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Enzyme Replacement Therapy for Fabry Disease

Maria Dolores Sanchez-Niño, PhD[1] and Alberto Ortiz, MD, PhD[1]

Abstract

Fabry disease is a rare X-linked disease caused by the deficiency of α-galactosidase that leads to the accumulation of abnormal glycolipid. Untreated patients develop potentially lethal complications by age 30 to 50 years. Enzyme replacement therapy is the current standard of therapy for Fabry disease. Two formulations of recombinant human α-galactosidase A (agalsidase) are available in most markets: agalsidase-α and agalsidase-β, allowing a choice of therapy. However, the US Food and Drug Administration rejected the application for commercialization of agalsidase-α. The main difference between the 2 enzymes is the dose. The label dose for agalsidase-α is 0.2 mg/kg/2 weeks, while the dose for agalsidase-β is 1.0 mg/kg/2 weeks. Recent evidence suggests a dose-dependent effect of enzyme replacement therapy and agalsidase-β is 1.0 mg/kg/2 weeks, which has been shown to reduce the occurrence of hard end points (severe renal and cardiac events, stroke, and death). In addition, patients with Fabry disease who have developed tissue injury should receive coadjuvant tissue protective therapy, together with enzyme replacement therapy, to limit nonspecific progression of the tissue injury. It is likely that in the near future, additional oral drugs become available to treat Fabry disease, such as chaperones or substrate reduction therapy.

Keywords

Fabry disease, genetics, chronic kidney disease, therapy, dose, agalsidase

Introduction

Fabry disease is the result of a genetic deficiency of the lysosomal enzyme α-galactosidase A, encoded by the GLA gene that leads to the accumulation of glycosphingolipids, such as globotriaosylceramide (Gb3) and globotriaosylsphingosine (Lyso-Gb3). Globotriaosylsphingosine is considered to contribute to end-organ damage as it promotes podocyte injury and vascular smooth muscle cell proliferation.[1-4] Disease manifestations usually develop in childhood and include neuropathic pain, hypohidrosis, angiokeratoma, and digestive symptoms.[5] Excess glycolipid deposition is present from birth, and the first evidence of Fabry kidney injury, that is, effacement of podocyte foot processes in renal biopsies and development of pathological albuminuria, can also be observed in children.[6] Adults develop potentially lethal progressive chronic kidney disease, leading to the need for dialysis or transplantation, cardiovascular complications including arrhythmia and heart failure, and stroke.[5,7,8] Symptoms are usually more severe in males because of the X-linked nature of the genetic defect, but females may also develop severe disease.[9]

Current pathogenesis-based therapy involves enzyme replacement therapy (ERT) by the intravenous administration of human recombinant α-galactosidase A (agalsidase), either agalsidase-α (Shire, Cambridge, MA, USA) or agalsidase-β (Genzyme, a Sanofi Company, Cambridge, MA, USA) every 2 weeks.[10] In addition, patients with Fabry disease should receive coadjuvant tissue protective therapy, together with ERT, to limit nonspecific progression of tissue injury. The best example to date is antiproteinuric therapy with renin–angiotensin blockers to protect the kidney.[11,12] It is our personal opinion that in the absence of better Fabry disease-specific evidence, antiproteinuric therapy should be started as soon as albuminuria becomes pathologic (ie, above 30 mg/g creatinine), as is the case for another proteinuric kidney disease of metabolic

[1] Dialysis Unit, IIS-Fundación Jiménez Díaz, UAM, REDINREN, FRIAT, Madrid, Spain

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Corresponding Author:
Alberto Ortiz, MD, PhD, Dialysis Unit, IIS-Fundación Jiménez Díaz, UAM, REDINREN, FRIAT, Avd Reyes Católicos 2, 28040 Madrid, Spain.
Email: aortiz@fjd.es

origin (ie, diabetic kidney disease)[13,14] and for another genetic proteinuric nephropathy (ie, Alport syndrome).[15,16] It is likely that in the near future, additional oral drugs became available to treat Fabry disease, such as chaperones or substrate reduction therapy.[17,18] In this article, we will focus on ERT, currently available agalsidase formulations, indications to start therapy, dosing, and efficacy.

