PROGRESSION OF NEPHROPATHY IN FABRY PATIENTS RECEIVING ENZYME REPLACEMENT THERAPY (ERT); RELATION TO ANTI-DRUG ANTIBODY (ADA) STATUS AND PROTEINURIA

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ABSTRACT

INTRODUCTION AND AIMS: Fabry disease (FD) is an X-linked multisystem lysosomal storage disorder, affecting both males and females caused by the deficient of α-galactosidase-A (α-Gal-A) activity. Long-term disease manifestations include progressive renal failure, hypertrophic cardiomyopathy, cardiac rhythm disturbances, stroke and death. Two enzyme replacement therapies (ERT) are commercially available, agalsidase-alfa (Shire) and agalsidase-beta (Genzyme). The clinical benefit of ERT may not be as robust as anticipated, especially in the subset of males with 'classic' Fabry disease. A combination of factors, including dose, dosing interval, the presence of anti-drug antibodies (ADA), estimated glomerular filtration rate (eGFR), age and the timing of ERT initiation, and proteinuria could explain less than optimal responses to currently available ERT.

METHODS: In an effort to identify Fabry patients who continue to progressively lose kidney function, despite receiving standard ERT therapy (agalsidase-beta), 37 patients were analyzed for annualized eGFR slope, anti α-Gal-A antibody, serum-mediated inhibitory activity towards agalsidase-beta, and proteinuria. All of these patients were screened for the BALANCE study (a head-to-head blinded comparison of pegunigalsidase-alfa to agalsidase-beta at 1 mg/kg every two weeks, NCT02795676) with change in eGFR as the primary end-point.

RESULTS: These FD patients, both males and females, experienced progressive loss of kidney function while being treated with agalsidase-beta (treated for 1 to 12 years). At the time of screening for the study, the average annualized eGFR slope was -8.1 (SD=6.6) mL/min/1·73m^2/year, the eGFR at the screening visit was 70.0 (SD=17.9) and 75.1 (SD=11.0) ml/min/1.73m^2 for males and females, respectively; the urine protein/creatinine ratio (UPCR) was 669 (SD=579) mg/gr for male and 45 (SD=25) mg/gr for females. Males initiated ERT treatment at 39 (SD=10) and females at 42 (SD=7) years of age. The male patients were at age of 43 (SD=11) and females at 47 (SD=9) years at screening. Of the 27 male patients, 15 (56%) were found to be ADA positive to agalsidase-beta, with titers ranging between 469 to 127933. These patients also had serum-mediated *in-vitro* enzyme inhibition with a mean of 84% (SD=11%) inhibition. None (0%) of the 10 females were ADA positive to agalsidase-beta. More ADA-positive male patients (10/14=67%) had significant proteinuria (UPCR) ≥500 mg/gr than ADA-negative males (5/12=45%), with similar results seen when they were stratified at 60 mL/min/1·73m^2.

FABRY DISEASE THERAPY

Unmet Clinical Need

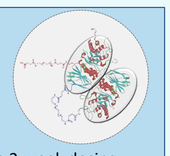
- **Continuous disease progression**
- **Immune response**
- **Infusion reactions**
- **Long-term efficacy**

Available Treatments for Fabry Disease

- **agalsidase beta (Fabrazyme®):** Approved in the US & the EU. Administrated IV at 1 mg/kg body weight, EOW (agalsidase beta USPI)
- **agalsidase alfa (Replagal®):** Approved in the EU. Administrated IV at 0.2 mg/kg body weight, EOW (agalsidase alfa SMPC)
- **Migalastat (Galafold®):** Approved in the EU. Administrated orally, 123 mg hard capsules every other day (Migalastat SMPC)

New ERT -Pegunigalsidase alfa

PEGylated, Chemically Modified α-Gal-A Enzyme



- PEGylated, covalently-linked homodimer composed of two subunits produced in plant cells. Subunits linked through a **2KDa PEG cross-linker**, contains additional **PEG moieties** bound to only one subunit through a lysine residue.
- Providing continuous presence of the enzyme throughout the 2-week dosing interval, without compromising the enzyme activity and internalization to target organ and cells.
- PEGylation potentially reduces immunogenicity by masking immunogenic epitopes together with its continues presence, has the potential to induce immune tolerance.

METHODS

BALANCE study is a Phase III study for Fabry disease. The study is a head-to-head blinded comparison of pegunigalsidase-alfa to agalsidase-beta at 1 mg/kg every two weeks, with change in eGFR as the primary end-point, NCT02795676.

