

# Expert Opinion

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## Advances in the management of Anderson-Fabry disease: Enzyme replacement therapy

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Anderson-Fabry disease (AFD) is a lysosomal storage disorder due to  $\alpha$ -galactosidase A ( $\alpha$ -Gal A) deficiency and the resultant accumulation of incompletely metabolised glycosphingolipids, primarily globotriosylceramide, within various tissues. It is an X-linked multisystem disorder characterised by progressive renal insufficiency, with added morbidity from cardio- and cerebro-vascular involvement, and associated with significant impact on quality of life and diminished lifespan. The disease manifests primarily in hemizygous males; however, there is increasing recognition that heterozygous (carrier) females may also develop disease-related complications although onset among affected women may be delayed. Until recently, treatment has been limited to symptomatic management of pain and other measures to alleviate the problems associated with end-stage complications from renal, cardiac and nervous system involvement. The availability of the recombinant enzyme offers the potential of a safe and effective targeted treatment approach. At the moment, two distinct enzyme formulations are approved in Europe (and in other countries) and both continue to undergo FDA evaluation in the US. Increasing knowledge of the natural history of AFD and greater experience with enzyme therapy should enable optimal patient care. The relative rarity and complexity of AFD necessitates a multi-disciplinary team approach that may be facilitated by a centralised registry.

**Keywords:** Anderson-Fabry disease, enzyme therapy, lysosomal storage disorder, renal failure,

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### 1. Introduction

AFD is a lysosomal storage disorder (LSD) named after two dermatologists, William Anderson and Johann Fabry, who independently in the 1890s described patients with the disorder that now bears their names [1]. It is an X-linked defect of glycosphingolipid (GSL) catabolism resulting from the deficient activity of  $\alpha$ -galactosidase A ( $\alpha$ -Gal A) due to a wide variety of causal mutations, which individually tend to occur as 'private' alleles (i.e., restricted to a single or few families) [2]. AFD is a multi-system disorder ensuing from the accumulation of globotriosylceramide (Gb<sub>3</sub>) in epithelial cells of the cornea, glomeruli and tubules of the kidney, cardiac myocytes, ganglion cells of the dorsal root and autonomic system, specific cortical and brainstem structures [2]. Lipid deposits are also evident in endothelial, perithelial and smooth-muscle cells of blood vessels and, to a lesser degree, histiocytic and reticular cells of connective tissue [2].

The clinical features of AFD include corneal and lenticular opacities, acroparesthesias, angiokeratomas, hypohidrosis and major end organ disease (kidneys, heart and brain) [3]. Acroparesthesia constitutes the earliest, major source of morbidity during the first two decades of life but it is often not attributed initially to AFD [4].

Indeed, unless there is a positive family history the diagnosis is often missed or significantly delayed until the phenotype has fully evolved. Most affected individuals have proteinuria and ultimately develop renal failure. The patients' clinical course also can be complicated by cardiac and cerebrovascular disease, which combined with renal failure, lead to early mortality. Organ dysfunction is likely due to a host of mechanisms triggered by the presence of excessive tissue Gb<sub>3</sub> storage, beyond the generalised vascular dysfunction and resulting ischaemic changes due to vascular occlusion.

The disease manifests primarily in affected hemizygous males and to some extent in heterozygous (carrier) females [5-8]. Variable expression may be partly explained by differences in residual enzyme activity and the variable penetrance in females may be partially attributed to the effect of Lyonisation (i.e., random inactivation of the normal or mutant allele bearing X-chromosome) [8]. The median survival for hemizygous males is 50 years (IQR 40 - 56 years) and for obligate females is 70 years (IQR 57 - 78 years) [7,8].

AFD is considered a rare pan-ethnic disorder with an estimated frequency of 1 in 117,000 male births [9]. However, recent studies suggest that the incidence may be underestimated, as certain patients with residual enzyme activity have disease characterised predominantly by cardiac involvement. Indeed, screening of patients with hypertrophic cardiomyopathy revealed that 3 - 9% of such patients had underlying  $\alpha$ -Gal A deficiency [10,11].

