

1890s by two European physicians, namely, Johan Fabry (from Germany) and William Anderson (from England), in the delineation of the phenotype resulting from deficiency of the lysosomal hydrolase AGAL [4]. The causal enzymatic defect was defined (in 1967) by Roscoe O Brady; a major proponent of enzyme therapy and a key investigator in the clinical trials involving agalsidase alfa directed by Raphael Schiffmann at the National Institutes of Health (NIH, MD, USA) [5].

The gene sequence (mapped to Xq22) which encodes AGAL was characterized by Robert J Desnick and colleagues (1986); their group at Mount Sinai Medical Center in New York also devised a means for the over-expression of the protein (agalsidase beta) in Chinese hamster ovary (CHO) cells – the source of an alternative formulation used in the treatment of AFD [6–8].

AFD is characterized by onset of acroparesthesias (i.e., recurrent or lancinating pains in the distal extremities) and bouts of abdominal pain and diarrhea in late childhood or adolescence. In the absence of a positive family history, the diagnosis is often missed. This may also be due to the typical absence of objective findings (e.g., normal electrophysiology test results) at presentation. Studies have shown the mean age of symptom onset (at ages 9 and 16 years in males and females, respectively) is relatively early but the diagnosis is significantly delayed (on average by about 10 years) [9,10]. Findings in this age group that may serve as useful clues and prompt consideration of the diagnosis include angiokeratomas (reddish-blue telangiectasias mostly distributed in the ‘bathing trunk’ region) and the presence of corneal or lenticular opacities (evident on slit-lamp eye exam).

In adulthood, major morbidity from AFD results from renal failure (end-stage renal disease [ESRD]), cardiac dysfunction (congestive heart failure, ventricular arrhythmias) and cerebrovascular events (such as transient ischemic attacks and stroke). Surveys of the European and US Renal Disease System database indicate that dialysis for ESRD was introduced most often among classically affected AFD patients between the ages of 35 and 45 years. Of interest in the US study, 5 out of 42 (12%) of the patients identified were women, including two who were over 60 years of age [12]. Significant disability and reduction in quality of life also derives from hearing loss, gastrointestinal disturbances (e.g., abdominal pain and diarrhea) and hypohydrosis, from the associated psychosocial stress [13].

There is wide heterogeneity in clinical expression, partly influenced by the underlying molecular gene defect and the corresponding residual enzyme activity, lyonization among females and eventually the patient’s renal status. Untreated AFD is associated with significant impairment in physical and functional well-being and leads to premature death (median survival is 50 and 70 years for hemizygous males and obligate females, respectively) [9,10].

Pathology

The incomplete metabolism of several glycosphingolipids (primarily globotriaosylceramide [GL₃]) represents the root cause of AFD [5]. As a consequence, widespread GL₃ deposits are found in the epithelial cells of the cornea, glomeruli and tubules of the kidney, cardiac myocytes, ganglion cells of the dorsal root (DRG) and autonomic nervous system (ANS), and specific cortical and brain stem structures [14]. The pattern of tissue GL₃ distribution is a major determinant in the oligosystemic expression of AFD. Thus, involvement of the DRG may mediate the pain crises and ANS disease may be the underlying cause of other symptoms such as abnormal sweating, gastrointestinal disturbances and cardiovascular instability. Conversely, hepatic dysfunction and disabling bone complications are not core disease features.

Pathologic changes often precede the onset of major organ dysfunction by many years. Histological examination of kidney tissue reveals the presence of lipid deposits in the podocytes and vascular endothelium, with regions of mesangial widening and glomerulosclerosis. The significance of vascular endothelial involvement is a subject of debate, as these changes are often noted in engrafted kidney that remains functional for several years [15]. Accordingly, renal function is maintained as long as there continues to be sufficient surviving nephrons that are able to compensate for those that are destroyed by the accumulating material.

It is likely that other mechanisms of disease play contributory roles and ultimately lead to the development of multiple end-organ failure. For instance, altered vascular reactivity and a prothrombotic state are considered to represent added risk factors for stroke in AFD [16].

