Standardising clinical outcomes measures for adult clinical trials in Fabry Disease: A global Delphi Consensus

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Abstract

Background

Recent years have witnessed a considerable increase in clinical trials of new investigational agents for Fabry disease (FD). Several trials investigating different agents are currently in progress; however, lack of standardisation results in challenges to interpretation and comparison. To facilitate the standardisation of investigational programs, we have developed a common framework for future clinical trials in FD.

Methods and findings

A broad consensus regarding clinical outcomes and ways to measure them was obtained via the Delphi methodology. 35 FD clinical experts from 4 continents, representing 3389 FD patients, participated in 3 rounds of Delphi procedure. The aim was to reach a consensus regarding clinical trial design, best treatment comparator, clinical outcomes, measurement of those clinical outcomes and inclusion and exclusion criteria. Consensus results of this initiative included: the selection of the adaptative clinical trial as the ideal study design and agalsidase beta as ideal comparator treatment due to its longstanding use in FD. Renal and cardiac outcomes, such as glomerular filtration rate, proteinuria and left ventricular mass index, were prioritised, whereas neurological outcomes including cerebrovascular and white matter lesions were dismissed as a primary or secondary outcome measure. Besides, there was a consensus regarding the importance of patient-related outcomes such as general quality of life, pain, and gastrointestinal symptoms. Also, unity about lysoGb3 and Gb3 tissue deposits as useful surrogate markers of the disease was obtained. The group recognised that cardiac T1 mapping still has potential but requires further development before its widespread introduction in clinical trials. Finally,

Patients with end-stage renal disease or renal transplant patients should be excluded unless a particular group for them is created inside the clinical trial.

Conclusion

This consensus will help to shape the future of clinical trials in FD. We note that the FDA has, coincidentally, recently published draft guidelines on clinical trials in FD and welcome this contribution.

Keywords

1. Introduction

Fabry disease (FD) is an X-linked lysosomal storage disorder caused by a mutation in the *GLA* gene which leads to a deficiency of the enzyme alpha-galactosidase A. This impairment leads to accumulation of mainly sphingolipids in cells from different tissues and organs, particularly globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3)[1]. This ultimately results in renal and cardiac dysfunction, potentially early stroke, and white matter lesions in the central nervous system as well as pain, and autonomic disturbance[2].

Two clinical phenotypes have been described: "classical" and "later-onset"[3]. These cannot be predicted exclusively by genotype[4, 5]. Classical FD is characterised by a multi-system involvement with early acroparaesthesia, sweating abnormalities and gastrointestinal disturbance, whereas most of the later onset phenotypes may be an attenuated form of the disease, commonly involving a single organ system, usually cardiac or sometimes renal[3].

The incidence of FD has been estimated at 1:50,000, however, new-born screening reveals rates that range from approximately 1:1,250 male live births[6] to around about 1:9000 live births depending on the geographical region, study and definition of FD[7-9] This may be due to suboptimal awareness, resulting in late diagnosis[10] particularly of later onset and milder forms of the condition[11].

Two different approaches are approved for treatment of FD: Intravenous enzyme replacement therapy (ERT) with Agalsidase alpha (Replagal©, Takeda) or Agalsidase beta (Fabrazyme©, Sanofi) and oral therapy with the reversible pharmacological chaperone Migalastat (Galafold©, Amicus) Several new therapies are in investigation including new intravenous enzymes, oral substrate reduction therapy and gene therapy[12].

Consequently, recent years have witnessed a considerable increase in clinical trial activity of new investigational agents for FD. Several trials investigating different agents are currently in progress. However, lack of standardisation of clinical end points results in challenges to interpretation and comparison and impedes the understanding of benefit of new interventions.

To facilitate a discussion of the standardisation of investigational programs, we have employed a Delphi consensus process to develop a common framework for clinical trial design in FD. There are three previous studies using Delphi methodology with experts in the field of FD to achieve consensus in various areas. The first one is a 2014 Delphi consensus[13] about best clinical practice in FD which included 15 well-known FD experts and was completed online. It reached consensus in different areas such as diagnosis, decision to start ERT, management of enzyme replacement infusions and adverse effects and follow up/monitoring of patients. The other studies, also published in 2014, tackled the problem of *GLA* gene variants of unknown significance (VUS) from different perspectives: one proposed an algorithm obtained through Delphi methodology to aid in the diagnosis and management of adults with left ventricular hypertrophy and VUS in the *GLA* gene another assessed the diagnosis of FD in patients with chronic kidney disease and VUS, , and a third one analysed definitive FD diagnosis in patients who present with non-specific symptoms such as neuronopathic pain and cornea verticillata[14-16].

Finally, and coincidentally with the ongoing process of this study, the Food and Drug Administration (FDA) released in August 2019 a draft guidance which provide recommendations to sponsors regarding clinical trial design for approving new drugs and therapies in Fabry Disease[17].