## 2. Enzyme replacement therapy for lysosomal storage disorders

Advances in the application of molecular genetic techniques have enabled development of directed protein therapies as an alternative to potentially curative procedures such as bone marrow transplantation, which can be associated with a high morbidity and mortality. To a large extent, this milestone has resulted in a shift in the management paradigm offered to patients with LSDs – from an approach that relied on predictive genetic counselling and palliative care to the genuine possibility of offering early diagnosis and active intervention.

In Gaucher disease (GD), the introduction of enzyme replacement therapy (ERT) established 'proof of concept' of rational drug design based on knowledge of the underlying enzyme deficiency. Slow substrate turnover, restricted sites of pathology and targeted macrophage delivery of the recombinant enzyme contributed to the success of this approach [12-15]. The absence of primary CNS involvement in Type 1 GD (the most common subtype) also obviated the need for breaching the blood-brain barrier to gain access to CNS sites of storage. These observations have provided the impetus for consideration of ERT for other storage disorders, such as AFD disease and mucopolysaccharidosis I and II.

The need to meet potential patient requirements (i.e., supply demands) has been addressed by industrial scale production of the relevant enzyme using recombinant genetic

techniques. This process was facilitated by the introduction of legislation providing several incentives to biotechnology/pharmaceutical companies to manufacture drugs for rare 'orphan' disorders. Orphan drug status is a designation given for therapies of disease where affected individuals number < 200,000 (in the US) or affect no more than 5/10,000 people (in Europe).

Ultimately, approval by the appropriate regulatory agencies is granted based on the outcome of clinical trials demonstrating safety and efficacy. The small number of eligible patients, heterogeneity of clinical expression, paucity of reliable surrogate markers which correlate with the pattern and severity of disease and the uncertainty *a priori* of being able to reverse disease (which creates a problem in the selection of the appropriate primary end points), represent confounding factors in the conduct of the clinical trials for rare disorders, particularly when the period of observation is short. When possible, investigations of safety and response in an animal model of disease prior to human clinical trials may provide useful insights. Extensive information on the natural history of disease is also important because it can serve as reference points for establishing whether a given therapeutic approach has been effective in reducing or eliminating the incidence of 'sentinel' events (i.e., disease-related complications).

### 2.1 Evaluation of $\alpha$ -Gal A ERT in mouse models of AFD

Preclinical ERT studies were conducted in the knockout mouse model of AFD, generated by homologous recombination and introduction of the null  $\alpha$ -Gal A allele in embryonic stem cells [16,17]. These experiments involved evaluations of the pharmacodynamic effects of recombinant human (rh)  $\alpha$ -Gal A and its pharmacokinetics. At least four rh $\alpha$ -Gal A glycoforms, which differed in the number of sialic acid and mannose-6-phosphate (M6P) residues, were evaluated in one study which revealed a longer circulatory half-life in the relatively higher sialylated preparations [18]. The repeated administration of the enzyme resulted in significant progressive reduction of Gb<sub>3</sub> in various organs (e.g., liver, heart, spleen and kidney) in a dose (i.e., direct with increasing dose)– and time (i.e., increased following repeated dosing)– dependent manner [18]. As Gb<sub>3</sub> accumulation in patients with AFD represents the initial insult to the cells and sets off the cascade of events that eventually leads to disease expression, these findings enable a reasonable expectation of stabilisation or improvement with ERT [18,19].

Additionally, the rate of tissue Gb<sub>3</sub> accumulation was analysed in the treated mouse model to ascertain the optimal frequency of rh $\alpha$ -Gal A administration. These studies suggested that dosing every 2 weeks is adequate to deplete tissue Gb<sub>3</sub> storage and prevent its re-accumulation [18]. However, species-specific metabolic differences may exist in the rate of substrate turnover and, thus, determination of the optimal dosing regimen requires independent confirmation in humans and further investigations through clinical trials designed to examine dose-frequency and tissue-response characteristics.

