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REVIEW

# Enzyme replacement therapy for Fabry disease: some answers but more questions

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**Abstract:** Fabry disease (FD) is a multisystem, X-linked disorder of glycosphingolipid metabolism caused by enzyme deficiency of α-galactosidase A. Affected patients have symptoms including acroparesthesias, angiokeratomas, and hypohidrosis. More serious manifestations include debilitating pain and gastrointestinal symptoms, proteinuria and gradual deterioration of renal function leading to end-stage renal disease, hypertrophic cardiomyopathy, and stroke. Heterozygous females may have symptoms as severe as males with the classic phenotype. Before 2001, treatment of patients with FD was supportive. The successful development of enzyme replacement therapy (ERT) has been a great advancement in the treatment of patients with FD and can stabilize renal function and cardiac size, as well as improve pain and quality of life of patients with FD. In this review, we have provided a critical appraisal of the literature on the effects of ERT for FD. This analysis shows that data available on the treatment of FD are often derived from studies which are not controlled, rely on surrogate markers, and are of insufficient power to detect differences on hard clinical endpoints. Further studies of higher quality are needed to answer the questions that remain concerning the efficacy of ERT for FD.

**Keywords:** Fabry disease, agalsidase α, agalsidase β, Replagal, Fabrazyme, critical appraisal, evidence-based medicine

## Introduction

Fabry disease (FD) is an X-linked inborn error of glycosphingolipid catabolism resulting from the deficient activity of the lysosomal hydrolase, α-galactosidase A (α-Gal A). The enzymatic defect leads to the accumulation of glycosphingolipids, mainly globotriaosylceramide (GL-3), in body fluids, in the lysosomes of endothelial, perithelial, and smooth-muscle cells of blood vessels, in ganglion cells, and in many cell types in the heart, kidneys, eyes, and most other tissues.[1]

Clinical manifestations in classically affected hemizygous males who have no detectable enzyme activity include early childhood or adolescent onset of pain (acroparesthesias) in the extremities, angiokeratoma in skin and mucous membranes, and hypohidrosis. Corneal and lenticular opacities are also seen as early findings. Gastrointestinal problems, such as diarrhea, constipation, and abdominal pain, are common. Endocrine abnormalities include thyroid disease and fertility problems in both males and females. With increasing age, proteinuria, hyposthenuria, and lymphedema appear. Severe renal impairment leads to hypertension and uremia. Death usually occurs from renal failure or from cardiac or cerebrovascular disease. Atypical hemizygotes with residual enzyme activity may have later onset of symptoms, and

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such symptoms may be limited to the heart in some cases (the 'cardiac variant').[1] Heterozygous females can be as severely affected as hemizygous males, although the range of symptoms varies widely. A frequent clinical finding in females is the characteristic whorl-like corneal epithelial dystrophy observed by slit-lamp microscopy (cornea verticillata).[1]

Confirmation of the clinical diagnosis in males requires the demonstration of deficient α-Gal A activity in plasma, leukocytes, or fibroblasts, or increased levels of GL-3 in plasma or urinary sediment. Heterozygous females may have intermediate or even normal levels of enzymatic activity and accumulated substrate, so accurate diagnosis of heterozygous females requires identification of a molecular lesion in the α-Gal A gene or by linkage analysis in families with an affected male.[1]

Before 2001, treatment of patients with FD was exclusively supportive. Advancement of molecular genetic techniques led to the development of enzyme replacement therapy (ERT). There are two forms of (ERT): agalsidase α (AGALA) (Replagal®; Shire Human Genetic Therapies Inc, Cambridge, MA) and agalsidase β (AGALB) (Fabrazyme®; Genzyme Corporation, Cambridge, MA). Table 1 compares the two forms of available ERT.[2,3] In this review, we have examined the literature on the effects of ERT for FD with the aim of providing a critical appraisal of the literature and its limitations.

## Methods

We formulated a comprehensive search strategy in an attempt to identify all relevant studies published in the English language. An Ovid search was conducted using the Ovid databases: MEDLINE® (1950 to present with daily update) and Embase (1980 to date). Details of the search strategy are presented in Table 2. After the exclusion of case reports, studies not on ERT effects, studies not on FD, and general reviews on FD, 41 studies were included in this review.[2, 4–7, 9–45] Abstracts were reviewed using the evidence grading system developed by Oxford Centre for Evidence-based Medicine – Levels of Evidence (March 2009).[46]

## Results

### Factors that complicate interpretation of data

The grading of the evidence available on ERT for FD is listed in Table 3. A summary of the effects of ERT on various Fabry-related endpoints is provided in Table 4. While reading through the information in the tables, it is important to remember that, regardless of whether a disease is rare or common, studies of adequate quality are needed to distinguish true findings from false findings. There is a real need to critically appraise the literature available on ERT for FD for several reasons listed below.

#### The high cost of ERT

Given the recommended dosage of AGALA and AGALB, the cost of treatment for a 70-kg patient exceeds US$200,000 per year, and therefore, accurate information on the effects of ERT on hard clinical outcomes, such as the need for dialysis and stroke, is needed to be able to calculate the cost-effectiveness of therapy.

