Long-term safety of enzyme replacement therapy with agalsidase alfa in patients with Fabry disease: post-marketing extension surveillance in Japan

Makoto Arakawa [*], Yoshinori Ikeda , Hiromichi Otaka , Sanghun Iwashiro

Japan Medical Office, Takeda Pharmaceutical Company Limited, Tokyo, Japan

ARTICLE INFO

ABSTRACT

Fabry disease is a rare inherited X-linked metabolic disorder in which deficient alpha-galactosidase A activity causes progressive build-up of globotriaosylceramide (Gb3) and multi-system dysfunction. Following approval of agalsidase alfa for Fabry disease in Japan in 2006, an 8-year all-case post-marketing surveillance (PMS) showed that the treatment was well tolerated and effective for managing disease progression in adult Japanese patients. The present nationwide prospective observational study extended the initial PMS by enrolling patients who continued agalsidase alfa treatment after the initial 8-year period in a 6.5-year extension survey. Patient information from the initial PMS and the extension survey was evaluated as a single data set (observation period: February 2007–September 2021). Of 493 patients in the initial PMS, 129 (45.0% male classic, 6.2% male non-classic, 48.8% female heterozygous phenotype) consented to participate in the extension survey and were included in the analysis. The mean duration of treatment was 9.6 years. A total of 145 adverse drug reactions (ADRs) occurred in 31 patients (24%), and 22 serious ADRs occurred in 12 patients (9.3%). Although serious cardiac, renal, or cerebrovascular adverse events decreased in frequency over time in male patients, serious cardiac events continued to occur in female patients, who showed higher incidence of cardiac complications at baseline. No new safety concerns were identified. Additionally, long-term agalsidase alfa treatment sustained the initial reduction in Gb3 concentrations without increasing the rate of anti-agalsidase antibody positivity. These findings suggest that agalsidase alfa treatment demonstrates continued safety and sustains patients' clinical course over the long term.

1. Introduction

Fabry disease is a rare inherited X-linked metabolic disorder in which mutations in the alpha-galactosidase A (GLA) gene cause deficient lysosomal GLA activity [1]. The decrease in GLA leads to progressive build-up of globotriaosylceramide (Gb3) (also called ceramide trihexoside) in different tissues and body fluids, resulting in wide-ranging multi-system symptoms that present with varying degrees of severity [1]. Patients are typically categorized according to two phenotypes: classic and non-classic [2]. Male patients with the classic phenotype have little to no GLA activity and typically develop symptoms such as acroparesthesias, hypohidrosis, angiokeratomas, and gastrointestinal dysfunction from childhood or adolescence [2]. Without treatment, these patients develop severe renal, cardiac, and cerebrovascular complications by adulthood with poor prognosis [3]. Male patients with the non-classic phenotype have residual GLA activity and typically develop milder symptoms that manifest later than in patients with the classic phenotype [2]. On the other hand, female patients who are heterozygous for GLA mutations experience a wider variety of symptoms that range from being as severe as those in male patients with the classic phenotype to completely absent [2].

As Fabry disease is progressive, patients require lifelong treatment to guard against organ dysfunction and enhance survival. The main treatment for Fabry disease is enzyme replacement therapy (ERT), which aims to replace the deficient GLA in patients [2,4]. Two formulations of ERT are currently available in Japan: agalsidase alfa and

agalsidase beta. Agalsidase alfa is a recombinant GLA produced from cultured human cells and is administered by intravenous infusion [5]. This preparation of agalsidase alfa was first approved in Europe in August 2001 and then in Japan as an orphan drug in October 2006. Clinical trials have shown that agalsidase alfa treatment is safe and effective for reversing the pathogenesis of the disease's major clinical symptoms [6]. Additionally, long-term survey findings from the Fabry Outcome Survey (FOS), an international survey initiated in 2001 [7,8], indicate that agalsidase alfa treatment slows renal decline and progression of left ventricular hypertrophy [9–12]. Similarly, a mandatory, 8-year, all-case post-marketing surveillance (PMS) of agalsidase alfa in Japanese adults with Fabry disease conducted from February 2007 to March 2015 showed that the treatment was well tolerated and effective for managing the progression of renal and cardiac dysfunction [13].

The present study aimed to extend the long-term safety and efficacy evaluation of agalsidase alfa in Japanese patients in a real-world setting by following the disease course of patients who continued treatment with agalsidase alfa after completion of the 8-year all-case PMS (hereafter referred to as the "initial PMS").

2. Materials and methods

2.1. Study design

This nationwide prospective observational study was conducted as an extension of the initial PMS of agalsidase alfa (Replagal, Takeda Pharmaceutical Company Limited, previously marketed by Sumitomo Pharma) performed in Japanese patients with Fabry disease from February 2007 to March 2015. The initial PMS was designed and conducted by Sumitomo Pharma, and the data were inherited by Takeda Pharmaceutical Company Limited. Details of the initial PMS are published elsewhere [13]. Briefly, patients were diagnosed with Fabry disease by a physician if they had deficient GLA activity or a GLA gene mutation. Clinical phenotype of Fabry disease in male patients (classic or non-classic) was determined based on the physician's judgment. All patients with Fabry disease who were administered agalsidase alfa were included in the survey. Agalsidase alfa was administered intravenously at 0.2 mg/kg over 40 min every 2 weeks according to the approved dose regimen. The initial PMS was performed for a maximum observation period of 8 years in accordance with Good Postmarketing Surveillance Practice (GPSP Ordinance) (Ministry of Health, Labour and Welfare [MHLW] Ordinance Related to Standards for Conducting Post-Marketing Surveys and Studies on Drugs; MHLW Ordinance No. 171 issued by MHLW on December 20, 2004). As the initial survey comprised a mandatory surveillance study, the need for informed consent was waived in compliance with Japanese health authority regulations.

Patients who continued to receive agalsidase alfa after the initial 8-year period were enrolled in a 6.5-year (March 2015–September 2021) extension survey. The observation period was from the beginning of the initial PMS (February 2007) to September 2021 or drug discontinuation, whichever was earlier. This study was conducted in accordance with the Declaration of Helsinki, GPSP, and Good Vigilance Practice (GVP) ministerial decrees. Written or verbal informed consent was obtained from all patients prior to starting the extension survey.

2.2. Outcome measures

2.2.1. Demographic and clinical characteristics

Anonymized patient information was collected using Electronic Data Capture. Data from the initial PMS and the present extension survey were combined and evaluated as a single data set. Patient demographic information was collected at the time of enrollment in the initial PMS, including date of birth, sex, Fabry disease phenotype, family history, symptoms of Fabry disease, and complications. Information on administration status of agalsidase alfa was continuously collected over the observation period.

2.2.2. Safety assessments

Safety information was also continuously collected during the observation period. Adverse events were coded according to System Organ Class (SOC) and Preferred Terms (PT) of the Japanese version of the Medical Dictionary for Regulatory Activities (MedDRA/J) Version 25.0. Adverse drug reactions (ADRs) were defined as adverse events judged to be related to agalsidase alfa therapy by each individual attending physician. Severity and date of onset were also determined by the attending physician.

2.2.3. Laboratory assessments

Plasma and serum samples were obtained from 4 mL of whole blood taken before treatment and at the discretion of the attending physician thereafter to measure Gb3 concentrations and the presence of anti-agalsidase alfa antibodies. Gb3 concentration was measured using high-performance liquid chromatography and reported as nmol/mL. Anti-agalsidase alfa immunoglobulin (Ig) G and IgE antibodies were detected using enzyme-linked immunosorbent assay, colorimetric reaction, and spectrophotometry. Samples were determined to be positive for anti-agalsidase alfa antibodies when the absorbance was at least twofold greater than that of the baseline sample.