The BALANCE study is enrolling Fabry patients who continue to progressively lose kidney function, despite receiving standard ERT therapy (agalsidase-beta):

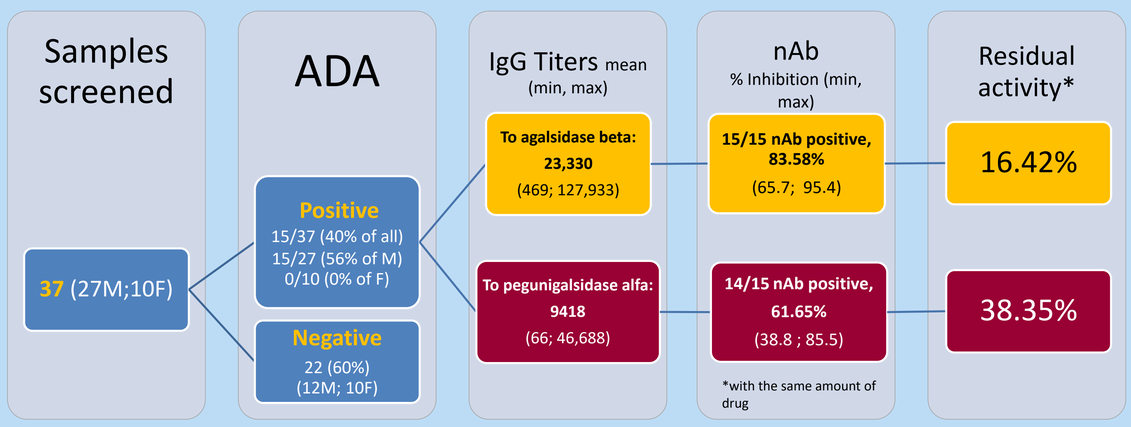
- The kidney function parameters for inclusion include eGFR (by CKD-EPI equation) at screening between 40 to 120 mL/min/1.73 m^2 (but not be higher than 120 mL/min/1.73 m^2 during 9 to 18 months before screening).
- Linear negative slope of eGFR of ≥ 2 mL/min/1.73 m2 based on at least 3 serum creatinine values over approximately 1 year (range of 9 to 18 months, including the value obtained at the screening visit).
- Urine protein to creatinine ratio (UPCR) > 0.5 g/g (0.5 mg/mg or 500 mg/g) should be treated with an Angiotensin converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) and ACE/ARB therapy should be stable for at least 4 weeks prior to screening.
- ADA status was not an inclusion/exclusion criterion.

At the time of the analysis, a total of 37 patients were screened for the study. These subjects were analyzed for the following parameters at the time of screening:

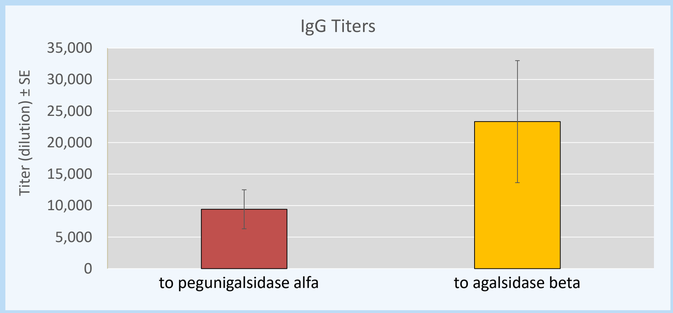
- estimated glomerular filtration rate (eGFR$_{CKD-EPI}$) based on serum creatinine
- Annualized linear eGFR slope
- Proteinuria by spot urine test for Urine Protein/Creatinine Ratio (UPCR)
- Anti α-Gal-A antibody using a designated validated ELISA
- Serum-mediated inhibitory activity towards agalsidase-beta using a designated validated enzymatic activity assay

