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Agalsidase alfa for the treatment of fabry disease: a closer look

Enzyme replacement therapies substitute particular enzymes in individuals who have deficient or absent levels of enzymatic activity due to inherited defects. Fabry disease, a rare X-linked genetic disorder yet common lysosomal storage disease, is due to the impaired activity of alpha-galactosidase A. Currently, there are two enzyme preparations available for the treatment of Fabry disease: agalsidase alfa (Replagal®), manufactured by Shire, and agalsidase beta (Fabrazyme®), manufactured by Genzyme. Here, we review the molecular characteristics and intracellular trafficking of the endogenous and exogenous enzymes as well as the pharmacological properties of Replagal. We also summarize clinical experiences with Replagal and provide insight into its therapeutic value for the treatment of Fabry disease.

Keywords: agalsidase alfa • enzyme replacement therapy • Fabry disease • lysosomal storage disease • Replagal®

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Fabry disease is a rare, X-linked disorder caused by mutations in the gene encoding the lysosomal enzyme α -Gal A (α -Gal A; E.C. 3.2.1.22). The deficiency of α -Gal A leads to intralysosomal deposition of glycosphingolipid products, primarily globotriaosylceramide (GL3) in multiple tissues and organs, including the vascular, endothelial and smooth muscle cells. GL3 accumulation in the skin, heart, eyes, autonomic nervous system and kidney ultimately leads to progressive tissue damage and end organ dysfunction [1,2].

Fabry disease has an estimated prevalence of 1 in 40,000 males. This figure may be an under representation, however, as data from newborn screening studies suggests the late-onset form may be under diagnosed [3,4]. The *GLA* gene for Fabry disease is located on the long arm of the X chromosome at position Xq22.11 and more than 500 mutations have been documented [5–10]. Although most of the mutations are single point mutations, they result in less than 1% residual α -Gal A activity in hemizygote males. Furthermore, it is now understood that females can also

be affected with the disease, although their presentation may be more variable than in males [11].

Individuals with Fabry disease suffer from a progressively debilitating disease and multisystemic symptoms that begin in childhood advance into adulthood. Early manifestations in adolescent males include corneal whorls, angiokeratomas, acroparesthesia, abdominal pain, diarrhea, constipation, hypohidrosis or anhidrosis [12]. Heart manifestations include left ventricular (LV) enlargement, arrhythmias and valvular involvement [13–16]. Microalbuminuria represents the first sign of kidney involvement and, ultimately, end-stage renal disease (ESRD) contributes to the high mortality rate in patients [17]. Other symptoms include transient ischemic attacks, strokes [18,19], hearing loss, vestibular dysfunction [20,21], ocular abnormalities [22] and lowered quality of life [2,12,23].

Background

Prior to 2001, there was no disease-specific treatment available for Fabry disease. Management for patients had traditionally been

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supportive and symptom based [24]. In 1973, Johnson and Brady showed that the infusion of purified α -Gal A, derived from human placenta, rapidly cleared GL3 from systemic circulation in patients with Fabry disease [25]. However, technical complications prevented further development of enzyme replacement therapy (ERT) for Fabry disease until 2001, when α -Gal A was produced using novel molecular biologic techniques based on recombinant DNA technology. Currently, there are two recombinant α -Gal A preparations available for treatment of Fabry disease: agalsidase alfa (Replagal®; Shire Human Genetic Therapies, MA, USA) and agalsidase beta (Fabrazyme®; Genzyme Corporation, MA, USA). Both enzyme preparations have been approved in the European Union by the European Agency for the Evaluation of Medicinal Products. Replagal was granted orphan designation by the European Medicines Agency in 2000, followed by marketing authorization under exceptional circumstances in 2001. It has since become available internationally, with the exception of the USA where it is under review, and has been approved for use in patients with a confirmed diagnosis of Fabry disease. Alternatively, Fabrazyme was approved by the US FDA in 2003. In June 2009, Genzyme reported a viral contamination in the manufacturing of Fabrazyme, which led to an international shortage of the therapy. Replagal was therefore granted an expanded access protocol so patients could receive an ERT in the USA during the shortage [26].

Both of the recombinant α -Gal enzymes are recommended to be administered via intravenous infusion once every two weeks. Regardless of the similar proteomic profile, however, the approved dose of Replagal is fivefold less than that of Fabrazyme. Replagal is administered at 0.2 mg/kg while Fabrazyme is administered at 1.0 mg/kg. This work will provide an overview of ERT in Fabry disease, summarize the clinical experience with Replagal and provide insight into the product's therapeutic value and safety profile.

Molecular & intracellular trafficking

α -Gal A is a homodimer, with each monomer made up of two domains that are able to function separately. One domain contains the active site at the N-terminal and the other domain contains a β -sandwich of anti-parallel β strands at the C-terminal of the protein [27]. As a result, the enzyme harbors two active sites that are separated by 43 Å. 3D structure analysis of the protein revealed that these active sites act independently [28].

Belonging to the glycoside hydrolase family, α -Gal A hydrolyses the terminal α -galactosyl moieties from glycolipids and glycoproteins, and most importantly GL3. Interestingly, both the substrate and its product have anomeric carbons with α configuration. As

proposed by Koshland, this net retention of configuration is the result of a double displacement reaction mechanism. The reaction requires two amino acid side chains, one that acts as a nucleophile and another that acts as an acid base catalyst. At first, the nucleophile residue attacks the anomeric center to displace the aglycone and form a glycosyl enzyme intermediate, while the other residue, acting as an acid catalyst, protonates the glycosidic oxygen as the bond cleaves. In the second step, this intermediate glycosyl enzyme gets hydrolyzed by water. At the same time the other residue, acting as a base catalyst, deprotonates the water molecule [29].

Guce and colleagues further investigated the catalytic cycle of α -Gal by x-ray crystallization and described four stages of the catalytic mechanism. The empty enzyme (Stage 1) incorporates water molecules at the approximate locations of the galactose binding sites. Kinetic studies demonstrated that the substrate-bound wild-type α -Gal (Stage 2) has a K_m of 8.3 ± 0.5 mM and a k_{cat} of 63.5 ± 0.1 s⁻¹, which are comparable to the two recombinant α -Gal administered in ERT. When the complex forms a covalent intermediate (Stage 3), it is in a 1S3 twist boat conformation that is distorted from the favored chair conformation of the sugar ring. When the second nucleophilic attack is completed, the ligand turns from the distorted state reached at Stage 3 to a low energy conformation (Stage 4) [28].