2.3. Statistical analysis

Demographics and disease-related data are presented as mean (standard deviation; SD) for continuous variables and number (percentage) for categorical variables. The analysis set included all patients who consented to participate in the extension survey. ADRs are presented as number (percentage) by SOC and PT and disease phenotype. The cumulative rate of serious adverse events (SAEs) of cardiac, renal, or cerebrovascular origin in patients with Fabry disease over the observation period are shown using Kaplan-Meier curves. Initial occurrence was adopted as an event in the curve. Cardiac events were classified under the SOC of "cardiac disorders", renal events were classified under the SOC of "renal and urinary disorders" with PT of "renal" or "urinary", and cerebrovascular events were classified under the SOC of "nervous system disorders" with PT of "blood", "stroke", or "infarction".

Mean (SD) plasma Gb3 concentrations are presented as a time series by the presence or absence of prior ERT and disease phenotype. Anti-agalsidase alfa antibody results are presented by measurement time point for patients who received the assessment and had at least one positive result. All calculations were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Patient disposition

A total of 493 patients participated in the initial PMS of agalsidase alfa beginning in February 2007, and 360 patients had continued treatment by the end of the survey in March 2015 (Fig. 1). Of these, 129 patients consented to participate in the extension survey, all of whom were included in the analysis set. Of the 129 patients, 41 (31.8%) discontinued the study before the end of the 6.5-year observation period. Reasons for discontinuation included switching to another drug (18 patients, 43.9%), changing hospitals (14 patients, 34.1%), adverse events (four patients, 9.8%), patient's preference for reasons other than adverse events (two patients, 4.9%), no hospital visit (two patients, 4.9%), insufficient effect (one patient, 2.4%), and death (six patients, 14.6%) (duplicate aggregation was permitted).

3.2. Demographic and baseline clinical characteristics

Of the 129 patients, 58 (45.0%) were male with the classic
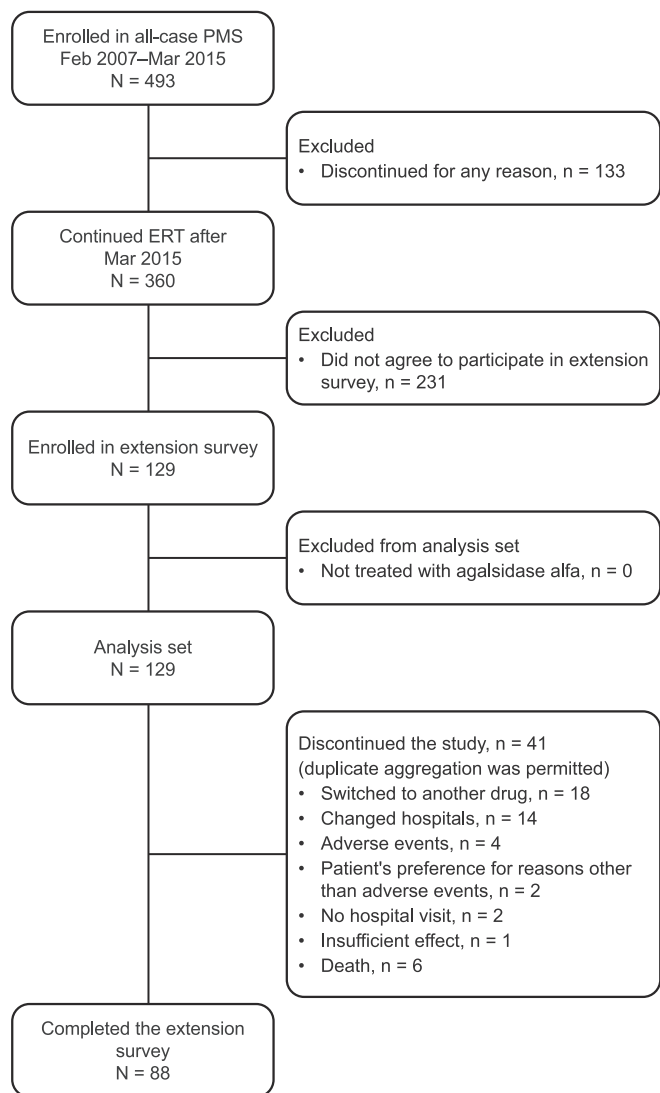
Fig. 1. Patient flow chart.
ERT, enzyme replacement therapy; PMS, post-marketing surveillance.

Table 1
Patient Demographics at the Start of the Initial PMS.

Variable	Total	Male (Classic Phenotype)	Male (Non-classic Phenotype)	Female (Heterozygous Phenotype)
N (%)	129 (100)	58 (45.0)	8 (6.2)	63 (48.8)
Age, years				
Mean (SD)	39.7 (17.5)	27.2 (13.1)	52.1 (12.2)	49.6 (14.0)
<20	22 (17.1)	18 (31.0)	0	4 (6.3)
≥20 to <30	16 (12.4)	15 (25.9)	0	1 (1.6)
≥30 to <40	22 (17.1)	16 (27.6)	2 (25.0)	4 (6.3)
≥40 to <50	22 (17.1)	5 (8.6)	1 (12.5)	16 (25.4)
≥50 to <60	31 (24.0)	3 (5.2)	3 (37.5)	25 (39.7)
≥60 to <70	14 (10.9)	1 (1.7)	2 (25.0)	11 (17.5)
≥70	2 (1.6)	0	0	2 (3.2)
Age at diagnosis, years, mean (SD)	36.1 (17.8)	23.6 (13.4)	48.1 (13.5)	45.9 (14.3)
Family history	103 (79.8)	47 (81.0)	3 (37.5)	53 (84.1)
Prior ERT				
Without	75 (58.1)	28 (48.3)	5 (62.5)	42 (66.7)
With	44 (34.1)	24 (41.4)	3 (37.5)	17 (27.0)
Unknown	10 (7.8)	6 (10.3)	0	4 (6.3)
Time from diagnosis to initial agalsidase alfa therapy, years, mean (SD)				
Total[a]	3.8 (6.1)	3.8 (5.6)	4.1 (5.7)	3.6 (6.5)
Naïve ERT	1.3 (3.8)	0.4 (0.4)	2.7 (5.2)	1.6 (4.8)
Duration of agalsidase alfa treatment, years, mean (SD)	9.6 (2.3)	9.7 (2.4)	9.3 (1.2)	9.6 (2.2)
Complications				
Neurological	66 (51.2)	45 (77.6)	1 (12.5)	20 (31.7)
Hypohidrosis	47 (36.4)	39 (67.2)	1 (12.5)	7 (11.1)
Gastrointestinal	12 (9.3)	8 (13.8)	1 (12.5)	3 (4.8)
Dermatological	30 (23.3)	25 (43.1)	1 (12.5)	4 (6.3)
Cardiac	71 (55.0)	24 (41.4)	5 (62.5)	42 (66.7)
Renal	35 (27.1)	15 (25.9)	5 (62.5)	15 (23.8)
Cerebrovascular	18 (14.0)	7 (12.1)	1 (12.5)	10 (15.9)
Ophthalmological	35 (27.1)	13 (22.4)	0	22 (34.9)
Auditory	12 (9.3)	9 (15.5)	1 (12.5)	2 (3.2)
eGFR, mL/min/m^2, mean (SD)	96.4 (32.8)	115 (32.4)	60.3 (28.5)	83.2 (21.9)
Patients with conventional treatment at baseline				
Dialysis	8 (6.2)	4 (6.9)	2 (25.0)	2 (3.2)

