Pegunigalsidase alfa, PEGylated α-Galactosidase-A enzyme in development for the treatment of Fabry disease, shows correlation between renal Gb_3 inclusion clearance and reduction of plasma Lyso-Gb_3

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ABSTRACT

Fabry disease is caused by the loss of function of the lysosomal enzyme α-Galactosidase A, which leads to accumulation of globotriaosylceramide (Gb_3/GL-3). Reduction in histological Gb_3 burden in renal peritubular capillaries (PTC) is considered an appropriate and objective surrogate endpoint likely to predict clinical benefit of treatment in Fabry disease. Pegunigalsidase alfa is a novel PEGylated enzyme in development for the treatment of Fabry disease with an enhanced pharmacokinetics.

Objectives: The phase I/II (NCT01678898/NCT01769001) dose ranging studies were designed to evaluate the safety, efficacy and pharmacokinetics of pegunigalsidase alfa administered IV every other week in adult, symptomatic, treatment naïve, male and female Fabry disease patients.

Methods: The Barisoni Lipid Inclusion Scoring System (BLISS) was employed to quantitatively assess patients' renal biopsies taken at baseline and at 6 months of treatment. BLISS methodology consists of counting the number of Gb3 inclusions per PTC; a decrease in the score is indicative of clinical improvement. Renal biopsies were available and evaluated in 13 out of 16 patients allocated in the three dose groups. Plasma globotriaosylsphingosine (Lyso-Gb_3) is a breakdown product of Gb_3, which is assessed as a specific biomarker of Fabry disease. Plasma Lyso-Gb_3 samples were available for all the patents that completed 12 or 24 month of treatment.

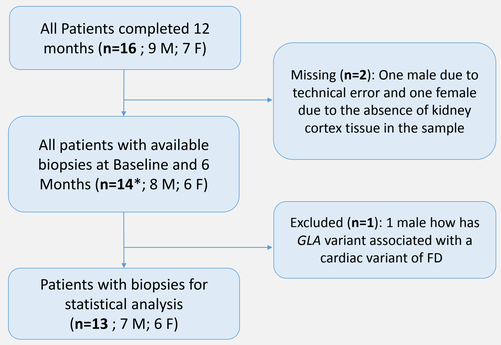
Results: Mean BLISS score at baseline was 4.3, proving an important renal involvement and was reduced to a mean score of 0.8 after 6 months (-67.8% ± 8.9 %) with an 86.5% reduction in the 1 mg/kg dose cohort. From the totality of the available biopsies (n=14, including one subject carries a cardiac *GLA* gene variant), 78.6% of patients reached ≥50% reduction in BLISS score. These results show a profound reduction in Gb_3 inclusions in PTC after 6 months of pegunigalsidase alfa treatment. A high correlation (r=0.800) between the reduction in plasma Lyso-Gb_3 and the reduction of kidney Gb_3 inclusions based on kidney biopsies was observed.

Conclusion: The correlation between the reduction of Gb_3 in the kidney and the reduction is plasma Lyso-Gb_3 is giving additional support to the potential effectiveness of pegunigalsidase alfa in treating Fabry disease.

STUDY DESIGN

PB-102-F01/F02 study is an open label study with adult symptomatic Fabry disease patients, which were either enzyme replacement therapy (ERT)-naïve or untreated for the last 6 months prior to screening to the study. A key parameter evaluated was renal Gb_3 burden assessed in kidney biopsies at baseline and after 6 months of pegunigalsidase alfa treatment with either one of three doses (0.2, 1.0 and 2.0 mg/kg). Of the 16 patients that completed one year of treatment, 14 patients had available biopsies at both time points. Two biopsy samples were unavailable due to technical reasons. In addition, biopsies from another patient were excluded from the statistical analysis since this patient carries a *GLA* variant (p.N215S) associated with a cardiac variant of Fabry disease with a rare renal manifestation[1,2] and had minimal histopathology Gb_3 inclusion findings at baseline.