#### Lack of accurate natural history data

The studies of natural history regarding FD are very heterogeneous,[47–53] resulting in imperfect understanding of the natural history of this rare disease. As most of the publications on FD do not contain a prospective, untreated control group, accurate natural history data are essential for determining the effect of therapy. For example, natural history data are discordant when considering the risk of stroke. Studies report that rates of stroke range from 4.2% to 27% in females and from 6.7% to 24% in males. Even more confusing, successive publications from the same registry cite conflicting rates of stroke.[47,48,50,51] The data on glomerular filtration rate (GFR) are just as confusing. For example, in one of the earliest reports on the natural history of FD, a retrospective chart review of males with FD estimated annual decline in estimated GFR at 12.2 mL/min per year.[54] Another study that summarized the results of three separate clinical trials that were conducted at different times and sites showed rates of decline ranging from 2.9 to 7 mL/min/

**Table 1** Comparison between characteristics of AGALA and AGALB

| | AGALA | AGALB |
|---|---|---|
| Production | Human cell line by gene activation[2,3] | Chinese hamster ovary cells by recombinant techniques[3] |
| Dose | 0.2 mg/kg/2 weeks | 1 mg/kg/2 weeks |
| Duration of an infusion | 40 min | 2–4 h |
| Premedication | None, unless patient has infusion reactions | Antipyretic and/or antihistamine |

**Abbreviations:** AGALA, agalsidase α; AGALB, agalsidase β.

**Table 2** Search strategy

| | Medline (Ovid SP) | PubMed | Embase |
|---|---|---|---|
| Search by disease (limit to human and English) | 2800 | 980 | 3766 |
| Limit to therapy | 557 | 195 | 1106 |
| Limit to clinical trial | 72 | 62 | 273 |
| Exclusions of case reports, studies not on ERT effect, not on FD, and general reviews on FD | 30 | 20 | 231 |
| Final included | 41 | | |

**Notes:** We formulated a comprehensive search strategy in an attempt to identify all relevant studies published in English language. An Ovid search was conducted using the Ovid databases: MEDLINE® 1950 to present with daily update and Embase (1980 to date). The following search terms were used: Fabry* disease, enzyme replacement, agalsidase, Replagal, and Fabrazyme. These terms were entered as MESH subject heading terms as well. ADJ2 = words closely adjacent within two words of each other; $ = truncation, any number of characters; and Boolean operator Or/And were used to combine search terms. Filters for randomized-controlled trials were used. In addition, reference lists of relevant published articles were searched to make the search as complete as possible.
**Abbreviations:** FD, Fabry disease; MESH, Medical Subject Headings; ERT, enzyme replacement therapy.

year/1.73 $m^2$ in untreated males.[44] As most of the reported literature on FD and ERT does not include a control group, the lack of accurate natural history information makes the effects of ERT difficult to interpret.

### Conflicting prevalence data

Several studies investigating FD in dialysis patients in the United States and European registries reported prevalence to be 0.0168% and 0.0188% respectively,[55] with the prevalence among dialysis males about 0.027% in both registries while a study in Austria reported higher results in dialysis males with a prevalence of 0.264% and prevalence in overall dialysis patients of 0.161%.[56] A recent systematic review demonstrated that the overall FD prevalence on dialysis was 0.33% in males and 0.10% in females.[57] These data may underestimate the prevalence of FD in females on dialysis, however, as 91% of the screening studies in women were performed using α-Gal A activity analysis as the primary screening method, which is unreliable for detection of FD in female patients.[57] Newborn screening studies showed that the incidence of Fabry mutations in Taiwan Chinese and Italian populations to be 1:1400 and 1:3100 males, respectively,[58,59] which is 15–30 times higher than previous estimates.[1] More accurate data on disease prevalence are needed to identify the degree of ascertainment bias which may be present in the large multinational registries that provide most of the available data on the effects of ERT therapy.

### Unknown impact of antibodies

Treatment of Fabry patients may induce the formation of neutralizing antibodies toward AGALA and AGALB, and this may influence the effects of therapy. Antibody formation is more common in males.[7] The significance of these antibodies on clinical endpoints, though, is unclear as most of the studies on this have evaluated only surrogate endpoints and not all studies report on the presence of antibodies. One trial in male patients showed that the urinary GL-3 levels failed to decline in patients with IgG antibodies, whereas a reduction could be detected in patients without IgG antibodies.[6,7] This is in contrast to a study showing that 1.0 mg/kg of AGALB did reduce cardiac mass in small group of patients who were antibody positive. Using a surrogate marker like GL-3, which is an unreliable indicator of disease severity, may contribute to the poor understanding of inhibitory effect of IgG antibodies.[53,60]

When evaluating the medical literature on the effects of ERT on disease activity in FD, it is important to look critically for certain points including 1) the presence of a concurrent control group rather than using conflicting retrospective natural history data, 2) clear delineation of the origins of the patient cohort including a discussion of the number of subjects who were excluded from analysis and the reasons for exclusion, 3) use of hard clinical endpoints, appropriate randomization and blinding techniques, and 4) clear description of the power of the study to detect a significant difference in the primary outcome. As these features seem to be a basic requirement of any data evaluating a therapeutic modality, many of these key points are missing in the available data on ERT for FD.

