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Diagnosis and treatment of Fabry disease

Diagnóstico y tratamiento de la enfermedad de Fabry

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Fabry disease is an X-linked hereditary disease resulting from mutations in the GLA gene leading to deficiency of the lysosomal enzyme α-galactosidase A and glycolipid accumulation (1). Two Fabry phenotypes have been recognized. Classical Fabry disease symptoms start in childhood, compromising the quality of life. Severe injury to the kidneys, heart and central nervous system develops in young adults and shortens the lifespan. Non-classic, late-onset Fabry disease results from milder enzyme deficiency and usually lacks the childhood manifestations. Males develop full-blown disease, while in females random X chromosome inactivation (Lyonization) results in a spectrum of phenotypes ranging from asymptomatic to a disease as severe as in males (2). The availability of enzyme replacement therapy (ERT) from the early 2000s has revolutionized Fabry disease treatment. However, add-on therapy aimed at symptoms relief and tissue protection is also required. The diagnosis of Fabry disease poses a series of diagnostic challenges that will be discussed in the present review, together with current issues of therapy, in the context of disease pathogenesis.

Pathogenesis of disease manifestations

A correct understanding of the pathogenesis is required for correct diagnosis and optimal treatment.

Fabry disease may be caused by more than 800 different defects of the GLA gene. This is an important concept since demonstration of the specific genetic defect may require diverse genetic diagnostic approaches. The most common defects are point mutations leading to amino acid substitutions or stop codons. However major deletions or more complex genetic defects, such as mosaicisms, may also occur.

The genetic defect results in low intralysosomal α-galactosidase A activity. In classical Fabry disease this is typically <1% of normal activity. Higher activity may be found in late-onset Fabry disease. In females random X chromosome inactivation may result in normal overall enzyme activity. However individual female cells may have a complete absence of enzyme.

Fabry disease is a lysosomal storage disease. Enzyme deficiency leads to progressive accumulation of glycolipids typically inside lysosomes. However, glycolipids also accumulate in the extracellular space. The best characterized accumulated glycolipids are globotriaosylceramides (Gb3) and globotriaosylsphingosines (lyso-Gb3). Lyso-Gb3 are more hydrosoluble molecules since they have lost a fatty acid chain. Both Gb3 and lyso-Gb3 have multiple different forms, but whether specific forms have differential roles in the pathogenesis of Fabry disease is still incompletely understood. While the literature commonly refers to glycolipid deposits as Gb3 deposits, most histological studies rely on morphology, and thus, do not assess specifically Gb3 deposits. Lyso-Gb3 is of particular interest since the relative increase in circulating levels in Fabry patients when compared to healthy controls is much higher than the increase in Gb3, there is no overlap in values between

Fabry males and normal controls, and very little overlap between Fabry females and normal controls (3). Thus, it has been suggested that assessment of circulating lyso-Gb3 may be used to support the pathogenicity of a GLA mutation. However, there is overlap between circulating Gb3 levels in late-onset Fabry males or in Fabry females and healthy controls. In addition, there is evidence for a pathogenic role of lyso-Gb3 in Fabry disease. Thus, lyso-Gb3 levels within the range found in Fabry disease promoted the proliferation of vascular smooth muscle cells and activated fibrogenic and inflammatory responses through autocrine activation of TGFβ1 and Notch1 in podocytes (3-5). These and other observations are changing concepts about the pathogenesis of tissue injury. Until recently it was thought that accumulation of glycolipids in endothelial cells led to mechanical obstruction of small vessels. However, a more complex pathogenesis appears to involve additional cell types (e.g. glomerular podocytes, cardiomyocytes, vascular smooth muscle cells and neurons) from the early stages of the disease and glycolipid recruitment of secondary mediators of tissue injury to cause tissue fibrosis (6). Detailed renal biopsy data show that glycolipid accumulation in podocytes far exceeds accumulation in endothelial cells (7). This is not unexpected, since endothelial cells turnover periodically decreases intracellular glycolipids, while podocytes are terminally differentiated cells that do not divide and unrelentingly accumulate glycolipids. In children the amount of glycolipids in podocytes is higher than in endothelial cells, podocyte (but not endothelial) glycolipids correlate with severity of albuminuria, and evidence of podocyte injury (foot process effacement) precedes albuminuria (7). In this regard, the earliest manifestations of Fabry nephropathy is pathological albuminuria, a manifestation of podocyte injury, which progressively increases to overt proteinuria as podocytes are lost and histological focal segmental glomerulosclerosis develops. This is followed by a progressive decrease of glomerular filtration rate with patients needing renal replacement therapy at a mean age of 40 years (8,9). Both the severity of proteinuria and the severity of focal segmental glomerulosclerosis are key prognostic factors both in natural history and in ERT patients (10,11). This sequence of events suggests that podocytes are key target cells in Fabry nephropathy, reminding of the pathogenesis of diabetic nephropathy. This has therapeutic consequences, since antiproteinuric therapy targeting the renal angiotensin system (RAS) may be nephroprotective. Proteinuria may reach nephrotic range but typically is not associated with nephrotic syndrome, resembling other causes of secondary focal segmental glomerulosclerosis. Full blown nephrotic syndrome should be studied by renal biopsy to exclude additional glomerular diseases even in patients are already diagnosed of Fabry disease.