(continued on next page)

phenotype, eight (6.2%) were male with the non-classic phenotype, and 63 (48.8%) were heterozygous female (Table 1). The mean (SD) age at enrollment in the initial PMS was 27.2 (13.1) years among male patients with the classic phenotype, 52.1 (12.2) years among male patients with the non-classic phenotype, and 49.6 (14.0) years among heterozygous female patients. Mean (SD) age at diagnosis of Fabry disease was 23.6 (13.4), 48.1 (13.5), and 45.9 (14.3) years, respectively. Mean (SD) duration of agalsidase alfa treatment was 9.6 (2.3) years, with a median (interquartile range) of 9.9 (8.0–11.4) years. A total of 44 (34.1%) and 75 (58.1%) patients indicated that they had and had not received prior ERT, respectively, while 10 (7.8%) patients reported unknown prior exposure. Complications at baseline were typically more frequent in male patients with the classic phenotype, except for those of cerebrovascular, ophthalmological, and cardiac origin, of which the latter occurred at a particularly higher incidence in heterozygous female patients (66.7% vs. 41.4%).

3.3. Safety outcomes

Over the course of the extension survey, 145 ADRs occurred in 31 patients (24%) (Table 2).

The most common ADRs overall were pruritus, urticaria, pain in extremity, and pyrexia (*n* = 4 patients each, 3.1%), followed by

Table 1 (*continued*)

Variable	Total	Male (Classic Phenotype)	Male (Non-classic Phenotype)	Female (Heterozygous Phenotype)
Renal transplantation	0	0	0	0
Pacemaker	8 (6.2)	0	1 (12.5)	7 (11.1)

Data are n (%), unless otherwise noted.

eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; PMS, post-marketing surveillance; SD, standard deviation.

[a] Including patients treated with pre-ERT therapy (agalsidase alfa or agalsidase beta in a clinical trial for approval).

headache and rash ($n = 3$ patients each, 2.3%). The incidence and most common types of ADRs varied among the three phenotypes. The most common ADRs were pain in extremity and pyrexia in male patients with the classic phenotype ($n = 4$ patients each), cerebellar infarction, headache, sinus node dysfunction, pruritus, urticaria, and chest discomfort ($n = 1$ patient each) in male patients with the non-classic phenotype, and abnormal hepatic function and rash ($n = 2$ patients each) in heterozygous female patients. Further, 46 ADRs occurred in 14 patients (10.9%) for the first time during the extension survey. The ADR events were cerebellar infarction, tinnitus, sudden hearing loss, atrial fibrillation, cardiac failure, pericarditis, sinus node dysfunction, shock, dyspnea, oropharyngeal discomfort, rash, urticaria, skin induration, muscular weakness, pain in extremity, injection site urticaria, malaise, edema peripheral, pyrexia, protein in the urine, increased Gb3, road traffic accident, and infusion-related reaction ($n = 1$ patient each).

A total of 22 serious ADRs occurred in 12 patients (9.3%). These included cardiac failure (four events in one patient); pyrexia, wheezing, chills, headache, and pain in extremity (one of each event in one patient); urticaria, pruritus, and cough (one of each event in one patient); atrial fibrillation and shock (one of each event in one patient); and carpal tunnel syndrome, cerebellar infarction, retinal vein occlusion, sudden hearing loss, pericarditis, sinus node dysfunction, chest pain, and traffic accident (one event in one patient each). Eight patients discontinued the extension study due to adverse events and/or death (two due to adverse events only, four due to death only, two due to adverse events that led to death). Causes of death among the six patients who died under treatment (four male, two female) were ventricular tachycardia ($n = 2$, 1.55%) and heart failure, sudden death, traffic accident, and vascular stenosis ($n = 1$ each, 0.78%). Traffic accident was the only event for which a causal relationship to agalsidase alfa was not ruled out by the reporting physician, as it was impossible to exclude it from among all possible causes of the accident, including a simple operational error, a symptom of Fabry disease, or agalsidase alfa treatment. Among the remaining two patients without death, one patient discontinued due to increased brain natriuretic peptide levels, and the other due to rash and protein in the urine.

Over the course of the 14.5-year PMS, SAEs of cardiac, renal, and cerebrovascular origin occurred in patients with all three disease phenotypes (Fig. 2). In male patients with the classic phenotype, these events increased through the end of the initial 8-year PMS before plateauing, with no additional cases reported in the extension survey (Fig. 2A). Likewise, no further events were noted in male patients with the non-classic phenotype (Fig. 2B). In heterozygous female patients, while new renal and cerebrovascular events had also ceased by year 5 of the initial PMS, in contrast, the incidence of cardiac events continued to occur during the extension survey (Fig. 2C).

3.4. Laboratory findings

Of the 129 patients, 121 had plasma Gb3 concentrations for analysis (eight patients had no Gb3 data). After excluding patients who reported unknown prior ERT exposure ($n = 10$), plasma Gb3 concentrations in

Table 2

Frequency of ADRs.[a]

	Analysis Set			
	Total ($N = 129$)	Male (Classic Phenotype) ($N = 58$)	Male (Non-classic Phenotype) ($N = 8$)	Female (Heterozygous Genotype) ($N = 63$)
Patients with ADRs[b], n (%)	31 (24.0)	18 (31.0)	4 (50.0)	9 (14.3)
Number of ADRs	145	116	6	23

ADR[a] by SOC and PT	Patients, n (%)			
Psychiatric disorders	1 (0.78)	1 (1.72)	0	0
Insomnia	1 (0.78)	1 (1.72)	0	0
Nervous system disorders	7 (5.43)	5 (8.62)	2 (25.0)	0
Carpal tunnel syndrome	1 (0.78)	1 (1.72)	0	0
Cerebellar infarction	1 (0.78)	0	1 (12.5)	0
Headache	3 (2.33)	2 (3.45)	1 (12.5)	0
Hypoesthesia	1 (0.78)	1 (1.72)	0	0
Tremor	1 (0.78)	1 (1.72)	0	0
Restless legs syndrome	1 (0.78)	1 (1.72)	0	0
Eye disorders	1 (0.78)	1 (1.72)	0	0
Retinal vein occlusion	1 (0.78)	1 (1.72)	0	0
Ear and labyrinth disorders	3 (2.33)	3 (5.17)	0	0
Tinnitus	2 (1.55)	2 (3.45)	0	0
Sudden hearing loss	1 (0.78)	1 (1.72)	0	0
Cardiac disorders	4 (3.10)	0	1 (12.5)	3 (4.76)
Atrial fibrillation	1 (0.78)	0	0	1 (1.59)
Cardiac failure	1 (0.78)	0	0	1 (1.59)
Palpitations	1 (0.78)	0	0	1 (1.59)
Pericarditis	1 (0.78)	0	0	1 (1.59)
Sinus node dysfunction	1 (0.78)	0	1 (12.5)	0
Vascular disorders	2 (1.55)	1 (1.72)	0	1 (1.59)
Hypotension	1 (0.78)	1 (1.72)	0	0
Shock	1 (0.78)	0	0	1 (1.59)
Hot flush	1 (0.78)	0	0	1 (1.59)
Respiratory, thoracic and mediastinal disorders	4 (3.10)	3 (5.17)	0	1 (1.59)

(*continued on next page*)

Table 2 (*continued*)