The genetic information of α -Gal A is encoded by the *GLA* gene that is localized on the X chromosome. As a result, hemizygous males are clinically affected more seriously than those females who are mutation carriers. *GLA* is 12 kilobases (kb) in length and includes 7 exons and 12 Alu repetitive elements that makes approximately 20% of the gene. DNA transcription gives rise to a 1.45 kb mRNA that encodes a protein of 429 amino acids, including a 31 amino acid-long signal peptide at the N-terminal [30]. From the endoplasmic reticulum, the polypeptide moves to the Golgi network for further processing. In the *cis*-Golgi network, a mannose-6-phosphate (M6P) tag is added to the oligosaccharide moieties of the protein that renders the protein for lysosomal transport. Moving forward in the apparatus, M6P moieties are recognized and bound by M6P receptor (M6PR) proteins in the *trans*-Golgi at a pH between 6.5 and 6.7. Via vesicle transport, α -Gal A is then shipped to the late endosomes where the slight change in acidity, at approximately pH 6.0, leads to the dissociation of the M6P containing enzyme from its receptor. Upon release, the protein is transferred to its final destination, the lysosome, where the acidic environment (pH 4.5–5.0) activates the enzyme (Figure 1).

Rarely, the endogenous enzyme is secreted into the extracellular milieu by exocytosis. This extracellular α -Gal A that harbors M6P moieties is recaptured by the plasma membrane by binding to M6PR present on the cell surface. After internalization, the protein gets incorporated into endosomal vesicles. Once these vesicles fuse with a lysosome, the enzyme dissociates from the receptor and becomes activated by the acidic pH. This pathway is considered to be one of the major uptake mechanisms utilized by ERT (Figure 2).

Both Fabrazyme and Replagal are produced by recombinant DNA technology and share the same amino acid sequence. Fabrazyme is produced in a Chinese hamster ovary mammalian cell system while Replagal is produced in a continuous human cell line of undisclosed origin. Differences between the two products are attributed to post-translational modification, specifically N-linked glycosylation, as a result of distinct production in different species. Higher levels of fucose, galactose, *N*-acetylglucosamine and mannose were detected in Replagal compared with Fabrazyme. Although both synthetic enzymes have the same amount of sialic acid on a mole to mole of protein basis, Fabrazyme is considered to be more highly sialylated simply due to its higher sialic acid to galactose ratio compared with Replagal. Moreover, Fabrazyme contains a higher level of M6P at a molar basis.

Since the M6PR is considered to be a major uptake mechanism for extracellular enzyme, binding assays and enzyme uptake experiments were performed to compare the two enzyme preparations. Lee and colleagues reported that Fabrazyme showed superior receptor binding affinity at all tested concentrations and enhanced cellular uptake at lower concentrations but comparable enzyme accumulation at higher concentrations compared with Replagal at equimolar concentrations [31]. One explanation for this phenomenon could be the presence of other alternative uptake mechanisms that are activated at higher enzyme concentrations [32].

Recently a new manufacturing process, a bioreactor (agalAF1) in place of the previously employed roller bottle process, was implemented to increase the effectiveness of the production, and to eliminate animal-sourced components [33]. However, there were no changes proposed for either the product information or consumer medicine information as a result of this change. Most data in clinical trials have been derived from agalsidase alfa produced by the roller bottle. While these two formulations are regarded to be different as a result of the manufacturing differences, it is expected that their biological and thus clinical effects are to be similar.

Lysosomes mediate a wide range of biological processes including autophagy. As one of the chief func-

tions of the lysosome, autophagy has been shown to be disrupted in many lysosomal disorders including Fabry disease. Some data suggest that dysregulated autophagy may play a role in the kidney dysfunction that is often seen in Fabry disease, demonstrating the complexity in the Fabry disease process [34,35].

Pharmacological characteristics

Several studies have demonstrated that the plasma concentration (T_{max}) of Replagal peaks at the end of transfusion with the serum levels declining in a biphasic manner thereafter [36–40]. The reported mean clearance of the enzyme ranges from 140 to 160 ml/kg while the clearance normalized for body weight averaged between 2.1 and 2.2 ml/min per kg [36]. Serum clearance is similar in males and females and a tendency for reduced clearance with increasing age has been demonstrated in a pediatric trial [36]. It has been shown that $t_{1/2}$ influenced by gender, with males having significantly longer $t_{1/2}$ than females (70 and 50 min in pediatric patients and 112 and 89 min in adults, respectively) [36]. Schiffmann and colleagues reported that the average clearance of Replagal was 65% higher than the calculated creatinine clearance, suggestive of non-renal modes of elimination, such as cellular uptake by various organs [39]. Furthermore, patients with ESRD showed biphasic plasma elimination and a similar pharmacokinetic profile for Replagal compared with patients that have normal kidney function [40]. Clearance in tissues is significantly lower than that in plasma. Tissue biopsies showed that 8–32% of the total administered α -Gal A dose was present in the liver about 2 days after infusion [39]. Immunohistochemical staining of liver biopsy samples revealed profound staining of the enzyme on Kupffer cells, sinusoidal endothelial cells and hepatocytes [39].

GL3, the substrate for α -Gal A, is routinely measured in blood and urine and is occasionally measured in biopsy samples. The level of GL3 is considered to be an indicator for treatment efficacy and has been used as surrogate marker in several studies [37,40]. Even short-term treatment with Replagal at 0.2 mg/kg every other week reduces plasma GL3 levels by 50% [38]. Notably, no significant difference in plasma GL3 clearance was shown among doses ranging 0.2 mg/kg every other week to weekly 0.4 mg/kg [38]. Patients on dialysis or who had received a transplant experienced similar level of GL3 clearance. Plasma GL3 levels decreased by 43% after a 27 weeks of agalsidase alfa treatment [40]. Hughes and colleagues investigated the efficacy of three different dosing regimens of Replagal for four weeks, 0.1 mg/kg weekly, 0.2 mg/kg biweekly and 0.2 mg/kg weekly, and found urine GL3 level reduction with the 0.2 mg/kg/week dose [41]. Changes in