Enzyme Replacement Therapy

Currently, 2 agalsidase preparations are available in many countries. Agalsidase-α is available in Europe, Canada, Latin America, and other countries, while agalsidase-β is available in those same countries and additionally in the United States. Another agalsidase formulation is in clinical development: PRX-102, a plant-derived chemically modified agalsidase, resulting in a cross-linked homodimer that has a prolonged stability.[19]

Agalsidase-α and agalsidase-β are produced in different cell types: human fibrosarcoma cells for agalsidase-α and cells from hamster ovary (CHO) for agalsidase-β.[10] CHO cells are the industry standard for the production of human recombinant proteins. However, the 2 forms of agalsidase share the amino acid sequence and there are no major pharmacodynamic–pharmacokinetic differences as a consequence of the different cellular origin.[10] Both enzymes are distributed throughout the body and taken up mostly by the liver, though they also have access to the key target organs in Fabry disease, such as the heart and kidney. Surprisingly, the approved dose for the 2 enzymes is markedly different. Thus, agalsidase-α should be administered at a dose of 0.2 mg/kg/2 weeks, while agalsidase-β should be administered at a dose of 1.0 mg/kg/2 weeks.[10,20,21]

Agalsidase is infused intravenously over 40 to 120 minutes, the longer duration corresponding to agalsidase-β, since a higher amount of protein is administered. Infusion usually takes place in a hospital environment, though patients who tolerate the infusions well may receive it at home. The main adverse effects are infusion reactions common to other protein-based medications. These reactions may involve shaking chills, fever, and even dyspnea or chest tightness and are usually easily managed by pretreatment with acetaminophen, antihistamines, and in rare cases, steroids, as well as by transitorily stopping the infusion and reinitiating it at a slower pace. Anaphylactic reactions are distinctly uncommon, but they have occurred and may preclude further therapy. Antiagalsidase antibodies may develop in males who are unable to synthesize the protein. In some cases, they may result in neutralization of enzyme activity.[22]

A key issue of ERT is the cost that, although different from country to country, may be in the range of €150 000 per year.

Indications to Start ERT

When discussing the indication to start therapy, we should remember the mechanism of action of ERT. Enzyme replacement therapy clears glycolipid deposits, thus preventing further tissue injury or allowing repair of reversible tissue injury. However, it has no impact on irreversible tissue injury that has already occurred. As an example, kidneys that have reached end-stage renal disease and require renal replacement therapy will not recover the function on ERT. As a consequence, it is expected that ERT is most effective when started early in the course of the disease, that is, before irreversible tissue injury takes place. Once tissue injury has occurred, it is expected that nonspecific factors favoring the progression of tissue injury will remain active, despite ERT and clearance of glycolipid deposits. An example from everyday clinical practice may better illustrate this concept. Diabetic nephropathy takes 10 to 20 years to develop following the diagnosis of diabetes. This means that for 10 to 20 years, the kidney is progressively being injured. By the time tissue injury becomes clinically apparent in the form of albuminuria or decreased glomerular filtration rate, treatment of the metabolic defect alone (for example with insulin) does not prevent the progression of kidney disease to end-stage kidney disease requiring dialysis or transplantation. This is so because once the kidneys have been injured, kidney injury progresses as a consequence of the loss of functional renal mass and kidney infiltration by inflammatory cells, even when the original cause of kidney injury has been treated or has disappeared. For this reason, diabetic patients with pathological albuminuria or decreased glomerular filtration rate required nephroprotective therapy with renal–angiotensin system blockers.[13,23] Available information suggests that Fabry disease behaves similar to diabetic nephropathy and that ERT alone may slow but does not prevent the progression of tissue injury once it is present.[24]

Several guidelines have been issued on the subject of when to initiate ERT in Fabry disease.[25-29] When should ERT be started in Fabry disease? The answer appears to be deceptively easy: before irreversible tissue injury occurs. Ideally, as soon as any tissue injury leading to symptoms occurs, since we do not have a clear idea of the point at which tissue injury becomes irreversible. However, how does this apply to an individual patient? This is a disease present from birth. Should ERT be started, implying intravenous infusions every 2 weeks, in the newborn? Or maybe later? But exactly when? Currently, there is no consensus answer to these questions. If we had an oral drug to treat Fabry disease, there would be no such discussion about when to start. However, this is an expensive drug that is uncomfortable for patients. There is a general agreement that for classical Fabry disease in males, therapy should be started in childhood as these patients are often symptomatic in childhood and symptoms means that there is already tissue injury. Neuropathic pain is thus an indication to start therapy, as is hypohidrosis. It may be argued that pain may be treated with antipain medication. However, this does not prevent further glycolipid accumulation and further tissue injury. Thus, in our view, an indication to start therapy is whenever pain requires antipain medication. Another organ that is already injured in childhood is the kidney. However, usual pediatric care does not disclose evidence of kidney injury: In childhood, pathological albuminuria is the first manifestation of Fabry nephropathy. However, albuminuria is not routinely assessed in a healthy