Another key manifestation of Fabry disease is cardiomyopathy. This is characterized by left ventricular hypertrophy that usually developing in the third decade of life and is followed by replacement fibrosis and the development of potentially life-threatening arrhythmia (atrial fibrillation and ventricular tachycardia) and heart failure. The increases heart size is related to myocyte hypertrophy and does not represent an equal volume of

accumulated glycolipids. It is thus hypothesized that glycolipids or secondary mediators of injury promote cardiomyocyte hypertrophy and fibrosis. In addition, coronary microvascular dysfunction may contribute to ischemia in the absence of major atherosclerotic lesions.

Central nervous system disease is characterized by white matter lesions presumably resulting from small vessel disease, as well as strokes. Again, the pathogenesis is unclear and small vessels injury, large vessels tortuosity leading to abnormal flow patterns and embolic events triggered by arrhythmia may all play a role.

Non-life-threatening disease manifestations include neuropathic pain (classically known as acroparesthesia, although this is pain and not paresthesia, and pain is not limited to acral regions), hypohidrosis, abdominal pain or diarrhea/constipation, and angiokeratoma (1). Neuropathic pain and digestive manifestations were classically considered consequences of ischemia due to endothelial involvement. However, these early childhood manifestations of Fabry disease usually develop when endothelial involvement is minimal. Typically, damage to small myelinated (Aδ) fibers and unmyelinated (C) fibers does not alter the electromiogram. Indeed, lyso-Gb3 activates voltage-dependent Ca^{2+} channels in sensory neurons triggering pain signals. This information may help to select the optimal anti-pain medication (12). Hypohidrosis may be the result of autonomic dysfunction or glycolipid accumulation in sweat gland cells. Autonomic dysfunction has also been suggested to contribute to gastrointestinal symptoms.

Diagnosis

Diagnosis and staging of Fabry disease usually involves 6 sequential steps: suspect the diagnosis, demonstrate the enzymatic defect, demonstrate the genetic defect, assess the burden of disease, confirm that signs and symptoms are indeed a consequence of Fabry disease, and study the family (**Table 1**). The diagnosis of Fabry disease is challenging. In fact the mean time from onset of symptoms to diagnosis is around 20 years.

When to suspect Fabry disease. Fabry disease should be suspected in patients with any of the features listed in table 1. In addition, symptoms of Fabry disease may not be spontaneously referred by the patients. A classical example is neuropathic pain. Childhood pain may improve with age or the patient become unwilling to speak about it after multiple failures to diagnose the origin, raising the suspicion of simulation. This history should be obtained by directed interrogation. Angiokeratoma are more frequent in the bath suit area and will not be visible unless the patient is naked. Thus, Fabry disease may also be suspected in patients with evidence of target organ injury even in the absence of classical signs and symptoms of Fabry disease. Potential scenarios include chronic kidney disease of unknown, especially if this is present in males under the age of 50 years, is accompanied by proteinuria, and hypertension is mild of absent (13). We must emphasize that a renal biopsy does not exclude Fabry disease unless electron microscopy has been performed. This is especially true when

focal segmental glomerulosclerosis is the diagnosis. Fabry disease should also be suspected in patients with unexplained left ventricular hypertrophy. It is a matter of discussion whether Fabry disease should be suspected in patients with unexplained stroke at young age in the absence of other manifestations of Fabry disease.

