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Fabry disease: current treatment and future perspective

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Fabry disease (FD), a rare X-linked lysosomal storage disorder, is caused by mutations in the *α-galactosidase A gene* gene encoding *α-galactosidase A* (α-Gal A). The functional deficiency of α-Gal A results in progressive accumulation of neutral glycosphingolipids, causing multi-organ damages including cardiac, renal, cerebrovascular systems. The current treatment is comprised of enzyme replacement therapy (ERT), oral pharmacological chaperone therapy and adjunctive supportive therapy. ERT has been introduced 20 years ago, changing the outcome of FD patients with proven effectiveness. However, FD patients have many unmet needs. ERT needs a life-long intravenous therapy, inefficient bio-distribution, and generation of anti-drug antibodies. Migalastat, a pharmacological chaperone, augmenting α-Gal A enzyme activity only in patients with mutations amenable to the therapy, is now available for clinical practice. Furthermore, these therapies should be initiated before the organ damage becomes irreversible. Development of novel drugs aim at improving the clinical effectiveness and convenience of therapy. Clinical trial of next generation ERT is underway. Polyethylene glycolylated enzyme has a longer half-life and potentially reduced antigenicity, compared with standard preparations with longer dosing interval. Moss-derived enzyme has a higher affinity for mannose receptors, and seems to have more efficient access to podocytes of kidney which is relatively resistant to reach by conventional ERT. Substrate reduction therapy is currently under clinical trial. Gene therapy has now been started in several clinical trials using *in vivo* and *ex vivo* technologies. Early results are emerging. Other strategic approaches at preclinical research level are stem cell-based therapy with genome editing and systemic mRNA therapy.

Key words: Fabry disease, Next-generation enzyme replacement therapy, Chaperone therapy, Substrate reduction therapy, Gene therapy, mRNA therapy.

Introduction

Fabry disease (FD, Online Medelian Inheritanc in Man #301500), a rare X-linked lysosomal storage disorder, is caused by mutations in the *α-galactosidase A* (*GLA*) gene encoding α-galactosidase A (α-Gal A). The functional deficiency of α-Gal A accumulates glycosphingolipids, including globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) in cells, which lead to tissue damage and progress to multi-organ failure involving the kidney, heart, and nervous systems [1].

The prevalence of classic FD, an X-linked disorder, is estimated at 1 in 40,000 to 1 in 117,000 males. The first newborn screening study (NBS) was conducted in Italy and revealed an unexpectedly high prevalence of male Fabry patients (1:3,100) with the vast majority, based on their genotypes, presumed to have the late-onset variant of the disease [2]. Subsequently, these findings were confirmed in Taiwan (1:1,250 male live births) [3]. Overall, the prevalence of late onset FD is 7-10 folds higher than classic FD. NBS studies revealed frequencies of the classic and late onset (non-classic) phenotypes of up to 1 in 22,570 males and

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1 in 1,390 males, respectively [3]. The results of high-risk group screening of FD recently revised the prevalence estimates as 0.21% males and 0.15% females among hemodialysis screenees, 0.25% males of renal transplant screenees, 0.94% males and 0.90% females among cardiac screenees, and 0.94% males and 0.90% females among stroke screenees [4].

α-Gal A is a homodimeric glycoprotein encoded by the *GLA* gene which is located on the long arm of the X chromosome. Numerous *GLA* mutations are currently reported in gene mutation databases. Missense, nonsense, consensus splice site, cryptic splicing, and frameshift mutations (small and large deletions and insertions) cause FD. In general, nonsense, consensus splice site, and most frameshift mutations result in little or no α-Gal A enzyme activity, and are associated with the classic phenotype. In contrast, a proportion of the missense mutations and rare cryptic splicing mutations can encode enzymes with residual α-Gal A activity, which may explain the late-onset, non-classic phenotypes. As of March, 2023, more than 1,100 different mutations of the *GLA* gene have been described in FD. FD is a pan-ethnic disease, but some countries have very common mutation, for instances, IVS4+919G>A in Taiwanese FD patients and p. N215S in UK (https://www.hgmd.cf.ac.uk/ac/index.php). These mutations cause late onset cardiac phenotype. Also, the presence of pseudo-deficiency allele may cause problems interpreting biochemical and clinical data. Particularly, in East Asian countries, the p.E66Q variant is common, highly prevalent as 1% of newborn showing about 30% of residual α-Gal A activity [1].