Interestingly, Gb<sub>3</sub> levels in plasma were shown to increase earlier (~ 40% of pretreatment levels at 2.5 - 3 weeks) when compared with the rate of tissue re-accumulation in the heart, spleen and liver (maximally decreased at 3 - 4 weeks) following a single dose (3 mg/kg) [18]. These observations imply differences in substrate flux between the plasma and tissue compartments and the possibility that the earlier rise in plasma Gb<sub>3</sub> levels may be confounded by GSLs newly synthesised in the liver and subsequently released into the circulation. Alternatively, the delayed tissue Gb<sub>3</sub> re-accumulation may suggest a prolonged tissue half-life for the rh $\alpha$ -Gal A and/or slow rate of substrate turnover. The latter raises the potential for increased interval between treatments (possibly using higher doses), if as speculated that plasma Gb<sub>3</sub> is not a reliable indicator of tissue substrate clearance. These hypotheses require further examination but are clearly relevant to determination of the ideal treatment regimen.

Histological evidence of clearance of Gb<sub>3</sub> deposits further demonstrates intracellular hydrolysis of accumulated substrate and prolonged tissue half-life of exogenously given rh $\alpha$ -Gal A [18,19]. Of interest, the biodistribution studies revealed ~ 95% of the administered rh $\alpha$ -Gal A was recovered in the liver [18]; an organ whose function remains intact in patients with AFD. The avid hepatic uptake may represent the presence in the liver of a high number of receptors (e.g., M6P- and asialo-receptors). This issue is an important consideration in the selection of the appropriate enzyme formulation as discussed below. Rational drug design demands minimal, non-specific competitive uptake to ensure delivery of a high concentration of the enzyme to the major organs (i.e., kidney and heart) that are compromised by AFD.

### 3. Clinical trials

There were two separate clinical trials, one led by the National Institute of Health (NIH) [20,21] and the other by the Mount Sinai School of Medicine study group (MSSG) [22,23]. A third trial was also conducted at the Royal Free Hospital (London, UK), which at this time has only been reported in abstract form [24]. The NIH and MSSG studies were conducted in two Phases (I/II and II/III) and employed a study design likely discussed with the appropriate regulatory agencies with the hope of achieving 'fast-track approval'. The need for two separate trials was prompted by the existence of two different enzyme formulations, agalsidase alfa (Replagal™, Transkaryotic Therapies, Inc.) and agalsidase beta (Fabrazyme™, Genzyme Corporation), distinguished by the cell type used for production of the rh $\alpha$ -Gal A. The NIH used agalsidase alfa, which is produced in a genetically engineered continuous human cell line. MSSG used agalsidase beta, which is produced using a Chinese hamster ovary (CHO) cell line.

The Phase I/II trials were conducted as single-centre, open-label, dose-escalation studies to assess the safety of rh $\alpha$ -Gal A infusions and determine an appropriate dose for subsequent trials designed to demonstrate clinical efficacy. The NIH

group gave a single (20 min) infusion of rh $\alpha$ -Gal A to five different groups of two patients (n = 10), using five different enzyme doses (ranging from 0.3 - 4.7 units/kg of body weight) [20]. Patients (n = 15) in the MSSG were divided into five groups of three and received a total of five doses (over approximately 4 - 6 h [0.83 ml/min]), which ranged from 0.3 - 3.0 mg/kg given either once every 14 days (3/5 groups) or 48 h (in two subsets of patients on 1 and 3 mg/kg) [22].

The NIH group demonstrated delivery of the exogenous enzyme to the sinusoidal endothelial cells, Kupffer cells and hepatocytes, with a tissue half-life in the liver > 24 h. These findings were associated with significant reduction of Gb<sub>3</sub> in the liver and shed renal tubular cells in the urine sediment but no significant change in the plasma Gb<sub>3</sub> levels [20]. The MSSG showed dose-dependent clearance of plasma Gb<sub>3</sub>, associated with decrease in tissue Gb<sub>3</sub> storage (liver > kidney) in some organs but a slight increase in others (e.g., heart) [22]. Additionally, the MSSG patients were reported to have less pain, increased ability to sweat and improvement in quality-of-life measures [22]. No drug related adverse events were observed by the NIH group, while the MSSG patients reported mild-to-moderate, transient increase in blood pressure and sero-conversion (i.e., development of anti-rh $\alpha$ -Gal A antibodies) in 8/15 patients; four of whom experienced hypersensitivity-type reactions necessitating the discontinuation of further infusions in two patients [22].