Screening of high risk populations. Diverse studies have screened for Fabry disease in high risk populations, including patients on hemodialysis, with unexplained left ventricular hypertrophy or with unexplained, early stroke (14-16). The frequency of Fabry disease in such screening programs has in general ranged from 0.1 to 1 % (1). A caveat is that the majority of genetic variants identified correspond to GLA single nucleotide polymorphisms, variants of a non-significance and late-onset disease associated mutations (15-16). In this regard, for some patients it is difficult to demonstrate that the genetic GLA variant is the cause of the disease that motivated the screening. This issue underlies the reluctance by many physicians to develop neonatal screening programs. In such programs in Taiwan, the US and Galicia (Spain), the frequency of GLA genetic variants in these screenings has been as high as 1 in 3000, with a 11 to 1 ratio of late onset/unknown significance to classical disease (1). By contrast the frequency of classical Fabry disease is estimated to be 1 in 40.000 to 1 in 120.000.

Enzymatic diagnosis. Demonstration of very low α-galactosidase levels is the basis for the diagnosis of Fabry disease in males. However in females a normal enzymatic activity does not exclude Fabry disease. An activity below 1% of normal is diagnostic of Fabry disease. Higher activity, in general below 10%, may also be observed in males with late-onset variants. The most common method is assessment of α-galactosidase enzymatic activity in dried blood spot (DBS). This greatly improves the logistics of collecting samples and sending them to an outside laboratory. Alternative methods involve assessment of enzymatic activity in plasma or leukocytes. Unavailability of the enzymatic assay in routine clinical labs at major hospitals is a major drawback for the early diagnosis of Fabry disease. The fact that at present only reference laboratory offer the test and that it cannot be ordered through the hospital computer system, hinders the use of the test. At present most diagnostic laboratories have set the cut off for positivity of the test to suspect Fabry disease at a screening level. That is, a possible Fabry disease is flagged at levels of enzyme activity below 30%, with the knowledge that this will result in false positive results and that the diagnosis will be confirmed at the genetic level.

Genetic diagnosis. The diagnosis should be confirmed at the genetic level. Sequencing of all exons and intron-exon boundaries is the initial standard approach. If this is normal but the enzyme activity is very low, additional genetic testing should be performed to exclude large deletions or more complex genetic defects.

Variants of an unknown significance. With the advent of screening studies and the use of a higher cut off point for enzyme activity assays for this purpose, the presence of milder mutations and genetic variants of a non-significance has increased. This poses a diagnostic challenge, especially when the only manifestation

potentially attributable to Fabry disease was the trigger of the screening (e.g. end-stage kidney disease) and the options to clearly establish the link between the genetic defect and the cause of tissue injury are limited. As an example, in a patient on dialysis that carries a variant of a non-significant and has no other manifestations of Fabry disease, a renal biopsy cannot be used to establish that Fabry disease was the cause of the kidney disease. A number of consensus reports have suggested the attitude when variants of a non-significant are found in specific clinical contexts (17). It has also been suggested that a high circulating lyso-Gb3 concentration supports the pathogenicity of the genetic defect.

Some variants of a non-significant merit specific comments because they appear frequently in genetic screenings.

D313Y (p.(Asp313Tyr)) is now recognized as a SNP present in 0.19% of the population, causing a pseudo-deficiency (1). That is, enzyme activity is low in plasma but normal in the lysosomes. However to this date, some authors consider that D313Y may be pathogenic.