child and many centers do not even assess albuminuria in patients with Fabry disease, relying instead on proteinuria strips. However, proteinuria strips may provide false-negative results when only pathological albuminuria but not pathological proteinuria is present or when urine is diluted because of high water ingestion. Thus, every child with Fabry disease should be routinely and repetitively tested for urinary albumin-to-creatinine ratio, and the appearance of pathological albuminuria should trigger the initiation of ERT. In this regard, there is recent evidence that the appearance of pathological albuminuria is a relatively late event in the course of Fabry nephropathy.[6,30] Thus, although pathological albuminuria usually develops in childhood, histological evidence of tissue injury usually precedes pathological albuminuria. Children with normoalbuminuria and Fabry disease may have glomerulosclerosis and podocyte foot process effacement as manifestations of tissue injury beyond glycolipid accumulation.[30] In this regard, renal biopsy may provide a more precise estimation of the degree of kidney injury than routine clinical laboratory assessments. Fabry cardiomyopathy is characterized by reduced myocardial contraction and relaxation tissue Doppler velocities, detectable even before the development of left ventricular hypertrophy. Tissue Doppler imaging may provide a preclinical diagnosis of Fabry cardiomyopathy, allowing early institution of ERT.[31] Despite these recommendations, most patients with Fabry disease receiving ERT today have started their therapy late: mean age at start of therapy in the Fabry registry is 40 years.[32-34] The reason for this late start in clinical practice is a combination of delays in the diagnosis of Fabry disease and unavailability of any therapy until quite recently. In addition to the indications to start therapy that we have already mentioned, ERT should be initiated, if it has not already been prescribed, whenever other evidence of tissue injury develops, including left ventricular hypertrophy, arrhythmia, heart failure, stroke, white matter lesions in the central nervous system, or decreased glomerular filtration rate. However, starting at this point is starting late as tissue injury is severe enough to result in clinical manifestations. Ideally, all classic males should be starting on ERT as soon as it is feasible at the earliest clinical manifestations of the disease.

Females with Fabry disease pose a different problem. While it is widely recognized that females may develop manifestations of Fabry disease as severe as males, when lyonization leads mainly to inactivation of the wild-type GLA allele, it is also true that some females with Fabry disease may be asymptomatic their whole life, when lyonization results in the inactivation mainly of the mutated GLA allele.[9] Thus, for females, it is not correct, in the current stage of knowledge, to start therapy in the absence of symptoms of Fabry disease or when these symptoms are mild, since we are unable to predict whether severe symptoms will ever develop. Thus, initiation of therapy for females may not be required until adulthood and we will rely on evidence of tissue injury to indicate ERT, such as neuropathic pain that requires medication or other laboratory or imaging evidence of injury to the kidney, heart, or central nervous system, including pathological albuminuria (>30 mg/g creatinine), decreased glomerular filtration rate (<60 mL/min/1.73 m^2), left ventricular hypertrophy (cardiac mass above normal for age), heart fibrosis as evidenced in heart magnetic resonance imaging, arrhythmia, heart failure, stroke, or white matter lesions in the central nervous system. Since having Fabry disease does not protect from other causes of kidney, heart, or central nervous system injury, it is reasonable to perform studies that support causality between Fabry disease and these manifestations, especially in older women. In the future, tools may be developed that allow prediction of which females will present severe Fabry disease. Thus, these patients will benefit from an early start of therapy, even in the absence of symptoms, while patients who remain asymptomatic will not be subjected to ERT. In this regard, a recent publication by Germain et al holds promise: Assessment of the degree of lyonization of the 2 GLA alleles was well correlated with the severity of the disease.[35]