In the current study, 32 FD clinical experts from 4 continents, representing 3389 FD patients, participated in 3 rounds of Delphi procedure. The aim was to reach a consensus regarding clinical trial design, best treatment comparator, clinical outcomes, measurement of those clinical outcomes and, inclusion and exclusion criteria

2. Methods

A Delphi methodology was followed to achieve consensus. In the first phase, pre-meeting, an expert in the field and one facilitator (D.A.H. and D.M.M.) reviewed all the clinical outcomes

hosen in phase 3 and open-label extension clinical trials that had been performed in FD previously.

Clinical outcomes were classified according to organ/system: cardiac, renal, neurologic, gastrointestinal, exercise, quality of life, biomarkers, and others. For each category, the clinical outcome was specified and annotated for type of outcome (primary/secondary outcome), unit of measure, number of studies, months of follow up and statistical significance. After reviewing the literature, internationally known experts were invited to participate in the Delphi Procedure. We collated a list of experts from recent Fabry publications and conference presentations from across the world. We approached them with a personal invitation and explanation by email. In total, there were 46 personal invitations. Of them, 27 were from European countries, 4 from Australia, 4 from South America, 9 from North America and 2 from Asia. The current Delphi panel is formed by the experts who answered the call.

The panel initially evaluated the clinical outcomes listed in the literature review providing free text on possible clinical outcomes, modalities of measurement and possible inclusion and exclusion criteria. Items which were chosen by more than a 10% of the experts were considered for further review in the second stage. In case no item reached 10%, the threshold would be 5%.

In the second survey, participants ranked the different items using a seven-point Likert scale following the guidance previously published[18].

Consensus was defined as:

-Strong consensus/High agreement if >75% of the participants chose 5 or more.

-Consensus/ Agreement if >75% of the participants chose 4 or more as 3 meant only a 50% of agreement.

- Disagreement consensus if >75% of the participants chose 1 or 2.

After analysing the results of the second survey, the panel met online to discuss the results and to evaluate if a global consensus was reached or further blinded rounds of survey were required.

3. Results

3.1 Previous Clinical Trials

The pre-meeting review of previous clinical trials detected 19 studies. Most of studies were from the early 2000's, with five studies that were still ongoing. 63% were phase 3 randomized clinical trials studies (RCT) and 37% were phase 3 RCT plus their phase 4 open-label extensions. The different treatments analysed in the studies were: Agalsidase alpha and beta (73%) migalastat (11%) lucerastat (5%) and PRX-102 (11%)

A total of 111 clinical outcomes were found. One study followed patients for 65 months. However, the other studies ranged from 6 to 35 months. 45 clinical outcomes were measured as primary endpoints (41%) and the rest were secondary. Only kidney endothelium Gb3 deposits, skin endothelium Gb3 deposits and pain at its worst via brief pain inventory (BPI) scale were evaluated in more than one study as a primary endpoint[19-24]. However, secondary outcomes were shared by several studies. Only 31 clinical outcomes reached statistical significance (28%) and when two completed studies looked at the same outcome, they never agreed on the significance. A summary of the literature review can be found on **Error! Reference source not found.**, with a more detailed analysis of the different studies in the supplementary information 1.

Organ	Clinical Outcomes	Clinical Outcomes Measured as Primary Endpoint	Clinical Outcomes Measured as Secondary Endpoint	Months of Follow Up	Clinical Outcomes Which Reached Statistical Significance
Cardiac	ECG changes Echocardiography measures MRI measures Blood pressure drop Clinical events	9 (36%)	23 (92%)	6,18,24, and 35	4 (16%)
Renal	Glomerular filtration rate Creatinine Urine analysis Clinical events Kidney pathological examination	8 (35%)	19 (82%)	6,18,24, and 35	4 (17%)
Neurologic	Clinical events Brain MRI Pain Cerebral blood flow Hearing loss Intradermal fibre density	11 (61%)	9 (50%)	5,6,18,24, and 35	8 (44%)
Gastrointestinal	Diarrhoea Pain and other symptomatology Stool characteristics	1 (17%)	5 (83%)	6	2 (33%)
Exercise	Treadmill stress test	6 (86%)	1 (14%)	18,24 and 35	0 (0%)

	Pulmonary function				
Quality of life	Questionnaires Pain medication use	0 (0%)	4 (100%)	5,6,18 and 24	1 (25%)
Biomarkers	Plasma Gb3 and lysoGb3 Urine Gb3 and lysoGb3 Gb3 in tissues Cystatin-C	7 (33%)	17 (81%)	5,6,18,24 and 35	8 (38%)
Other	Oxide nitric pathway Alpha galactosidase activity Severity score Body weight Clinical events Treatment related outcomes	4 (44%)	5 (56%)	6,24 and 35	4 (44%)

Table 1 Summary of the literature review

In summary:

Cardiac outcomes included ECG changes, echocardiography measures, MRI measures and clinical events. Of all the cardiac clinical outcomes, only left ventricular mass index (LVMI), left ventricular mass, stroke volume, diastolic blood pressure drop and clinical events were evident as end points. LVMI measured via echocardiography was most frequent. All other measures were employed as secondary endpoints. Statistical significance was achieved in the reduction of QRS duration, reduction of LVMI and interventricular septum measured by echocardiography and diminishing LVMI measured by MRI[23, 25, 26].