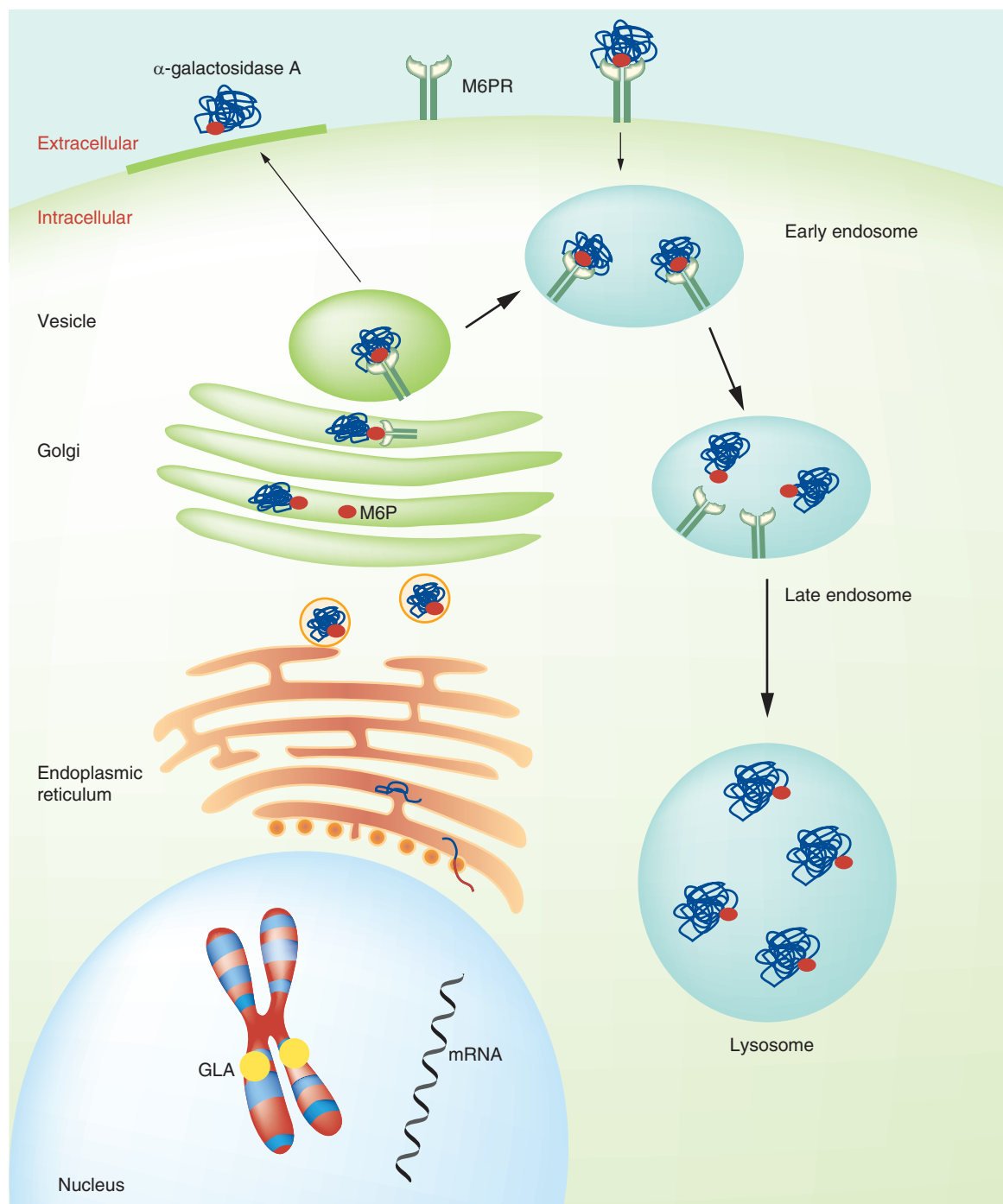


Figure 1. Intracellular trafficking of α -galactosidase A. Following DNA transcription, mRNA of α -galactosidase A is translated to a polypeptide chain by ribosomes on the surface of the endoplasmic reticulum. Then the protein moves to the Golgi network. M6P moieties are added to the enzyme in the *cis*-Golgi and recognized by M6PR in the *trans*-Golgi cisternae. While the majority of the protein is shipped to the lysosome by endosomal vesicles, a small portion of the enzyme gets secreted by exocytosis. Following M6PR binding, the mislocalized enzyme can be incorporated into endosomal vesicles and delivered to the lysosome. M6P: Mannose 6-phosphate; M6PR: Mannose-6-phosphate receptor.

GL3 levels have been studied in several pediatric trials [36,42–43]. Boys presented with higher levels of plasma and urine GL3 at baseline compared with girls. Upon treatment, both plasma and urine GL3 were reduced

to within normal levels after 12–23 weeks of Replagal therapy.

Although several studies have followed the changes in GL3 levels in Fabry patients, none have assessed its

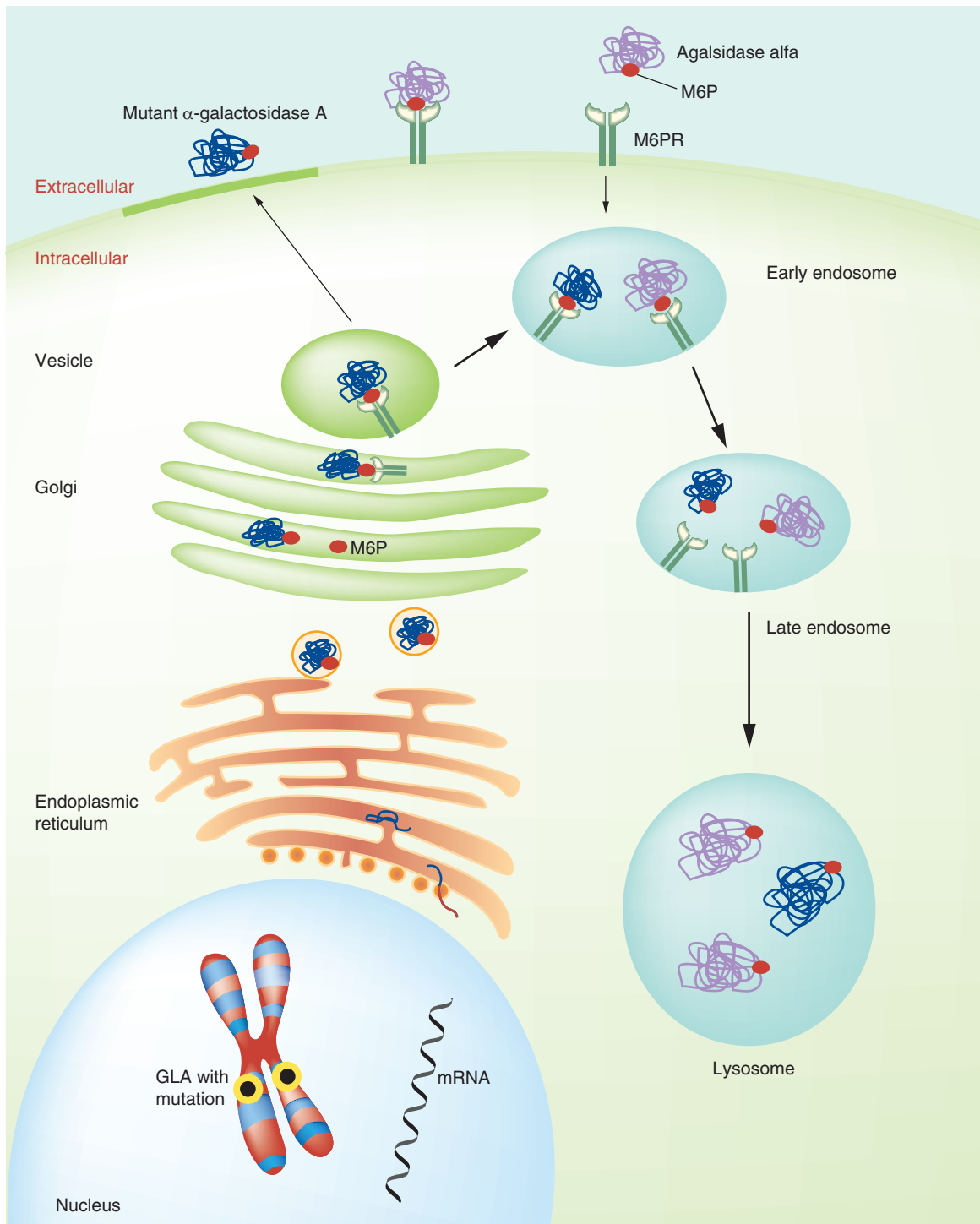


Figure 2. Enzyme replacement therapy. In Fabry disease, the endogenous enzyme dysfunction of α -galactosidase A is corrected by enzyme replacement therapy. Agalsidase alfa binds to M6PRs located on the outside surface of the cell membrane. Following endocytosis, the drug is transported to the lysosomes. M6P: Mannose 6-phosphate; M6PR: Mannose-6-phosphate receptor.

long-term predictive value. However, in a 12-month follow-up study of patients treated with Replagal, GL3 levels did not appear to be useful biomarkers for predicting glomerular filtration rate or LV mass index [44]. While both plasma and urine levels of GL3 are

commonly evaluated, urine GL3 is a more sensitive but less specific test. One drawback of following urine GL3 levels is that the substrate level is often normalized to creatinine. As a result, changes in creatinine level, due to such factors as physical activity or signifi-

cant weight loss, alter the urine GL3 readout without having changes in the substrate level.