Interestingly, patients in the MSSG study on the highest dose-frequency (Groups D and E, on 1 and 3 mg/kg rh $\alpha$ -Gal A q 48 h) showed a response pattern that was counterintuitive. Decline in plasma Gb<sub>3</sub> levels in these patients occurred later; to levels that were less than those observed in patients on the biweekly dosing schedules. Furthermore, although Gb<sub>3</sub> levels in the liver were uniformly decreased for all treatment groups, heart and kidney Gb<sub>3</sub> levels dramatically increased in a patient (#13) from Group E on the highest dose [22]. As significant Gb<sub>3</sub> hydrolysis is not expected to occur in plasma, the observed changes suggest the possibility that the high levels of administered enzyme may have interfered with the transport mechanism which facilitate delivery of plasma Gb<sub>3</sub> to its cellular sites of metabolism. The paradoxical findings for the heart and kidney may simply reflect tissue-site sampling differences between baseline and post-treatment biopsies. Alternatively, it is possible that enzyme delivery to these tissue sites was inadequate because of saturation of the relevant receptors involved with intracellular uptake in the heart and kidneys. If this were the case, potentially non-specific distribution of the enzyme to other cells could occur. It is likely that competitive uptake by endothelial cells (directly interfaced with the circulation and offering a greater surface area) and the liver are also relevant events. Obviously, the limited data on this subject does not permit a definitive conclusion. These concerns require careful investigation as delivery of a high concentration of functional enzyme to relevant target sites of pathology likely represent a major determinant of response. Although the law of mass

**Table 1. Summary of patient profiles and the clinical assessments used in studies of ERT for AFD<sup>1</sup>.**

	Agalsidase alfa	Agalsidase beta
<b>Design</b>		
Entry Criteria	≥ 18 years: Neuropathic pain (NIH); Left ventricular hypertrophy (RFH) <sup>2</sup>	≥ 16 years: Serum creatinine ≤ 2.2 mg/dl (MSSG)
Number of patients:		
α-Gal A-treated	14 males (34.0 years) 7 males (34.6 years) <sup>2</sup>	27 males; 2 females (32.0 years)
Placebo	12 males (34.4 years) 8 males (36.9 years) <sup>2</sup>	29 males (28.4 years)
Enzyme source and dose (given every 2 weeks)	Human cells; 0.2 mg/kg; 40 min infusion; no premedications	Chinese hamster ovary cells; 1.0 mg/kg; 4 - 6 h infusion (0.25 mg/min); premedication (1000 mg acetaminophen, 25 - 50 mg hydroxyzine)
Outcome Measures:		
Primary	Effect on neuropathic pain (assessed by Brief Pain Inventory) while off analgesics; decreased cardiac Gb <sub>3</sub> and ventricular size (cardiac mass index assessed by MRI) <sup>2</sup>	% of patients with clearance of renal interstitial capillary microvascular endothelial Gb <sub>3</sub> deposits
	GFR; renal histology (glomerular count, glomerular and tubulointerstitial morphology, glycolipid inclusions)	GFR; Clearance of microvascular endothelial Gb <sub>3</sub> deposits in heart and skin
Secondary	Effect on pain based on period off analgesics	Effect on pain using Short-form McGill Pain Questionnaire; Quality of life (Short-Form General Health Survey SF-36)
	Gb <sub>3</sub> in plasma, kidney tissue, 24 h urine sediment; cardiac tissue <sup>2</sup>	Gb <sub>3</sub> in plasma, kidney, heart, skin, 24 h urine sediment
Duration of the study	24 weeks randomised, 24 weeks open label; followed by extension study	20 weeks randomised, 24 weeks open label; followed by extension study
<b>Findings</b>		
Safety <sup>3</sup> :		
Infusion reactions	10%	~ 50%
Developed antibodies	55% at 6 months	83% at 1 year; Subsequently, IgE antibodies noted in at least one patient <sup>4</sup>
Pain	Consistent and progressive decline in pain scores (p = 0.02), compared with placebo; 4/11 discontinued analgesics <i>versus</i> none on placebo (p = 0.03)	Treated group had change from baseline, but no different from placebo (p > 0.05)
Quality of life (QOL)	Pain related QOL improved (p = 0.05), compared with placebo	Compared with baseline: significant improvement in physical and emotional roles for treated group, and in physical role and body-pain components for placebo group. No difference between the two groups.