Another population that is problematic from the point of view of indication of therapy is composed of patients with nonclassical, also called late-onset, Fabry disease. In recent years, it has become apparent that not all of the more than 800 GLA mutations are associated with the same severity of Fabry disease.[36,37] Thus, some mutations are clearly associated with late-onset disease, such as N215S.[38] These patients may lack the classical pediatric manifestations of Fabry disease including neuropathic pain, hypohidrosis, and angiokeratoma. They usually do not develop Fabry nephropathy, but they do have cardiac manifestations that may be symptomatic from the fifth decade of life. It is clearly likely that these patients will not benefit from start of therapy in childhood, but the best timing to initiate ERT and whether ERT prevents severe clinical events remain unknown. Current pathophysiological knowledge suggests that ERT should be started before irreversible tissue injury and that may mean before the development of left ventricular hypertrophy. On the other hand, these patients do not have endothelial accumulation of glycolipids. Rather, glycolipids accumulate in long-lived cells such as cardiomyocytes and podocytes.[38] Thus, not all currently available agalsidase formulations may be appropriate for these patients. Although they may be considered having less severe disease and then expected to benefit from lower-dose agalsidase, we do know that lower-dose agalsidase may not be able to clear podocytes as effectively as higher-dose agalsidase, though both formulations are able to clear endothelial cells.[6] Thus, lower-dose agalsidase may not be appropriate for disease limited to podocytes and cardiomyocytes. The issue of nonclassical Fabry disease is currently unsolved, and in part this is due to the fact that for many GLA mutations, it is unknown whether the phenotype is classical or nonclassical or may be both in different families, depending on as yet uncharacterized factors.[32,39-41] The individual physicians may get some insights from the clinical manifestations of the patient family. If neuropathic pain, hypohidrosis, and angiokeratoma are present from childhood, it will probably be classical Fabry disease. However, mutations linked to nonclassical Fabry disease are more common in the general population and are the most frequent mutations found

in screening programs for patients at risk for Fabry disease, such as those on renal replacement therapy, with left ventricular hypertrophy, or with stroke.[37,42-44] Thus, when this type of screening identifies a patient with a previously undescribed mutation, it is difficult to offer therapeutic advice, especially if there is no tissue confirmation that the clinical manifestation leading to the screening effort was indeed caused by Fabry disease.

Dosing and Efficacy of ERT

Agalsidase-α and agalsidase-β obtained a market authorization based on placebo-controlled randomized clinical trials (RCTs) that assess short-term effects on pain (primary end point for agalsidase-α) or endothelial glycolipid deposits (secondary end point for agalsidase-α, primary end point for agalsidase-β).[20,21] However, both patients and physicians expect the drugs to prevent the long-term consequences of Fabry disease, namely, end-stage kidney disease, severe cardiovascular events, and stroke. There is no clinical trial–derived evidence that agalsidase-α prevents these events, since no clinical trial with such a primary point has been published. By contrast, agalsidase-β showed, in the largest placebo-controlled clinical trial of agalsidase performed to date, that it reduces the incidence rate of hard end points and severe clinical events (end-stage kidney disease, severe cardiovascular events, stroke, death) by 40% within a 3-year time frame in patients who already had moderately severe kidney disease.[45] Baseline clinical characteristics of the groups (placebo and agalsidase-β) were unbalanced.[45] Patients taking agalsidase-β were older and had 53% higher proteinuria, the main risk factor for the progression of chronic kidney disease in Fabry nephropathy.[33,34] After adjustment for this imbalance, the decrease in the incidence rate of events was 61% ($P = .034$). Thus, both drugs clear endothelial deposits and they are expected to improve any clinical manifestations derived from endothelial glycolipid deposition, but only agalsidase-β has been shown to reduce the incidence rate of severe events.

We have additional information on the efficacy of both drugs derived from registry data.[24,34,46-48] Since Fabry disease is rare, it is not feasible to perform the kind of trials that will establish efficacy on hard end points in an intention-to-treat basis as compared to placebo. Thus, regulatory authorities requested both companies selling agalsidase to maintain a registry that allows evaluating performance (safety and efficacy) of the drug in clinical practice. Data derived from both registries have reported stabilization of disease manifestations, especially if ERT is started early in the course of the disease. However, only the Fabry registry has reported in a large (>1000 patients) observational analysis that, consistent with the placebo-controlled clinical trial results, agalsidase-β 1.0 mg/kg/2 weeks is associated with a 50% decreased incidence rate of hard end points, defined as severe clinical events (end-stage kidney disease, severe cardiovascular events, stroke, death) over time, contrary to the expected findings of an increasing rate of severe clinical event over time, associated with aging, in natural history patients.[32] This is also consistent with long-term follow-up data of patients enrolled in the pivotal agalsidase-β trial.[24,49] These studies also illustrated that renal biopsies may detect more severe kidney disease than suggested by the assessment of glomerular filtration rate. By contrast, no long-term follow-up is available for patients in the pivotal agalsidase-α trial.