R118C has been found in diverse screening efforts mainly in the Iberian Peninsula (16). Most individuals with this genetic variant appear not to have manifestations of Fabry disease. However the prevalence of R118C was 10-fold higher in young Portuguese stroke patients than in the general population. It is possible that in presence of a permissive genetic background some patients may develop clinical manifestations. In a Spanish hemodialysis screening program R118C (p.Arg118Cys) was found in 0.14 % of hemodialysis patients (15), while the frequency of this variant in the Iberian peninsula general population was 0.10 % (p=1.0, Fisher exact test)(16), suggesting that this GLA genetic variant was not the cause of the kidney failure, despite the authors interpreting the result as indicative of a higher than expected prevalence of Fabry disease in hemodialysis patients. In this regard, late-onset Fabry disease is mainly characterized by cardiomyopathy, and severe kidney disease is, in general, distinctly uncommon (1), with the potential exception of R363H (p.(Arg363His)).

A143T (p.(Ala143 Thr)) has been observed in 1 in 5000 newborns in Missouri and pathogenicity has been questioned. However A143T has also been reported to be the only genetic defect found in the descendants of the original patients described by Anderson in the United Kingdom (in the United Kingdom Fabry disease is referring to as Anderson-Fabry disease).

Thus, we suggest that individuals carrying genetic variants of unknown significance be followed periodically until we develop additional tools that allow a better assessment of risk for future clinical problems.

Treatment

Treatment of Fabry disease should be based on our current understanding of the pathogenesis. Conceptually Fabry disease manifestations result from the sequential development of three related but ultimately independent problems that each requires a specific therapeutic approach (**Table 2**). The first problem is the enzyme deficiency that is currently treated with ERT, although molecular chaperones will soon be available. The second problem is tissue injury, which requires specific adjuvant therapeutic approaches on top of ERT in order to minimize symptoms and prevent non-specific progression of tissue injury. The third problem is organ failure that may require renal replacement therapy or kidney or heart transplantation on top of ERT. Several consensus reports provide guidance (18-20).

Enzyme replacement therapy

ERT currently consists of the biweekly intravenous administration of recombinant human α-galactosidase A (agalsidase). Cell membrane receptors bind circulating enzyme and internalize it to the lysosome, where it becomes active. Two forms of agalsidase are available in most of the world (**Table 3**), although agalsidase-β is the only one available in the United States as the Food and Drug Administration did not approve agalsidase-α. Strikingly, the label dose of agalsidase-α and agalsidase-β is 5-fold different (0.2 vs 1.0 mg/kg/2 weeks)(21). Although this is a sensitive issue since there are underlying commercial interests, current evidence summarized in **Table 3** suggest that the lower dose allows a faster infusion of the enzyme, but is limited by a lower intracellular enzymatic activity (22) that may negatively impact its efficacy in cleaning glycolipid deposits in some cell types such as podocytes (23). In this regard, for the purpose of the discussion we will use the term low dose for 0.2 mg/kg/2 weeks, high dose for 1.0 mg/kg/2 weeks and intermediate dose for dosing regimens that fall in between in terms of accumulated dose over two weeks, independently of the agalsidase form used.

Efficacy. When discussing the evidence supporting the efficacy of ERT we should remember that this is a rare disease. Thus, appropriately powered randomized placebo-controlled trials (RCT) were performed to assess the efficacy of therapy on the clearance of glycolipid deposits or pain (24,25). However longer term studies addressing hard end-points such as severe clinical events, changes in the slope of glomerular filtration rate or changes in left ventricular hypertrophy are more difficult to conduct. To put the problem in perspective, over 1500 patients were randomized to demonstrate nephroprotection by RAS blockade in diabetic nephropathy, and over 9000 patients to demonstrate the benefit of statins over cardiovascular outcomes in patients with chronic kidney disease. However, the largest published placebo-controlled RCT studying agalsidase enrolled 82 patients (26). Given the current availability of agalsidase, no further placebo-controlled trial is ethically possible. The evidence for the efficacy of the ERT can be summarizes as follows (**Table 3**):