ADR[a] by SOC and PT	Patients, n (%)			
Cough	2 (1.55)	2 (3.45)	0	0
Dyspnea	1 (0.78)	1 (1.72)	0	0
Wheezing	1 (0.78)	1 (1.72)	0	0
Laryngeal discomfort	1 (0.78)	0	0	1 (1.59)
Oropharyngeal discomfort	1 (0.78)	1 (1.72)	0	0
Gastrointestinal disorders	2 (1.55)	0	0	2 (3.17)
Eructation	1 (0.78)	0	0	1 (1.59)
Nausea	1 (0.78)	0	0	1 (1.59)
Hepatobiliary disorders	2 (1.55)	0	0	2 (3.17)
Hepatic function abnormal	2 (1.55)	0	0	2 (3.17)
Skin and subcutaneous tissue disorders	11 (8.53)	7 (12.07)	2 (25.0)	2 (3.17)
Papule	1 (0.78)	1 (1.72)	0	0
Pruritus	4 (3.10)	3 (5.17)	1 (12.5)	0
Rash	3 (2.33)	1 (1.72)	0	2 (3.17)
Urticaria	4 (3.10)	3 (5.17)	1 (12.5)	0
Skin induration	1 (0.78)	1 (1.72)	0	0
Musculoskeletal and connective tissue disorders	6 (4.65)	5 (8.62)	0	1 (1.59)
Back pain	1 (0.78)	0	0	1 (1.59)
Muscular weakness	1 (0.78)	1 (1.72)	0	0
Pain in extremity	4 (3.10)	4 (6.90)	0	0
General disorders and administration site conditions	11 (8.53)	7 (12.07)	1 (12.5)	3 (4.76)
Chest discomfort	2 (1.55)	0	1 (12.5)	1 (1.59)
Chest pain	1 (0.78)	0	0	1 (1.59)
Chills	1 (0.78)	1 (1.72)	0	0
Injection site urticaria	1 (0.78)	1 (1.72)	0	0
Malaise	2 (1.55)	2 (3.45)	0	0
Edema	1 (0.78)	0	0	1 (1.59)
Edema peripheral	1 (0.78)	1 (1.72)	0	0
Pyrexia	4 (3.10)	4 (6.90)	0	0
Investigations	3 (2.33)	3 (5.17)	0	0
Blood lactate dehydrogenase increased	1 (0.78)	1 (1.72)	0	0
Blood alkaline phosphatase increased	1 (0.78)	1 (1.72)	0	0
Globotriaosylceramide increased	1 (0.78)	1 (1.72)	0	0

Table 2 (*continued*)

ADR[a] by SOC and PT	Patients, n (%)			
Alanine aminotransferase increased	1 (0.78)	1 (1.72)	0	0
Gamma-glutamyltransferase increased	1 (0.78)	1 (1.72)	0	0
Protein urine present	1 (0.78)	1 (1.72)	0	0
Injury, poisoning and procedural complications	2 (1.55)	2 (3.45)	0	0
Road traffic accident	1 (0.78)	1 (1.72)	0	0
Infusion related reaction	1 (0.78)	1 (1.72)	0	0

Data are n (%), unless otherwise noted.

ADR, adverse drug reaction; PT, Preferred Term; SOC, System Organ Class.

[a] ADRs were coded using the Japanese version of the Medical Dictionary for Regulatory Activities Version 25.0.

[b] Multiple episodes of the same PT or SOC in the same patient were counted only once.

111 patients were analyzed. Plasma Gb3 concentrations achieved during the first six months of agalsidase alfa treatment were sustained over the 14.5-year observation period regardless of prior exposure to ERT (Fig. 3). In male patients with the classic phenotype, mean (SD) baseline plasma Gb3 levels were initially high at 9.5 (4.0) nmol/mL in those without prior ERT exposure but declined rapidly to 5.2 (1.6) nmol/mL after 6 months of treatment and remained stable thereafter. In those with prior ERT exposure, baseline Gb3 was 5.3 (2.4) nmol/mL, where it remained relatively unchanged for the entire observation period. Male patients with the non-classic phenotype and heterozygous female patients showed relatively smaller changes in plasma Gb3. Male patients with the non-classic phenotype with and without prior ERT exposure had a mean (SD) baseline plasma Gb3 concentration of 3.8 (1.7) nmol/mL and 3.3 (0.5) nmol/mL, respectively, where levels remained steady for the entire observation period. Likewise, heterozygous female patients with and without prior ERT exposure showed a mean (SD) baseline plasma Gb3 concentration of 3.9 (1.2) nmol/mL and 4.0 (1.6) nmol/mL, respectively, where it remained at a steady level until the final measurement.

Among the 129 patients included in the extension survey, 781 IgG anti-agalsidase alfa antibody tests (mean [SD] 6.1 [2.7] per patient, range 0–12) were conducted in 128 patients, and 23 tests from six patients were positive for anti-agalsidase alfa antibodies (Fig. 4). All six patients were male with the classic phenotype. One of these patients reported an ADR of non-serious malaise after testing positive for anti-agalsidase alfa IgG, and three patients became negative for anti-agalsidase alfa antibodies on subsequent testing (one patient ceased testing, while two patients underwent at least one more test after receiving a negative result). No IgE anti-agalsidase alfa antibodies were detected in any patients.

4. Discussion

This nationwide prospective observational study extended the initial 8-year PMS of agalsidase alfa in Japanese patients with Fabry disease to a total of 14.5 years. This is the longest surveillance of agalsidase alfa treatment in clinical practice in Japan, with a mean duration of treatment of 9.6 years. Long-term agalsidase alfa treatment sustained reduced Gb3 concentrations without increasing the rate of anti-agalsidase antibody positivity. Further, no new safety concerns were identified. Overall, these findings suggest that agalsidase alfa treatment demonstrates continued safety and sustains patients' clinical course over the long term.