## Grading of evidence

Seventy-one percent of the studies were graded as Grade 4 or higher (see Table 3). Only one study achieved Grade 1,[25] although several achieved Grade 2.[4,5,10,11,17,18,24,34,44]

The single Grade 1 study[25] has a placebo-controlled and blinded design, but nonetheless has significant limitations. The primary endpoint of this study was to show the effects of ERT on a composite clinical endpoint, which included renal, cardiac, and neurological events. Although the effects of AGALB on the composite outcome were of borderline significance ($P = 0.06$), secondary analyses of protocol-adherent patients adjusted for baseline proteinuria demonstrated a more

**Table 3** Grading of evidence of clinical trials of the ERT

| Study | Drug and dose[a] | Design/duration | Total number in study | Number in each study arm | Age (mean or range; years) | Gender | Duration (months) | Primary outcome | Grading system (OCEBM)[b] |
|---|---|---|---|---|---|---|---|---|---|
| Banikazemi et al[25] | AGALB | RCT: placebo-controlled, double-blind | 82; 74 protocol adherent | ERT: 51<br>Placebo: 31 | ERT: 46.9<br>Placebo: 44.3 | 72M; 10F<br>ERT: 45M; 6F<br>Placebo: 27M; 4F | 35 | Composite outcome composed of renal, cardiac, and neurologic events | 1b |
| Bierer et al[24] | AGALB | RCT: double-blind, placebo-controlled | 15; 6 in randomized arm with serial testing; 9 with baseline assessment but not randomized | ERT:<br>4 Placebo: 2 | 32 (20–47) | 14M; 1F<br>R: 5M; 1F<br>NR: 9M; 0F | 18 | Cardiopulmonary exercise characteristics and baseline and impact of ERT on cardiopulmonary exercise tolerance | 2a |
| Eng et al[5] | AGALB | RCT: multicenter, double-blind, placebo-controlled with open-label follow-up | 58 | ERT: 29<br>Placebo: 29 | ERT: 16–48<br>Placebo: 17–61 | ERT: 27M; 2F<br>Placebo: 29M; 0F | 5 (double-blind)<br>6 (open-label) | Clearance of GL-3 in renal microvascular endothelial | 2a |
| Hajioff et al[15] | AGALA | RCT: placebo-controlled with open-label follow-up | 15 | ERT: 7<br>Placebo: 8 | ERT: 36.4<br>Placebo: 36.9 | 15M; 0F | 6 placebo-controlled<br>24 open-label | Hearing loss | 2a |
| Hajioff et al[26] | AGALA | RCT: double-blind study placebo-controlled then open-label extension | 15 | ERT: 7<br>Placebo: 8 | 16–56 | 15M; 0F | 6 (placebo-controlled)<br>36 open-label | Hearing loss | 2a |
| Hughes et al[34] | AGALA | Randomized, double-blind, placebo-controlled | 15 | ERT: 7<br>Placebo: 8 | >18, mean or range not specified | 15M; 0F | 6 | Myocardial GL-3 content | 2a |
| Moore et al[10] | AGALA | RCT: double-blind, placebo-controlled | 26 | ERT: 14<br>Placebo: 12 | Fabry: 19–47<br>Control: 21–48 | Fabry: 26M; 0F<br>Control: not specified | 6 | Resting rCBF | 2a |