Another biomarker for Fabry disease is the recently identified deacylated globotriaosylceramide (lyso-GL3) [45]. This cationic amphiphile is readily water soluble due to its large polar sugar moiety and, unlike GL3, does not distribute into the organic phase during organic solvent extraction. *In vitro* experiments showed that lyso-GL3 inhibits α -Gal A and promotes proliferation of smooth muscle cells, thus providing a possible explanation for the vascular changes that present in Fabry disease. Detected in plasma and in urine samples, lyso-GL3 is a highly specific and, in some patients, a more sensitive marker for disease activity. Lyso-GL3 is not only more abundantly present in the plasma than GL3, but it also subsides more slowly after initiation of ERT. Elevated plasma levels of lyso-GL3 are detected in almost all male and adult female patients with Fabry disease. However, young, presymptomatic heterozygous female carriers, patients with R112H and those with P60L mutations may have normal plasma lyso-GL3 levels [46].

Lyso-GL3 provides a promising alternative for following disease progression, especially in heterozygous female patients whose GL3 might be in normal range. Furthermore, lyso-GL3 levels were found to correlate with LV mass changes as well as with hazard ratios to develop strokes or white matter lesions in both genders [47]. While plasma lyso-GL3 does not correlate with total disease severity in Fabry males, lifetime exposure to lyso-GL3 does correlate with disease manifestations in both male and female patients [46]. In most cases, plasma lyso-GL3 acts as a useful clinical tool in the diagnosis of Fabry disease and in predicting disease severity.

There is an unmet need for identifying and utilizing clinically relevant biomarkers for Fabry disease. Auray-Blais and colleagues have reported the presence of additional analogs of GL3 and lyso-GL3 in patients with Fabry disease, some of which are excreted at higher levels than the substrate itself. Further testing is needed to select analogs with clinically relevant values [48,49].

Efficacy

The clinical efficacy of Replagal has been studied in six randomized clinical trials [41,50–54], fourteen open-label studies [15,36–38,42–43,52–53,55–60], and a few descriptive studies [21,61–62]. Data extracted from the Fabry Outcome Survey (FOS) has provided additional information on the effects of Replagal [20,63–72]. FOS, which is owned and operated by Shire, is a global observational database for patients with Fabry disease. FOS aims to increase the understanding of the nature of the disease

and improve management of all patients with Fabry disease, including women and children. Renal, cardiac and neurological end points were most commonly studied.

In 2001, Schiffmann and colleagues published the first randomized, double-blind, placebo-controlled Phase II/III trial with Replagal [50]. The trial examined the efficacy of 0.2 mg/kg intravenous Replagal administered every other week in 26 adult men. Patients randomized to Replagal received 12 doses over a 22 week time period. This trial demonstrated statistically and clinically significant differences in improvement of neuropathic pain, the study's primary end point. Significant findings were also demonstrated in the secondary end points of creatinine clearance and cardiac conduction, suggesting that the therapeutic effects of the drug are widespread. Several other randomized, controlled trials and open-label studies have further supported the efficacy and safety of Replagal [52,57–58,73].

Renal & cardiac outcome measures

In most Fabry patients, kidney dysfunction becomes apparent by the third decade of life and invariably progresses to ESRD without treatment. Schiffmann and colleagues followed the estimated glomerular filtration rate (eGFR) in adult men who had over four years of treatment with Replagal [57]. The eGFR remained stable in subgroups of patients with stage I (eGFR >90 ml/min) or stage II (eGFR 60–89 ml/min) chronic kidney disease at baseline. In contrast, in the subgroup of patients with stage III chronic kidney disease (eGFR 30–59 ml/min), the slope of the decline in eGFR was reduced relative to comparable historical controls, suggesting an ERT-mediated mitigation of renal function decline in this susceptible population [57]. During the 24 month follow-up period after switching from every other week to weekly infusions, the mean rate of change in eGFR was observed to slow from -8.0 ± 0.8 ml/min/1.73 m²/yr to -3.3 ± 4.7 ml/min/1.73 m²/yr [58]. In a pooled analysis of three prospective randomized trials and their open-label extension studies, West and colleagues found that the mean rate of change in GFR for the entire study population of 108 males was -4.8 ± 10.6 ml/min/1.73 m²/yr [59]. After eight hyperfiltration patients were removed from analysis, the mean rate of change of eGFR was -2.9 ± 8.7 ml/min/1.73 m²/yr. Additionally, Feriozzi and colleagues document sustained effectiveness of Replagal out to 5 years in adults with stable eGFR as measured by the CKD-Epi formula [74]. These data demonstrate that treatment with Replagal may prevent deterioration of renal function in patients with Fabry disease.

Tondel and colleagues looked at the clearance of

GL3 deposits from glomerular endothelial and mesangial cells. They report for the first time the complete clearance of the GL3 deposits from glomerular endothelial and mesangial cells by Replagal. Additionally, the higher drug dose appeared to give better GL3 clearances in children although there were no clinical differences reported between those on Replagal and Fabrazyme [75].

Several observational studies have used the FOS database, each with slightly different patient populations, treatment durations and primary end points, to evaluate renal function [63,65,68–69]. An early FOS study demonstrated Replagal stabilized renal function following one to two years of ERT in patients with mild (stage II) to moderate (stage III) renal dysfunction [65]. Another study found a slight reduction in eGFR during 2 years of ERT [69]. Kidney transplant recipients in this study also demonstrated no increase in proteinuria, consistent with previous work by Schwarting and colleagues [65] on the effect of Replagal in patients with chronic kidney disease. In a study of males by Schwarting, eGFR declined with stage I/II renal disease but remained stable in stage III [68].

A few studies have examined the effect of Replagal on kidney function in females [37,55]. Baehner and colleagues found no deterioration in renal function in female patients treated with Replagal for more than 2 years [37]. Average kidney function, as assessed by eGFR and proteinuria, remained constant over 4 years during a separate study of 36 females [55]. These results support the necessity of early initiation with ERT in Fabry patients, as the drug appears to stabilize kidney function in patients with moderate kidney disease while having less effect in patients with advanced disease (Table 1).