Classic FD patients are characterized by absent or severely reduced (<1% of mean normal) α-Gal A activity with substantial globotriaosylceramide (GL-3) accumulation in various cells such as vascular endothelial cells, cardiomyocytes, smooth muscle cells, and podocytes. Symptoms usually develop in childhood or adolescent, eventually suffering from progressive multiorgan failure. They are mostly males, experiencing multisystem symptoms such as acroparesthesia, hypohidrosis, angiokeratoma, corneal opacity, hearing loss, and progress to renal failure, cardiovascular disease, and stroke. Chronic neuropathic pain and episodic severe pain crises are the first symptoms, typically manifest during childhood. Symptoms such as angiokeratomas, nonspecific gastrointestinal symptoms, hypohidrosis, and asymptomatic corneal clouding (cornea verticillata) are accompanying early presentations. Early renal pathologic change begins without clinical symptom or subtle microalbuminuria at a young age, indicating renal injury. Morbidities of multiple vital organs are usually observed even in relatively young male adult patients. They are progressive involving chronic kidney disease (CKD) with progression to renal failure and left ventricular hypertrophy (LVH) with myocardial fibrosis and arrhythmias, transient ischemic attacks (TIAs), strokes, hearing loss, and eventually cause premature death. The spectrum of disease in heterozygous female patients ranges from being asymptomatic or having mild, late-onset phenotypes to the severe phenotype (as observed in male patients with the classic disease phenotype). Female patients typically develop disease complications at older ages than male patients, although renal failure may manifest at a similar mean age in female patients with a skewed X inactivation pattern and predominant expression of the mutant *GLA* allele. However, a larger group of patients has late-onset phenotypes with varying levels of residual α-Gal A activity, age of onset, and manifestations [5-9].

Non-classic FD are late-onset and mild phenotypes, who show reduced but some residual α-Gal A activity, usually higher than 1-2% of the enzyme activity. Non-classic, late-onset Fabry disease usually presents two clinical phenotypes, cardiac and renal type without evident neuronopathic pain, or angiokeratoma [1,10].

Current therapy for Fabry disease

1. Enzyme Replacement Therapy

1) Preparations of Enzyme Replacement Therapy

Enzyme replacement therapy (ERT) with recombinant human α-Gal A significantly reduces Gb3/Lyso-Gb3 accumulation and improves the clinical outcomes of FD. The first ERT for Fabry disease, agalsidase beta (Fabrazyme®) was approved by the US Food and Drug Administration (FDA) in 2003. In Korea, first ERT with this enzyme was initiated in 2004. This treatment is administered intravenously at 1.0 mg/kg body weigh every two weeks and is designed to reduce the accumulation of GL-3 in the body, which can help to alleviate symptoms and slow the progression of the disease. It is produced in Chinese hamster ovary (CHO) cells. Infusion duration is about 3-4 hours. Depending on individual tolerance, the infusion duration may be gradually reduced to 90 minutes.

The other form of ERT is agalsidase alfa (Replagal®) is administered at 0.2 mg/kg body weight every other week by intravenous (IV) infusion and is approved in many countries throughout the world, though not by the US FDA. It is produced in a human cell line (human fibrosarcoma cells HT-1080), with the infusion duration of approximately 40 minutes In 2014, Fabagal®, an agalsi-

dase beta manufactured by ISU Abxis (Seongnam-si, Gyeonggi-do, South Korea), was approved in South Korea only, which is produced in CHO cells [1,11-13].

2) Effectiveness of Current Enzyme Replacement Therapy

The effectiveness of Fabrazyme has been evaluated in several clinical studies and real-world data [14-21]. The first clinical study has proven its efficacy and safety in 2001, leading to the approval by US FDA. In randomized, placebo-controlled double-blind study, it demonstrated 20 of the 29 patients in the recombinant α-galactosidase A group (69 percent) had no microvascular endothelial deposits of globotriaosylceramide after 20 weeks, as compared with none of the 29 patients in the placebo group (P<0.001), with the reduction of microvascular endothelial deposits of globotriaosylceramide in the skin (P<0.001) and heart (P<0.001) [22].

In the same year, the result of double-blind placebo-controlled trial of agalsidase alfa was published. Mean BPI neuropathic pain severity score declined from 6.2 (0.46) to 4.3 (0.73) in patients treated with α-gal A vs no significant change in the placebo group (P=0.02). In the kidney, glomeruli with mesangial widening decreased by a mean of 12.5% for patients receiving agalsidase alfa vs. a 16.5% increase for placebo (P=0.01). Mean inulin clearance decreased by 6.2 mL/min for patients receiving α-gal A vs. 19.5 mL/min for placebo (P=0.19). Mean creatinine clearance increased by 2.1 mL/min (0.4 mL/s) for patients receiving α-gal A vs. a decrease of 16.1 mL/min (0.3 mL/s) for placebo (P=0.02). In patients treated with α-gal A, there was an approximately 50% reduction in plasma glycosphingolipid levels, a significant improvement in cardiac conduction, and a significant increase in body weight. However, US FDA does not approve it, because US FDA required further clinical trial but the company withdrew FDA application [23].

However, a systematic review and meta-analysis on the natural course of FD and the effectiveness of ERT described that it is effective in reducing LVH, but has a limited effect on renal function. There is still unmet need for improved treatment options. Thirty-one studies were systematically reviewed while six studies were included in the meta-analysis. In patients with a glomerular filtration rate (GFR)>60 mL/min/1.73 m^2, decline of renal function was similar for treated and untreated patients. Only ERT treated males with a GFR<60 mL/min/1.73 m^2 had a slower rate of decline in renal function, possibly attributable to anti-proteinuric therapy. Regardless of LVH at baseline, left ventricular (LV) mass remained stable or increased in males despite ERT, however at a slower rate compared to untreated male patients. In ERT treated females with LVH, LV mass decreased, and remained stable in females without LVH. White matter lesions (WMLs) cannot be prevented by ERT. Stroke, cardiac and end-stage renal complications develop, though the incidence of new complications seems to be reduced during ERT [5].