| Moore et al[11] | AGALA | RCT: double-blind, placebo-controlled | 26 | ERT: 14<br>Placebo: 12 | Fabry: 19–47<br>Control: 21–48 | Fabry: 26M; 0F<br>Control: not specified | 6 | rCBF following visual stimulation and acetazolamide challenge | 2a |
| Schiffmann et al[4] | AGALA | RCT: double-blind, placebo-controlled | 26 | ERT: 14<br>Placebo: 12 | ERT: 34<br>Placebo: 34.4 | 26M; 0F | 6 | Neuropathic pain assessed with BPI | 2a |
| Thurberg et al[17] | AGALB | RCT: multicenter, placebo-controlled, double-blind then open-label follow-up | 58 | ERT: 29<br>Placebo: 29 | ERT: 16–48<br>Placebo:17–61 | ERT: 27M; 2F<br>Placebo: 29M; 0F | 5 (double-blind)<br>30 (open-label) | GL-3 clearance characteristics of each cell type | 2a |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Vedder et al[6] | AGALB and a AGALA both given at 0.2 mg/kg/ 2 weeks | RCT: comparative trial of two commercial products, not blinded | 34 | AGALA: 18<br>AGALB: 16<br>10 from each included in primary endpoint analysis | AGALA: 19–60<br>AGALB: 24–76 | AGALA: 9M; 9F<br>AGALB: 9M; 7F | 24 | Reduction in left ventricular mass | 2a |
| West et al[44] | AGALA | Combination of data from three RCT: double-blind, placebo-controlled | 108 | ERT and placebo: 42<br>ERT only: 51<br>Placebo only: 15 | 18–54 | 108M; 0F | 12 | GFR | 2a |
| Baehner et al[16] | AGALA | Nonrandomized, single-center, open-label study | 15 | One arm: ERT | ≥18 | 0M; 15F | 13 | Safety, clinical efficacy, and pharmacokinetic profile | 4 |
| Beck et al[12] | AGALA | Observational study from registry | 545 | ERT: 314 (203M; 111F) | 25.2M; 32.8F | 281M; 264F | 12–24 | Renal function (assessed by estimated GFR), heart size (assessed by echocardiography), pain (assessed by the BPI), and quality of life (assessed by the European Quality of Life Questionnaire EQ-5D) | 4 |
| Buechner et al[40] | Not specified | Retrospective study | 43 | ERT: 24<br>No ERT: 19 | 19–74 | 25M; 18F | Retrospective study | Clinical and radiological CNS findings | 4 |
| Dehout et al[45] | AGALA | Questionnaire study | 11 | ERT: 11 | 17.4–45.8 | 9M; 2F | 12 | Frequency and severity of abdominal pain | 4 |
| Eng et al[9] | AGALB ; dose-ranging study from 0.3 mg/kg/2 weeks to 3 mg/kg/38 h | Nonrandomized, open-label, single-center | 15 | ERT: 15 | 18–45 | 15M; 0F | 5 doses | Dose-ranging study | 4 |
| Eto et al[43] | AGALB | Open-label, multicenter, nonrandomized | 13 | ERT: 13 | 16–34 | 13M; 0F | 5 | Efficacy and safety | 4 |
| Feriozzi et al[39] | AGALA | Observational study from registry | 165 | One arm: ERT | 18.4–68.0 | 115M; 50F | 36 | Renal function | 4 |
| Germain et al[38] | AGALB | Open-label, phase III extension study | 58 | One arm: ERT, 44 completed the study, 14 withdrew | 17–62 | 56M; 2F | 54 | long-term safety and efficacy | 4 |