The incidence of ADRs in the present extension survey (24.0%) is comparable to that reported in the initial PMS of Japanese patients
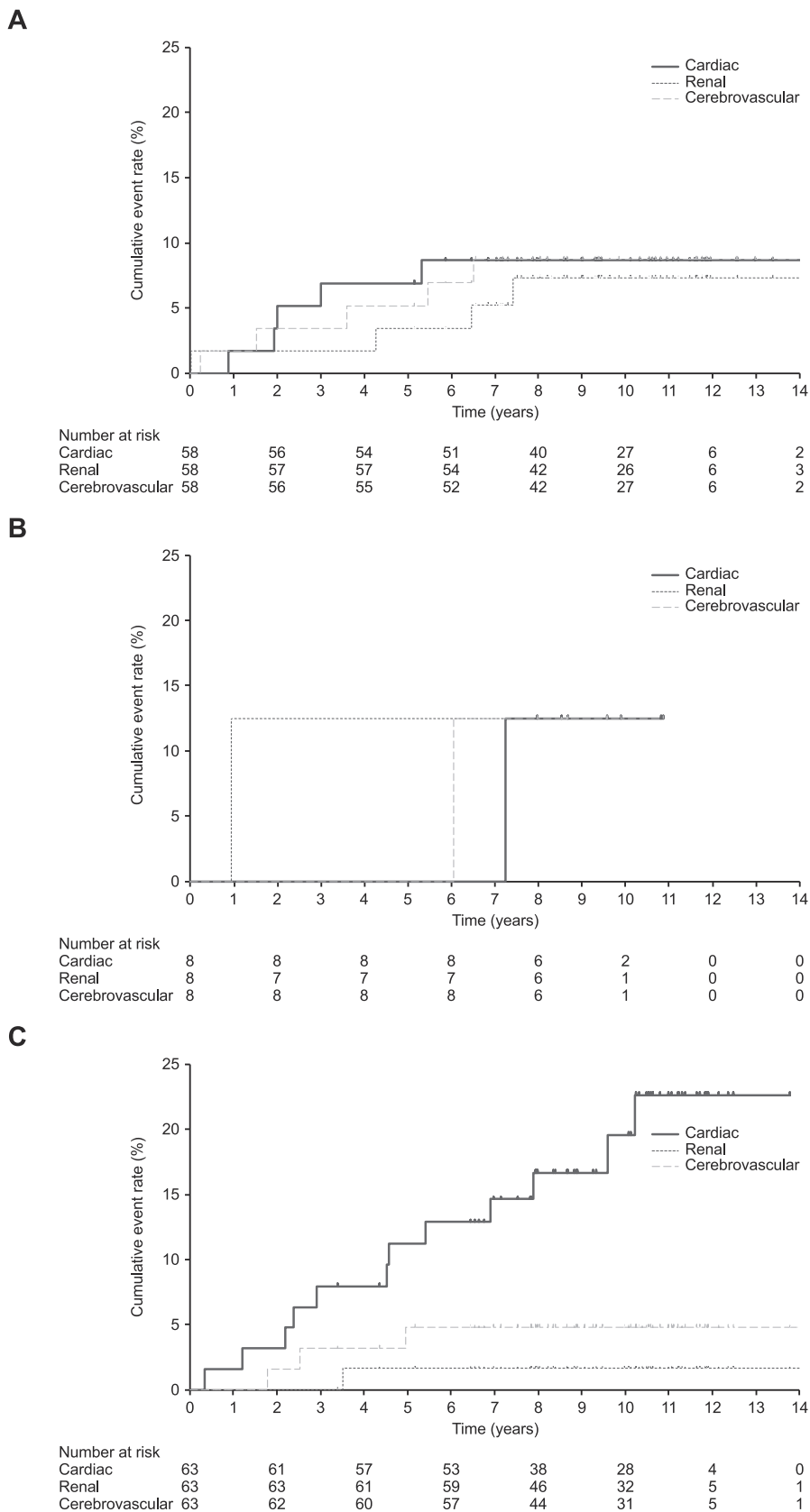
Fig. 2. Kaplan-Meier curves of the cumulative rate of serious adverse events of cardiac, renal, or cerebrovascular origin in patients with Fabry disease during treatment with agalsidase alfa. (A) Male patients with classic Fabry disease. (B) Male patients with non-classic Fabry disease. (C) Female patients with Fabry disease.
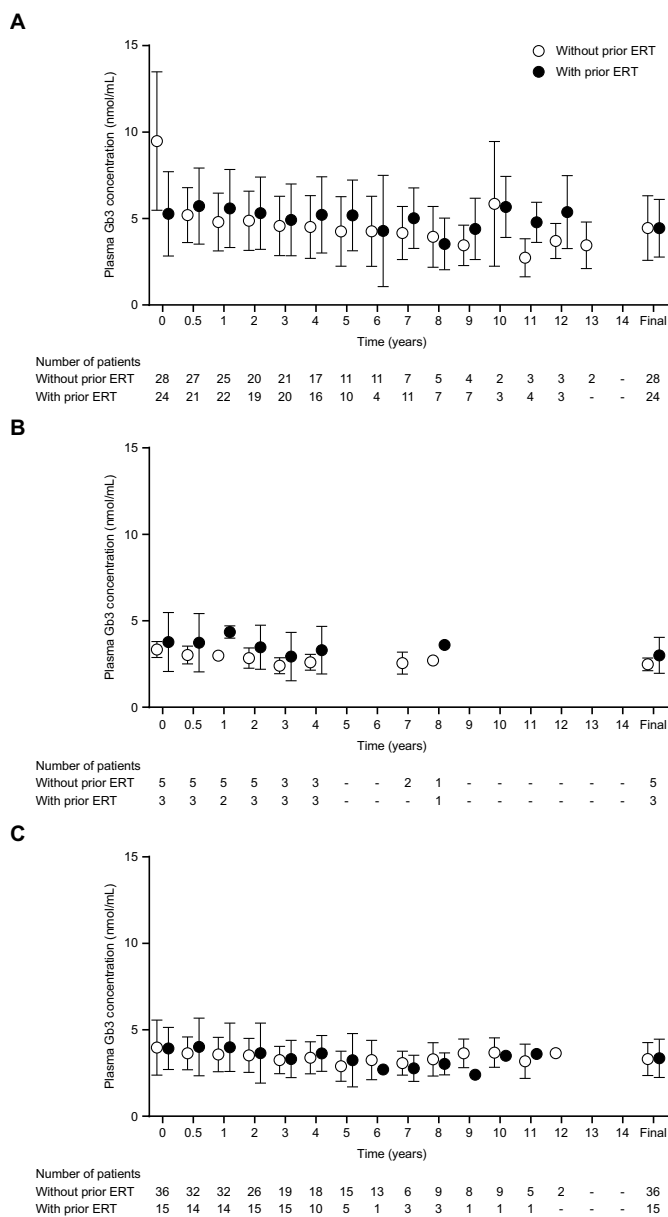
Fig. 3. Plasma Gb3 titer over time in patients with Fabry disease receiving treatment with agalsidase alfa with or without prior ERT. (A) Male patients with classic Fabry disease. (B) Male patients with non-classic Fabry disease. (C) Female patients with Fabry disease.

"-"in the tables indicates there were no data available at those time points. ERT, enzyme replacement therapy; Gb3, globotriaosylceramide.

(24.5%) [13]. In the present study, the most frequent events aggregated by SOC were "skin and subcutaneous tissue disorders" and "general disorders and administration site conditions" at 8.5%, followed by "nervous system disorders" at 5.4% (Table 2). The respective incidences in the initial PMS were 5.1%, 10.3%, and 5.1% (unpublished data, submitted to the Pharmaceuticals and Medical Devices Agency), indicating that while "general disorders and administration site conditions" and "nervous system disorders" occurred at a similar rate, "skin and subcutaneous tissue disorders" were slightly more frequent in the extension survey. Of the 14 patients who developed ADRs for the first time in the extension survey, three had ADRs in the "skin and subcutaneous tissue disorders" SOC. Whether patients are at increased risk of such ADRs with continued treatment is unclear and warrants further investigation.

In contrast, the incidence of ADRs observed here is slightly lower than that reported in the 5-year analysis of findings from the ongoing real-world FOS (33.9%, 188/555 patients) performed predominantly in European countries [14]. The most frequent ADRs also differed. While pruritus, urticaria, pain in extremity, and pyrexia ($n = 4$ patients each, 3.1%) were the most common ADRs in the present survey, among these only pyrexia was considered frequently reported in FOS ($n = 12$ patients, 2.2%). In contrast, infusion-related reactions ($n = 35$ patients, 6.3%) were most common in FOS but were only reported in one patient (0.8%) in the present survey. Interestingly, however, the incidence of serious ADRs was higher in the present survey (22 serious ADRs in 12 patients, incidence of 9.3%) compared with FOS (10 serious ADRs, incidence not reported, but no >1.8%). The discrepancies between the present PMS and FOS may be due to differences in definitions used to identify ADRs and their severity. Alternatively, the discrepancies may suggest differences in ADRs in Japanese compared with European populations, although further analyses are needed to confirm this possibility. Nevertheless, both real-world studies reported no additional safety concerns, suggesting that agalsidase alfa is a generally safe treatment.