There have been lots of discussions and conflicting data about which preparation is more effective. One systemic review paper including 77 cohort studies recruiting 15,305 subjects, reported that agalsidase beta is associated with a significantly lower incidence of renal, cardiovascular, and cerebrovascular events than no ERT, and a significantly lower incidence of cerebrovascular events than agalsidase alfa. The pooled proportions were as follows: a) for renal complications, agalsidase alfa 15.3% [95% CI 0.048, 0.303; I2=77.2%, P=0.0005]; agalsidase beta 6% [95% CI 0.04, 0.07; I2=not applicable]; and untreated patients 21.4% [95% CI 0.1522, 0.2835; I2=89.6%, P<0.0001]. Effect differences tended to favor agalsidase beta over agalsidase alfa or untreated patients [24].

Switch studies data during agalsidase beta shortage showed that a switch from agalsidase beta to alfa was generally safe. However, some of papers reported a significant loss of renal function, not reversed even by higher dosages, and an increase in lyso Gb3 levels after the switch; on the contrary, the switch-back to agalsidase beta resulted in a decrease in lyso-Gb3 levels. These results confirm a dose-dependent effect of agalsidase on Gb3 clearance and suggest the importance of dose in FD treatment and recommend a meticulous surveillance in patients with dose reduction [25].

An international cohort study on comparison of effectiveness of agalsidase alfa versus agalsidase beta in FD patients was conducted in 387 FD patients. It revealed a similar clinical event rate for both enzymes (HR 0.96, P=0.87). The decrease in plasma lysoGb3 was greater following treatment with agalsidase beta, specifically in men with classic FD, persisting in the presence of antibodies. The risk to develop antibodies was higher for patients treated with agalsidase beta (OR 2.8, P=0.04). left ventricular mass index (LVMI) decreased in a higher proportion following the first year of agalsidase beta treatment (OR 2.27, P=0.03), while eGFR slopes were similar [26].

A review paper on clinical relevance of globotriaosylceramide accumulation in FD and the effect of agalsidase beta in affected tissues reported that agalsidase beta is effective in substantially clearing Gb3 in a range of cells from the tissues affected by FD. Agalsidase beta has also been shown to slow renal decline and lower the overall risk of clinical progression, demonstrating an indirect link between treatment-related Gb3 clearance and sta-

bilization of FD [25].

3) Influencing Factors on the Effectiveness of Enzyme Replacement Therapy

Phenotype (classic vs. non-classic FD), patient's sex, and initiation age of ERT are all contributing factors, influencing the effectiveness of ERT. Another factor influencing the response to ERT is the generation of anti-drug antibodies (ADAs) against recombinant alpha-galactosidase A (r-αGal A). ADAs can negatively influence ERT effectiveness by pharmacodynamic as well as pharmacokinetic alterations. ADAs inhibit the catalytic activity of r-αGal A *in vitro* (iADAs) in about half of classic male FD patients, resulting in a less robust reduction of plasma lysoGb3 levels as well as with increased excretion of urinary Gb3 levels. The presence of high iADAs may lead to an accelerated decline of renal function. Whether ADAs inhibit enzyme uptake in the target cells remains to be elucidated. Another factor contributing to the limited effectiveness of ERT is the inefficient biodistribution. The vast majority of infused r-αGal A goes to the liver, whereas cardiomyocytes and podocytes, both critical cell in FD, are resistant to uptake of r-αGal A [27,28].

4) Initiation of Enzyme Replacement Therapy

Plasma lysoGb3, deacylated form of Gb3 levels are strongly correlated with phenotype, revealing high levels in classic FD patients and lower levels in late-onset, non-classic FD patients [11,29]. It is also correlated with disease severity in late-onset FD patients and in female patients with classic FD, not in the male patients with classic FD. However, plasma levels of lysoGb3 decrease substantially during ERT in male patients with classic FD, often into the ranges of late-onset, non-classic FD male patients or female patients. Reduction of lysoGb3 is varying, depending on age at initiation of ERT. It is lower in patients who started treatment before the age of 25 years, compared to those who started later in life. Therefore, earlier starting treatment, before the evident organ damage, especially in male patients with classic FD is critical. However, the exact timing of treatment initiation is unclear. Decision for initiation of ERT is even more complex given the phenotype variability and disease severity in non-classic male FD, as well as in female patients.

For classic FD males, consensus was achieved that ERT is recommended as soon as there are early clinical signs of kidney, heart, or brain involvement, but may be considered in patients of ≥16 years in the absence of clinical signs or symptoms of organ involvement [27]. However, it is logical to assume younger the ERT begins, the better the outcome since asymptomatic pediatric FD patients revealed already Gb3 deposits and podocyte changes in renal biopsy specimen [21,30]. Dr. Desnick (from Icahn School of Medicine at Mount Sinai, New York) recommends asymptomatic classic male FD patients may start to receive ERT at the age of 8 (personal communication).

Symptomatic females with classic mutation and males with non-classic (late-onset) FD should be treated as soon since there are early clinical signs of kidney, heart, or brain involvement, while treatment may be considered in females with non-classic FD with early clinical signs as follows;

Renal function impairment: decreased GFR (<90 mL/min/1.73 m^2 adjusted for age >40 years [GFR category ≥G2], persistent albuminuria >30 mg/g [albuminuria category A2 or A3]), podocyte foot process effacement or glomerulosclerosis on renal biopsy, moderate or severe GL-3 inclusions in a range of renal cell types.