We should also mention recent evidence suggesting that the dose of agalsidase is important, derived from histological studies in children assessing podocyte glycolipid deposition, studies on the progressive loss of glomerular filtration rate in response to doubling of the cumulative dose of agalsidase-α, and a head-to-head clinical trial of agalsidase-α 0.2 mg/kg/2 weeks versus agalsidase-β 1.0 mg/kg/2 weeks, which is ongoing in Canada.[6,50,51]

A case series of children and young adults with Fabry disease who had a baseline renal biopsy and a second renal biopsy performed after 5 years of ERT, with either agalsidase-α or agalsidase-β, disclosed that only patients receiving the highest cumulative dose over those 5 years (that is patients on agalsidase-β 1.0 mg/kg/2 weeks) cleared glycolipids from podocytes and a decrease in albuminuria was only observed in patients who cleared podocytes.[6] In contrast to the findings in podocytes, any dose of ERT cleared the endothelium as had been demonstrated in pivotal clinical trials by the 2 enzyme formulations.[6]

Schiffmann et al recently reported the 10-year follow-up of patients who were losing glomerular filtration rate at a significant rate while on agalsidase-α 0.2 mg/kg/2 weeks for 2 to 4 years and were then switched to agalsidase-α 0.2 mg/kg/weekly, effectively doubling the cumulative agalsidase-α dose.[51] This resulted in slower progression of chronic kidney disease. However, it is likely that the switch occurred too late: 50% of the patients ended up requiring renal replacement therapy. They may have benefited from a higher dose from an earlier time point in the course of the disease.

A preliminary report of the Canadian Fabry Disease Initiative clinical trial disclosed that patients randomized to agalsidase-α 0.2 mg/kg/2 weeks had a 50% higher incidence rate of severe clinical event than those randomized to agalsidase-β 1.0 mg/kg/2 weeks.[50] Although this difference may potentially have a huge clinical impact, the difference was not statistically significant since the study was greatly (almost 6-fold) underpowered. To illustrate the impact of an adequately powered trial on statistical significance, the Study of Heart and Renal Protection (SHARP) trial of statins versus placebo in patients with chronic kidney disease observed a 17% reduction in severe events and this was highly significant, but it required the randomization of almost 10 000 patients who were followed for 5 years.[52]

Additional evidence of dose will likely be derived from publications on the consequence of the agalsidase shortage, which developed in 2009. During this period, issues with the fabrication of agalsidase-β precluded adequate dosing of hundreds of patients throughout the globe who were switched to agalsidase-α 0.2 mg/kg/2 weeks or lower-dose agalsidase-β. In the largest report to date, the switch to a lower dose of enzyme was associated with a faster loss of renal function.[53]

When Not to Initiate or When to Stop ERT in Classically Affected Males

Given the high cost of ERT and the fact that it is uncomfortable for patients, there is some debate about when not to initiate ERT if end-organ damage is advanced or even when to discontinue ERT. In general, clinical judgment and in-depth discussion with patients and family members are required when considering these decisions. A European Fabry Working Group consensus document addressed these issues.[25] This document was very cautious but identified some situations in which stopping ERT may be considered. These included patients with end-stage Fabry disease or other comorbidities, leading to a life expectancy of <1 year; cognitive decline of any cause; lack of response for 1 year when the sole indication for ERT is neuropathic pain; stopping ERT; combined end-stage renal and heart disease, without an option for renal transplantation; and noncompliance. However, it should be emphasized that end-stage kidney disease alone is not the reason to stop therapy, since these patients are still at risk of heart disease and stroke and may have symptoms related to Fabry disease such as neuropathic pain. In the same situations, not initiating ERT should be considered. The Dutch group has the most extensive published experience stopping ERT.[54]

Conclusion

Enzyme replacement therapy is the only therapy that is marketed for and has proven both efficacy and safety in Fabry disease. Thus, ERT should be the basis of Fabry disease therapy and should not be replaced by symptomatic therapy that improves the symptoms but allows glycolipid accumulation and tissue injury to progress to irreversible organ damage.