Renal outcomes consisted of estimated glomerular filtration rate (eGFR) by various methods, measured glomerular filtration rate (mGFR) and creatinine levels. In addition, urine analysis of factors such as microalbuminuria, proteinuria over 24h, protein/creatinine ratio, albumin/creatinine ratio, variation in proteinuria of more than 33% and proteinuria defined as more than 300mg of protein in 24h. Time to dialysis and kidney transplant were also included. Finally, in this category outcomes relating to renal pathology (other than Gb3 deposits) such as number and state of glomeruli in biopsies and presence or not of tubular pathology were included. eGFR, eGFR slope, mGFR and time to dialysis and transplant were regarded as primary endpoints in different studies. No primary endpoint reached statistical significance. However, some secondary endpoints such an increase in creatinine clearance, reduction of protein/creatinine ratio in urine and an increase of normal glomeruli with a reduction of glomeruli with mesangial widening reached statistical significance in some studies[23, 27].

Neurologic outcomes encountered, included clinical neurologic examination, time to event (stroke and transient ischemic attack), cerebral MRI scan analysis, hearing loss improvement and pain analysis. They also included more novel outcomes such as analysis of cerebral blood flow by echography or positron emission tomography (PET) scan and intradermal nerve fibres density. 61% of neurological outcomes were regarded as primary[20, 23, 27-31] and 44% reached statistical significance. There was a reduction of average pain measured by BPI item 3 to 6 and pain at its worst measured by BPI, improvement of all the echography items regarding blood flow (pulsatility index, mean velocity, resistance index and peak velocity) improvement of blood flow measured by PET scan and, finally, improvement of hearing loss[23, 28-30].

Gastrointestinal outcomes were taken into consideration in three studies, two still ongoing[20, 22, 32]. The outcomes consisted of clinical symptoms such as diarrhoea, abdominal pain at its worst, general symptoms evaluated by the irritable bowel syndrome scale (IBSS) and other symptoms reported via Likert scale. Furthermore, stool was specifically analysed in the two ongoing studies: One looked at number of days with stools with a Bristol stool scale of 6 or 7, and the other study looked at stool frequency. All the outcomes were followed for 6 months and only general symptoms reported via Likert scale were considered as a primary endpoint. The endpoints evaluated by the study which had finished, reached statistical significance. They were improvement of diarrhoea and improvement of other symptoms measured via Likert scale[22].

Exercise outcomes consisted of exercise tolerance via treadmill stress test evaluating different parameters and pulmonary function changes. All the exercise outcomes except for the treadmill stress test in two studies, were evaluated as primary endpoints. None of them reached statistical significance[27, 32, 33].

Quality of life was assessed in a general way through SF-36 and EQ-5D-5L questionnaires along with the frequency of pain medication use. Quality of life specifically linked to pain was measured in one study through analysing question 9 of the BPI questionnaire[23]. All quality of life outcomes were evaluated as secondary outcome[21, 26, 32, 34]s. 50% of the quality of life

outcomes were being evaluated by studies that still were ongoing. In the other half only the pain related quality of life measured by the question 9 in BPI reached statistical significance.

Plasma biomarkers used as outcomes included Gb3, cystatin C, LysoGb3 in plasma and urine, Gb3 in different units of measure, 24h Gb3 and Gb3 measured in different tissue samples (kidney endothelium, cardiac endothelium, skin endothelium, glomeruli, mesangial space, skin smooth muscle, skin perineurium and finally a 50% reduction of interstitial capillary accumulated Gb3) The most frequent employed biomarker in plasma was lysoGb3; in urine 24h Gb3;and in tissue kidney endothelium Gb3 deposits. While in plasma only Gb3 measured in µg/mL was evaluated as primary outcome, in tissue all except for podocytes Gb3, mesangial Gb3 and glomeruli Gb3 were evaluated as primary outcomes. In urine, none of them were evaluated as primary outcome. Statistical significance was reached in plasma measurement in Gb3 measured in ng/µL and nmol/mL as well as lysoGb3 reduction measured in ng/mL and nmol/L[22, 25, 27]. In urine, a reduction of Gb3 and 24h Gb3 reached statistical significance[21, 22]. Reduction of Gb3 tissue deposits, in cardiac, kidney and skin endothelium achieved statistical significance.