Central nervous symptoms and signs: silent strokes, cerebral white matter lesions (on brain Magnetic Resonance Imaging [MRI]).

Cardiac lesions; asymptomatic cardiac disease (cardiomyopathy or arrhythmia, cardiac fibrosis on contrast cardiac MRI).

5) Cessation of Enzyme Replacement Therapy

ERT should not be withheld from FD patients with severe renal insufficiency (GFR <45 mL/min/1.73 m^2) and from those on dialysis or with cognitive decline, but cautiously evaluated on an individual basis. Cessation of ERT may be considered in patients with end stage FD or other co-morbidities with a life expectancy of <1 year, those with cognitive decline of any cause. Also, stopping ERT may be justified in case of lack of response for 1 year when the only indication for ERT is neuropathic pain, patients with end stage renal disease without an option for renal transplantation, in combination with advanced heart failure (NYHA class IV). Poor adherence is another indication of cessation of ERT [31].

2. Pharmacological Chaperone Therapy

A pharmacological chaperone molecule, migalastat (Galafold®) has recently been made available for the treatment of FD, and is approved in the United States and Europe for use as a first-line therapy in FD patients with amenable *GLA* gene variants in 2016 but second-line therapy in Korea currently. Amenable mutations are characterized by some changes of a gene (for example missense, small-in frame insertion) associated with residual or minimal enzymatic activity and specifically a documented response in an *in vitro* assay: about 30-35% of FD patients have an amenable mutation.

Migalastat (1-deoxygalactonojirimycin) is an analogue of the terminal galactose of Gb3, stabilizing amenable mutant forms of the α-Gal A enzyme. Migalastat augments and stabilizes the lysosomal activity of the α-Gal A by facilitating the trafficking of amenable mutant forms of α-Gal A enzyme from the endoplasmic reticulum to lysosomes. The amenability needs to be verified by a laboratory test assaying α-Gal A migalastat-induced activity in human embryonic kidney (HEK) cells transfected with DNA plasmids containing *GLA* variants. Migalastat amenable mutations exhibits at least a 1.2-fold increase and an absolute activity>3% over wild type alpha-galactosidase. A activity in the presence of the 10 μmol/L migalastat.

The amenability of mutations can be easily checked by reviewing a published set of known genetic variants or by downloading a website listing amenable mutations (http://www.galatoldamenabilitytable.com). The drug is administrated orally 123 mg once every other day [32,33].

The effectiveness of migalastat has been investigated in two main clinical trials; FACET and ATTRACT. The FACETS trial was a placebo-controlled, double-blind trial that evaluated the efficacy and safety of migalastat in patients with FD and with amenable mutations who were ERT-naïve [34]. The aim of the ATTRACT study was to investigate the efficacy and safety of migalastat every other day in patients with FD who had previously been treated with ERT. The results of open-label extension of the randomized, phase III ATTRACT study demonstrated sustainable, long-term stability of renal function and reduction in LVMI without new safety concern in FD patients with amenable mutations [35].

Chaperone therapy might be favored for obese patients with an amenable mutation because chaperone therapy is dose-fixed, independently of body weight, in contrast to ERT, to avoid additional cost.

3. Palliative and Adjunct Therapy

Palliative therapy for Fabry disease is a treatment approach that aims to alleviate symptoms and improve quality of life for patients with the disease. There are several palliative therapies that are commonly used for Fabry patients, including:

Pain management: Neuronopathic pain is a common symptom of FD, hampering daily ordinary life. The combination of ERT or pharmacological chaperone and analgesics results in effective pain relief. First line option of current pain management includes carbamazepine, gabapentin, pregabalin, and serotonin and norepinephrine reuptake inhibitors (e.g., duloxetine, venlafaxine). Nonsteroidal anti-inflammatory drugs are not effective. Tramadol, lidocaine, and topical capsaicin patch may be the second line option. Use of strong opioids should be the last resort.

Cardiac care: Patients with FD can develop cardiac complications such as LVH, which can lead to heart failure. Cardiac care may include the use of beta-blockers, ACE inhibitors (ACEi), and diuretics to control hypertension and heart failure. If symptomatic bradycardia/chronotropic incompetence or significant AV conduction impairment, consider permanent cardiac pacing. If evidence of atrial fibrillation, lifetime anticoagulation is recommended. Implantable cardioverter-defibrillator should be considered in case of malignant arrhythmia.

Reno-protective medication: Patients with FD can develop renal complications such as proteinuria, which can lead to CKD. Renal care may include the use of ACEi, angiotensin receptor blockers (ARBs), and diuretics to control hypertension and CKD. Since a high sodium diet minimizes the effect of ACEi and ARBs, a low sodium diet is recommended.

Gastrointestinal management: Metoclopramide and H-2 blockers alleviated the symptoms of delayed gastric emptying and epigastric discomforts. FD patients (especially pediatric FD) complain of dysmotility and diarrhea, which should be managed with dietary changes (increased fiber intake, more frequent and smaller meals) and pharmacotherapy.

Auditory impairment: Hearing aids and cochlear implants are needed.