Enzyme replacement therapy should be started as soon as feasible in males with classical Fabry disease. However, for females, Fabry manifestations must develop before the indication of therapy. The correct timing of ERT initiation for non-classical or late-onset Fabry disease is unclear.

Most countries have 2 agalsidase formulations available: agalsidase-α and agalsidase-β. Strikingly, the authorized dose of agalsidase-β is 5 times higher than the authorized dose of agalsidase-α. Recently, accumulated evidence suggests that the higher dose (1.0 mg/kg/2 weeks) may be advantageous at least for some patients and it is the only dose that has shown to decrease the incidence rate of severe clinical events in a placebo-controlled clinical trial. In this regard, there is evidence from a placebo-controlled RCT and from a large (>1000 patients) observational study supporting the efficacy of agalsidase-β 1.0 mg/kg/2 weeks in preventing hard end points related to heart and renal disease, while such evidence is not available for agalsidase-α 0.2 mg/kg/2 weeks. It is likely that in the near future, additional oral drugs become available to treat Fabry disease, such as chaperones or substrate reduction therapy. Oral agents may facilitate early initiation of therapy.

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References

1. Sanchez-Nino MD, Sanz AB, Carrasco S, et al. Globotriaosylsphingosine actions on human glomerular podocytes: implications for Fabry nephropathy. *Nephrol Dial Transplant*. 2011; 26(6):1797-1802.
2. Sanchez-Nino MD, Carpio D, Sanz AB, Ruiz-Ortega M, Mezzano S, Ortiz A. Lyso-Gb3 activates Notch1 in human podocytes. *Hum Mol Genet*. 2015;24(20):5720-5732.
3. Weidemann F, Sanchez-Nino MD, Politei J, et al. Fibrosis: a key feature of Fabry disease with potential therapeutic implications. *Orphanet J Rare Dis*. 2013;8:116.
4. Aerts JM, Groener JE, Kuiper S, et al. Elevated globotriaosylsphingosine is a hallmark of Fabry disease. *Proc Natl Acad Sci U S A*. 2008;105(8):2812-2817.
5. Germain DP. Fabry disease. *Orphanet J Rare Dis*. 2010;5:30.
6. Tondel C, Bostad L, Larsen KK, et al. Agalsidase benefits renal histology in young patients with Fabry disease. *J Am Soc Nephrol*. 2013;24(1):137-148.
7. Ortiz A, Oliveira JP, Waldek S, Warnock DG, Cianciaruso B, Wanner C; Fabry Registry. Nephropathy in males and females with Fabry disease: cross-sectional description of patients before treatment with enzyme replacement therapy. *Nephrol Dial Transplant*. 2008;23(5):1600-1607.
8. Ortiz A, Cianciaruso B, Cizmarik M, et al. End-stage renal disease in patients with Fabry disease: natural history data from the Fabry Registry. *Nephrol Dial Transplant*. 2010;25(3):769-775.
9. Wilcox WR, Oliveira JP, Hopkin RJ, et al. Females with Fabry disease frequently have major organ involvement: lessons from the Fabry Registry. *Mol Genet Metab*. 2008;93(2):112-128.
10. Desnick RJ. Enzyme replacement therapy for Fabry disease: lessons from two alpha-galactosidase A orphan products and one FDA approval. *Expert Opin Biol Ther*. 2004;4(7):1167-1176.
11. Warnock DG, Thomas CP, Vujkovac B, et al. Antiproteinuric therapy and Fabry nephropathy: factors associated with preserved kidney function during agalsidase-beta therapy. *J Med Genet*. 2015;52(12):860-866.
12. Perez-Gomez MV, Sanchez-Nino MD, Sanz AB, et al. Horizon 2020 in diabetic kidney disease: the clinical trial pipeline for add-