Finally, more difficult to classify outcomes included antidrug IgG antibodies, treatment failure, time to death, time to a composite of clinical events (renal, cardiac, and/or neurologic) body weight change, disease severity score [Mainz severity scale index (MSSI)], alpha galactosidase A activity, and measurement of nitric oxide pathway both at plasma and through skin biopsy. Except for the nitric oxide pathway outcomes, time to death and time to composite clinical events, all the clinical outcomes were evaluated as secondary outcomes. Statistical significance was reached as a decrease of time to the composite clinical events, an increase in body weight and an improvement in both nitric oxide pathway outcomes[23, 27, 35].

3.2 Delphi survey first round results.

32 experts answered all phases and were part of the Delphi panel. They came from all around the world and represented all specialities involved in the clinical care of FD patients (**Error! Reference source not found.** and **Error! Reference source not found.**). The mean number of

FD patients who were being followed by the panel was 113 patients with a mean time working

on the field of FD of 16 years.

Nationalities	Frequency	Percent
Argentina	2	6.25
Australia	3	9.38
Brazil	1	3.13
Canada	3	9.38
Czech Republic	1	3.13
Denmark	1	3.13
France	1	3.13
Germany	2	6.25
Italy	2	6.25
Netherlands	1	3.13
Norway	2	6.25
Portugal	2	6.25
Spain	2	6.25
Switzerland	1	3.13
UK	5	15.63
USA	3	9.38

Table 2 Panel's nationalities

Specialty	Frequency	Percent
Biochemistry	2	6.25
Cardiology	4	12.50
Clinical Genetics	2	6.25
Endocrinology	1	3.13
Internal Medicine	3	9.38
Metabolic specialist	2	6.25
Nephrology	9	28.13
Neurology	3	9.38
Pediatrics	6	18.75

Table 3 Panel's medical specialities

Results which passed the designated threshold in phase one are summarised in **Error!**

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Item	Above Threshold	Below Threshold
Ideal methodology Fabry disease trial	Adaptative design Randomized controlled trial	Open label Registry Crossover studies
Ideal treatment comparator	Agalsidase beta Agalsidase alfa Placebo Best supportive care Migalastat ERT (both)	
How to evaluate renal outcomes	GFR measured by nuclear medicine or iohexol Estimated GFR by CKD-EPI Proteinuria 24h	Albumin/creatinine ratio Albuminuria Renal clinical events (Need for dialysis, renal transplant)
How to evaluate neurologic outcomes	Stroke and TIA clinical events White matter lesions	Deafness Changes in neurological examination
How to evaluate quality of life outcomes	Multidimensional questionnaire Work compliance SF-36	EDQ5
How to evaluate cardiac outcomes	Left ventricular mass index Cardiac clinical events T1 mapping	IVS E/E´ LVEF ECG changes Gadolinium late-enhancement Troponin
How to evaluate exercise tolerance	Cardiopulmonary function test Tolerance questionnaire Treadmill exercise test 6MWT	
How to evaluate substrate outcomes	LysoGb3 plasma LysoGb3 urine	Not useful

How to evaluate gastrointestinal outcomes	Diarrhoea scale Multidimensional gastrointestinal scale	Abdominal pain Number of bowel movements/day IBSS
How to evaluate pain outcomes	Pain scale BPI	Neurophysiological studies Number and use of pain medication
How to evaluate other outcomes	Tissue Gb3 deposits	Depression Other biomarkers (TnT, BNP) Migraine-like headache
How to evaluate clinical Events	Mainz scale	
Fabry disease trial duration	24 months 36 months 60 months	More than 5 years
Important inclusion criteria	Disease-causing mutations Male gender Symptomatic Fabry patients Increased LysoGb3 in plasma Proteinuria 24h	Decreased AGAL activity Baseline kidney biopsy with substrate accumulation Renal disease More than 18 years old Naïve patients Classical phenotype
Important exclusion criteria	Variant of unknown significance Non-compliance Advanced cardiac disease End-stage renal disease Stroke Female gender Renal transplant	Previous FD treatment Children

Table 4 First Delphi survey results

3.3 Delphi survey second round results.

A second survey composed from items that reached the significance threshold in the first survey was sent to the Delphi panel to rate in a Likert Scale from 1 to 7. Following the consensus definition explained above, consensus results of this initiative are summarised in **Error! Reference source not found.** and they included: the selection of the adaptative clinical trial as the ideal study design and agalsidase beta as ideal comparator treatment. Renal and cardiac outcomes, such as glomerular filtration rate, proteinuria and left ventricular mass index, were prioritised as primary outcomes, whereas neurological outcomes including cerebrovascular and white matter lesions were dismissed as a primary or secondary outcome measure. There was also consensus regarding the importance of patient-related outcomes such as general quality of life, pain, and gastrointestinal symptoms and unity regarding lysoGb3 and Gb3 tissue deposits as useful surrogate markers of the disease was obtained. The group recognised that cardiac T1 mapping still has potential but requires further development before its widespread introduction in clinical trials. Finally, regarding inclusion and exclusion criteria, enrolled individuals should not have variants of unknown significance nor advanced disease and it is desirable to be males with symptomatic FD and elevated lysoGb3.