A small number of randomized trials and observational studies have looked at the effects of Replagal on cardiac end points in patients with Fabry disease. Hughes and colleagues examined LV mass as measured by MRI in 15 adult males [16]. Ten of the 15 patients had LV hypertrophy (LVH) at baseline. LV mass and LV mass index (LVMI) were reduced to a significantly greater extent following 6 months of treatment with Replagal compared with placebo. Additionally, while not statistically significant, a mean 20% reduction in myocardial GL3 content was shown, as assessed by serial transvenous endomyocardial biopsies. This compares to a mean 10% increase in patients receiving placebo. These results were confirmed in a 2 year open-label extension study [16]. The QRS complex duration was also shortened in patients treated with Replagal versus placebo [50].

MacDermot and colleagues reported that approximately 15% of female patients with Fabry disease died

of cardiomyopathy in their study [19], demonstrating that cardiomyopathy is a significant clinical syndrome in heterozygote females. Baehner and colleagues followed fifteen females for 55 weeks on Replagal therapy [37]. Every patient who had a LVMI greater than 124 g/m² at baseline demonstrated a decline in LVMI following ERT. The significant decrease in QRS complex duration was consistent with reductions in LV wall thickness and mass [37].

Several studies from FOS have also addressed cardiac end points. Two studies demonstrated reduced LV size in patients who had an enlarged heart at baseline [63,71]. In patients with baseline cardiac hypertrophy, treatment resulted in a sustained reduction in LVMI after 5 years and a significant increase in midwall fractional shortening after 3 years. In patients without baseline hypertrophy, LVMI and midwall fractional shortening remained stable [71].

Kampmann completed a pooled analysis of 45 patients enrolled in FOS who received treatment with Replagal for 3 years [70]. Of 14 patients with baseline LVH, LVMI decreased significantly after 1 year in nine patients, and after 3 years in ten patients. Of the 31 patients without baseline LVH, LVMI significantly increased after 1 year and there was no significant change from baseline in ten patients after 3 years. This suggested that ERT was associated with a significant reduction in LVMI in patients with baseline hypertrophy. Hughes and colleagues found that LV mass had a significant decrease in women but not in men [72].

The impact of Replagal on cardiac end points has been studied less frequently in pediatric patients. Ries and colleagues followed heart rate variability in 24 children [42]. Heart rate variability, determined by 2-h ambulatory monitoring, was reduced in the boys compared with the girls at baseline. All indices of heart rate variability improved significantly in the boys after 6 months of ERT [42]. Furthermore, LV mass indexed to height remained stable throughout a 4-year study of 17 children [56]. The rate of increase in cardiac wall thickness remains unknown in untreated children making correlation of treatment difficult with these findings. Table 2 outlines the findings of left ventricular mass index (Table 2).

For patients with advanced disease process, ERT will have limited efficacy. Two recent studies prospectively followed renal, cardiac and cerebrovascular outcomes in adults receiving ERT, either Replagal or Fabrazyme. Weidenmann and colleagues found that despite ERT, clinically meaningful events including sudden cardiac death, continue to develop in patients with advanced Fabry disease [76,77]. Similarly, Rombach and colleagues conclude that while the risk of developing a first or second complication declines with increasing

Table 1. Clinical outcome measures in subjects treated with agalsidase alfa: renal outcome measures.

Study type	Patients (n)	ERT frequency	Length of therapy (months)	eGFR (ml/min/1.73 m ²)		Proteinuria (mg/24h)		Ref.
				Baseline	Post-therapy	Baseline	Post-therapy	
FOS	Transplant recipients (20)	Biweekly	24	59.2	51.1	–	–	[69]
OL	Males (11)	Biweekly	41	77.8	53.7	1217	1485	[58]
		Weekly	24	53.7	50.6	1485	1271	
OL EXT	Children (17)	Biweekly	48	121.0	121.7	–	–	[56]
OL EXT	Adults (24)	Biweekly	12	108.7 (CKD I) 78.6 (CKD II) 47.1 (CKD III)	105.6 (CKD I) 77.9 (CKD II) 39.8 (CKD III)	353	–	[57]
			24	–	112.6 (CKD I) 83.0 (CKD II) 40.5 (CKD III)	–	–	
			36	–	114.6 (CKD I) 74.5 (CKD II) 32.9 (CKD III)	–	543	
			48	–	101.1 (CKD I) 69.7 (CKD II) 24.8 (CKD III)	–	–	
FOS	Adults (165) (M:115, F:50)	Biweekly	36 month	M115.0 (CKD I) 76.5 (CKD II) 49.1 (CKD III) F103.2 (CDK I) 72.5 (CDK II) 49.5 (CDK III)	M98.3 (CKD I) 66.3 (CKD II) 42.8 (CKD III) F96.4 (CDK I) 67.3 (CDK II) 46.3 (CDK III)	–	–	[68]

CKD I: eGFR ≥ 90 ml/min/1.73 m²; CKD II: eGFR = 60–89 ml/min/1.73 m²; CKD III: eGFR < 59 ml/min/1.73 m².

CKD: Chronic kidney failure; DB: Double blinded; eGFR: Estimated glomerular filtration rate; EXT: Extension; F: Female; FOS: Fabry outcome survey; LV: Left ventricular; M: Male; OL: Open label.

treatment, the use of ERT in advanced Fabry disease seems of doubtful benefit [77]. Thus, long term ERT, even combined with optimal supportive care, does not appear to prevent progression, but instead may lower the risk of developing additional complications.

Cerebrovascular outcomes

While recombinant α -Gal A preparations do not cross the blood–brain barrier, ERT appears to reverse the exaggerated cerebrovascular response in Fabry disease. Two randomized studies with Replagal used cerebrovascular responses as primary end points. Abnormal resting cerebral blood flow induced by visual stimulation and acetazolamide decreased significantly following ERT for 18 months. Chronic alteration of the nitric oxide pathway has also been implicated in CNS involvement in Fabry disease. Protein nitration, which may be critical, was shown to be reversible with ERT [78].

Strokes are a severe complication of Fabry disease and occur in approximately 7% of Fabry patients [79]. However, data are sparse on the effectiveness of ERT on strokes as strokes have not been used as a outcome measure in the trials of Replagal. Addition-

ally, the effect of Replagal on white matter changes still remains unclear as white matter lesions have been shown to both disappear and appear after treatment with Replagal [61,62].

In limited studies, peripheral nervous system pathology is represented by intraepidermal nerve fiber density [80] and nerve conduction [53]. There was no significant difference between 6 months of treatment and placebo [80]. However, after 18 months of therapy, there was a significant decline in intraepidermal nerve fiber density [53].