on therapies on top of renin angiotensin system blockade. *J Clin Med*. 2015;4(6):1325-1347.
13. American Diabetes Association. 9. Microvascular complications and foot care. *Diabetes Care*. 2016;39(suppl 1):S72-S80.
14. Schievink B, Kropelin T, Mulder S, et al. Early renin-angiotensin system intervention is more beneficial than late intervention in delaying end-stage renal disease in patients with type 2 diabetes. *Diabetes Obes Metab*. 2016;18(1):64-71.
15. Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F. Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. *J Am Soc Nephrol*. 2013; 24(3):364-375.
16. Gross O, Licht C, Anders HJ, et al. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. *Kidney Int*. 2012;81(5):494-501.
17. Ashe KM, Budman E, Bangari DS, et al. Efficacy of enzyme and substrate reduction therapy with a novel antagonist of glucosylceramide synthase for Fabry disease. *Mol Med*. 2015;21:389-399.
18. Warnock DG, Bichet DG, Holida M, et al. Oral migalastat HCl leads to greater systemic exposure and tissue levels of active alpha-galactosidase A in Fabry patients when co-administered with infused agalsidase. *PLoS One*. 2015;10(8):e0134341.
19. Kizhner T, Azulay Y, Hainrichson M, et al. Characterization of a chemically modified plant cell culture expressed human alpha-galactosidase-A enzyme for treatment of Fabry disease. *Mol Genet Metab*. 2015;114(2):259-267.
20. Schiffmann R, Kopp JB, Austin HA III, et al. Enzyme replacement therapy in Fabry disease: a randomized controlled trial. *JAMA*. 2001;285(21):2743-2749.
21. Eng CM, Guffon N, Wilcox WR, et al; International Collaborative Fabry Disease Study Group. Safety and efficacy of recombinant human alpha-galactosidase A—replacement therapy in Fabry's disease. *N Engl J Med*. 2001;345(1):9-16.
22. Lenders M, Stypmann J, Duning T, Schmitz B, Brand SM, Brand E. Serum-mediated inhibition of enzyme replacement therapy in Fabry disease. *J Am Soc Nephrol*. 2016;27(1):256-264.
23. Fernandez-Fernandez B, Ortiz A, Gomez-Guerrero C, Egido J. Therapeutic approaches to diabetic nephropathy—beyond the RAS. *Nat Rev Nephrol*. 2014;10(6):325-346.
24. Germain DP, Charrow J, Desnick RJ, et al. Ten-year outcome of enzyme replacement therapy with agalsidase beta in patients with Fabry disease. *J Med Genet*. 2015;52(5):353-358.
25. Biegstraaten M, Arngrimsson R, Barbey F, et al. Recommendations for initiation and cessation of enzyme replacement therapy in patients with Fabry disease: the European Fabry Working Group consensus document. *Orphanet J Rare Dis*. 2015;10:36.
26. Eng CM, Germain DP, Banikazemi M, et al. Fabry disease: guidelines for the evaluation and management of multi-organ system involvement. *Genet Med*. 2006;8(9):539-548.
27. Ortiz A, Oliveira JP, Wanner C, Brenner BM, Waldek S, Warnock DG. Recommendations and guidelines for the diagnosis and treatment of Fabry nephropathy in adults. *Nat Clin Pract Nephrol*. 2008;4(6):327-336.
28. Linthorst GE, Germain DP, Hollak CE, et al; European Medicines Agency. Expert opinion on temporary treatment recommendations for Fabry disease during the shortage of enzyme replacement therapy (ERT). *Mol Genet Metab*. 2011;102(1):99-102.
29. Terryn W, Cochat P, Froissart R, et al. Fabry nephropathy: indications for screening and guidance for diagnosis and treatment by the European Renal Best Practice. *Nephrol Dial Transplant*. 2013;28(3):505-517.
30. Tondel C, Kanai T, Larsen KK, et al. Foot process effacement is an early marker of nephropathy in young classic Fabry patients without albuminuria. *Nephron*. 2015;129(1):16-21.
31. Pieroni M, Chimenti C, Ricci R, Sale P, Russo MA, Frustaci A. Early detection of Fabry cardiomyopathy by tissue Doppler imaging. *Circulation*. 2003;107(15):1978-1984.
32. Ortiz A, Abiose A, Bichet DG, et al. Time to treatment benefit for adult patients with Fabry disease receiving agalsidase beta: data from the Fabry Registry. *J Med Genet*. 2016;53(7):495-502.
33. Wanner C, Oliveira JP, Ortiz A, et al. Prognostic indicators of renal disease progression in adults with Fabry disease: natural history data from the Fabry Registry. *Clin J Am Soc Nephrol*. 2010;5(12):2220-2228.
34. Warnock DG, Ortiz A, Mauer M, et al. Renal outcomes of agalsidase beta treatment for Fabry disease: role of proteinuria and timing of treatment initiation. *Nephrol Dial Transplant*. 2012; 27(3):1042-1049.
35. Echevarria L, Benistan K, Toussaint A, et al. X-chromosome inactivation in female patients with Fabry disease. *Clin Genet*. 2016;89(1):44-54.
36. van der Tol L, Svarstad E, Ortiz A, et al. Chronic kidney disease and an uncertain diagnosis of Fabry disease: approach to a correct diagnosis. *Mol Genet Metab*. 2015;114(2):242-247.
37. Ferreira S, Ortiz A, Germain DP, et al. The alpha-galactosidase A p.Arg118Cys variant does not cause a Fabry disease phenotype: data from individual patients and family studies. *Mol Genet Metab*. 2015;114(2):248-258.
38. Meehan SM, Junsanto T, Rydel JJ, Desnick RJ. Fabry disease: renal involvement limited to podocyte pathology and proteinuria in a septuagenarian cardiac variant. Pathologic and therapeutic implications. *Am J Kidney Dis*. 2004;43(1):164-171.
39. Lenders M, Weidemann F, Kurschat C, et al. Alpha-galactosidase A p.A143 T, a non-Fabry disease-causing variant. *Orphanet J Rare Dis*. 2016;11(1):54.
40. Saito S, Ohno K, Sakuraba H. Fabry-database.org: database of the clinical phenotypes, genotypes and mutant alpha-galactosidase A structures in Fabry disease. *J Hum Genet*. 2011;56(6):467-468.
41. Abstracts 4th Update on Fabry Nephropathy: Biomarkers, Progression and Treatment Opportunities. *Nephron*. 2015;130:77-91.
42. Herrera J, Miranda CS. Prevalence of Fabry's disease within hemodialysis patients in Spain. *Clin Nephrol*. 2014;81(2): 112-120.
43. Monserrat L, Gimeno-Blanes JR, Marin F, et al. Prevalence of fabry disease in a cohort of 508 unrelated patients with hypertrophic cardiomyopathy. *J Am Coll Cardiol*. 2007;50(25): 2399-2403.
44. Baptista MV, Ferreira S, Pinho-E-Melo, et al. Mutations of the GLA gene in young patients with stroke: the PORTYSTROKE study—screening genetic conditions in Portuguese young stroke patients. *Stroke*. 2010;41(3):431-436.