Item	Consensus	No Consensus
Ideal methodology Fabry disease trial	Adaptative design	Randomized controlled trial
Ideal treatment comparator	Agalsidase beta	Agalsidase alfa Placebo Best supportive care Migalastat ERT (both)
Main primary clinical outcomes	Cardiac outcomes Cardiac clinical events Renal Outcomes Renal clinical events Composite clinical events Quality of life	Substrate Neurologic outcomes Neurologic clinical events
Main secondary clinical outcomes	Renal outcomes Quality of life Composite clinical events Cardiac clinical events Renal clinical events	Cardiac outcomes Substrate Neurologic outcomes Neurologic clinical events
Exploratory outcomes	Gastrointestinal outcomes Pain Exercise tolerance	
How to evaluate renal outcomes	GFR measured by nuclear medicine or non-radioactive product Estimated GFR by CKD Proteinuria 24h	
How to evaluate neurologic outcomes	Stroke and TIA clinical events	White matter lesions
How to evaluate quality of life outcomes	Multidimensional questionnaire	Work compliance SF-36
How to evaluate cardiac outcomes	Left ventricular mass index Cardiac clinical events	T1 mapping
How to evaluate exercise tolerance	Cardiopulmonary function test	Tolerance questionnaire Treadmill exercise test 6MWT
How to evaluate substrate outcomes	LysoGb3 plasma	LysoGb3 urine
How to evaluate gastrointestinal outcomes	Diarrhea scale Multidimensional gastrointestinal scale	
How to evaluate pain outcomes	Pain scale BPI	
How to evaluate other outcomes	Tissue Gb3 deposits	
How to evaluate clinical Events		Mainz scale
Fabry disease trial duration	36 months 60 months	24 months
Important inclusion criteria	Disease-causing mutations Male gender Symptomatic Fabry patients Increased LysoGb3 in plasma	Proteinuria 24h
Important exclusion criteria	Variant of unknown significance Non-compliance Advanced cardiac disease	End-stage renal disease Stroke Female gender Renal transplant

Table 5 Consensus results

3.4 Delphi survey final panel discussion.

After feedback about the results was given by the facilitator, there was a meeting of the panel. In the meeting, the panel assessed all the items where consensus was met, those which nearly reached consensus and agreed the results. The panel also addressed with detail consensus results which provoked some discussion, such as neurologic complications, the role of female FD patients and VUS in clinical trials

4. Discussion

This is the first expert global consensus regarding FD and clinical trials design detailing every aspect of the clinical trial, from the methodology to the clinical outcomes. As with previous consensus on other aspects in FD, it was performed via Delphi methodology.

While the FDA recently released guidance regarding clinical trials, we believe our results expand upon and complement that guidance.

4.1 Clinical trial design

Given the rarity of the disease the FDA guidance suggest the use of a single well-controlled trial to approve a new drug for FD to detect a clinically meaningful effect on core clinical aspects of the disease. The definition of a well-controlled trial is given in the document 21 CFR 314.126 which gives recommendation on how to design a good clinical trial taking into consideration an appropriate control group, stratifying at randomization by prognostic factors such as sex and age. The specific clinical trial design is not discussed, stating that any well-controlled clinical trial should "provide sufficient details of study design, conduct and analysis to allow critical evaluation and a determination of whether the characteristics of a well-controlled study are present." However, while no specific clinical trial is recommended, all the recommendations are easier to achieve as a randomized controlled trial (RCT).

The Delphi panel consensus agreed that while RCT have been widely used, there has been increasing understanding that in the field of rare diseases they are difficult to perform[36]. It is

obvious that rare diseases' clinical trials have fewer potential patients for enrolment but difference in randomization, masking and the use of active comparators has also been found compared to other more common disease areas[37].

Therefore, different clinical trial designs have been increasingly identified to try to solve some of these problems (e.g. adding a run-in phase to the trial). One of them is adaptive clinical trials which allow flexibility through shifting and redesigning the protocol while it is ongoing[38]. While this design is not free of difficulties[39], it may be more relevant to achieve meaningful results in rare diseases than standard RCT. The Delphi panel feel an adaptive design suits better FD due to the complex diversity of clinical outcomes and the small number of patients that are available to participate in clinical trials.

4.2 Best treatment comparator

The FDA does not give a recommendation on this matter. In the 21 CFR 314.126 document they state that a comparison of a new drug with a proven treatment is one acceptable strategy to conduct a new clinical trial. The trial can have two groups (new treatment and active treatment) or more than two (for example, new treatment, active treatment and placebo or other treatments or dosages) The Delphi panel reached a consensus specifying one treatment, among others instead of giving general recommendations. The Delphi panel acknowledges that finding an ideal comparator for a potential new FD drug is challenging. However, due to the wider availability and more global experience with Agalsidase beta (Agalsidase alpha is not approved in the United States) and some indications of better biochemical, substrate, and LVMI outcomes, the panel decided to choose only Agalsidase beta as recommended comparator for a FD clinical trial[40-45].