Although several insights have been gained from investigations of the pathogenesis of AFD, our knowledge remains incomplete. As examples, the reason(s) for the mainly vertebrobasilar distribution of the cerebrovascular lesions (i.e.,

large-vessel ectasia) and the common finding of significant concentric left ventricular hypertrophy despite the small fraction of cardiac GL₃ storage (accounting for <2% of heart tissue weight) is unclear.

Genetics

AFD is an X-linked trait with an estimated prevalence of one in 117,000 males [17]. Disease frequency is probably higher, based on recent surveys of patient populations with renal failure and on dialysis and those with late-onset hypertrophic cardiomyopathy which revealed that 0.16 and 6% of screened cases have deficient AGAL activity [18,19]. Increased recognition of disease-related symptoms among heterozygous females has led to the proposal that affected families should be counseled that AFD is an X-linked disorder with variable penetrance and expressivity in females [20]. Delayed onset of disease and the attenuated expression noted in the majority of female carriers are partly attributable to lyonization, that is, the random inactivation of one X chromosome early in embryogenesis.

Over 300 mutations of the AGAL gene have been described, with most resulting in mis-sense, non-sense and splicing gene defects or large and small gene rearrangements; generally confined in frequency to few families [21]. Certain mis-sense mutations are associated with residual enzyme activity, which leads to atypical disease presentation such as predominant cardiac involvement (cardiomyopathy and cardiac conduction defects) in the absence of or preceding renal failure among males and females in their 50s or 60s. Specific mutations which result in the loss of the encoded enzyme (as a consequence of its diversion from the lysosome and subsequent delivery to the unstable-protein degradation system) may be responsive to enzyme enhancement approaches [22]. The latter strategy involves the use of chemical chaperones that assist in stabilizing the defective enzyme so that it can achieve its functional conformation within the lysosome [23].

Agalsidase alfa

The recombinant formulation manufactured by Transkaryotic Therapies, Inc. (TKT, MA, USA) is produced in a continuous human cell line through gene activation; a proprietary technique involving homologous recombination and the modification of regulatory DNA sequences to turn on (activate) the endogenous production of proteins [24].

The active enzyme product is a homodimer, consisting of two subunits approximately 50 kDa; each whose amino acid composition is identical to the endogeneous human enzyme (containing three N-linked glycosylation sites) [101]. The primary mode of cellular uptake of agalsidase alfa is via the mannose-6-phosphate (M6P) receptors, which recognize the corresponding ligand (M6P) on the processed protein (~1.8 mol/mol protein). Sialylation of the carbohydrate residues is an added characteristic of the drug, intended to minimize nonspecific uptake of the enzyme through asialoglycoprotein receptors present in hepatic cells. To appreciate the influence of sialylation on the circulatory half-life of recombinant AGAL see [25].

As AFD is considered an orphan disorder, development of agalsidase alfa and its use in human trials was considered under an accelerated ('fast-track') approval process. Regulatory approval for commercial use of agalsidase alfa was granted in 2001 by the European Agency for Evaluation of Medicinal Products [(see 102)]. A comprehensive review by the Endocrinology and Metabolic Advisory Committee and the Center for Biologic Evaluation and Research (in 2003) led the FDA to conclude the available clinical data supporting the use of agalsidase alfa provided at that time was insufficient for marketing approval in the USA [103,104].

Clinical trials

Table 1 provides a summary of the clinical trials that have been conducted involving male and female patients with AFD and the use of agalsidase alfa.

In the initial phase of investigations, which was conducted at a single center (namely, the NIH), a single infusion of agalsidase alfa (with doses ranging from 0.007–0.1 mg/kg) was administered to five groups of two men (n = 10) with AFD [3]. This study established delivery of the exogenous enzyme to the sinusoidal endothelial cells, Kupffer cells and hepatocytes, with a tissue half-life in the liver of greater than 24 h. Plasma half-life ranged from 42 to 117 min. These findings were associated with a significant reduction of GL₃ in the liver and shed renal tubular cells in the urine sediment, but no significant change in the plasma GL₃ levels. Interestingly, 28 days after treatment mean GL₃ levels in urine had decreased by 38% from baseline. No drug-related adverse events were observed and the patients experienced no liver toxicity and did not exhibit any antibodies against agalsidase alfa.