45. Banikazemi M, Bultas J, Waldek S, et al; Fabry Disease Clinical Trial Study Group. Agalsidase-beta therapy for advanced Fabry disease: a randomized trial. *Ann Intern Med*. 2007;146(2):77-86.
46. Germain DP, Weidemann F, Abiose A, et al. Analysis of left ventricular mass in untreated men and in men treated with agalsidase-beta: data from the Fabry Registry. *Genet Med*, 2013;15(12):958-965.
47. Waldek S, Germain DP, Wanner C, Warnock DG. Enzyme replacement therapy for Fabry's disease. *Lancet*. 2010; 375(9725):1523-1524.
48. Mehta A, Beck M, Elliott P, et al. Enzyme replacement therapy with agalsidase alfa in patients with Fabry's disease: an analysis of registry data. *Lancet*. 2009;374(9706):1986-1996.
49. Germain DP, Waldek S, Banikazemi M, et al. Sustained, long-term renal stabilization after 54 months of agalsidase beta therapy in patients with Fabry disease. *J Am Soc Nephrol*. 2007;18(5): 1547-1557.
50. Sirrs SM, Bichet DG, Casey R, et al. Outcomes of patients treated through the Canadian Fabry disease initiative. *Mol Genet Metab*. 2014;111(4):499-506.
51. Schiffmann R, Swift C, Wang X, Blankenship D, Ries M. A prospective 10-year study of individualized, intensified enzyme replacement therapy in advanced Fabry disease. *J Inherit Metab Dis*. 2015;38(6):1129-1136.
52. Baigent C, Landray MJ, Reith C, et al; SHARP Investigators. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. *Lancet*. 2011;377(9784):2181-2192.
53. Lenders M, Canaan-Kuhl S, Kramer J, et al. Patients with Fabry disease after enzyme replacement therapy dose reduction and switch-2-year follow-up. *J Am Soc Nephrol*. 2016;27(3):952-962.
54. Arends M, Linthorst GE, Hollak CE, Biegstraaten M. Discontinuation of enzyme replacement therapy in Fabry disease in the Dutch cohort. *Mol Genet Metab*. 2016;117(2):194-198.