4.3 Clinical outcomes

The FDA guidance classifies clinical outcomes according to core clinical disease manifestations. Those core clinical manifestations are renal disease, cardiovascular disease, cerebrovascular disease, gastrointestinal symptoms, and pain.

Furthermore, the FDA specifies that paediatric patients should have appropriate clinical outcomes to assess disease severity and burden. The same concept applies to late-onset FD.

This Delphi consensus organised clinical outcomes in an alternative way. Clinical outcomes were organised in primary endpoint clinical outcomes, secondary and exploratory outcomes reflecting the importance of different organs involved in FD. It is no surprise to see renal and cardiac outcomes dominate both primary and secondary outcomes. Whilst clinical outcomes that were more subjective such as gastrointestinal involvement or pain were classified as exploratory; the Delphi panel indicated that quality of life must remain an important primary outcome measure.

Conversely, and in distinction from the FDA, neurologic outcomes were dropped from the list. The Delphi panel acknowledged that the pathophysiology of these events is still not fully understood, and this could lead to difficulty interpreting the results of clinical trials. Whilst several efforts have been made to elucidate the mechanisms that trigger neurologic events in FD patients[40] , there is no consensus for the incidence of these complications[43, 44, 46] or their long-term implications[47] Furthermore, some studies suggest that stroke and WML progress slowly to be able to see differences in the timespan of a clinical trial[48], which would difficult the measure of the clinical endpoint.

Therefore, the Delphi panel concluded that, until better knowledge of the neurologic involvement in FD, it is reasonable to focus on other outcomes rather than in central nervous manifestations of the disease in primary studies attempting to bring new agents to patient benefit. However central neurological outcomes should be recorded in any clinical trial as a safety endpoint. Further understanding of the pathophysiology and potential for prevention of stroke in FD remains an urgent research need and long-term post marketing follow up of new interventions should further evaluate their effect on stroke.

Finally, while the Delphi panel did not specifically address paediatric outcomes, it understands there might be some special arrangements in paediatric clinical trials, and this could be the subject of a further exercise.

4.4 Measurement of cardiac outcomes

The FDA guidance is more restrictive in this category than in other clinical outcomes. The guidance recommends using only clinical symptoms and exercise capacity as cardiac clinical outcomes indicating that more evidence is needed to support the use of left ventricular mass index or global longitudinal strain as a surrogate marker.

The Delphi panel agreed that in the clinical trial scenario, despite some uncertainty regarding the predictive value of LVMI, given the availability of imaging modalities to monitoring of heart structure and the multiple factor impacting on exercise capacity, left ventricular mass index measured via cardiac MRI should be used to measure cardiac outcomes, alongside cardiac clinical events. It is worth pointing out that the role of native cardiac T1 mapping did not reach consensus. Although native cardiac T1 mapping is an increasingly used technique with promising applications in FD, it has been mainly used for diagnosis[49] and there are still some issues with standardisation of the results which are being addressed[50] and with potential interpretation of changes in response to therapy.

Furthermore, at the panel meeting, experts considered that in the near-future, loop recorder devices could be used to determine whether arrythmias are a useful surogate marker of disease. The ability of loop recorders to detect otherwise undocumented arrythmias may clarify this, specifically for the arrythmias that might occur at an early disease stage[51]. This is currently the subject of active investigation[52].

4.5 Measurement of renal outcomes

The guidance from the FDA states that sponsors could use the effect on a specified magnitude of renal function loss either by measured GFR or an accepted estimating equation of GFR. Although they recommend stratifying the loss of renal function according to the various stages of renal disease enrolling patients with different levels of renal function, they do not mention patients already on end-stage renal disease or dialysis They suggest the clinical outcome time to end-stage renal disease or dialysis should be assessed, although this would require a trial of significant duration.

While GFR decline is an accepted clinical outcome, the FDA guidance states that there is insufficient evidence to support the use of proteinuria as a surrogate endpoint. This decision is based on lack of clear evidence about whether treatment effects on proteinuria can predict treatment effects on other renal outcomes in FD.

Finally, the FDA recommends a thorough optimisation of angiotensin enzyme converter inhibitors and/or angiotensin receptor 2 antagonists in each patient before being enrolled in a clinical trial.

