BL eGFR × ln (BL UPE) | 1 | 0.10 ± 0.13 | 0.75 | 0.4617 |
| BL eGFR × CFB at month 12 ln (UPE) | 1 | 0.12 ± 0.27 | 0.43 | 0.6755 |

Agalα, agalsidase alfa; BL, baseline; CFB, change from BL; CKD, chronic kidney disease; DF, degrees of freedom; eGFR, estimated glomerular filtration rate; EXT, extension study; $Gb_3$, globotriaosylceramide; Pbo, placebo; RCT, randomized controlled trial; UPE, urine protein excretion.

[a]Pbo→agalα subgroup: 6 months of placebo (RCT) followed by 12 months of agalα (EXT). [b]Agalα→agalα subgroup: 6 months of agalα (RCT) followed by 6 months of agalα (EXT). [c]CKD 2/3 subgroup: stage 2/3 CKD before beginning agalα treatment.

retained in the model as a predictor of renal function in the overall renal analysis population, with statistically significant interaction effects detected with baseline eGFR. In addition, baseline urine protein excretion predicted renal function in the placebo→agalα and agalα→agalα subgroups, and CFB in urine protein excretion at month 12 was found to be a predictor in the placebo→agalα subgroup.

Currently available published evidence does not support the use of plasma or urine $Gb_3$ concentrations as predictive biomarkers for disease progression in patients with Fabry disease. For example, in a cross-sectional analysis in a population of 96 Dutch patients with Fabry disease, concentrations of $Gb_3$ in plasma and urine did not correlate with any clinical symptoms assessed, including renal dysfunction.[8] The findings of the present analyses are consistent with these studies. Although baseline urine $Gb_3$ excretion was found to be a significant predictor, this may have been of a reflection of baseline Fabry disease severity before agalα treatment initiation; e.g., patients with more severe Fabry disease at baseline may have experienced worse disease outcomes than patients with less progressed Fabry disease before treatment. To assess formally whether plasma and urine $Gb_3$ concentrations would be predictive biomarkers over the longer term for the clinical outcome in the natural history of Fabry disease, a longitudinal study in untreated subjects would be necessary.

The present study has several limitations. Because three trials were analyzed, the pooled patient population was heterogeneous because of differences in inclusion/exclusion criteria. These trials were not designed to evaluate renal function as a primary end point. The primary end point of one trial (TKT010) was changed from neuropathic pain to renal function after 15 months. The original inclusion criteria of this trial, however, were not modified to coincide with this change. In addition, study subjects with Fabry disease were not required

to have abnormal baseline eGFR and LVMI, which might leave them at lower risk of developing progressive kidney disease or cardiac deterioration during the course of these relatively short-term studies. Although a few significant predictors of renal function were found in the overall analysis population (e.g., baseline eGFR, age at start of agalα, baseline urine $Gb_3$ excretion, baseline and CFB at month 12 in urine protein excretion), further research is needed to determine if the lack of statistically significant differences in these same predictors in the subgroups were due to any specific characteristic(s) of these subgroups or were attributable to the patient number being too small to detect an effect. One indication that the patient numbers may have been insufficient in the subgroups is that only the renal analysis population and the placebo→agalα subgroup retained CFB at month 12 in urine protein excretion as a statistically significant predictor. Furthermore, a backward-elimination regression model is not a hypothesis-driven model; nevertheless, we consider it the best choice for these exploratory *post hoc* analyses; however, the follow-up period on ERT was relatively short, so additional analyses may be needed to assess long-term effects. Finally, although some studies support globotriaosylsphingosine as a potentially useful biomarker for monitoring Fabry disease, this evidence has been generated in relatively recent years.[6,21,22] At the time of the design of the clinical trials included in the current report, globotriaosylsphingosine was not considered a potential surrogate marker of Fabry disease progression or response to treatment and, thus, was not included as a measured end point.

The biological reason why changes in plasma or urine $Gb_3$ concentrations are not useful as biomarkers is not clear. Blood and urine $Gb_3$ concentrations are not mechanistically directly related to the outcome measures used in the current study; urine $Gb_3$ excretion is mostly derived from the renal collecting system, whereas the outcome (eGFR) reflects glomerular function. Likewise, changes in plasma $Gb_3$ do not necessarily represent its turnover in the heart muscle. Because renal function in most patients was not in the rapidly declining stage, the possibility exists that significant reduction in urine or plasma $Gb_3$ concentrations could not be reflected in changes in eGFR over a relatively short period of 12 months. Another possible explanation for our findings is that $Gb_3$ concentrations may not participate in the pathogenic process of renal glomerulopathy or hypertrophic cardiomyopathy of Fabry disease. Therefore, $Gb_3$ may be an example of a biomarker that is not entirely in the causal pathway of the disease process.[23]

In conclusion, the lack of correlation between CFB at month 12 of urine and plasma $Gb_3$ concentrations and renal outcomes suggests that changes in their levels do not predict clinical progression in Fabry disease; however, the discordant baseline urine $Gb_3$ excretion results in the subgroup analyses raises some question as to whether such a relationship exists and requires additional study. Other molecules, for example, globotriaosylsphingosine or reactive oxygen species, may also likely be active participants in the Fabry pathogenic cascade in the kidney and the heart.[21,24,25]

## SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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## DISCLOSURE

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