(*Continued*)

**Table 3** (*Continued*)

| Study | Drug and dose[a] | Design/duration | Total number in study | Number in each study arm | Age (mean or range; years) | Gender | Duration (months) | Primary outcome | Grading system (OCEBM)[b] |
|---|---|---|---|---|---|---|---|---|---|
| Gupta et al[32] | AGALA | Prospective, open-label, placebo-controlled, nonrandomized | 49; 27 patients; 22 normal controls | 3 years after ERT: 22; ERT-naive: 5; healthy control: 22 | 3 years after ERT: 40.6; ERT-naive: 33.8; healthy control: 36 | 22M; 0F | | Skin impedance measurements | 4 |
| Hilz et al[21] | AGALB : 0.9–1.1 mg/kg/2 weeks | Prospective, placebo-controlled with open-label follow-up | 47; 22 Fabry and 25 normal controls | ERT: 22 Control: 25 | ERT: 27.9 Control: 29 | 22M; 0F | 5 (placebo-controlled phase); 18 (open-label phase) | Function of C-, Adelta-, and Abeta-nerve fibers and intradermal vibration receptors | 4 |
| Hoffmann et al[42] | AGALA | Observational study from registry | 120 | One arm: ERT | Not specified | 73M; 47F | 12–24 | BPI and health-related quality of life | 4 |
| Hoffmann et al[41] | AGALA | Observational study from registry | 752 | One arm: ERT | 33.6M; 37.3F | 353M; 393F | 24–36 | BPI | 4 |
| Imbriaco et al[29] | AGALB | Prospective, nonrandomized, open-label | 11 | One arm: ERT | 22–54 | 8M; 3F | 45 | LV function and myocardial signal intensity | 4 |
| Jardim et al[13] | AGALA | Nonrandomized, open-label, prospective study | 8 | One arm: ERT | 24–47 | 7M; 1F | 12 | Clinical and radiological CNS findings | 4 |
| Kosch et al[23] | AGALB | Open-label, uncontrolled, crossover study looking at timing of ERT with dialysis | 10 | ERT during dialysis and ERT between dialysis sessions | 45 | 10M; 0F | 2 dialysis sessions | Activity of α-Gal A in plasma | 4 |
| Lubanda et al[31] | AGALB 1 mg/kg/2 weeks × 6 months then 0.3 mg/kg/2 weeks × 18 months | Prospective, open-label study | 21 | One arm: ERT | 19.2–55.3 | 21M; 0F | 24 | GL-3 clearance | 4 |
| Palla et al[14] | AGALA | Nonrandomized, open-label | 21 | One arm: ERT | 22–71 | 13M; 8F | 12 | Peripheral vestibular function | 4 |
| Pisani et al[37] | AGALB | Nonrandomized, open-label, prospective study | 8/8 | One arm: ERT | 26–60 | 7M; 1F | 24 | Changes in symptoms and the echocardiographic evaluation of patients on dialysis | 4 |
| Schiffmann et al[2] | AGALA dose-ranging study 0.3–4.7 μ/kg single dose | Nonrandomized, small-number, single-dose, open-label study | 10 | One arm: ERT | 21–46 | 10M; 0F | Single dose | GL-3 clearance, pharmacokinetics, and safety | 4 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Schiffmann et al[19] | AGALA | This is a follow-up of previous RCT by Schiffmann et al.[2] Open-label, nonrandomized | 26 | One arm: ERT | 19–47 | 26M; 0F | 36 | Pain, warm and cold sensation, and sweating | 4 |
| Schiffmann et al[36] | AGALA | Single-center, prospective, open-label an extension of previous RCT | 25 | One arm: ERT | 36.8 | 25M; 0F | 48–54<br>20: completed 48<br>9: completed 54 | Safety and renal effects as well as the practicality of home infusions | 4 |
| Schiffmann et al[27] | AGALA | Prospective, open-label, nonrandomized | 17 | One arm: ERT | 7.3–18.4 | 16M; 1F | 42 | Safety | 4 |
| Schwarting et al[35] | AGALA | Observational study from registry | 201 | One arm: ERT | 20–60 | 131M; 70F | 55 | Renal function | 4 |
| Thofehrn et al[28] | AGALA | Open-label, nonrandomized | 9/9 | One arm: ERT | 34.55 | 7M; 2F | 36 | Proteinuria | 4 |
| Vedder et al[7] | AGALA at 0.2 mg/kg/2 weeks and AGALB at 0.2 mg/kg/2 weeks and 1 mg/kg/2 weeks | Comparative-trial, nonrandomized, open-label | 52 | AGALA<br>0.2 mg/kg: 18<br>AGALB<br>0.2 mg/kg: 13<br>AGALB<br>1 mg/kg: 21 | AGALA<br>0.2 mg/kg: 19–62<br>AGALB<br>0.2 mg/kg: 25–73<br>AGALB<br>1 mg/kg: 27–70 | AGALA<br>0.2 mg/kg:<br>10M; 8F<br>AGALB<br>0.2 mg/kg:<br>8M; 5F<br>AGALB<br>1 mg/kg:<br>10M; 11F | 12 | Occurrence of α-Gal A antibodies and their effect on urinary and plasma GL-3, and plasma chitotriosidase | 4 |
| Weidemann et al[20] | AGALB | Prospective, open-label, nonrandomized study | 16 | One arm: ERT | 24–57 | 15M; 1F | 12 | LV end-diastolic thickness of the posterior wall | 4 |
| Whybra et al[30] | AGALA | Nonrandomized, open-label, prospective | 36 | One arm: ERT | 14–76 | All female | 48 | Safety and tolerability | 4 |
| Wilcox et al[22] | AGALB | Open-label, nonrandomized extension of previous study | 58 | One arm: ERT | 16–61 | 56M; 2F | 30–36 | GL-3 clearance, safety profile, and kidney function | 4 |
| Wraith et al[33] | AGALB | Open-label study | 16 | One arm: ERT | 8–16 | 14M; 2F | 12 | GL-3 clearance | 4 |

**Notes:** [a]Except where otherwise specified, the dose of AGALB was 1 mg/kg/2 weeks and dose of AGALA was 0.2 mg/kg/2 weeks; [b]Grading system OCEBM, Oxford Centre for Evidence-based Medicine.[46]

**Abbreviations:** RCT, Randomized-controlled trial; GL-3, globotriaosylceramide; M, male; F, female, R, randomized; NR, not randomized; GFR, glomerular filtration rate; AGALA, agalsidase α; AGALB, agalsidase β; rCBF, regional cerebral blood flow; CNS, central nervous system; BPI, Brief Pain Inventory; LV, left ventricular; α-Gal A, α-galactosidase A; ERT, enzyme replacement therapy.