Table 1. Agalsidase alfa in clinical trials.

Study	Design	n	Duration
NIH clinical study (MD, USA)			
TKT001	Open-label, dose-escalation (0.007 to 0.1 mg/kg) safety study	10	Single dose
TKT003	Randomized, double-blind, placebo-controlled; involving patients selected for neuropathic pain	26	6 months
TKT006	Open-label, maintenance study for patients completing TKT003	25	1 year
TKT010	Randomized, double-blind, placebo-controlled	80	6 months
TKT011	Open-label, maintenance study for patients completing TKT006	24	1 year
RFH clinical study (London, UK)			
TKT005	Randomized, double-blind, placebo-controlled; Involving patients with left ventricular hypertrophy	15	6 months
University of Mainz clinical study (Germany)			
TKT014	Open-label, safety and efficacy study for female patients	15	3–6 months
Total	Multi-dose studies [§]	136	> 2 years

NIH: National Institutes of Health; RFH: Royal Free Hospital; TKT: Transkaryotic Therapies, Inc.

[§]All trials subsequent to TKT001 used 0.2 mg/kg of agalsidase alfa every 2 weeks.

The absence of a demonstrable relationship between the plasma GL₃ response and the administered enzyme dose (for the dosages examined) led to consideration of a higher dose (of 0.2 mg/kg every 2 weeks) in a subsequent trial involving 26 AFD patients – divided into two groups; with one or the other on placebo or agalsidase alfa for 6 months [26]. This trial was followed by an extension phase which lasted for another 6 months during which time all patients received the recommended dose of agalsidase alfa.

In the latter study, reduction in neuropathic pain (measured by the Brief Pain Inventory [BPI]) was chosen as the primary end point, supplemented with information regarding the use of pain medication. Although a treatment effect was initially reported, a careful examination of the data relating to neuropathic pain revealed several confounding factors that raised some reservations regarding its interpretation [103]. Issues brought forth included the types of pain medications taken by the patients for symptomatic relief while receiving agalsidase alfa, and the temporal relationship between the pattern of analgesic-use and the appraisal of pain symptoms (which the protocol required had to be carried out while off medication).

Additional assessments included evaluation of renal function and pathology. Stabilization in renal function (based on creatinine clearance) at the 6-month time point was noted among the treated patients (when compared with the group

on placebo), which showed no correlation with the change in mean serum creatinine levels. The latter observation may be related to the absence of significant renal insufficiency among the study patients at baseline (i.e., study entry). Patients in this trial were selected based on the presence of neuropathic pain. There were several concerns regarding the clinical significance of the renal findings associated with the rapid deterioration in renal function noted in the group on placebo, although this reversed during the following 6 months (open-label phase of the study). For comparative purposes, a review of the historical data on renal function (based on glomerular filtration rate [GFR]) in adult male patients with AFD was conducted; which indicated a mean rate of decline in GFR of 12.2 ml/min/yr, associated with progression from onset of chronic renal insufficiency to ESRD over a mean of 4 ± 3 years (range: 1–13 years) [27]. However, the inherent bias associated with medical publications was cited as a potential problem limiting meaningful analysis of the study results relating to changes in renal function.

There were also changes in kidney GL₃, associated with a significant and persistent decrease in plasma and urine sediment GL₃ levels; of approximately 20–50% at 6 months and by approximately 50–80% after 12 to 18 months of treatment. The GL₃ clearance was noted to be 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). Unfortunately, a lack of rigor in the scoring of the histopathologic findings and lipid infiltration in the kidney undermined the attribution of significance to these observations.