1. According to placebo-controlled RCTs, agalsidase decreases circulating glycolipids and clears endothelial cells for the most part within six months (**Figure 1.A.1 and A.2**) and improved neuropathic pain (24,25).
2. High dose (1.0 mg/kg/biweekly) ERT clears glycolipid deposits in podocytes (23). This evidence was obtained from case-series.
3. According to a placebo-controlled RCT, high dose (1.0 mg/kg/biweekly) ERT decreases the incidence of severe clinical events in patients with more advanced disease (**Figure 1.A.3**) (26). Pre-specified adjustment for baseline imbalances on disease severity was performed. This is further supported by recent registry data (**Figure 1.B)** (27). Overall the decrease in the incidence of severe clinical events hovers around 50%.
4. According to registry data, case series, and post-hoc analysis of placebo controlled RCTs, ERT improves hypohidrosis and gastrointestinal symptoms and stabilizes cardiac, renal and central nervous system disease, especially if started early.
5. A controversial issue is dose. **Figure 1** summarizes data from RCTs and large (>1000 patients) registry studies (24-27). Besides case series and reports suggesting that any of the current dosing regimens clears endothelial cells but only high dose (1.0 mg/kg/2 weeks) clears podocytes (23) and that some patients require higher cumulative doses (either 1.0 g/kg/2 weeks or 0.2 mg/kg/week) to slow progression of kidney disease (28), an ongoing RCT is testing head-to head agalsidase alfa 0.2 mg/kg/2 weeks vs agalsidase beta 1.0 mg/kg/2 weeks with a primary end-point of severe clinical events . The latest update of the trial was presented at the Garrod 2016 symposium (**Figure 1.A.4**). Despite significantly less severe Mainz Severity Score Index at baseline for patients on agalsidase alfa, the number of events per enrolled patient was double in alfa than in beta: alfa 45 events/69 patients (0.65); beta 15/45 (0.33) at 8 years of follow-up (http://garrodsymposium.com/garrod2016/posters/#p104; accessed July 18, 2016). However, the difference was not statistically significant since the study was grossly underpowered (n randomized=114, estimated sample size >600). The fact that the study is so underpowered makes it unlikely that statistically significant differences can be demonstrated, leaving individual physicians to integrate the available information into their dosing decision-making process.

Safety. As with other protein-based biologicals, infusion reactions are the main side effect and can usually be managed with premedication with acetaminophen, ant-histaminics or corticoids. Anaphylactic reactions requiring withdrawal of ERT are exceptional. Male patients with absent or very distorted proteins may develop anti-agalsidase antibodies cross-reactive to both agalsidase alfa and beta. Antibodies may be neutralizing and may decrease the efficacy of therapy.

Monitoring. The efficacy of therapy should be monitored at two levels: glycolipid deposits and evidence of tissue injury. Currently there is no optimal marker of glycolipid deposits and sometimes repeat tissue (usually

kidney) biopsy may required to confirm clearance of glycolipids, specially from hard-to-clear cells such a podocytes (23). Clearance of podocytes may take years and in some patients lower doses may not achieve podocyte clearance (23). Circulating lyso-Gb3 is currently the best non-invasive marker and ideally should be normalized. In addition, tissue injury should be monitored (**Table 1**). A suboptimal response may be due to development of neutralizing anti-agalsidase antibodies.

Additional therapeutic approaches for the enzyme deficiency

On April 1, 2016, the European Medicines Agency (EMA) recommended granting a marketing authorization in the European Union for the chaperone migalastat. Phase 3 RCT data remain unpublished as of July 17, 2016. Migalastat is an oral agent that increases the enzymatic activity for a subset of mutations ("amenable" mutations), decreasing glycolipid deposition in patients with those mutations. The exact place of migalastat in the treatment of Fabry disease remains unclear.

Additionally, oral agents that reduce the generation of glycolipids (substrate reduction therapy), gene therapy and extended half-life agalsidase molecules are also under clinical development.