Pulmonary care: Smoking should be strongly discouraged. Bronchodilators may helpful to relieve airway obstruction.

Physical therapy: Regular physical therapy can help to improve joint mobility and muscle strength, and reduce pain in patients with Fabry disease.

Occupational therapy: Occupational therapy can help patients with Fabry disease to manage daily activities and improve their independence.

Psychological support: Patients with FD may experience emotional and psychological distress due to their illness and the impact it has on their daily lives. Psychological support may include counseling, support groups, and therapy.

It is important to note that palliative therapy is not curative, and long-term monitoring and treatment is required, also the therapy may not be effective for all patients, and the response to therapy can vary depending on the specific mutation and other factors. Additionally, it is important to consult with a specialist in the field before starting any palliative therapy [1,33].

Novel Therapeutic Strategies and Future Perspectives

1. Next Generation Enzyme Replacement Therapy

The underlying mechanism of r-α-Gal A uptake variation between different cell types remains yet unknown. The mannose 6-phosphate (M6P) mediated endocytosis is the main mechanism of r-αGal A uptake, recent studies demonstrate that other pathways play roles as well. Especially, endothelial cells may use different uptake mechanism from fibroblasts since blocking the M6P receptor inhibited r-αGal A uptake in fibroblasts, but endothelial cells are able to uptake r-α-Gal A. Non-M6P dependent endocytic pathways play role clearing Gb3 because membranes of endothelial cells lack M6P receptors. In podocytes which are relatively resistant to ERT, enzyme uptake is partly via M6P receptors, along with two other receptors: megalin and sortilin. However, there are unknown additional uptake mechanisms. Current r-αGal A preparations are not able to penetrate the blood-brain barrier. Although there is some accumulation of Gb3 in the brain of FD patients, the clinical significance remains unclear, as the main complications like TIAs and cerebrovascular accidents are most likely caused by vascular pathology. Future r-α-Gal A enzymes must carry the property of more efficient uptake into various cell types, preferably crossing the blood brain barrier as well as prolonged effect with efficient bio-distribution.

While both first-generation ERT preparations are produced in mammalian cell lines, next generation ERT preparations aim to prolong therapeutic effects and avoid disadvantages of mammalian cell line production such as expensive production costs and the risk of contamination. Recent approaches aim to generate r-αGal A in plant-derived cell lines. Two plant-derived ERT agents (Pegunigalsidase alfa®, moss-αGal®) are currently under clinical trials.

Pegunigalsidase alfa is a novel polyethylene glycolylated and covalently cross-linked form of r-αGal A, developed as ERT for FD and produced in tobacco cells (tobacco plant cell-based ProCellEx System). It targets not only sustaining plasma half-life, but also improving long-term therapeutic tolerance by reducing ADA formation by pegylation [36]. Pegunigalsidase seems to be functionally equivalent to the currently available ERTs, with in vitro stability and a ten-fold increase in half-life in male Fabry mice compared to approved drugs. Clinical phase I/II trials also reported a reduction of Gb3 accumulation in renal biopsy tissue with long plasma half-life (80 hours) and reduced immunogenicity, allowing monthly infusion interval. Three phase III studies are currently underway (BALANCE [NCT02795676], BRIDGE, and BRIGHT [NCT03180840]). BALANCE is a multicenter, randomized, actively controlled, direct comparison study (head-to-head) to evaluate the safety and efficacy of pegunigalsidase alfa (1 mg/kg) compared to the approved dose of agalsidase beta (1 mg/kg) focusing on renal function. BRIDGE is an open switch-over study to assess the safety and efficacy of the switch from an approved dose of agalsidase alfa (0.2 mg/kg) to pegunigalsidase alfa (1 mg/kg). BRIGHT is an open switch-over study to evaluate the safety and efficacy of the switch from an approved dose of agalsidase alfa (0.2 mg/kg) or agalsidase beta (1 mg/kg) every 2 weeks to a higher dose of pegunigalsidase alfa (2.0 mg/kg) every 4 weeks. First preliminary data of the phase III BRIDGE trial (NCT03018730) report that therapy switch from agalsidase alfa to pegunigalsidase alfa was safe, well-tolerated and resulted into stabilization, or at least slower progression of renal failure (eGFR slope improvement from −5.1 to 0.23 mL/min/1.73 m^2/year in both male and female).

Moss-αGal is a r-αGal A expressed in Physcomitrella patens, which is a genetically modified moss. Its uptake is not mediated by mannose6-phosphate receptor but through the mannose receptor. It is more preferentially targeted to renal cells than algalsidase alfa. A phase I study showed good safety and tolerability of Moss-aGal in six women after a single intravenous dose of 0.2 mg/kg. Phase II and III studies are in preparation [13,28,32,37].

2. Substrate Reduction Therapy

Two different SRT molecules (Ibiglustat/Venglustat®, Lucerastat®) have been developed and are currently evaluated in both pre-clinical and clinical trials. While venglustat is still at an early stage of approval and only few data have been published so far [38]. Preliminary data suggest gradual clearance of Gb3 from skin capillary endothelium and a gradual reduction of plasma lyso-Gb3 in treatment-naive patients. Lucerastat is currently undergoing clinical evaluation in the randomized multi-center double-blind clinical phase III MODIFY-trial (NCT03425539). Promising initial results from phase I/II clinical trials demonstrate that lucerastat therapy has been safe over a 12-week long oral medication. Besides, a significant reduction of plasma biomarkers was observed, including glycosphingolipids, glucosylceramide, lactosylceramide, and globotriaosylceramide. Both SRT agents are promising oral therapeutics for FD patients regardless of genotypes [28,37,39].