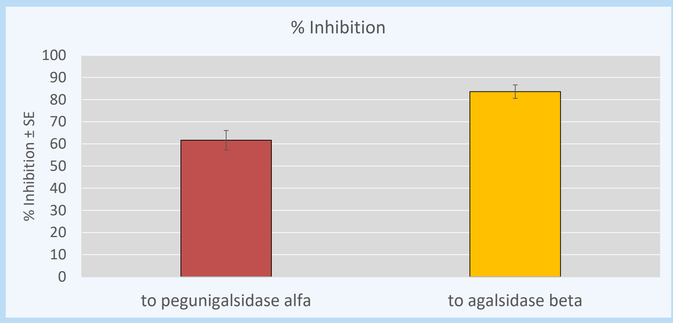
ADA RESULTS

Antibody Assessment for samples of FD patients previously treated with ERT



1. The data presents the baseline characteristics of FD patients screened for the BALANCE study. Patients who continue to progressively lose kidney function, despite receiving standard ERT therapy (agalsidase-beta) for 1-12 years.
2. Assessment of FD patients for the presence of anti agalsidase-beta antibodies and test the cross reactivity of these antibodies to pegunigalsidase alfa.
3. Results show that 56% of the screened male patients were ADA positive (40% of the entire screened population including females).
4. All anti agalsidase-beta ADA positive are neutralizing (nAb) with mean inhibition of 83.6%.
5. **Current experience with pegunigalsidase-alfa ERT** *(Warnock et al, WORLD Symposium 2017):*
   - 19% ADA positive
   - Not all had neutralizing activity
   - All became to be negative after 1 Y of treatment
6. **Cross reactivity:**
   - Binding: All ADA positives samples for agalsidase-beta reacted to pegunigalsidase-alfa, but to a lower extent (i.e., lower titers)
   - Neutralizing: 14 of the 15 nAb positive to agalsidase-beta neutralizes pegunigalsidase-alfa activity, but left about double percent "effective" enzyme when tested in-vitro with the same amount of enzyme:
     - Agalsidase-beta was inhibited by 83.4% leaving 16.4% of effective enzyme, and
     - Pegunigalsidase-alfa was inhibited by 61.6% leaving 38.4% effective enzyme





RENAL FUNCTION *Vs.* ADA STATUS

Baseline characteristics of FD patients previously treated with ERT

	Female		Male ADA-		Male ADA+	
	Mean	SD	Mean	SD	Mean	SD
ADA Status	Negative		Negative		Positive	
n	10		12		15	
On Pre-medication (n)	3		5		6	
Age started ERT	**42**	7	**37**	10	**40**	10
Duration Previous treatment (years)	**4.4**	3.4	**5.8**	3.9	**3.8**	2.8
eGFR$_{CKD-EPI}$ at Screening	**75.1**	11.0	**69.7**	20.0	**70.2**	17.0
eGFR Slope at Screening	**-9.4**	9.8	**-7.1**	3.5	**-7.9**	6.0
UPCR (mg/gr)	**45**	25	**547**	620	**767**	546

The baseline characteristics of FD patients previously treated with agalsidase beta ERT were compared based on gender and ADA status. The baseline characteristics were found to be similar in the parameters of age of ERT treatment initiation, duration of treatment, eGFR levels at time of screening and eGFR annualized slope at time of screening.
UPCR was significantly different between male and female but not between ADA positive to ADA negative.

FD patients previously treated with ERT stratified by UPCR and ADA status and related to eGFR

N	UPCR < 500mg/gr		UPCR ≥ 500*mg/gr	
ADA Status	Negative	Positive	Negative**	Positive
eGFR$_{CKD-EPI}$ ≥ 60 mL/min/1·73 m^2	4 (57%)	3 (43%)	3 (27%)	8 (73%)
eGFR$_{CKD-EPI}$ < 60 mL/min/1·73 m^2	3 (60%)	2 (40%)	1 (33%)	2 (67%)

*All treated with ACEi/ARBs

** eGFR data of 1 patient was not available

FD patients previously treated with agalsidase beta ERT were stratified based on UPCR and ADA status related to eGFR. The UPCR levels were stratified by ≥ 500 mg/gr *Vs.* <500 mg/gr based on the cut off level indication an important proteinuria; and eGFR $_{CKD-EPI}$ by ≥ 60 mL/min/1·73 m^2 *Vs.* <60 mL/min/1·73 m^2 and compared between ADA positive and ADA negative.

As can be seen in the table above, the distinct group (between the 8 stratification groups) was the group of patients with ADA positive, UPCR ≥ 500 mg/gr and eGFR $_{CKD-EPI}$ by ≥ 60 mL/min/1·73 m^2.

None (0%) of the 10 females were ADA positive to the agalsidase-beta. More ADA-positive male patients (10/14=71%) had significant proteinuria (UPCR) ≥500 mg/gr than ADA-negative males (5/12=42%), with similar results seen when they were stratified at 60 mL/min/1·73m^2.

CONCLUSIONS

A) The BALANCE study screening strategy identified Fabry patients with severe, progressive Fabry nephropathy despite long-term ERT.

B) Progressive Fabry nephropathy in females with classic phenotype was found not to be associated with severe proteinuria or ADA status.

C) Severe proteinuria was more frequently observed among ADA positive, classic phenotype, males.

D) Neutralizing antibodies may contribute to the progressive loss of kidney function and substantial proteinuria in Fabry patients, which may limit the success in controlling proteinuria and/ or stabilizing renal function.

E) The current data show that pegunigalsidase alfa activity is less inhibited by serum mediated neutralizing antibodies.

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