**Table 1. Summary of patient profiles and the clinical assessments used in studies of ERT for AFD<sup>1</sup> (continued).**

	<b>Agalsidase alfa</b>	<b>Agalsidase beta</b>
<b>Gb<sub>3</sub> clearance</b>	<b>Change in mean levels:</b>	<b>Change in median levels:</b>
Plasma	54% decrease	Undetectable (< 1.2 ng/ml) after week 20
Kidney tissue	20.5% decrease	23.3% decrease
Urine Sediment	28.9% decrease; 50% decrease <sup>2</sup>	50% decrease
Kidney histology (Reported differently)	Improved: 21% increased fraction of normal glomeruli (p = 0.01); 33% decreased mesangial widening (p = 0.01)	Improved: 69% had "0" scores (i.e., no or trace microvascular endothelial Gb <sub>3</sub> deposits; p < 0.001); also noted in skin (p < 0.001) and heart (p < 0.001).
Renal function	Treatment arm had stable GFR measured by creatinine clearance (p = 0.02) and inulin clearance (p = 0.19), compared with placebo	Serum creatinine concentration no different than placebo; baseline GFR provided with no follow up data, although reported as unchanged (p = 0.19)
Cardiac change	Improved cardiac conduction (i.e., decrease in QRS-complex duration [p = 0.047]); one patient in placebo group developed right bundle branch block that resolved with open-label treatment; Reduction (4.2%) in mean cardiac mass after six months (p = 0.041), compared with increase on placebo <sup>2</sup>	No significant change from baseline
Other	Increased body weight (p = 0.02); Increased resting regional cerebral blood flow reversed with treatment <sup>5</sup>	

4-MU: Methylumbelliferone; AFD: Anderson-Fabry disease; ELISA: Enzyme-linked immunosorbent assay; ERT: Enzyme replacement therapy; Gb<sub>3</sub>: Globotriaosylceramide; GFR: Glomerular filtration rate; MRI: Magnetic resonance imaging; MSSG: Mount Sinai Study Group; NIH: National Institutes of Health; RFH: Royal Free Hospital; QOL: Quality of life.

<sup>1</sup>Modified from [43]. Except where indicated the results of the study using agalsidase alfa were obtained from [21], and those reported for the study using agalsidase beta are from [23].

<sup>2</sup>Separate study (n = 15 patients) conducted at Royal Free Hospital, London, UK [24].

<sup>3</sup>In both studies safety monitored by antibodies to  $\alpha$ -Gal A, electrocardiogram, echocardiography, and clinical monitoring for adverse events. (Data from the European labels of the products.

<sup>4</sup>[25].

<sup>5</sup>[28].

action would lead to expectations of greater substrate clearance within cells when more enzyme is given, therapeutic responses at the organismal level are subject to enzyme bio-availability and distribution and ultimately the concentration of enzyme within the subcellular compartment(s) of substrate hydrolysis. Thus, the relationship between dose and response may not be direct and this may be an issue when comparing two drugs that may share the same amino acid sequence but differ in glycosylation pattern.

The Phase II/III trials were randomised, placebo-controlled, double-blind studies with fundamental differences in patient entry criteria, administered dose and primary and secondary end points (as shown in the Table 1) [21,23]. Additional information was also reported for patients who subsequently enrolled in an open-label extension phase [25,26].

Entry criteria with respect to neuropathic pain differed between the two trials. After 6 months, patients in the NIH group reported significant improvement in neuropathic pain

[21]. Although treated patients in the MSSG reported pain relief, the response was no different than that seen in the placebo arm [23]. The MSSG patients were not selected for pain symptoms and remained on analgesics throughout the study. It is possible that failure to ascribe significant pain response to agalsidase beta was influenced by this factor and the instrument used for assessment of pain.