3. Gene Therapy

There are several ongoing clinical trials for gene therapy. These trials are using different approaches such as adeno-associated

virus (AAV) vectors, and lentiviral vectors.

The first interventional, multicenter, multinational, open-label study (NCT03454893, NCT02800070, AVR-RD-01, AvroBio) is based on the lentiviral *ex vivo* transduction of hematopoietic stem cells. This approach aims to use a CD34+ cell-enriched fraction that contains cells transduced with a lentiviral LV vector including human *GLA* cDNA. A total of 20 classic FD male patients (≥16 years old), who had not previously received ERT and/or chaperone therapy within 3 years of the time of screening, received a one-time intravenous administration of AVR-RD-01 and were observed for 64 weeks. Moreover, a long-term follow-up study for 14 years of participants administered AVR-RD-01 is underway (NCT04999059) [40].

Three further phase I/II clinical studies (NCT04046224, NCT04040049) are based on AAV *in vivo* transduction of hepatocytes, using these cells as r-αGal A producing platform. *In vivo* gene therapy utilizes hepatotropic AAV vectors AAV2/6 and AAVS3 for the transfer of the *GLA* gene to hepatocytes. STAAR is a multicenter, open-label, dose-finding study for the AAV2/6 vector-based drug ST-920 (NCT04046224, Sangamo).

The second clinical study on AAV-based gene therapy is also still in the recruitment phase (NCT04040049; FLT190; Freeline Therapeutics), based on a platform that is also designed for future treatment of other rare diseases including hemophilia A and B and Gaucher disease. FLT190 is composed of a codon-optimized *GLA* transgene with a liver-specific promoter. The construct, covered by a synthetic capsid, demonstrates higher transduction efficiency in human hepatocytes compared to wild-type AAV serotypes [41].

A third clinical study on AAV-based gene therapy utilizes an attenuated AAV (4D-310; 4D Molecular Therapeutics). Preclinical studies in mice verified that the novel capsid 4D-C102 was especially efficient in transducing human cardiomyocytes. Since myocardial cells are hard to reach with ERT, this approach will be promising for FD patients with predominant cardiac manifestations [28,33,37,42,43].

The first reports available so far have shown that the concept in general is valid, leading to a prompt substantial rise of α-Gal A levels in the first patients after treatment. However, it currently remains largely unclear whether these initial effects will be long-lasting, or repetitive gene therapy will be needed. Future results of further clinical trials will evaluate benefits but also risks, such as the development of neutralizing antibodies and immunologic reactions.

4. Stem Cell Therapy with Genome Editing

Generation of a CRISPR/Cas9-corrected-hiPSC line (DDLA-Bi001-A) from FD-derived induced pluripotent stem cells (iPSCs) having *GLA* gene mutation (c.803_806del) was reported recently. *GLA* mutation (1268fs*1 (c.803_806del)) of FD iPSCs was corrected using the CRISPR-Cas9 gene editing system. The corrected FD-iPSCs retained normal morphology, karyotype, expression of pluripotency-associated markers, trilineage differentiation potential, and α-Gal A activity [44].

5. mRNA Therapy

Preclinical study using mRNA-based therapy (Moderna Inc.) was conducted in different species evaluating a systemically delivered mRNA encoding human α-Gal A for treatment of FD. Pharmacokinetics and bio-distribution of α-Gal A were characterized after a single administration of lipid nanoparticles formulated mRNA in wild-type CD1 mice. It showed a prolonged half-life of α-Gal A in plasma, liver, kidney, and heart. A single intravenous administration of human α-Gal A mRNA in FD mice demonstrated a dose-dependent elevation of α-Gal A activity with concomitant reduction of lysoGb3 in plasma and tissues (liver, kidney, heart, spleen). Repeat administration (every other week and monthly) of human α-Gal A mRNA in FD mice resulted in significant substrate reduction in a dose dependent manner. It demonstrated sustained functional α-Gal A protein in plasma after each dose, without production of ADA. Taken together, these preclinical proof-of-concept studies indicate that systemic mRNA therapy could be a potential treatment for FD [45,46].

Conclusion

Diverse novel therapeutic strategies are under development for the treatment of FD. Some are either already evaluated in clinical trials or emerging soon. While ERT in FD has proven its effectiveness over many years, the availability of oral chaperone therapy led to a significant improvement at least of quality of life in many FD patients. However, many needs of FD patients remain unmet. Current therapies should be initiated early enough to prevent irreversible organ damage, and maintained lifelong. Also, there are possibility of immunogenicity, amenability limitation, and extremely high cost. With novel therapy options such as not only oral substrate reduction therapy but also gene therapy just emerging, these options are potentially promising in satisfying the current unmet needs of FD patients.