Analysis of SAEs of cardiac, renal, and cerebrovascular origin in the present survey showed that while all these events decreased in frequency in male patients with long-term agalsidase alfa administration, cardiac events continued to occur over time in female patients. This may be explained by the higher proportion of female patients with cardiac complications at enrollment in the initial PMS compared with male patients with the classic phenotype (66.7% vs. 41.4%) and the presence of an association between the severity of baseline cardiac dysfunction and the risk of cardiac events in patients treated with agalsidase alfa [15]. Given the difficulty of Fabry disease diagnosis [1] and the lower levels of Gb3 in female patients [16], necessitating genetic screening, female patients are typically diagnosed with Fabry disease at an older age than male patients with the classic phenotype [17]. The delay in diagnosis and the relatively advanced age of female patients could make it more likely that they will already have underlying cardiac damage at diagnosis. Indeed, the mean age at diagnosis and at study inclusion in the initial PMS differed by >20 years between female patients and male patients with the classic phenotype. The proportion of ERT-naïve patients was also higher in female patients than in male patients in the present survey. These findings suggest the need for early diagnosis and early initiation of treatment in female patients.

Although >20 years have passed since the first approval of ERTs such as agalsidase alfa for the treatment of Fabry disease, the ideal time to initiate treatment remains unclear. Given that the first symptoms of the classic phenotype of Fabry disease typically manifest in childhood [18], early treatment may be important. In fact, early treatment has been found to alleviate many of the disease's symptoms and stabilize, and in some cases reverse, disease progression [18]. Findings from FOS have also demonstrated that prompt treatment is linked to significant reductions in cardiovascular events in both male and female patients [19,20]. However, as mentioned above, diagnosis is often delayed in female patients. Further, despite findings that male and female patients respond similarly to agalsidase alfa [21], evidence from Spain and Germany suggests that, even when found to fulfill the criteria for ERT initiation, female patients with Fabry disease are less likely to receive ERT than male patients [22,23]. Thus, in addition to strategies to promote early diagnosis, improved adherence to treatment guidelines may also be needed to ensure that female patients receive more timely therapy.

As reported in the initial PMS [13], agalsidase alfa treatment decreased plasma Gb3 concentrations in male patients with the classic phenotype without prior ERT exposure. Longer-term surveillance revealed no obvious elevation in plasma Gb3 regardless of prior ERT exposure. Agalsidase alfa treatment has previously been shown to reduce plasma Gb3 in male [24,25] and female [26,27] patients. The variability in baseline plasma Gb3 levels among the three phenotypes in the present survey is also in line with previous findings that male patients have higher pretreatment plasma Gb3 levels than female patients

No.	Age group	Sex	Type	Prior ERT	0	6 m	1 y	2 y	3 y	4 y	5 y	6 y	7 y	8 y	9 y	10 y	11 y	12 y	13 y	14 y
1	<20	Male	Classic	Yes		+	+	+	+	+	+			+						
2	≥30 to <40	Male	Classic	Yes		+	+	+	+	+						—	—			
3	≥40 to <50	Male	Classic	Yes		+	+	+	+											
4	≥50 to <60	Male	Classic	Yes		+	+	+	+			+								
5	<20	Male	Classic	No					+				—							
6	≥50 to <60	Male	Classic	No	—	—	—	+	—	—	—	—	—							

Fig. 4. Anti-agalsidase alfa antibody positivity over time. Antibody measurements were conducted at the discretion of the physician. All 129 patients had antibody levels measured at least once; only those with at least one positive measurement are shown. A blank square indicates no measurement at that time point. ERT, enzyme replacement therapy; m, months; y, years.

[16]. Therefore, findings from this extension survey indicate that agalsidase alfa therapy sustains reduced plasma Gb3 levels over the long term.

In the present survey, six of 58 males with the classic phenotype (10.3%) developed anti-agalsidase alfa antibodies. This incidence is low compared with a previous study reporting that more than half of male patients with the classic phenotype develop anti-drug antibodies against agalsidase alfa and beta [28]. As suggested by van der Veen et al. [29], patients with this most severe phenotype often develop anti-drug antibodies following ERT because the absence of the native enzyme causes their immune system to recognize the recombinant enzyme as a foreign body. Although the small sample size makes it impossible to draw conclusions on whether development of these antibodies had any impact on the safety of the therapy in the present cohort, one of the six patients who developed anti-agalsidase antibodies reported an ADR of non-serious malaise, while two patients discontinued the survey, one due to an adverse event of vascular stenosis that led to death and the other due to switching to another drug. In addition to safety implications, anti-agalsidase alfa antibodies can inhibit agalsidase alfa activity, potentially affecting efficacy [28]. However, analysis of Gb3 concentrations in the six patients who developed anti-agalsidase alfa antibodies was inconclusive of a clear relationship between these markers. Nevertheless, the observation that three of the six patients who developed anti-agalsidase alfa antibodies subsequently tested negative suggests that they may have developed low titers, which have been shown to have no significant effect on the safety or efficacy of agalsidase alfa treatment [25]. Therefore, our data suggest there was a low incidence of anti-Gb3 antibodies following agalsidase alfa treatment, and that if they do occur, they do not have a significant impact on the subsequent clinical course or safety in Japanese patients with Fabry disease.

This study has several strengths. First, it is the longest real-world surveillance of agalsidase alfa treatment in Japan to date, following patients for up to 14.5 years. Because patients with Fabry disease require lifelong treatment, such long-term real-world studies are highly valuable to both patients and physicians. In addition to the need for early diagnosis and early treatment initiation, we believe that it is necessary to continue to monitor the prognosis of patients receiving ERT to ensure the treatment remains safe and effective. Second, the study examined Japanese patients, who, along with Asian populations more generally, are under-represented in Fabry disease studies. Although the majority of studies on Fabry disease have been conducted in White populations [for a review, see [30]], there is evidence that Japanese patients with Fabry disease harbor at least one unique pathogenic mutation [31]. Continued research in this population will improve awareness, diagnostics, and treatment efforts. Finally, we conducted separate analyses by phenotype and sex to provide a true representation of the variability of the disease across these populations.

A number of limitations also warrant mention. First, this study only included patients from the initial PMS who continued to receive agalsidase alfa. This may have led to some bias in the population demographics, such as a low proportion of severe cases that could have resulted in poor control or death, cases with many adverse events, and patients with low satisfaction. Second, the relatively small sample size of male patients with the non-classic phenotype may have biased estimates of the treatment's safety and effect on markers of disease activity in this population. Third, the relatedness of adverse events to agalsidase alfa treatment and their severity were determined by individual attending physicians, which may have led to heterogeneous classification. Likewise, the timing of Gb3 measurement and anti-agalsidase alfa antibody tests was determined by attending physicians. Fourth, the observational nature of this study meant there was no comparator group, making it impossible to determine the degree to which agalsidase alfa treatment contributed to suppressing disease progression compared to no treatment. Finally, the effectiveness of treatment was assessed based on changes in plasma Gb3, which has been used as an efficacy endpoint in many clinical trials [6,32–34], as increases in plasma Gb3 are a pathogenic hallmark of Fabry disease. However, recent investigations have suggested that plasma Gb3 may not be a useful marker of response to treatment, with levels shown to be highly variable in some patients, particularly those with the N215S mutation and heterozygotes [35]. Further, there is no evidence of a correlation between elevated Gb3 and clinical symptoms [16]. Additionally, we did not assess lyso-Gb3 levels, which were identified as a potentially useful biomarker of Fabry disease after this survey was initiated [36]. Future investigations of drug effectiveness would benefit from assessing other markers such as lyso-Gb3 or globotriaosylsphingosine [37].