Significant differences marked by stabilisation of renal function observed in the NIH treated group compared to placebo were largely accounted for by a more rapid deterioration of function observed in the latter group. However, the decline in renal function observed in the placebo group reversed during the open-label phase [21]. There were no reports of deterioration in renal function among the MSSG patients. As the deterioration in renal function associated with AFD has not been shown to spontaneously reverse, preservation of renal function (as noted in the treated patients) should be considered a major advance in the management of these patients.

Both studies also examined changes in kidney Gb<sub>3</sub> and histology and reported decreases in Gb<sub>3</sub> levels in kidney tissue and urine sediment. In addition, the NIH group noted significant changes in the glomerular architecture, while the MSSG reported significant changes in the lipid deposits within renal interstitial capillaries [21,22]. These observations were associated in both studies with significant decrease in plasma Gb<sub>3</sub>. Interestingly, there were differences in the extent of lipid clearance within the various renal compartments. Clearance was greatest in the capillary endothelial cells that reside in the interstitium, while deposits in the glomerular podocytes appeared most resistant (i.e., least responsive to therapy). These findings may indicate differences in enzyme access to various tissue-sites within a single organ, enzyme dose-cellular response thresholds and/or structural state/composition of the accumulated substrate or its turnover. Assessment of therapeutic benefit ultimately should be dependent less on structural changes but more on clinical outcome (e.g., preservation, restoration or improvement of renal function and resolution of pain).

Additionally, the NIH group reported several other findings among their treated patients, including significant increase in body weight and decrease in QRS complex duration on electrocardiography [21]. In separate reports, they also noted that treatment with agalsidase alfa led to significant decrease in abnormal cerebral perfusion and vascular reactivity based on H<sub>2</sub><sup>15</sup>O positron emission tomography and the resolution of cerebrovascular hyperdynamia (i.e., abnormally increased blood flow in the absence of arterial stenosis) by transcranial Doppler study [26-29]. These changes may signify a reduced risk of stroke, an hypothesis that requires further investigation. Furthermore, in the study conducted at the Royal Free Hospital that included 15 patients with hypertrophic cardiomyopathy, treatment with agalsidase alfa was shown to lead to a significant reduction ( $p = 0.041$ ) in cardiac mass [24]. Cerebrovascular dynamics and hypertrophic cardiomyopathy and the effect of enzyme therapy using agalsidase beta for these AFD-related complications were not specifically evaluated in the MSSG study.

With the exception of one patient with a positive skin test in the MSSG group (and thus was unable to continue therapy), patients in both studies tolerated the entire course of treatment, none withdrew due to antibody formation, and antibody formation did not influence therapeutic outcome. However, there was a higher frequency of breakthrough (i.e., despite pre-medication) adverse reactions encountered in the MSSG patients, although this observation did not preclude further treatments [23]. More recently, a patient on agalsidase beta was reported to have developed IgE antibodies [25].

#### 4. Expert opinion

ERT for AFD appears to be safe and effective; a conclusion also reached by the European Agency for the Evaluation of Medicinal Products, enabling the marketing of both agalsidase alfa and agalsidase beta in Europe in June 2001. Authori-

sation for the use of either or both agents among AFD patients in the US remains to be determined.

Establishment of therapeutic guidelines for the use of ERT among patients with AFD requires a thorough understanding of disease mechanisms and the rationale for therapy. Although intracellular Gb<sub>3</sub> storage represents the initial insult to the cells, it is likely that a cascade of pathological mechanisms are involved in the development of disease-related complications as suggested by recent studies which revealed an imbalance in vascular reactivity and the propensity for a pro-thrombotic state among patients with AFD [30,31]. These observations suggest that palliative measures may continue to be important and that other pharmaceutical agents may be necessary in conjunction with ERT to effect complete patient response or disease control.