Pain, disease severity, quality of life & other outcome measures

Pain is one of the most prevalent and debilitating symptoms in Fabry disease with an onset early in childhood. Data from clinical trials and FOS demonstrate that ERT with Replagal reduces pain. In the first randomized controlled trial, Schiffmann and colleagues reported that Replagal reduced the severity of neuropathic pain [50]. The open-label extension study used the brief pain inventory (BPI) and demonstrated a reduction in pain [53]. The BPI rates pain on scale of

0–10, with 0 rated as ‘no pain’ and 10 rated as ‘worst pain’. A one-unit reduction in the BPI score is considered statistically significant. After 6 months of therapy, the BPI reduced from 6.9 to 4.5. In women, again using the BPI, the ‘pain at its worst’ score was reduced from 4.6 ± 2.9 at baseline to 3.3 ± 2.9 after 12 months and remained reduced through 4 years [55]. Another study, TKT010, attempted to use pain measures as an outcome measure in adult males with Fabry disease on Replagal. However, initial data analysis were not significant so the study end point was changed from pain to renal function as reported in the paper by West [59]. Interestingly, one recent trial comparing three altered dosing intervals did not find significant differences for the outcome of BPI score [41].

The FOS database has been queried using pain and measures of quality of life (QOL) as end points. Beck and colleagues showed improved pain scores and improved QOL scores [63]. Mean QOL utility score was improved and maintained after 2 years. In a later publication, Hoffman and colleagues completed a

large retrospective analysis over 3 years of therapy to assess the effects of Replagal on pain [67]. They noted a reduction in pain with Replagal after 3 years of ERT, including a statistically significant reduction in male patients and in children after 12 months of treatment [67]. Finally, Mehta and colleagues used FOS to show significant score changes of the BPI and QOL measures after 5 years of therapy [71].

Baehner and colleagues demonstrated significant improvement in QOL measures in a female population [37]. BPI scores decreased significantly in one study of children receiving ERT [56] and in another the BPI and related QOL scores decreased in most pediatric patients [81].

The Mainz Severity Score Index (MSSI), a measure of total disease burden, was developed as a tool for monitoring disease progression and assessing the effects of ERT in a population of patients from different treatment centers. Significant improvements in total MSSI were seen in two studies. Parini and colleagues saw improvement from baseline total MSSI

Table 2. Clinical outcome measures in subjects treated with agalsidase alfa: change in left ventricular mass index.

Study type	Patients (n)	ERT frequency	Length of therapy (months)	Change in LV mass compared with baseline	Ref.
DB	M (15)	Biweekly	6	Therapy: -6.4 g/m^2	[16]
OL	M (10)	Biweekly	24	No change	
FOS	-52 -17	Biweekly Biweekly	12 24	Significant decrease in LV mass ($p < 0.05$) particularly in patients with the greatest degree of hypertrophy	[62]
FOS	F (38)	Biweekly	12	$>85 \text{ g/m}^2$: -33.8 g/m^2 $60\text{--}85 \text{ g/m}^2$: -18.9 g/m^2 $48\text{--}60 \text{ g/m}^2$: -9.7 g/m^2 $<48 \text{ g/m}^2$: -4.5 g/m^2	[54]
	F (37)	Biweekly	24	$>85 \text{ g/m}^2$: -33.8 g/m^2 $60\text{--}85 \text{ g/m}^2$: -19.8 g/m^2 $48\text{--}60 \text{ g/m}^2$: -12 g/m^2 $<48 \text{ g/m}^2$: -5.4 g/m^2	
	F (36)	Biweekly	36	$>85 \text{ g/m}^2$: -35.6 g/m^2 $60\text{--}85 \text{ g/m}^2$: -18.1 g/m^2 $48\text{--}60 \text{ g/m}^2$: -9 g/m^2 $<48 \text{ g/m}^2$: -3.7 g/m^2	
	F (36)	Biweekly	48	$>85 \text{ g/m}^2$: -40 g/m^2 $60\text{--}85 \text{ g/m}^2$: -14.3 g/m^2 $48\text{--}60 \text{ g/m}^2$: -7.3 g/m^2 $<48 \text{ g/m}^2$: -4.5 g/m^2	
OL pediatric	M (19) F (5)	Biweekly	6	-1.0 g/m^2 (M) -3.2 g/m^2 (F)	

DB: Double blinded; ERT: Enzyme replacement therapy; F: Female; FOS: Fabry outcome survey; LV: Left ventricular; M: Male; OL: Open label.

in a population of patients receiving treatment for more than a year, with a median of almost 3 years [82]. Another study found the total MSSSI was reduced after 1 year and improved over 4 years [55]. Significant changes were additionally noted in the MSSSI cardiovascular [55,82] and neurologic subscales [55,82], but were not seen in the renal MSSSI scale [55,82] or general MSSSI scale [55].

Hearing loss, usually high-frequency sensorineural hearing loss, is a common manifestation of Fabry disease in adults. Replagal was shown to have a positive effect on mild-to-moderate high-frequency hearing loss after long-term use of therapy [52,83]. High-frequency sensorineural hearing loss improved above baseline by 4 dB at 42 months [83]. This was again demonstrated in the open-label extension study [52]. Hearing loss improved above baseline by 2.1 dB at 18 months and by 4.9 dB at 40 months. A total of 26 patients with hearing loss were identified in FOS [20]. In patients with mild-to-moderate loss at baseline, hearing thresholds improved significantly, by 4–7 dB at most frequencies. However, it was identified that patients with normal hearing or severe loss had no change in hearing levels. ERT with Replagal appears to reverse the hearing deterioration in patients with Fabry disease. This improvement is gradual, however, suggesting the need for long-term ERT [52,83]. Vestibular and auditory function was studied in thirty-seven patients treated with Replagal over 5 years [21]. The authors reported an improvement in vestibular function within the first year of ERT, whereas auditory function stabilized.

Gastrointestinal symptoms, primarily diarrhea and abdominal pain, are prominent in males and females with Fabry disease. One open-label study used severity and frequency of abdominal pain to evaluate the

efficacy of Replagal [60]. The frequency and severity of abdominal pain was reduced after 6 months of treatment with six out of 11 patients experiencing improvement. Similarly, data from FOS reported a reduced prevalence of abdominal pain, with a statistically significant reduction after 1 year in males and children [66] (Table 3).

Anhidrosis and hypohidrosis are common symptoms in both adult and pediatric patients with Fabry disease. A few studies have demonstrated improvements in sweat function or volumes in adults [41,53] and children [43], although one of these studies noted improvement, but only with weekly dosing [41].

It is now well understood that female heterozygotes for a *GLA* mutation can be symptomatic with Fabry disease [84,85]. Baehner and colleagues studied fifteen adult female patients [37]. After 6 months of treatment, a significant reduction in LV mass indexed to body surface area was seen (148 g/m² at baseline to 124 g/m²). Furthermore, there was a significant improvement in QOL. This study was extended to evaluate the efficacy and tolerability in 36 women with Fabry disease [55]. Pain scores improved and the total MSSSI was significantly reduced after 12 months of treatment and continuously improved over 4 years. Additionally, average kidney function remained constant during the study [55].