In conclusion, this extension survey of the initial PMS of agalsidase alfa treatment in Japanese patients with Fabry disease confirmed the ERT's long-term safety and suppressive effects on disease markers in real-world clinical practice. These findings suggest that agalsidase alfa treatment demonstrates continued safety and sustains patients' clinical course over the long term.

Funding

CRediT authorship contribution statement

Makoto Arakawa: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Yoshinori Ikeda:** Writing – review & editing, Visualization, Conceptualization. **Hiromichi Otaka:** Writing – review & editing, Validation, Supervision. **Sanghun Iwashiro:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Makoto Arakawa reports financial support, administrative support, article publishing charges, equipment, drugs, or supplies, statistical analysis, and writing assistance were provided by Takeda Pharmaceutical Company Limited. Yoshinori Ikeda reports financial support, administrative support, article publishing charges, equipment, drugs, or supplies, statistical analysis, and writing assistance were provided by Takeda Pharmaceutical Company Limited. Hiromichi Otaka reports financial support, administrative support, article publishing charges, equipment, drugs, or supplies, statistical analysis, and writing assistance were provided by Takeda Pharmaceutical Company Limited. Sanghun Iwashiro reports financial support, administrative support, article publishing charges, equipment, drugs, or supplies, statistical analysis, and writing assistance were provided by Takeda Pharmaceutical Company Limited. Makoto Arakawa reports a relationship with Takeda Pharmaceutical Company Limited that includes: employment. Yoshinori Ikeda reports a relationship with Takeda Pharmaceutical Company Limited that includes: employment. Hiromichi Otaka reports a relationship with Takeda Pharmaceutical Company Limited that includes: employment. Sanghun Iwashiro reports a relationship with Takeda Pharmaceutical Company Limited that includes: employment and equity or stocks. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets, including the redacted study protocol, redacted statistical analysis plan, and individual participants data supporting the results reported in this article, will be made available within three months from initial request, to researchers who provide a methodologically sound proposal. The data will be provided after its de-identification, in compliance with applicable privacy laws, data protection and requirements for consent and anonymization.

References

[1] A. Mehta, M. Beck, F. Eyskens, C. Feliciani, I. Kantola, U. Ramaswami, et al., Fabry disease: a review of current management strategies, QJM 103 (2010) 641–659, https://doi.org/10.1093/qjmed/hcq117.

[2] R.J. Desnick, R. Brady, J. Barranger, A.J. Collins, D.P. Germain, M. Goldman, et al., Fabry disease, an under-recognized multisystemic disorder: expert recommendations for diagnosis, management, and enzyme replacement therapy, Ann. Intern. Med. 138 (2003) 338–346, https://doi.org/10.7326/0003-4819-138-4-200302180-00014.

[3] K.D. MacDermot, A. Holmes, A.H. Miners, Anderson-Fabry disease: clinical manifestations and impact of disease in a cohort of 98 hemizygous males, J. Med. Genet. 38 (2001) 750–760, https://doi.org/10.1136/jmg.38.11.750.

[4] D.P. Germain, A. Fouilhoux, S. Decramer, M. Tardieu, P. Pillet, M. Fila, et al., Consensus recommendations for diagnosis, management and treatment of Fabry disease in paediatric patients, Clin. Genet. 96 (2019) 107–117, https://doi.org/10.1111/cge.13546.

[5] O. Azevedo, M.F. Gago, G. Miltenberger-Miltenyi, N. Sousa, D. Cunha, Fabry Disease Therapy: State-of-the-Art and Current Challenges, Int. J. Mol. Sci. 22 (2020), https://doi.org/10.3390/ijms22010206.

[6] C.M. Eng, N. Guffon, W.R. Wilcox, D.P. Germain, P. Lee, S. Waldek, et al., Safety and efficacy of recombinant human alpha-galactosidase a replacement therapy in Fabry's disease, N. Engl. J. Med. 345 (2001) 9–16, https://doi.org/10.1056/nejm200107053450102.

[7] M. Beck, U. Ramaswami, E. Hernberg-Ståhl, D.A. Hughes, C. Kampmann, A. B. Mehta, et al., Twenty years of the Fabry outcome survey (FOS): insights, achievements, and lessons learned from a global patient registry, Orphanet J. Rare Dis. 17 (2022) 238, https://doi.org/10.1186/s13023-022-02392-9.

[8] R. Giugliani, D.-M. Niu, U. Ramaswami, M. West, D. Hughes, C. Kampmann, et al., A 15-Year Perspective of the Fabry Outcome Survey, Journal of Inborn Errors of Metabolism and Screening 4 (2016), https://doi.org/10.1177/2326409816666298, 2326409816666298.

[9] M. Beck, D. Hughes, C. Kampmann, S. Larroque, A. Mehta, G. Pintos-Morell, et al., Long-term effectiveness of agalsidase alfa enzyme replacement in Fabry disease: a Fabry outcome survey analysis, Mol Genet Metab Rep. 3 (2015) 21–27, https://doi.org/10.1016/j.ymgmr.2015.02.002.

[10] C. Kampmann, A. Perrin, M. Beck, Effectiveness of agalsidase alfa enzyme replacement in Fabry disease: cardiac outcomes after 10 years' treatment, Orphanet J. Rare Dis. 10 (2015) 125, https://doi.org/10.1186/s13023-015-0338-2.

[11] U. Ramaswami, M. Beck, D. Hughes, C. Kampmann, J. Botha, G. Pintos-Morell, et al., Cardio- renal outcomes with long- term Agalsidase alfa enzyme replacement therapy: a 10- year Fabry outcome survey (FOS) analysis, Drug Des. Devel. Ther. 13 (2019) 3705–3715, https://doi.org/10.2147/dddt.S207856.

[12] M. Cybulla, K. Nicholls, S. Feriozzi, A. Linhart, J. Torras, B. Vujkovac, et al., Renoprotective Effect of Agalsidase Alfa: A Long-Term Follow-Up of Patients with Fabry Disease, J. Clin. Med. 11 (2022), https://doi.org/10.3390/jcm11164810.

[13] H. Sasa, M. Nagao, K. Kino, Safety and effectiveness of enzyme replacement therapy with agalsidase alfa in patients with Fabry disease: post-marketing surveillance in Japan, Mol. Genet. Metab. 126 (2019) 448–459, https://doi.org/10.1016/j.ymgme.2019.02.005.

[14] A. Mehta, M. Beck, P. Elliott, R. Giugliani, A. Linhart, G. Sunder-Plassmann, et al., Enzyme replacement therapy with agalsidase alfa in patients with Fabry's disease: an analysis of registry data, Lancet 374 (2009) 1986–1996, https://doi.org/10.1016/s0140-6736(09)61493-8.

[15] S. Feriozzi, A. Linhart, U. Ramaswami, V. Kalampoki, A. Gurevich, D. Hughes, Effects of Baseline Left Ventricular Hypertrophy and Decreased Renal Function on Cardiovascular and Renal Outcomes in Patients with Fabry Disease Treated with Agalsidase Alfa: A Fabry Outcome Survey Study, Clin. Ther. 42 (2020) 2321–2330. e2320, https://doi.org/10.1016/j.clinthera.2020.10.007.

[16] A.C. Vedder, G.E. Linthorst, M.J. van Breemen, J.E. Groener, F.J. Bemelman, A. Strijland, et al., The Dutch Fabry cohort: diversity of clinical manifestations and Gb3 levels, J. Inherit. Metab. Dis. 30 (2007) 68–78, https://doi.org/10.1007/s10545-006-0484-8.