AFD is a multisystem disorder associated with wide variability in clinical expression particularly among women. Plasma or leukocyte  $\alpha$ -Gal A enzyme activity and genotype do not appear to be dependable predictors of the subsequent phenotype (i.e., pattern and severity) in affected hemizygous males and heterozygous (carrier) females. However, some patients with certain  $\alpha$ -Gal A gene mutations associated with residual but low levels of enzyme activity do appear to have a more limited disease expression (e.g., cardiac variant) and/or delayed onset of symptoms and a less severe intensity (e.g., later onset of chronic renal insufficiency, lower renal Gb<sub>3</sub> content and lower scores for renal histological damage) [32-34].

Among asymptomatic patients, the decision to initiate therapy is confounded by the variability in clinical expression and absence of surrogate markers that reliably predict the pattern of organ involvement and disease severity. These are important considerations because it is likely that delayed treatment, at a stage when advanced tissue changes (e.g., glomerular sclerosis) are established may not lead to full recovery and represent persistent sources of morbidity. Early intervention offers the possibility of mitigating the disease process.

Studies have shown that AFD is associated with significant impact on quality of life and that palliative efforts (e.g., analgesics for neuropathic pain) do not bring satisfactory relief [35,36]. Despite the introduction of renal dialysis and transplantation, median cumulative survival time remains substantially reduced [7,8]. These observations underscore the importance of extra-renal morbidity resulting from cardiac, and cerebrovascular involvement and the need for specific and targeted treatments, such as ERT. Thus, the reports of cardiac and cerebrovascular changes noted in the patients on agalsidase alfa may bode well for patients at risk of progressive disease, even after renal failure has set in. Functional cardiac and cerebrovascular assessments were apparently not part of the study design to assess the efficacy of agalsidase beta. As the agalsidase beta trial employed a higher dose, it would have been interesting to note whether a cardiac dose-dependent response pattern does exist [23]. However, it should be noted that patients enrolled at the Royal Free Hospital on agalsidase

alfa (at 0.2 mg/kg) showed a positive outcome with reduction in cardiac mass [24].

In symptomatic patients, the decision to commence treatment is clear given the current safety profile and therapeutic responses observed in the clinical ERT trials. However, the issue of the appropriate therapeutic regimen (i.e., ideal enzyme formulation, dose and frequency of administration) to achieve optimal benefit with minimal risk and at reasonable costs remains to be established. These concerns obviously require greater clinical experience with the use of these biological agents and necessitate additional information regarding enzyme bioavailability and rate of substrate turnover. Pharmacokinetic and pharmacodynamic data also may help decipher whether there are differences between the current enzyme preparations that may be of clinical significance as a consequence of distinct post-translational modification (e.g., glycosylation, sialylation and phosphorylation of mannose residues); chemical properties which may influence not only access to tissue storage sites but also development of antibodies to the recombinant enzyme. Insights regarding this matter cannot be drawn from the current reports and additional investigations may be required. At present, the two biotechnology companies that manufacture rh $\alpha$ -Gal A are engaged in a patent dispute [37].

Although information is available on specific enzyme activity *in vitro* (i.e., rate of substrate hydrolysis), limited data are given on enzyme targeting (i.e., proportion of administered enzyme which remains functional in the various organs of pathology), tissue half-life and effectiveness in clearing tissue Gb<sub>3</sub> in humans. Enzyme uptake likely occurs through multiple specific receptors. The widespread distribution of these receptors may influence clinical response, as non-specific tissue uptake would unlikely reduce the systemic substrate burden. For instance, degree of sialylation influences the fraction of circulating enzyme taken up by the liver and could have an effect on therapeutic responses at extra-hepatic sites. Organ substrate redistribution has not been demonstrated; thus, intracellular hepatic Gb<sub>3</sub> clearance is unlikely to significantly reduce renal or cardiac Gb<sub>3</sub> storage. Thus, adjuvant therapy to limit the amount of enzyme taken up by the liver, or delivery mechanism designed to bypass or limit hepatic uptake may lead to greater uptake by the other organs, as suggested by the finding that administration of mannans to block mannose receptors prolonged the circulatory half-life and markedly increased enzyme uptake by the kidney in the mouse model treated with agalsidase beta [18].