**Table 4** Summary of the results of clinical trials of the ERT on Fabry-related outcomes

| Outcome | AGALA | No. of patients and duration in months | AGALB | No. of patients and duration in months | Clinical comments and limitations of studies for both drugs |
|---|---|---|---|---|---|
| Renal | Stabilized renal function in patients with a mild or moderate deterioration in renal function at baseline[12,28,35,36,39,44]<br>Long-term stabilization confirmed[35,36]<br>Proteinuria category (1 or ≥1 g/day) at baseline significantly predicted the rate of decline of GFR during treatment[44] | 9–545; 12–55 | Clearance of microvascular endothelial deposits of GL-3[5,17,22,38] and stabilized kidney function[55]<br>Long-term stabilization up to 54 months[38]<br>ERT can be performed during hemodialysis[23]<br>Reduced the frequency of and delayed the time to clinical renal events[25]<br>In dialysis patients, ERT is safe and effective in improving global quality of life[37]<br>Proteinuria category (1 or ≥1g/day) at baseline significantly predicted the rate of decline of GFR during treatment[38] | 8–95; 5–54 | Evidence is convincing for both drugs that decline of renal function can be stabilized or slowed<br>Few double-blind RCT<br>Most studies measure surrogate endpoints rather than clinical renal endpoints, such as death, dialysis, and transplantation |
| Cardiac | Reduced left ventricular size in patients who had an enlarged heart at baseline[12,34] | 15–545; 6–24 | Clearance of microvascular endothelial deposits of GL-3[5]<br>Decreased left ventricular hypertrophy and improved regional myocardial function[20,29]<br>Improvement in exercise tolerance[24] | 11–58; 5–45 | Data is convincing for both drugs that rate of increase of LV mass can be stabilized or slowed<br>Few data available on clinical cardiac endpoints, such as cardiac death, admission for pacemaker, incidence of significant arrhythmia, etc |
| Neurological | Does not cross blood–brain barrier<br>Corrected abnormally elevated cerebral blood flow and exaggerated cerebrovascular response.[10,11] Decrease in nitrotyrosine staining, which was increased in dermal and cerebral vessels of FD patients[10,11]<br>Patients suffer from stroke during treatment[36] | 25–36; 6–54 | Does not cross blood–brain barrier<br>Some patients suffered from stroke during treatment[22]<br>Variable progression of MRI abnormalities while on treatment[6] | 34–58; 24–36 | Studies to date measure surrogate endpoints, such as cerebral blood flow and white matter lesions, but the evidence on white matter lesions is conflicting as there are case reports suggesting that they both improve and deteriorate[13]<br>Stroke was part of a composite clinical outcome in one study, but no study has yet been published with the power to detect a significant effect of ERT on stroke as a primary outcome |
| Pain and peripheral neuropathy | Significant decline in pain score[4,12,42]<br>Modest but significant improvement in the clinical manifestations of the small-fiber neuropathy[19]<br>Pain severity classification shifted toward lower severity[41] | 26–752; 6–36 | Improves function of C-, A-, and A-nerve fibers and intradermal vibration receptors in Fabry neuropathy[21] | 47; 23 | Data is convincing that improvements in pain occur but do not always translate into reduction in analgesic requirements<br>In some studies, concomitant use of antipain medications made the inference of improvement solely due to ERT difficult |

| | | | | | |
|---|---|---|---|---|---|
| Quality of life | Significantly improved[12,42] | 120–545; 24 | Both ERT and placebo group improved[5] | 58; 5 | Data is convincing for both drugs that quality of life improves; studies use tools to measure quality of life which are not disease specific |
| GI symptoms | Severity and frequency of abdominal pain decreased[45] | 11; 12 | Significant improvement in abdominal pain and vomiting compared with baseline[33] | 16; 12 | Improvement in GI symptoms is a common clinical finding, but studies carried out to date are of small size and inadequately controlled |
| Hearing | Improved vestibular function but the difference is not significant[14,26]<br>Gradual reversion of the hearing deterioration[15] | 15–21; 6–36 | – | – | Effects of ERT on chronically progressive sensorineural hearing loss may differ from those on sudden acute hearing loss, both of which occur in Fabry patients<br>Small sample size<br>Clinical significance of small changes in auditory function not clear |
| Skin and sweat function | No significant difference on ERT[32]<br>Improved[19] | 26–47; 36 | Clearance of microvascular endothelial deposits of GL-3[5] | 58; 5 | Most studies are observational and use surrogate biomarkers<br>No comprehensive studies on the effect of ERT on angiokeratoma |

**Abbreviations:** AGALA, agalsidase α; AGALB, agalsidase β; ERT, enzyme replacement therapy; GFR, glomerular filtration rate; GL-3, globotriaosylceramide; FD, Fabry disease; LV, left ventricular; MRI, magnetic resonance imaging; GI, gastrointestinal.

pronounced treatment effect compared with the placebo group ($P = 0.034$). Although these data are encouraging, the raw data suggest that the effects of therapy on the composite outcome were primarily driven from one of the renal endpoints which was, in fact, a surrogate measure (33% increase in serum creatinine) rather than hard renal endpoints like dialysis or transplantation. The 33% increase in serum creatinine comprised 10/14 events in the AGALB group and 7/13 events in the placebo group. Another possible limitation of this study is that only about one-third of the patients in each group were receiving antiproteinuric therapy with angiotensin-converting enzyme (ACE) inhibitors or angiotensin-receptor blockers (ARBs). As therapy directed at the renin-angiotensin system is beneficial in Fabry nephropathy,[61] the underutilization of such supportive therapies may have served to increase the perceived benefit of ERT.

To measure the outcome of interest, 98% of the studies used surrogate endpoints. Surrogate measures are often used when the disease is so rare or the desired outcome is so far in the future that it would take an unreasonably long follow-up period to obtain a sufficient number of outcomes. Even though the association between the surrogate measure and the true outcome may be biologically plausible, using the surrogate measure may produce misleading results if the association with the true outcome is not based on hard endpoints. The surrogate marker used in the first large study of AGALB was GL-3.[5] This trial demonstrated that therapy with AGALB led to clearance of GL-3 from biopsy specimens of the kidney, heart, and skin. Although these results were used to gain approval for AGALB in the United States, subsequent studies have shown that the relationship between GL-3 and clinical endpoints are less clear.[53,62]