[17] A. Mehta, R. Ricci, U. Widmer, F. Dehout, A. Garcia de Lorenzo, C. Kampmann, et al., Fabry disease defined: baseline clinical manifestations of 366 patients in the Fabry outcome survey, Eur. J. Clin. Investig. 34 (2004) 236–242, https://doi.org/10.1111/j.1365-2362.2004.01309.x.

[18] C. Ellaway, Paediatric Fabry disease, Transl Pediatr. 5 (2016) 37–42. doi: https://doi.org/10.3978/j.issn.2224-4336.2015.12.02.

[19] D. Hughes, A. Linhart, A. Gurevich, V. Kalampoki, D. Jazukeviciene, S. Feriozzi, Prompt Agalsidase alfa therapy initiation is associated with improved renal and cardiovascular outcomes in a Fabry outcome survey analysis, Drug Des. Devel. Ther. 15 (2021) 3561–3572, https://doi.org/10.2147/dddt.S313789.

[20] R. Parini, G. Pintos-Morell, J.B. Hennermann, T.R. Hsu, N. Karabul, V. Kalampoki, et al., Analysis of renal and cardiac outcomes in male participants in the Fabry outcome survey starting Agalsidase alfa enzyme replacement therapy before and after 18 years of age, Drug Des. Devel. Ther. 14 (2020) 2149–2158, https://doi.org/10.2147/dddt.S249433.

[21] D.A. Hughes, M. Barba Romero, C.E. Hollak, R. Giugliani, P.B. Deegan, Response of women with Fabry disease to enzyme replacement therapy: comparison with men, using data from FOS–the Fabry outcome survey, Mol. Genet. Metab. 103 (2011) 207–214, https://doi.org/10.1016/j.ymgme.2011.03.022.

[22] M. Barba-Romero, G. Pintos-Morell, Gender Differences in the Application of Spanish Criteria for Initiation of Enzyme Replacement Therapy for Fabry Disease in the Fabry Outcome Survey, Int. J. Mol. Sci. 17 (2016), https://doi.org/10.3390/ijms17121965.

[23] M. Lenders, J.B. Hennermann, C. Kurschat, A. Rolfs, S. Canaan-Kühl, C. Sommer, et al., Multicenter female Fabry study (MFFS) - clinical survey on current treatment of females with Fabry disease, Orphanet J. Rare Dis. 11 (2016) 88, https://doi.org/10.1186/s13023-016-0473-4.

[24] D.A. Hughes, P.M. Elliott, J. Shah, J. Zuckerman, G. Coghlan, J. Brookes, et al., Effects of enzyme replacement therapy on the cardiomyopathy of Anderson-Fabry disease: a randomised, double-blind, placebo-controlled clinical trial of agalsidase alfa, Heart 94 (2008) 153–158, https://doi.org/10.1136/hrt.2006.104026.

[25] R. Schiffmann, J.B. Kopp, H.A. Austin 3rd, S. Sabnis, D.F. Moore, T. Weibel, et al., Enzyme replacement therapy in Fabry disease: a randomized controlled trial, JAMA 285 (2001) 2743–2749, https://doi.org/10.1001/jama.285.21.2743.

[26] F. Baehner, C. Kampmann, C. Whybra, E. Miebach, C.M. Wiethoff, M. Beck, Enzyme replacement therapy in heterozygous females with Fabry disease: results of a phase IIIB study, J. Inherit. Metab. Dis. 26 (2003) 617–627, https://doi.org/10.1023/b:boli.0000005658.14563.77.

[27] C. Whybra, E. Miebach, E. Mengel, A. Gal, K. Baron, M. Beck, et al., A 4-year study of the efficacy and tolerability of enzyme replacement therapy with agalsidase alfa in 36 women with Fabry disease, Genet. Med. 11 (2009) 441–449, https://doi.org/10.1097/GIM.0b013e3181a23bec.

[28] G.E. Linthorst, C.E. Hollak, W.E. Donker-Koopman, A. Strijland, J.M. Aerts, Enzyme therapy for Fabry disease: neutralizing antibodies toward agalsidase alpha and beta, Kidney Int. 66 (2004) 1589–1595, https://doi.org/10.1111/j.1523-1755.2004.00924.x.

[29] S.J. van der Veen, W.J. Vlietstra, L. van Dussen, A.B.P. van Kuilenburg, M.G. W. Dijkgraaf, M. Lenders, et al., Predicting the Development of Anti-Drug Antibodies against Recombinant alpha-Galactosidase A in Male Patients with Classical Fabry Disease, Int. J. Mol. Sci. 21 (2020), https://doi.org/10.3390/ijms21165784.

[30] M. Fuller, P.J. Meikle, J.J. Hopwood, Epidemiology of lysosomal storage diseases: An overview, in: A. Mehta, M. Beck, G. Sunder-Plassmann (Eds.), Fabry Disease: Perspectives from 5 Years of FOS, Oxford PharmaGenesis, Oxford, 2006. https://www.ncbi.nlm.nih.gov/books/NBK11603/.

[31] H. Sakuraba, T. Tsukimura, T. Togawa, T. Tanaka, T. Ohtsuka, A. Sato, et al., Fabry disease in a Japanese population-molecular and biochemical characteristics, Mol Genet Metab Rep. 17 (2018) 73–79, https://doi.org/10.1016/j.ymgmr.2018.10.004.

[32] Y. Eto, T. Ohashi, Y. Utsunomiya, M. Fujiwara, A. Mizuno, K. Inui, et al., Enzyme replacement therapy in Japanese Fabry disease patients: the results of a phase 2 bridging study, J. Inherit. Metab. Dis. 28 (2005) 575–583, https://doi.org/10.1007/s10545-005-0575-y.

[33] O. Goker-Alpan, K. Nedd, S.P. Shankar, Y.H. Lien, N. Weinreb, A. Wijatyk, et al., Effect and tolerability of Agalsidase alfa in patients with Fabry disease who were treatment Naïve or formerly treated with Agalsidase Beta or Agalsidase alfa, JIMD Rep. 23 (2015) 7–15, https://doi.org/10.1007/8904_2015_422.

[34] L. Goláň, O. Goker-Alpan, M. Holida, I. Kantola, M. Klopotowski, J. Kuusisto, et al., Evaluation of the efficacy and safety of three dosing regimens of agalsidase alfa enzyme replacement therapy in adults with Fabry disease, Drug Des. Devel. Ther. 9 (2015) 3435–3444, https://doi.org/10.2147/dddt.S80928.

[35] E. Young, K. Mills, P. Morris, A. Vellodi, P. Lee, S. Waldek, et al., Is globotriaosylceramide a useful biomarker in Fabry disease? Acta Paediatr. Suppl. 94 (2005) 51–54, discussion 37-58, https://doi.org/10.1111/j.1651-2227.2005.tb02112.x.

[36] H. Maruyama, K. Miyata, M. Mikame, A. Taguchi, C. Guili, M. Shimura, et al., Effectiveness of plasma lyso-Gb3 as a biomarker for selecting high-risk patients with Fabry disease from multispecialty clinics for genetic analysis, Genet. Med. 21 (2019) 44–52, https://doi.org/10.1038/gim.2018.31.

[37] S.M. Rombach, N. Dekker, M.G. Bouwman, G.E. Linthorst, A.H. Zwinderman, F. A. Wijburg, et al., Plasma globotriaosylsphingosine: diagnostic value and relation to clinical manifestations of Fabry disease, Biochim. Biophys. Acta 1802 (2010) 741–748, https://doi.org/10.1016/j.bbadis.2010.05.003.