References

1. Ortiz A, Germain DP, Desnick RJ, Politei J, Mauer M, Burlina A, et al. Fabry disease revisited: management and treatment recommendations for adult patients. Mol Genet Metab 2018;123:416-27.
2. Spada M, Pagliardini S, Yasuda M, Tukel T, Thiagarajan G, Sakuraba H, et al. High incidence of later-onset Fabry disease revealed by newborn screening. Am J Hum Genet 2006;79:31-40.
3. Matern D, Gavrilov D, Oglesbee D, Raymond K, Rinaldo P, Tortorelli S. Newborn screening for lysosomal storage disorders. Semin Perinatol 2015;39:206-16.
4. Doheny D, Srinivasan R, Pagant S, Chen B, Yasuda M, Desnick RJ. Fabry disease: prevalence of affected males and heterozygotes with pathogenic GLA mutations identified by screening renal, cardiac and stroke clinics, 1995-2017. J Med Genet 2018;55:261-8.
5. Rombach SM, Smid BE, Linthorst GE, Dijkgraaf MG, Hollak CE. Natural course of Fabry disease and the effectiveness of enzyme replacement therapy: a systematic review and meta-analysis: effectiveness of ERT in different disease stages. J Inherit Metab Dis 2014;37:341-52.
6. Ries M, Gupta S, Moore DF, Sachdev V, Quirk JM, Murray GJ, et al. Pediatric Fabry disease. Pediatrics 2005;115:e344-55.
7. Wang RY, Lelis A, Mirocha J, Wilcox WR. Heterozygous Fabry women are not just carriers, but have a significant burden of disease and impaired quality of life. Genet Med 2007;9:34-45.
8. Wilcox WR, Oliveira JP, Hopkin RJ, Ortiz A, Banikazemi M, Feldt-Rasmussen U, et al.; Fabry Registry. Females with Fabry disease frequently have major organ involvement: lessons from the Fabry Registry. Mol Genet Metab 2008;93:112-28.
9. Laney DA, Peck DS, Atherton AM, Manwaring LP, Christensen KM, Shankar SP, et al. Fabry disease in infancy and early childhood: a systematic literature review. Genet Med 2015;17:323-30.
10. Germain DP. Fabry disease. Orphanet J Rare Dis 2010;5:30.
11. Turkmen K, Baloglu I. Fabry disease: where are we now? Int Urol Nephrol 2020;52:2113-22.
12. Hwang S, Lee BH, Kim WS, Kim DS, Cheon CK, Lee CH, et al. A phase II, multicenter, open-label trial to evaluate the safety and efficacy of ISU303 (Agalsidase beta) in patients with Fabry disease. Medicine (Baltimore) 2022;101:e30345.
13. Simonetta I, Tuttolomondo A, Daidone M, Miceli S, Pinto A. Treatment of Anderson-Fabry disease. Curr Pharm Des 2020;26:5089-99.
14. Beck M, Ramaswami U, Hernberg-Ståhl E, Hughes DA, Kampmann C, Mehta AB, et al. Twenty years of the Fabry Outcome Survey (FOS): insights, achievements, and lessons learned from a global patient registry. Orphanet J Rare Dis 2022;17:238.
15. Lee CL, Lin SP, Niu DM, Lin HY. Fabry disease and the effectiveness of enzyme replacement therapy (ERT) in left ventricular hypertrophy (LVH) improvement: a review and meta-analysis. Int J Med Sci 2022;19:126-31.
16. Ramaswami U, Beck M, Hughes D, Kampmann C, Botha J, Pintos-Morell G, et al.; FOS Study Group. Cardio- renal outcomes with long-term agalsidase alfa enzyme replacement therapy: a 10- year Fabry Outcome Survey (FOS) analysis. Drug Des Devel Ther 2019;13:3705-15.
17. Sheng S, Wu L, Nalleballe K, Sharma R, Brown A, Ranabothu S, et al. Fabry's disease and stroke: Effectiveness of enzyme replacement therapy (ERT) in stroke prevention, a review with meta-analysis. J Clin Neurosci 2019;65:83-6.
18. Sasa H, Nagao M, Kino K. Safety and effectiveness of enzyme replacement therapy with agalsidase alfa in patients with Fabry disease: post-marketing surveillance in Japan. Mol Genet Metab 2019;126:448-59.
19. Ortiz A, Kanters S, Hamed A, DasMahapatra P, Poggio E, Maski M, et al. Agalsidase beta treatment slows estimated glomerular filtration rate loss in classic Fabry disease patients: results from an individual patient data meta-analysis. Clin Kidney J 2020;14:1136-46.
20. Banikazemi M, Bultas J, Waldek S, Wilcox WR, Whitley CB, McDonald M, et al.; Fabry Disease Clinical Trial Study Group. Agalsidase-beta therapy for advanced Fabry disease: a randomized trial. Ann Intern Med 2007;146:77-86.
21. Tøndel C, Thurberg BL, DasMahapatra P, Lyn N, Maski M, Batista JL, et al. Clinical relevance of globotriaosylceramide accumulation in Fabry disease and the effect of agalsidase beta in affected tissues. Mol Genet Metab 2022;137:328-41.
22. Eng CM, Guffon N, Wilcox WR, Germain DP, Lee P, Waldek S, 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;345:9-16.
23. Schiffmann R, Kopp JB, Austin HA 3rd, Sabnis S, Moore DF, Weibel T, et al. Enzyme replacement therapy in Fabry disease: a randomized controlled trial. JAMA 2001;285:2743-9.
24. El Dib R, Gomaa H, Ortiz A, Politei J, Kapoor A, Barreto F. Enzyme replacement therapy for Anderson-Fabry disease: a complementary overview of a Cochrane publication through a linear regression and a pooled analysis of proportions from cohort studies. PLoS One 2017;12:e0173358.
25. Riccio E, Pisani A. New insights in efficacy of different enzyme replacement therapy dosages in Fabry disease: switch studies data following agalsidase beta shortage. Clin Genet 2023;103:371-6.
26. Arends M, Biegstraaten M, Wanner C, Sirrs S, Mehta A, Elliott PM, et al. Agalsidase alfa versus agalsidase beta for the treatment of Fabry disease: an international cohort study. J Med Genet 2018;55:351-8.
27. Germain DP, Altarescu G, Barriales-Villa R, Mignani R, Pawlaczyk K, Pieruzzi F, et al. An expert consensus on practical clinical recom-