The most commonly reported side effects (~10%) were infusion-related reactions that were mild to moderate in severity and consisted primarily of fever and chills. These problems decreased in frequency and severity with time, either spontaneously or following the introduction of appropriate premedications prior to subsequent infusions. A low-titer antibody response was observed in 55% of treated patients, with over 80% demonstrating evidence of immunologic tolerance (based on reduction in antibody titers). However, in a subset of patients who seroconverted, there were indications that plasma GL₃ levels tended to rise. The observed decline in antibody titers over time suggests that these findings may not prove to have lasting therapeutic implications – although this requires further follow-up.

On a personal note, TKT's inability to obtain regulatory approval in the USA may have been the end result of a combination of unfavorable positions, including their focus on clinical response as the primary end point and brief period of observation; compounded by the relative limited experience of certain stakeholders – in the conduct of clinical trials for a multifaceted lysosomal storage disorder such as AFD and in their interactions with the FDA. Inadvertently, these factors may have been negatively influenced by the prospect of sole market exclusivity – in a winner-take-all proposition – and the best of intentions (i.e., demonstrating clinical benefit, beyond changes in a surrogate marker that would have been potentially sufficient for a conditional approval). In any case, the difficulty of defining *a priori* which aspect of AFD would be most responsive to treatment and the challenges of measuring changes in disease states represented additional confounding elements.

For orphan diseases associated with life-threatening complications, a surrogate marker considered likely to predict therapeutic benefit may be adequate grounds for accelerated approval – as obtained for agalsidase beta [105]. In such cases, the drug sponsor is required to provide – in the postmarketing phase – additional clinical information relating to the use of their product [28]. Full approval is ultimately contingent on establishing that the treatment is safe and effective in a clinically meaningful sense.

In practice – Fabry outcome survey & other reports

The Fabry Outcome Survey (FOS) is a European database established to collect information on the natural history of AFD and the long-term safety and efficacy of agalsidase alfa treatment [29]. At present, there are 60 participating FOS centers representing 11 European countries. The Fabry International Research Exchange (FIRE) is the corresponding observational database for investigators based in the Americas and Australia. Data collection and analysis through the FOS and FIRE programs are sponsored by TKT Europe-5S (Danderyd, Sweden) and its parent company TKT Inc. (MA, USA), respectively.

The most recent report from FOS describes the observations made in a cohort of 545 patients with AFD, including 281 (52%) males and 264 (48%) females [30]. Close to 60% of the subjects (n = 314) enrolled in this program were on enzyme therapy. In summary, the report noted that treatment with agalsidase alfa (using 0.2 mg/kg body weight over 40 mins administered every 2 weeks) for a period of at least 12 and 24 months in 60 and 30% of cases, respectively, resulted in the following:

- Stabilization of renal function (based on estimated GFR) in patients with mild (GFR between 60 and 90 ml/min/1.73 m²) or moderate (between 30 and 60 ml/min/1.73 m²) deterioration in renal function at baseline
- Reduction in left ventricular size (based on echocardiography) in patients with an enlarged heart (mean ventricular wall thickness >11 mm and left ventricular mass >50 g/m^{2.7}) at baseline
- Improvements in pain scores and quality of life (based on responses to the BPI and European Quality of Life Questionnaire EQ-5D)
- Infusion-related reactions (IRRs) that were usually mild and characterized by fever, malaise, or skin rash), noted in 12% of treated patients; estimated to have received a total of 14,800 infusions (an incidence of IRR of approximately 0.7%)

In the past year, several observations involving a smaller number of AFD patients receiving agalsidase alfa have also been made, including:

- Relief of gastrointestinal symptoms (primarily abdominal pain and diarrhea) following 6 months of treatment (n = 11) [31]