Interestingly, decreased plasma Gb<sub>3</sub> levels were observed (despite the fact that  $\alpha$ -Gal A is not highly active at plasma pH). This finding has led some investigators to suggest that monitoring changes in plasma Gb<sub>3</sub> may be a useful means of following tissue Gb<sub>3</sub> clearance. GSLs in plasma are bound to lipoproteins and hydrolysed intracellularly following their uptake through endocytosis. Kinetic studies show that 25% of the plasma GSL pool is newly synthesised each day, with a portion also derived from the turnover of senescent erythro-

cytes [2]. Turnover times for plasma GSLs were calculated to be from 4 - 8 days and the turnover rates were from 1 - 6  $\mu$ mol/day [38]. Earlier studies using plasmapheresis or phlebotomies did not appear to alter the natural history of the disease [39-41]. This is likely because vascular Gb<sub>3</sub> represents only a small proportion of total visceral Gb<sub>3</sub> storage. These observations suggest that reliance on plasma Gb<sub>3</sub> as a surrogate marker of response may be confounded by several factors. On the other hand, changes in urinary Gb<sub>3</sub> concentration may be a more sensitive marker and specifically useful in monitoring changes in body (i.e., kidney) substrate burden. About 75% of the urinary cells shed by an affected individual are derived from desquamated lipid-laden distal tubular epithelial cells [42]. Thus, renal tissue Gb<sub>3</sub> clearance with ERT would be anticipated to lead to progressive decline in urinary Gb<sub>3</sub> excretion. It would be ideal to have a non-invasive (surrogate) means of monitoring therapeutic response but further studies are necessary to clarify the functional significance of changes in serial plasma and/or urinary Gb<sub>3</sub> levels and whether either or both are useful in monitoring treatment response or assessing rate of disease progression.

Antibodies to rh $\alpha$ -Gal A developed in a proportion of patients receiving agalsidase alfa or agalsidase beta, although the incidence was higher with the latter drug. This difference may be due to the greater amount of agalsidase beta given per infusion. Alternatively, the breakthrough adverse reactions encountered with the use of agalsidase beta may be attributed to glycosylation differences between the two formulations. (As noted above post-translational modifications are species-specific and expected to differ between CHO cells and human cells. The functional implications of these differences, if any, remain to be determined). In any case, sero-conversion was anticipated given that most AFD patients are negative for cross-reacting immunological material (CRIM-negative). However, it will be important to determine whether neutralising antibody subtypes or additional IgE type antibodies develop; the latter may be of grave concern because of the potential for life-threatening episodes with anaphylaxis. Eventually, there will be greater appreciation of the spectrum of adverse reactions resulting from administration of rh $\alpha$ -Gal A and the relationship of adverse events to the administered dose. At this time, the trials suggest an acceptable safety profile for the use of ERT in AFD.

The cost of ERT for AFD has not been established globally but if it compares with the cost of ERT for GD, it is going to be expensive and possibly prohibitively so in certain parts of the world. The annual cost of treatment in Europe where the product is commercially available is estimated to be about US\$160,000. While healthcare-policy makers need to control the overall cost of healthcare delivery, this role needs to take into account societal obligations to ensure equitable access to novel therapies. The value of any treatment approach needs to be weighed against the costs associated with extensive resource utilisation (e.g., associated with dialysis or transplantation for renal failure, pacemaker insertion for heart block and chronic

care for stroke) and loss of productivity due to reduced lifespan [43]. These concerns raise the necessity of determining the effect of therapy on health-related quality of life, a process that can be facilitated through rare disease registries that foster evidenced-based care. If alternative therapeutic options including galactose infusion, gene therapy and substrate synthesis inhibitors prove to be safe and efficacious, the cost of ERT may be reduced (e.g., by alteration in dose size and administration frequency) [44-46].

There is often criticism of collaborations between academia and industry and of commercial incentives in drug development. However, it is certain that without the assistance of biotechnology companies, ERT for LSDs would have been

delayed or may never have come to fruition given the economics of a ‘constrained market’.

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