Adjuvant therapy

As indicated in **table 2**, adjuvant therapy should be added to ERT for symptomatic treatment or to prevent non-specific progression of tissue injury. The aim of adjuvant therapy should not be to delay or avoid the initiation of ERT, since it does not address the key pathogenic mechanisms, glycolipid accumulation. The only approach tested in a clinical trial is RAS blockade to lower proteinuria (29). As is the case for others causes of CKD, proteinuria is a key prognostic factor in ERT-naïve and ERT Fabry patients (10,11). Decreasing proteinuria to below 0.5g/day using RAS blockers was associated with slower progression of CKD, especially in younger patients (29). Given that patients may be hypotensive or not hypertensive, RAS blockade for control of proteinuria should be initiated at the lowest possible dose taken at night-time. Detailed discussion of additional adjuvant and symptomatic therapies can be found in recent reviews (12,13,18,19).

Conclusions

In conclusion, Fabry disease is a treatable rare disease that shortens lifespan. As a rare disease, diagnosis is often delayed. Once suspected, the diagnosis of classical Fabry disease is straightforward, given the characteristic clinical manifestations, very low enzymatic activity and genetic defect. However, there is still discussion about the pathogenicity and penetrance of some late onset variants which frequently pop up in screening efforts. The cornerstone of therapy is ERT, but adjuvant therapy is frequently needed to minimize

symptoms and prevent non-specific disease progression and complications, thus requiring a multidisciplinary team. Novel approaches to increase enzyme activity will be marketed soon.

Note added after completing the manuscript: On August 11 2016, the results of the migalastat phase 3 randomized controlled trial were published: Germain DP, Hughes DA, Nicholls K, Bichet DG, Giugliani R, Wilcox WR et al. Treatment of Fabry's Disease with the Pharmacologic Chaperone Migalastat. N Engl J Med. 2016;375:545-55

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Conflict of interest: Alberto Ortiz is consultant for Genzyme a Sanofi company and has received speaker fees from Shire. Maria Dolores Sanchez Niño has received speaker fees from Genzyme a Sanofi company.

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Table 1. Diagnosis of Fabry disease

1. **Suspect the diagnosis**
   - **Individual patient (<u>any</u> of the following):**
     Family history: neuropathic pain, heart disease, kidney disease, stroke. On mother side for males.
     Patient history: neuropathic pain, proteinuric kidney disease at early age (<50 years for males), arrhythmia, heart failure, stroke. Additionally, heat or cold intolerance, exercise intolerance, missing school as a child
     Physical exam: Fabry facies, angiokeratoma (bathing suit distribution), cornea verticillata (slit lamp), vascular tortuosity conjunctiva, low-normal blood pressure in CKD
     Diagnostic test findings: white matter lesions or pulvinar sign (cerebral MRI), short P-wave duration, PQ-interval and QRS width in EKG, characteristic late enhancement pattern in cardiac MRI
   - **Screening of high risk populations**
     Renal replacement therapy or CKD of unknown cause (ERBP recommendation for individual patient assessment)(13)
     Unexplained left ventricular hypertrophy
2. **Demonstrate the enzymatic defect**
   Current standard: dried blood spot (DBS)
   Alternatives: plasma leukocytes
3. **Demonstrate the genetic defect**
   Whole gene or exon-intron boundary sequencing
   Additional genetic tests as required
   Genetic report should be coupled to information about potential pathogenicity of the mutation
4. **Assess the burden of disease** (at diagnosis and follow-up)
   Kidney: urinary albumin/creatinine ratio, estimated glomerular filtration rate
   Heart: echocardiography, heart MRI, heart rhythm Holter
   Central nervous system: MRI
   Other as clinically indicated: pain scales, audiometry
5. **Confirm that signs and symptoms are a result of Fabry disease**.
   Biopsy: e.g. renal with finding of extensive glycolipid deposits. Electron microscopy mandatory.
   Plasma lyso-Gb3
6. **Study the family**
   Siblings
   Maternal side and daughters for male patients
   Both maternal and paternal sides and sons and daughters for females

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CKD: chronic kidney disease; EKG: electrocardiogram, ERBP: European Renal Best Practice; MRI: magnetic resonance imaging.