Many of the publications include data obtained by cross-sectional surveys,[47,48] database registries[12,35,40,42,43,49–52] or historical cohorts,[53] which are subject to different sources of bias including selection bias, ascertainment bias, reporting bias, survivor bias (based on the early death of more severely affected patients), incomplete and missing data (leading to misclassification), and importantly, the absence of simultaneous controls. There are two large multinational registries: the Fabry Outcome Survey (FOS) sponsored by Shire Human Genetic Therapies, manufacturer of AGALA, and the Fabry Registry sponsored by Genzyme Corporation, manufacturer of AGALB. There are numerous publications from these registries which contribute to the medical literature on FD.[12,35,39,41,42,63] As these registries are able to combine large number of patients from around the world with different genetic backgrounds, they provide

valuable information on the progression of Fabry-related complications and the effects of ERT, and can also help to define some of the less frequent manifestations of an already rare disease. However, there are some problems with the data inherent in both the registries in that data collection is voluntary and, therefore, incomplete. This results in publications where the total number of patients included in the studies is often less than the total number of eligible patients, which can compromise conclusions drawn from these studies. For example, one study from the FOS includes only 201 patients, while at the time of analysis, 608 patients (358 receiving ERT) were enrolled in the registry.[35] Another publication included only 71 men and 59 women, while at time of analysis, 3182 patients were enrolled in the registry.[63] Although it is admittedly difficult to perform high-quality randomized studies in diseases of low prevalence, it is not impossible in that such studies have been done in other types of kidney diseases with similar prevalence to FD.[64]

## Effects of ERT

The major effects of ERT on different organ systems are summarized in Table 4 along with the limitations of the studies from which these effects have been determined. In summarizing the literature to date on ERT for FD, some conclusions can be drawn. It is clear that FD is a multisystem, progressive disorder in both males and females.[49] It is clear that ERT is an effective treatment for neuropathic pain in FD.[4] It is also clear that ERT can stabilize renal function or at least slow the decline of renal function in many patients with Fabry nephropathy[12,25,28,35,36,38,41,44] and stabilize or improve surrogate parameters like cardiac size in those with cardiomyopathy.[12,20,29,34]

# Discussion

## Unanswered questions about the treatment of FD

There are many unanswered questions such as the following:

1. What is the role of risk factor modification in the prevention of Fabry-related complications? A cross-sectional study showed high prevalence of uncontrolled hypertension among adult patients with FD who are included in the FOS registry database.[65] However, the cross-sectional nature of the study makes it impossible to infer a role of risk factor modification from this type of publication. A report describes an open-label, nonrandomized, prospective evaluation of the effects of ACE inhibitor and ARB therapy that were shown to have beneficial effects on proteinuria and renal function in FD patients who are receiving AGALB given at 1 mg/kg/2 weeks.[61] However, this observational study has a small sample size and should be confirmed in a larger population. At present, an open-label, prospective, observational study (Fabrazyme and ARBs and ACE Inhibitor Treatment – FAACET) is exploring the hypothesis that titration of ACE inhibitors and ARBs to reduce urine protein excretion to $<$500 mg/day in Fabry patients receiving AGALB (1 mg/kg every 2 weeks) will slow the progression rate of decline of GFR compared to controls. Details of this study are available at http://www.clinicaltrials.gov/.
2. What is the pathophysiology of the Fabry vasculopathy? One comprehensive review article showed that smooth muscle cells are a key player in the vasculopathy of FD. It concludes that the proliferation of smooth muscle cells and GL-3 storage result in higher intima-media thickness, increased reactive oxygen species production as well as enhanced nitric oxide production, which may result in different findings with respect to endothelial-activation markers, which can be severely enhanced in the context of other vascular risk factors.[66] However, this article showed that most studies carried out on evaluating Fabry vasculopathy were limited to case reports or case-control studies making it difficult to infer causality.
3. What is the role of ERT in the primary prevention setting? It has been established that pediatric patients have a significant disease burden with renal dysfunction and that cardiac involvement is detectable in adolescents with FD.[67,68] Another case series showed that signs of cardiac involvement are evident at an early age. Seven of 20 children included in this study, aged from 6.2 to 17.4 years, had left ventricular hypertrophy.[69] Although it is clear that pediatric patients do have detectable disease, ERT would only be required in the primary prevention setting if disease manifestations are irreversible. It is clear that some disease manifestations, like renal and cardiac involvement, can be stabilized if diagnosed early in the disease course. What is not clear, though, is the reversibility of other Fabry-related complications including the risk of stroke. The extent to which ERT can reverse progressive organ damage must be determined separately for each organ system. This question is critical when trying to establish the appropriate age of initiation of ERT. Currently, there is an ongoing pediatric primary prevention study to study the effectiveness of two alternative dosing AGALB dosing regimens in treatment of naive, male pediatric patients (details are available at http://www.clinicaltrials.gov/).