The Delphi panel acknowledged that evaluating renal clinical outcomes is complex and it is one of the clinical outcomes with more variability in its measures across different clinical trials. Consensus was reached in the same items as the FDA guidance, but being more specific: monitoring of GFR evolution via estimated GFR by CKD-EPI, recognising the potential variability of this formula depending on muscularity (which is important in patients experiencing substantial pain) and that it is not suitable for children. A potential alternative of using measured GFR either by nuclear medicine or using non-radioactive substances such as iohexol was recommended. The Delphi panel also agreed on inclusion of all stages of renal function although patients with end-stage renal disease or renal transplant patients should be excluded unless a specific group for them is created inside the clinical trial.

Conversely, the Delphi panel reached consensus about the use of 24h proteinuria as a primary clinical outcome. While this was the result from the Delphi procedure, there were some concerns regarding this outcome which were assessed at the final online meeting. First, the panel recommends the measure of both total protein and albumin. Albuminuria is considered an early and sensitive surrogate marker of renal involvement, although renal changes have been witnessed without increased urine albumin and there can be differences between males and females[53]. Secondly, some experts considered collecting urine for a 24h period practically complex, highly variable, and unpopular among patients. In addition, the volume of urine might interfere with the results.

A solution put forward was the use of protein/creatinine and albumin/creatinine ratio of the first void urine in the morning, or a median of three separated void urine collections[54]. However, the fact that protein/creatinine ratio did not achieve consensus might indicate some specialists feel the ratio to be more complex to use than the raw proteinuria or albuminuria number, and its use could be less common outside nephrology clinics.

Nevertheless, the Delphi panel considered this an inexpensive, easy to measure outcome that while it is true there are limitations for albuminuria and proteinuria as a surrogate marker endpoint in a clinical trial, they are the cornerstone of treatment of most, if not all, proteinuric nephropathies[55] and therefore should be measured in FD clinical trials, and patients with high or progressive proteinuria should not be excluded.

Finally, the Delphi panel agreed on stabilising and monitoring angiotensin-converting enzyme inhibitors and other drugs used for renal and cardiac dysfunction that can have an impact on renal outcomes prior to the enrolment of the patient in the clinical trial

4.6 Measurement of gastrointestinal and pain outcomes

The FDA classifies pain and gastrointestinal symptoms as neuropathic pain due to their physiopathology. Nevertheless, it states that they might be enough to establish drug effectiveness if an improvement in abdominal or general pain, diarrhoea or constipation is observed through well-defined and reliable instruments[56].

While effort has been exerted to unveil the physiopathology of pain and gastrointestinal manifestations in FD[57], their subjectivity remains a challenge. Besides, some common tools to evaluate pain in FD pains, such as BPI does not record appropriately FD pain crisis. Hence, although the Delphi panel did not wish to undervalue the effect of these manifestations, on patients with Fabry disease[58, 59] their effects were felt to impact on overall quality of life which as such must remain as a primary clinical outcome, evaluating gastrointestinal and pain outcomes as exploratory outcomes. Finally, when measuring pain as a clinical outcome in a clinical trial, the panel would recommend optimising and standardising analgesic medication of patients prior to inclusion in any trial.

4.7 Gb3 in tissue and LysoGb3 as a substrate biomarker

Gb3, can be detected in histological samples from several organs and is a direct detection of damage and active disease. Widely used for diagnosis, due to its direct relationship with organ damage the FDA recognises its use when looking at the reduction in renal interstitial capillaries. It would act as a surrogate marker that can support an accelerated approval of a drug.

On the other hand, plasma lysoGb3 has gained some acceptance as a substrate related biomarker which may have greater significance than its obvious pharmacodynamic interpretation[60]. Although most patient's plasma lysoGb3 diminishes after treatment and a higher plasma lysoGb3 is correlated with a severe phenotype[61] the long-term corollaries of its reduction are as yet unproven. Further studies are warranted regarding this issue.

LysoGb3 analogues may also be detected in urine[62]. However, these are not yet widely available. Therefore, the Delphi panel agreed on monitoring plasma lysoGb3 levels during the clinical trial duration but did not agree a consensus on the measurement of urinary lysoGb3 or other analogues. The panel recommends Gb3 detection in tissue as a reliable surrogate marker of the disease.

4.8 Fabry Disease trial duration

The FDA does not state a specific duration of a FD clinical trial. It recommends that if the clinical trial's aim is to observe a reduction in disease's progression, it should allow enough time to observe disease progression in the control group. For example, in case of renal outcomes, it should be 24 months.

The Delphi panel acknowledges that FD is a disease with a slow but steady rate of progression. While it can cause vital complications, usually they are developed over many years[3]. Therefore, to be able to see clinical differences, clinical trials should ideally last long enough to witness these differences.

In this sense, the panel recognises that some commonly used markers of the disease, such as cardiac clinical events, might have a too slow event rate to be practical for pre-authorisation

clinical trials and potentially impede the development of a beneficial intervention. There is therefore evidently a need to explore new and existing biomarkers of disease which responds within a practical timeframe and therein to understand their long-term relationships with clinical events.