In 2011, Hughes and colleagues used FOS to report on the effectiveness of Replagal in women compared with men [72]. Their cohort of 78 women and 172 men were treated for 4 years. Measures of pain, health-related QOL, cardiac structure and function and renal function changed to a similar extent in women and men during treatment, with the exception of LV mass, which only reduced significantly in women [72]. Overall, the study showed that both genders respond to Replagal in

Table 3. Clinical outcome measures in subjects treated with agalsidase alfa: gastrointestinal symptoms.

Study type	Length of therapy (months)	Patients	Abdominal pain		Patients	Diarrhea		Ref.
			Baseline	Post-therapy		Baseline	Post-therapy	
Questionnaire	6	11 M: 9, F: 2	10	7	11 M: 9, F: 2	6	3	[59]
Interviews	12	62 (A: 48, C: 14) (M: 41, F: 21)	30 (A: 2, C: 9)	24 (A: 19, C: 5)	60 (A: 48, C: 12) (M: 39, F: 21)	16	11	[65]
	24	58 (A: 48, C: 10) (M: 33; F: 25)	25 (A: 17, C: 8) (M: 15, F: 10)	17 (A: 12, C: 5) (M: 12, F: 5)	57 (A: 47, C: 11) (M: 32, F: 25)	16 (A: 11, C: 5) (M: 13, F: 3)	15 (A: 12, C: 3) (M: 11, F: 4)	

CKD I: eGFR ≥90 ml/min/1.73 m²; CKD II: eGFR = 60–89 ml/min/1.73 m²; CKD III: eGFR <59 ml/min/1.73 m².

A: Adult; C: Children; CKD: Chronic kidney failure; DB: Double blinded; eGFR: Estimated glomerular filtration rate; EXT: Extension; F: Female; FOS: Fabry outcome survey; LV: Left ventricular; M: Male; OL: Open label.

a similar way, suggesting there should be no difference in the criteria for assessment of treatment in women and men [72].

Preclinical animal studies do not indicate any direct or indirect harmful effects on fertility and embryonic development with the use of Replagal. Studies are limited, but to date no harmful effects have been noted in pregnancy for either the mother or the newborn child [86,87]. Studies have not been performed to assess the amount of Replagal capable of crossing the placental or into human milk, but the amounts are predicted to be low based on biochemical properties of the enzyme.

Outcome measures in pediatric patients

Data are limited on the use of Replagal in the pediatric population. To date, two trials and one extension study have focused on children with Fabry disease who have been treated with Replagal. Ries and colleagues examined the therapeutic effect of Replagal in 24 children who were enrolled in a 6 month noncomparative multicenter trial [42]. After 6 months of treatment, three out of four children showed reduction in baseline microalbuminuria [42]. Of these, a total of 17 adolescents went on to complete a 3.5 year extension study [56]. Results showed an increase in the clearance of urine GL3, particularly in boys. In four children with microalbuminuria at study initiation, three showed a decrease in microalbuminuria values. Estimated eGFR remained stable in a pediatric extension trial [56]. BPI scores decreased significantly. Additionally, kidney function and LVM indexed to height remained stable throughout.

In another trial of 13 children [43], boys who had an above normal baseline plasma GL3 had a reduction to within normal levels after 12 to 23 weeks of Replagal. The BPI and pain related QOL score decreased in most patients. Higher volumes of sweat were recorded in most of the patients during the period of treatment [43].

More recently in 2011, Ramaswami and colleagues published from the FOS database on 64 boys and 34 girls who had been treated with Replagal for at least 6 months [81]. The prevalence of symptoms tended to be reduced after 12 to 24 months of ERT in patients who experienced symptoms at baseline. In the entire population, nonsignificant decreases in the prevalence of gastrointestinal problems in boys and pain crises in girls were observed after 12 to 24 months. While the data on these short-term studies in children appear to be promising, long-term studies are necessary to determine the clinical outcome in pediatric patients who initiate ERT early in disease progression.

ERT requires a lifetime commitment and is extremely costly. A case report by Kampmann [88] and colleagues discusses the successful long-term ERT in a

young adult with Fabry disease. However, it remains to be determined if ERT initiated early in patients with Fabry disease is able to prevent major organ failure in adulthood. Furthermore, in the past two decades, two variant types of Fabry disease with manifestations primarily involving the heart [89–92] or kidneys [93,94] have been reported. Additional studies needed to address the efficacy of ERT in the subsets of patients with these renal and cardiac variants. A recent cost effectiveness study in a Dutch Fabry population predicts the affordability of ERT is at stake due to the high costs and limited efficacy in advanced patients [95]. While expert panels suggest that ERT be initiated as early as possible in all males with Fabry disease, including children and those with end-stage renal disease and renal transplantation, as well as in females with significant disease [24,96], survival data are required to provide evidence for the long-term use of ERT in all patients with Fabry disease.

Immune response & tolerance

Clinically, the development of an immune response is anticipated in any recombinant human protein therapy [97]. This is thought to be more common when the native protein is absent, as is the case in many male patients with Fabry disease. The immune response that results in the development of antibodies against the infused proteins may affect the clinical outcome of ERT by the development of hypersensitivity, anaphylactoid or febrile reactions, or may lead to the development of cytokine release and a generalized inflammatory response or immune complex formation. Furthermore, the mounted immune response may lead to inactivation or degradation of the recombinant enzyme or may change the pharmacokinetic properties of the therapeutic protein.

Immunogenicity of recombinant human- α -Gal A has been assessed in different clinical trials [39,98]. Although Phase III trials for both Replagal and Fabrazyme show that the majority of patients develop an antibody response during the course of therapy, there are only rare case reports for the development of IgE antibodies. The seroconversion is mainly due to an IgG response, and may develop in patients treated with either Replagal or Fabrazyme. In fact, these IgG antibodies often cross react [99], although one case is documented in which the patient developed antibody response to only Fabrazyme and not against Replagal [100]. The observation that antibodies exhibit *in vitro* neutralization suggests the presence of an immunogenic epitope close enough to the catalytic site to prevent proper functioning of the enzyme when bound to the antibody.

A diversity of host factors and various properties

of the therapeutic protein influence immunogenicity. Structural similarity of the therapeutic agent to the innate protein and the previous history of exposure to other protein therapies that have similar structural properties are some of the key factors and can make a protein more or less immunogenic [101]. Both Replagal and Fabrazyme are derived from the same DNA sequence yet display C-terminal heterogeneity, most likely due to proteolytic processing of the mature full length protein [31]. The removal of C-terminal residues was suggested in an attempt to increase the specific activity of the enzyme [102]. However, more recent studies suggest that C-terminal differences may not result in different immunogenic properties.

Protein glycosylation may impact not only the efficacy but also the immunogenicity of a therapeutic protein. As mentioned earlier, Replagal and Fabrazyme both have similar types and locations of oligosaccharide attachments, although they differ in the number of sialic acid residues. For example, glycosylation sites at Asn108 and Asn184 are similar in both enzyme preparations [31]. On the other hand, Replagal contains a higher amount of complex chains and thus a lower sialic acid to galactose residue ratio. In addition, Replagal has a higher degree of sialylation and phosphorylation, but Fabrazyme contains a higher amount of M6P oligomannose side chains.