- Improvements in cardiac hypertrophy and systolic function in a 35-year old Japanese male patient after 6 months [32]. Specifically, the following changes were noted on cardiac magnetic resonance imaging (MRI): wall thickness decreased from 15 to 12 mm, left ventricular ejection fraction increased from 49 to 60%, and left ventricular mass (LVM) index decreased from 110.8 g/m² to 96.6 g/m² and normalized
- Decreased LVM from baseline at weeks 27 and 41, and a significant reduction in QRS durations at week 27 (n = 15 female patients) [33]. Furthermore, there was a significant improvement in quality of life and no deterioration in renal function over the 13- to 41-week period of observation. None of the patients in this study developed antibodies or experienced an IRR to agalsidase alfa
- White matter (WM) lesions (i.e., asymmetric widespread pattern of deep WM lesions that were hyperintense on T2- and FLAIR-weighted MR images) that were absent, remained normal (n = 3/7); while those present at baseline were either unchanged (n = 2) or worsened (n = 2; siblings aged 36 and 47 years and comprised the oldest patients in the group) [34]. Furthermore, the older patient in the sib-pair from the last group experienced a transient ischemic attack (at month 6) which resolved without neurologic sequelae. In this report, the follow-up brain MRI was obtained after 12 months of treatment
- Improvement in the clinical manifestations of the small fiber neuropathy, including pain relief, reduction in the threshold for warm and cold sensation in the foot and increased sweat excretion (based on quantitative sudomotor axon reflex testing [QSART] and confirmed by thermoregulatory sweat testing) at 36 months [35]
- Gradual improvement in hearing (by 4.0 db) at 42 months (n = 15) [36,37]

A report of note were changes in disease severity score (based on the Mainz Severity Score Index [MSSI]) that were observed in 39 AFD patients (including 24 males and 15 females) following 1 year of agalsidase alfa treatment [38]. The MSSI is a scoring system composed of four sections that cover the general, neurological, cardiovascular, and renal signs and symptoms of AFD [38].

An added reason for optimism derives from earlier observations that suggest agalsidase alfa

treatment may improve CNS-related outcome as a consequence of the reversal or resolution of the cerebrovascular hyperdynamicity (which is characteristically observed in the untreated AFD patient) [39,40]. Whether these observations translate to reduction in risk of stroke remains to be established.

Expert opinion

Enzyme-replacement therapy (ERT) for the lysosomal storage disorders represents an approach to correct the primary metabolic defect (i.e., deficient intracellular substrate hydrolysis); which is anticipated to result in long-term clinical benefits, barring safety concerns related to antibody formation (or other factors) and potential limitations in either the achievement of a sufficient concentration of the enzyme within affected tissues, or the reversibility of the disease process.

In AFD, the use of agalsidase alfa, when administered at 0.2 mg/kg of body weight every 2 weeks, appears to halt and possibly reverse disease progression. However, the full extent of treatment effect, its modification of the natural history of the disease and resultant improvement in patients' lives and ultimately the extension of their life-spans remain to be determined. Furthermore, increased experience is required to ascertain the factor(s) which influence treatment response, or lack thereof. Disease-related aspects may include the presence of pre-existing tissue damage, such as glomerulosclerosis in the kidneys and calcified aortic valves in the heart, which are not likely reversible by ERT and may necessitate the institution of palliative measures when progressive and present a threat to the patients wellbeing. The presence or absence of renal failure (and the steps taken – dialysis or kidney transplantation – to address this problem) may also impact on substrate turnover, as a fraction of the body's substrate burden is excreted in urine. Treatment-related considerations that may have a bearing on overall outcome may include the pattern of use of adjunctive measures such as aspirin for stroke prevention and anti-arrhythmic agents to reduce the risk of sudden cardiac death.

The issues relating to the overall management of patients with AFD are complex and there are current limitations in the establishment of definitive therapeutic guidelines due to scant knowledge regarding the natural history of the disease and the relevant mechanism(s) of pathology leading from lipid

deposition to specific disease-related complications. These concerns are further confounded by the fact that storage material can be present in several tissues for decades (e.g., podocytes in the kidneys) without leading to functional impairment until such time as the involved organ's reserve has been exhausted. The elucidation of these matters is important in shaping the decisions that will have to be made regarding treatment, with respect to the appropriate time to intervene and the identification of the patients most likely to benefit from treatment. From this body of knowledge will come informed decisions as to whether treatment will have to be started in pediatric patients (before 18 years) when the incidence of organ failure is relatively low (to prevent what may be inevitable), or in adults as a salvaging option. Furthermore, there is the added matter of which dose and frequency of enzyme administration will prove to be most advantageous. A careful analysis of the therapeutic strategy ultimately implemented is essential, not only because it may help to determine the best approach leading to the most favorable results, but also clarify any potential differences in health-economic implications. The latter is among the major challenges confronting health policy makers, following the introduction of novel treatments.