Table 2. Treatment of Fabry disease

1. ERT (or other therapies aimed at increasing enzyme activity)

Treatment initiation

Initiate in all classically affected males, in childhood if feasible

Individualize decision for non-classical males and for females: decision usually based on development of signs or symptoms of tissue injury

Monitoring

Glycolipid deposits: plasma lyso-Gb3, consider biopsy if suboptimal response to therapy

Tissue injury: see table 1: assessment of burden of disease

Anti-agalsidase antibodies

ERT dose modification

Consider increasing the dose up to 1.0 mg/kg/2 weeks if suboptimal response to initial dose

2. Treat tissue injury and associated symptoms

Potentially lethal disease manifestations

Kidney injury: antiproteinuric therapy with RAS blockers. For other aspects of kidney injury and its consequences, KDIGO recommendations for chronic kidney disease apply.

Arrhythmia, heart failure: as clinically indicated

Stroke: as clinically indicated

Non-lethal manifestations: as clinically indicated

Pain

Gastrointestinal symptoms

Replace nutritional vitamin deficiency

Other

3. Treat organ failure

Kidney transplantation or dialysis

Heart transplantation

ERT: enzyme replacement therapy; RAS: renin-angiotensin system; KDIGO: kidney disease | improving global outcomes at www.kdigo.org/.

Table 3. Widely available agalsidase preparations

	Agalsidase alfa	Agalsidase beta
Product characteristics		
Nature	Human recombinant protein	Human recombinant protein
Obtained from	Human fibrosarcoma cells	CHO cells
Availability	Worldwide, excluding USA	Worldwide and USA
Placebo-controlled trials and long-term follow-up		
Dose-finding phase 1 trials (mg/kg): dose tested (21)	0.007; 0.014; 0.028; 0.056; 0.1 (single dose)	0.1; 1.0; 3.0 (5 consecutive doses, every 2 weeks)
Dose tested in phase 2/3 placebo-controlled trials (mg/kg/2 weeks)(24,25)	0.2	1.0
Publication of 10 year follow-up outcome of patients enrolled in phase 2/3 placebo-controlled trials	No	Yes (30)
Patients from phase 2/3 RCT that required doubling the cumulative dose (weekly administration) for suboptimal clinical response after end of RCT	12/41 (29%)(28)	None reported
Phase 4 placebo-controlled trial, primary end-point: severe clinical events	No	Yes (26)
Head-to-head comparison		
CFDI Phase 4 alfa 0.2 vs beta 1.0 mg/kg/2 weeks RCT. Severe clinical events per enrolled patient at 8 years of follow-up*	0.65	0.33
Label information		
Administration	Intravenous	Intravenous
Infusion time (minutes)	40	>120
Recommended dose (mg/kg/2 weeks)	0.2	1.0
Intracellular enzyme activity on label dose and dose interval		
Intracellular α-Gal A activity in leukocytes, AUC over 2 weeks (mean (95% CI), hr[nmol/hr/mg]) at label recommended dose (22**)**	396 (299-493)	3709 (2517-4900)

AUC: Area under the curve; CFDI: Canadian Fabry disease initiative, CHO: Chinese hamster ovary, industry standard; CI: confidence interval, RCT: randomized controlled trial.

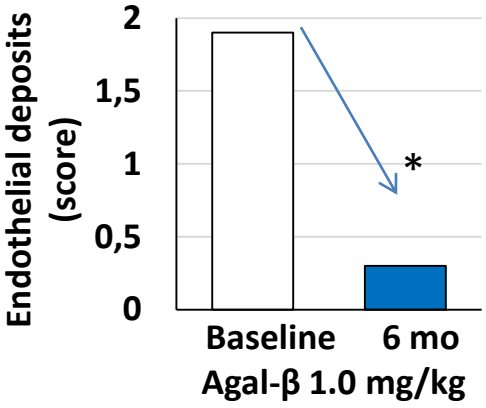
* Patients randomized to agalsidase alfa 0.2 mg/kg/2 weeks had milder disease severity at randomization than those randomized to agalsidase beta 1.0 mg/kg/2 weeks (http://garrodsymposium.com/garrod2016/posters/#p104; accessed July 18, 2016).