The rate of antibody formation could also be related to the administered dose and the infusion frequency. Antibodies are more often produced in patients receiving higher doses of ERT. At a dose of 1.0 mg/kg, there were higher rates of antibody formation when compared with the 0.2 mg/kg dosing schedule. Further increasing the dose may also counter the inhibitory effect of antibody formation, and hence may yield a better clinical response, as observed in some patients that switched from 0.2 mg/kg to 1.0 mg/kg dose and had a better urine GL3 clearance [103]. However, the effect of neutralizing antibodies on GL3 clearance and other outcome measures still needs to be verified [104].

Clinically, predicting an immune response in individual patients is difficult, especially when diverse mutations play a role in disease pathology [98]. Various missense mutations may alter either the processing or stability of the enzyme, which may result in deficient or even a complete lack of enzymatic activity despite detectable protein levels. Multiple molecular mechanisms, such as recombinant arrangements, frame shift mutations or splice junction defects, may result in lack of production of enzyme protein. The most common method for detection of endogenous enzyme protein is assessing cross-reactive immunologic material (CRIM) by using an antibody against the enzyme protein, and CRIM status has been correlated with development of

immune response. Although it has been hypothesized that patients with residual endogenous proteins and are thus CRIM (+) have an attenuated response to the recombinant protein, CRIM status was suggested to not be predictive of antibody development [101].

The variability in the phenotypes and the difference in disease progression make it difficult to assess the actual impact of antibodies on disease outcomes. It was shown that increasing IgG titers against the infused enzyme correlated with the amount of dermal capillary endothelial GL3 deposits [105]. Finally, Rombach and colleagues reported an increase in lysoGb3 in both plasma and urine and correlated these levels with an increased risk of stroke and cardiac hypertrophy [47].

Safety

Replagal was found to be well-tolerated and safe, even in home infusion settings [57,106]. The most common side effects were mild-to-moderate infusion-related reactions, such as rigor, fever, nausea, vomiting, headache, chest pain, flushing, pruritus, rhinitis, tremor, dyspnea, somnolence as well as acroparesthesia. The majority of adverse events, such as constipation, abdominal pain crisis and hearing loss, overlap with symptoms frequently observed during disease progression. Long-term data suggest that approximately 24% of male patients develop IgG antibodies after 3 to 12 months of Replagal therapy, although no IgE antibodies or anaphylactic reactions are observed. On the other hand, female patients do not seem to develop antibodies and develop infusion reactions with much lesser frequency. Over time, some male patients develop immunological tolerance to Replagal, as only 17% of the recipients were found to be IgG antibody positive after 12 to 54 months of treatment. Studies confirmed no safety issues with switching from Fabrazyme to Replagal [107,108].

Conclusion

ERT for Fabry disease has been commercially available for 12 years. According to the orphan drug policy in the USA, only one drug can be approved for 7 years with marketing exclusivity (USA FDA CDER: Accelerated Development Review and definition [109]). In the USA, only Fabrazyme was approved by the FDA for the treatment of Fabry disease with orphan drug status in 2003. Alternatively, both Replagal and Fabrazyme were approved by the European Medicines Agency for the Evaluation of Medicinal products in 2001, albeit at different doses. Still, there are gaps in the literature as data is sparse on the effect of ERT in different populations, including females, children and in patients with both minimal and advanced disease load.

The recent shortage of ERT supply has demon-

strated the value of having multiple therapeutic options available on the market. Ethical concerns exist about enrolling patients in placebo-controlled trials when treatments are available. Natural history registries and surveys, such as FOS, add to our knowledge about the progression of this disease, both for patients on therapy and for patients naive to therapy. However, it should be acknowledged that registries have their own biases. Patients enrolled in registries may not be a representative sample of patients who are otherwise being clinically treated for the disease. Additionally, while natural history registries are helpful, they have their own limitations such as incompleteness and variable quality of the data [110]. Further data to unravel the basic pathology of Fabry disease are needed to enhance the understanding of ERT in Fabry disease and its long-term outcomes for the

treated patients.

There are still many questions that need to be answered about when to initiate therapy, when to consider switching therapies and whether to combine therapeutic regimens, such as chaperone therapy, substrate reduction therapy and gene transfer therapy, all of which exist in experimental stages, either animal or Phase I–III clinical studies.

The lack of α -Gal A in the lysosomes is implemented as the culprit in Fabry disease, thus the exogenous replacement of the deficient enzyme in quantities adequate for equal distribution in different organ systems is expected to treat most of the disease manifestations. However, it is known that recombinant α -Gal A is unstable in peripheral blood, is not well incorporated into target organs, such as the heart, and does not penetrate the blood–brain barrier. Furthermore, there are multiple pathways

Executive summary

Background

- Fabry disease, a rare X-linked genetic disorder, is due to the impaired activity of alpha galactosidase A.
- Individuals with Fabry disease suffer from a progressively debilitating condition and multisystemic symptoms that begin in childhood and advance into adulthood.
- The main organ systems affected include the nervous, cardiovascular and urinary systems.
- Two enzyme replacement therapies are available to treat patients with Fabry disease, agalsidase alfa (Replagal®) and agalsidase beta (Fabrazyme®).

Molecular characteristics & intracellular trafficking

- α -galactosidase A hydrolyses the terminal alpha-galactosyl moieties from glycolipids and glycoproteins, and most importantly GL3.
- The endogenous enzyme traffics into the lysosome and gets activated by acidic pH.

Pharmacological characteristics

- The recombinant human enzyme, Replagal, is taken up by M6PR present on the cell surface and gets incorporated into lysosomes.
- The level of plasma and urine GL3 are often used as indicators for the efficacy of enzyme replacement therapy in patients with Fabry disease.
- Lyso-GL3 is a more sensitive marker for disease progression in many patients.

Efficacy

- Enzyme replacement therapy has been effective in slowing the progression of Fabry disease.
- Primary end points of renal, cardiac and neurological function have demonstrated significant improvements from baseline assessments.
- Additional end points such as pain, quality of life, gastrointestinal symptoms and hearing have also shown improvement.
- Studies in women and children have demonstrated similar effects.

Immune response & tolerance

- Immune response may affect the clinical outcome of ERT.
- Protein glycosylation may impact the efficacy and the immunogenicity of a therapeutic protein.
- Phenotype variability and differences in diseases progression make it difficult to assess the actual impact of antibodies on disease outcomes.

Safety

- Long-term Replagal treatment has been demonstrated to be safe and tolerable, and no additional safety concerns have been raised in women or children.

Conclusion & future perspective

- While we have 12 years of clinical trial and registry data published for the use of Replagal in the treatment of Fabry disease, further data to unravel the basic pathology of Fabry disease are needed to enhance the understanding of ERT in Fabry disease and its long-term outcomes for the treated patients.
- New treatments targeting alternate pathways with different approaches are in development.

involved in the pathophysiology of Fabry disease [111] that are beyond enzymatic deficiency and storage of abnormal lipid substrate. Soon, there will be various approaches to treat Fabry disease that may be used as an alternative or in conjunction with existing ERTs.

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