In any case, patient care limited to enzyme therapy alone appears to be inadequate and careful attention will have to be given to pain management, the use of angiotensin-converting enzyme inhibitors and antihypertensive medication (to delay progressive loss of renal function), and other approaches as needed to optimize outcome. The pattern of resource utilization (e.g., chronic use of pain medication, dialysis or kidney transplant for ESRD, pacemaker insertion for cardiac conduction problems) will also need to be monitored to determine the balance of healthcare expenditure. Ultimately, the impact of enzyme therapy on the patients' physical and functional well-being will determine the standard of care.

A twofold parallel approach, one directed at establishing the ideal means of defining the pattern and severity of disease and a second at accurately measuring the rate and magnitude of response to treatment (if any), will be critical in the conduct of future case-controlled and dose-optimization studies. One instrument has been developed by the Mainz group as a specific measure for objectively assessing the severity of

AFD and for monitoring ERT-related treatment effects [39]. Future observational studies must be hypothesis-driven and include further investigations of alternative enzyme dose-frequency regimens. One study design can include measurement of the time to develop a clinically significant (sentinel) event (i.e., deterioration in renal [based on rise in serum creatinine, need for intervention with dialysis or kidney transplantation], and/or cardiac dysfunction [arrhythmia requiring medical treatment or pacemaker/defibrillator placement, heart failure, myocardial infarction], CNS complication (transient ischemic attack [frequency/severity], stroke [debilitation therefrom]), or death (lifespan). Whether these clinical investigations should include invasive procedures such as a kidney biopsy remains to be determined. Preliminary studies appear to suggest that the proportion of sclerosed glomeruli detected in a section of kidney tissue (and the functional kidney status based on GFR) at baseline may be important predictors of renal response. Furthermore, the presence and persistence of antibodies to the infused enzyme and its influence on ultimate clinical outcome will also need to be tracked. These various surveillance efforts will be fostered by full engagement of caregivers with the registry program, a postregulatory commitment fostered by the drug manufacturer.

Outlook

Unless careful studies are undertaken – with patients stratified according to baseline disease state and detailed clinical information on outcomes are provided – it may never be possible to determine the answers to such fundamental questions on the subject of which enzyme formulation may be preferred, or whether differences in enzyme glycosylation and/or sialylation are truly minor [42]. Furthermore, opinion leaders who exert a major influence on the conduct of patient care and research and those who educate potential healthcare providers are exhorted to declare their potential conflict(s) of interest for a fair and full perspective on the recommendations that are put forth. Enzyme therapy for AFD holds so much promise and already there are clear indicators of its disease-modifying effects. On final analysis, we must still ask ourselves whether – in the arena of orphan drug development for AFD – our patients' best interests have been well served by the existence of two competing products.

Highlights

- Agalsidase alfa is a recombinant formulation of human α -galactosidase A (AGAL); enzyme-replacement therapy for Anderson–Fabry disease (AFD).
- Renal failure and cardio/cerebrovascular complications represent the major drivers of morbidity in untreated patients with AFD.
- Wide variability in clinical AFD expression is due to a combination of factors, including broad heterogeneity of causal mutations and the presence or absence of residual enzyme activity, lyonization among females (for this X-linked trait), and eventually the patient's renal status.
- Agalsidase alfa safely and effectively halts, stabilizes and/or improves several cardinal features of AFD.
- A comprehensive approach to patient management must include a thorough baseline assessment (to ascertain pattern and severity of disease), and a multifaceted therapeutic plan, incorporating adjunctive treatments such as anticoagulation (for stroke prevention), angiotensin-converting enzyme inhibitors (for renoprotection) and analgesics (for the acroparesthesia).

Recommended reading

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