Legend to figure

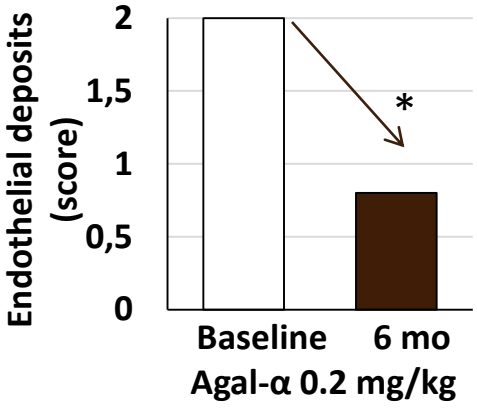
Figure 1. Efficacy of enzyme replacement therapy (ERT) with agalsidase (Agal). Results from major randomized controlled clinical trials and Registry studies.
A) Randomized controlled trials.
A.1) Phase 3 placebo-controlled clinical trial for agalsidase beta (total patients randomized: 58, follow-up 6 months). Endothelial deposits in kidney biopsies (25).
A.2) Phase 2 placebo-controlled clinical trial for agalsidase alfa (total patients randomized: 26, follow-up 6 months). Endothelial deposits in kidney biopsies (24).
A.3) Phase 4 placebo-controlled clinical trial for agalsidase beta (total patients randomized: 82, follow-up 3 years). Severe kidney, cardiac, central nervous system events or death (26).
A.4) Phase 4 head-to-head controlled clinical trial for agalsidase beta versus alfa (Canadian Fabry Disease Initiative, total patients randomized: 114, sample size calculation: >600, follow-up 8 years). Severe kidney, cardiac, central nervous system events or death (http://garrodsymposium.com/garrod2016/posters/#p104; accessed July 18, 2016).
B) Registry data. Fabry Registry, patients treated with agalsidase beta. Severe kidney, cardiac, central nervous system events or death (27). Incidence of severe clinical events in first 6 months of ERT compared to next 5 years (1044 patients, follow-up 5 years). Note that as patients get 5 years older during the study, an increased incidence rate of events would be expected but the opposite was observed. S
In all trials ERT was used at label dose: 0.2 mg/kg/2 weeks for agalsidse alfa and 1.0 mg/kg/2 weeks for agalsidase beta. * Statistically significant difference, n.a. Not available, given the nature of the study. Arrows indicate that the same population is followed over time.

A) Randomized controlled trials

A.1)



A.2)



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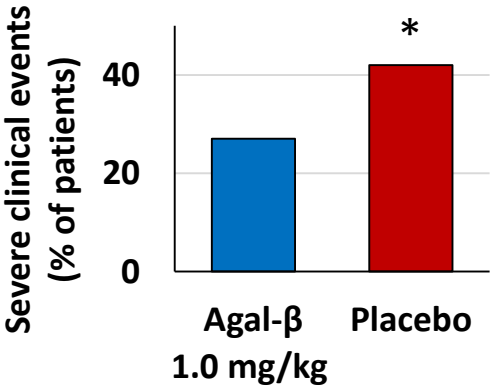
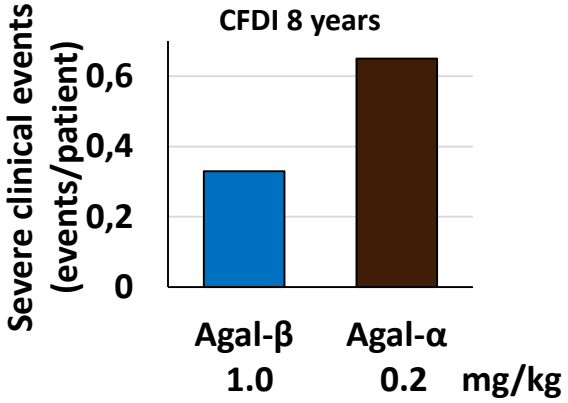


A.4)



B) Registry data