Meanwhile, the Delphi panel agreed on a 36 and 60-months duration for the initial study with additional open-label data collection.

4.9 Inclusion and exclusion criteria – Chronic renal disease and transplant.

The FDA guidance draft specifies that confirmed FD patients through biochemical (plasma and leukocytes alpha galactosidase A activity) and genetic testing of *GLA* gene are eligible to enrol in a clinical trial. Both females and males should be considered for enrolment. In addition, it states that children should be considered too.

Regarding genotype, the FDA considers that in the case of variants of unknown significance if there is biochemical or histopathological evidence of FD they can be accepted to participate in a clinical trial.

Similarly, the Delphi panel reached a consensus on inclusion criteria which demonstrate that the patient has FD with ongoing storage and organ effects, as it can be seen in table 4. Assuming all the candidates entering the study have a confirmation of FD following published recommendations[25], the panel felt that more specific inclusion criteria should be taken into consideration including male gender.

The selection of male gender as inclusion criteria was addressed at the online meeting. This decision was mainly driven by the need to evidence clear changes in the affected patients. The more severe phenotype of the disease tends to be more frequent in males and therefore, improvement is more easily observed and rapidly evaluated in these patients. However, new drugs being developed as treatment of Fabry disease should be suitable for both genders, a broad spectrum of disease stages and different genotypes.

Consequently, the panel agreed that a stepwise clinical trial should be recommended, accordingly the adaptive clinical trial design. In this design, the first step would be to test new drugs in males with classical FD. Once benefit is observed, then females and, afterwards patients with late-onset phenotypes will be included. Therefore, the panel wants to highlight female gender is not an exclusion criteria, but given the inter-patient variability in females, a significant clinical effect is more likely to be detected in males, hence, a tiered approach would be desirable.

Other inclusion criteria where consensus was agreed were recognised disease-causing mutations, symptomatic patients (ongoing organ damage) and increased lysoGb3 in plasma.

Unlike the FDA, the panel reached consensus on exclusion of VUS since the aetiology of clinical findings were not clear in such patients. Other exclusion criteria where consensus was met were advanced cardiac disease (defined by the extension of fibrosis and left ventricular hypertrophy specially with the presence of concomitant heart failure, arrhythmias or myocardial ischemia) due to the difficulty in reversing fibrosis and advanced myocardial remodelling, and non-compliance patients. The panel recommended that patients with end-stage renal disease or transplant should participate in a designated group and should not be excluded.

5. Conclusions

This Delphi driven consensus aimed to set a framework for future clinical trials in adult FD thought standardisation of different elements: clinical trial design, treatment comparator, clinical outcomes endpoints, duration, inclusion, and exclusion criteria.

Following Delphi methodology, the panel of experts reached a consensus on all the areas. Main recommendation of this initiative are:

1. The selection of the adaptive clinical trial as the ideal study design

2. Agalsidase beta as the ideal comparator treatment.

3. Renal and cardiac outcomes should be prioritised as primary or secondary outcomes, whereas neurological outcomes, including cerebrovascular and WML, should be considered in longer phase 4 studies.

4. Patient-related outcomes such as pain, gastrointestinal symptoms and, especially quality of life, are vital and consequently, quality of life outcomes should be included as a primary endpoint.

5. The most appropriate measure of renal outcomes are evolution of GFR (CKD-EPI or measured), 24h proteinuria and albuminuria and protein or albumin/creatinine ratios.

6. The most appropriate measure of cardiac outcomes are LVMI measured by MRI and clinical events. T1 mapping lacks sufficient standardisation and prognostic value to be currently recommended. It is recognised that loop recorder devices might be a future asset to any FD clinical trial because their ability to register previously undetected arrhythmia events, specially some that might happen at an early stage.

7. Plasma lysoGb3 and Gb3 tissue deposits are useful surrogate markers of the disease which may shorten the duration of clinical trials. However, further work is required to fully understand the relation of biomarkers and surrogates to clinical outcomes.

8. Duration of at least 36 to 60 months is recommended due to the slow rate of progression of the disease. An additional open-label phase should follow.

9. The demonstration of FD with ongoing organ damage is the foundation of clinical trial inclusion criteria. These are a pathogenic genetic variant, increased lysoGb3 in plasma, evidence of clinical disease and male gender. A stepwise adaptative approach should include the subsequent inclusion of females and patients with late onset disease in any study where effect is demonstrated in the first round with classical males.

10. Exclusion criteria include variants of unknown significance, advanced cardiac disease and non-compliance.

11. End-stage renal disease or renal transplant is not considered an exclusion criterion. However, these patients should be included in a specific study group.

This consensus may allow more rapid clinical trial development and implementation to understand therapeutic effects of new agents in FD and allow comparability and reduction of unwarranted variation in clinical study design. Finally, further similar work is required considering clinical trial design in children and evaluation of new organ-specific measurement techniques as they become available.

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