4. What is the appropriate dose of ERT? There are few dose ranging studies for either product. One small study by Vedder et al[7] suggested that 1.0 mg/kg of AGALB resulted in a more robust decline in GL-3 than does infusion of AGALA or AGALB at a dose of 0.2 mg/kg. The authors conclude that the higher dose of AGALB overcomes the negative effects of antibody formation. Lubanda et al[31] showed that in kidney interstitial capillary endothelium, the GL-3 clearance was achieved in 100% of patients with 1.0 mg/kg of dose compared to 90% with 0.3 mg/kg of dose. More information on the effects of different dosing regimens is needed for both AGALA and AGALB.
5. Are the two existing ERT products equivalent? A single head-to-head trial using AGALB and AGALA at the same dose showed no difference in surrogate endpoints, such as reduction of the left ventricular mass, GFR, pain and decline in GL-3 levels, treatment failure, and antibody formation.[6] However, there was no influence of antibodies on the reduction of urine GL-3 levels in patients treated with AGALB in contrast to the attenuated response seen in the pooled cohort treated with either AGALA or AGALB at 0.2 mg/kg/2 weeks.[7] An ongoing independent observational study known as the Canadian Fabry Disease Initiative (details are available at http://clinicaltrials.gov/) includes an arm, where patients newly started on ERT are randomized to one of the two commercially available products at product monograph doses,[70] and it will provide more data in the future on the relative effects of the two products.

## Future developments

Although the ERT is a step forward in the management of FD, the requirement for frequent infusions, the enormous cost for lifelong therapy, the inability of ERT to traverse the blood–brain barrier, and uncertainty about the long-term effectiveness on hard clinical endpoints in Fabry patients make other modalities of treatment candidates for consideration. Two such novel approaches are chaperone therapy[71–75] and gene therapy.[76]

Chaperone therapy is a novel approach that uses small molecules that specifically bind to and stabilize the functional form or shape of a misfolded protein in the endoplasmic reticulum (ER) of a cell. When a protein (enzyme) is misfolded because of a genetic mutation, it becomes unable to adopt the correct functional shape. This misfolded protein is recognized by the quality control system in the cell and is destroyed, leading to a decreased amount of enzyme that gets transported from the cell's ER to the cell's lysosome, and hence, reduced enzyme activity. The binding of the chaperone molecule helps the protein fold into its correct shape. This allows the protein to be properly trafficked from the ER and distributed to the lysosome in the cell, thereby increasing enzyme activity and cellular function and reducing substrate and stress on cells.[77] The advantage of such an approach includes better biodistribution of therapeutic agents, and such agents are able to traverse through the blood–brain barrier unlike ERT. Chaperone therapies can be administered orally, which may reduce the impact on quality of life caused by the need for biweekly infusions of ERT. In a trial of 27 patients with FD, treated for up to 2 years with 1-deoxygalactonojirimycin (DGJ) or Migalastat, the drug was safe and well tolerated. Migalastat increased the leukocyte, kidney, and skin α-Gal A activities and reduced the substrate (GL-3) levels in the urine and kidney biopsies of 24 patients.[78] Furthermore, the chaperone response of patients was similar to that predicted by models of in vitro responsiveness of α-Gal A gene mutations,[78] suggesting that there may be an easy way to determine which patients would be appropriate for the use of chemical chaperones. In another study, the response of T cells in normal individuals or in Fabry patient's to treatment with DGJ showed 28% increase in α-Gal A activity, whereas the response in Fabry individuals was mutation dependent ranging from no increase to fully normal activity.[71] Although these studies are promising, long-term trials looking at hard clinical endpoints are required.

Promising results have also been achieved in gene therapy experiments with the mouse model of FD. Adult Fabry model mice have been successfully treated by various viral vectors. Using adeno-associated viral vectors, long-term enzymatic and functional corrections in various organs of the Fabry mouse have been attained.[79,80] One study showed a single neonatal injection was effective to inhibit GL-3 accumulation in mice. If these data can be replicated in humans, this approach may be useful to prevent major organ failure developing later in life in patients with FD.[76] The advantages of gene therapy include persistent correction after a single procedure and cross-correction by enzymes secreted by organs. However, much work is still needed before this can be translated into the clinical setting.[78]

## Conclusion

ERT for FD is a major step forward for patients and has revolutionized care for patients with this fatal disease. However, as the field moves forward, questions need to be answered, some of which stem from the fact that most of the studies

are observational and/or uncontrolled. The availability of registries for FD currently is an excellent step to collect a large sample size. These registries could be used to draw participants for possible randomized-controlled studies which could generate Grade 1 data. Observational information from those registries, although useful to generate hypotheses, should never replace data from randomized-controlled trial. Crossover studies are a useful approach, but they present ethical challenges given that, at the current time, disease-modifying therapy for FD other than ERT is not available outside the clinical trial setting. In future, innovative approaches to research in rare diseases will be needed to obtain data of high quality while ensuring that there are no undue delays in translating the results of laboratory research into the clinical setting.

## Disclosure

Dr Sirrs has received speaking fees and has attended conferences with travel support sponsored by both Shire Human Genetic Therapies and Genzyme Corporation. She is also an investigator in the Canadian Fabry Disease Initiative, which receives funding from both Shire Human Genetic Therapies and Genzyme Corporation. Dr Alfadhel has no relevant disclosures.

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