mendations and guidance for patients with classic Fabry disease. Mol Genet Metab 2022;137:49-61.
28. Lenders M, Brand E. Fabry disease: the current treatment landscape. Drugs 2021;81:635-45.
29. Simonetta I, Tuttolomondo A, Daidone M, Pinto A. Biomarkers in Anderson-Fabry disease. Int J Mol Sci 2020;21:8080.
30. Spada M, Baron R, Elliott PM, Falissard B, Hilz MJ, Monserrat L, et al. The effect of enzyme replacement therapy on clinical outcomes in paediatric patients with Fabry disease - a systematic literature review by a European panel of experts. Mol Genet Metab 2019;126:212-23.
31. Biegstraaten M, Arngrímsson R, Barbey F, Boks L, Cecchi F, Deegan PB, 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.
32. Feriozzi S, Hughes DA. New drugs for the treatment of Anderson-Fabry disease. J Nephrol 2021;34:221-30.
33. Oder D, Müntze J, Nordbeck P. Contemporary therapeutics and new drug developments for treatment of Fabry disease: a narrative review. Cardiovasc Diagn Ther 2021;11:683-95.
34. 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.
35. Feldt-Rasmussen U, Hughes D, Sunder-Plassmann G, Shankar S, Nedd K, Olivotto I, et al. Long-term efficacy and safety of migalastat treatment in Fabry disease: 30-month results from the open-label extension of the randomized, phase 3 ATTRACT study. Mol Genet Metab 2020;131:219-28.
36. Lenders M, Pollmann S, Terlinden M, Brand E. Pre-existing anti-drug antibodies in Fabry disease show less affinity for pegunigalsidase alfa. Mol Ther Methods Clin Dev 2022;26:323-30.
37. van der Veen SJ, Hollak CEM, van Kuilenburg ABP, Langeveld M. Developments in the treatment of Fabry disease. J Inherit Metab Dis 2020;43:908-21.
38. Peterschmitt MJ, Crawford NPS, Gaemers SJM, Ji AJ, Sharma J, Pham TT. Pharmacokinetics, pharmacodynamics, safety, and tolerability of oral venglustat in healthy volunteers. Clin Pharmacol Drug Dev 2021;10:86-98.
39. Deegan PB, Goker-Alpan O, Geberhiwot T, Hopkin RJ, Lukina E, Tylki-Szymanska A, et al. Venglustat, an orally administered glucosylceramide synthase inhibitor: assessment over 3 years in adult males with classic Fabry disease in an open-label phase 2 study and its extension study. Mol Genet Metab 2023;138:106963.
40. Saleh AH, Rothe M, Barber DL, McKillop WM, Fraser G, Morel CF, et al. Persistent hematopoietic polyclonality after lentivirus-mediated gene therapy for Fabry disease. Mol Ther Methods Clin Dev 2023;28:262-71.
41. Shaimardanova AA, Solovyeva VV, Issa SS, Rizvanov AA. Gene therapy of sphingolipid metabolic disorders. Int J Mol Sci 2023;24:3627.
42. Domm JM, Wootton SK, Medin JA, West ML. Gene therapy for Fabry disease: progress, challenges, and outlooks on gene-editing. Mol Genet Metab 2021;134:117-31. Erratum in: Mol Genet Metab 2023;139:107541.
43. Simonetta I, Tuttolomondo A, Di Chiara T, Miceli S, Vogiatzis D, Corpora F, et al. Genetics and gene therapy of Anderson-Fabry disease. Curr Gene Ther 2018;18:96-106.
44. Choi JB, Seo D, Do HS, Han YM. Generation of a CRISPR/Cas9-corrected-hiPSC line (DDLABi001-A) from Fabry disease (FD)-derived iPSCs having α-galactosidase (GLA) gene mutation (c.803_806del). Stem Cell Res 2023;66:103001.
45. Felis A, Whitlow M, Kraus A, Warnock DG, Wallace E. Current and investigational therapeutics for Fabry disease. Kidney Int Rep 2019;5:407-13.
46. Zhu X, Yin L, Theisen M, Zhuo J, Siddiqui S, Levy B, et al. Systemic mRNA therapy for the treatment of Fabry disease: preclinical studies in wild-type mice, Fabry mouse model, and wild-type non-human primates. Am J Hum Genet 2019;104:625-37.