Flow diagram of patient disposition related to kidney biopsy



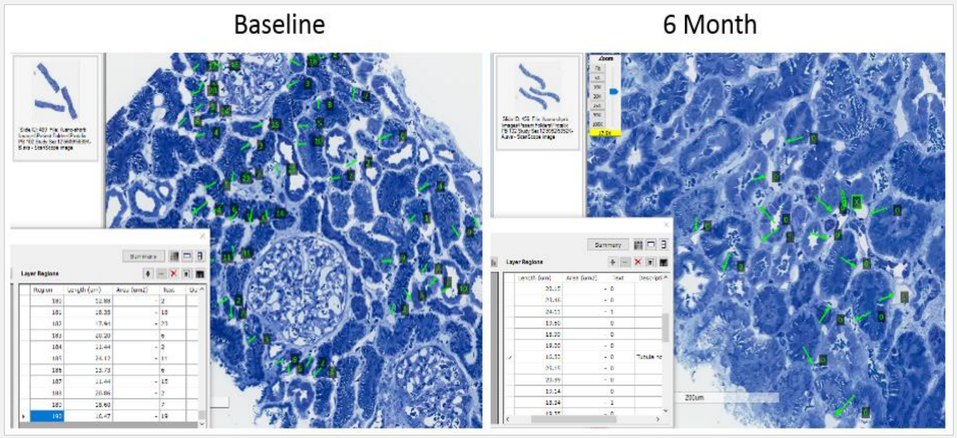
*Available for responder analysis

METHODS AND RESULTS

Kidney Gb_3 Scoring System (BLISS): Scoring for Gb_3 inclusions performed according to Barisoni Lipid Inclusion Scoring System (BLISS) methodology[3], which is a quantitative, reproducible method to evaluate Gb_3 inclusions in cortical peritubular capillaries (PTC) on Whole Slide Imaging (WSI). This method was developed to optimize the detection of Gb_3 inclusions in Fabry patients in a quantitative manner, based on the actual number of Gb_3 inclusions counted in each PTC.

Initially, all biopsies and WSI were image digitalized and numbered, ensuring randomization and blinding based on multiple levels prior to annotating and scoring by the blinded pathologists. Following, the analysis was done by three pathologists, one serve as the annotator and the other two served as reader/scorer for each biopsy on a rotation basis, so that each pathologist scored 2/3 of the renal biopsies and served as annotator for the remaining 1/3. Pathologists were blinded to whether a biopsy was baseline or post-treatment and to the dose of treatment. Annotation was performed by drawing an arrow pointing at each capillary, which automatically numbered the capillaries (consecutive enumeration); the two readers then scored the annotated WSIs. Finally, the overall score for a kidney biopsy was the mean of approximately 300 capillaries scored by two "blinded" pathologists.

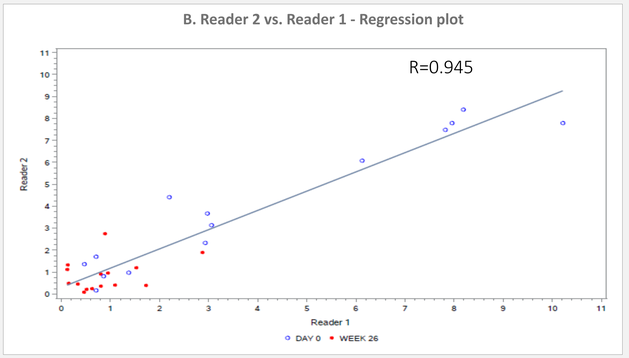
A. Screen Shot of the Digital Microscopy Images of Patient Biopsies Before and After the Treatment



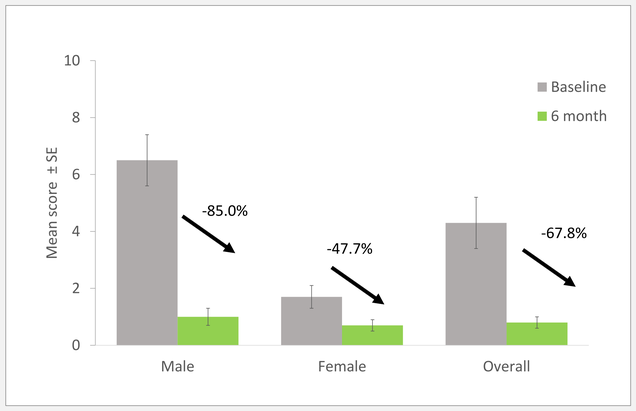
In **Panel A**, is an example of the digital microscopy images (of lower or higher magnification) with the electronic scoring sheet linked to the annotated WSI. The scoring pathologists recorded the number of Gb_3 inclusions for each capillary. As scoring progressed, the number of Gb_3 inclusions recorded for each capillary appeared on the WSI next to the arrow pointing to the enumerated annotated capillary. In this slide, at baseline, there are higher scores of Gb_3 PTC inclusions compering to the biopsies taken after 6 month of treatment, demonstrating reduction in Gb_3 kidney burden.

Evaluation of BLISS assay variation: The overall score for a biopsy of a patient at a visit was the mean score of approximately 300 individual capillaries of two readers. The assay variation was evaluated by various statistical means, among which, the inter-reader variability was evaluated, by comparing the mean score of each biopsy (overall 2 biopsies per patient, baseline and 6 months) by regression plot of the biopsy mean score given by reader 2 versus reader 1. The scatter plot of the biopsy scores as was given by the two readers at the two time points, show correlation of R=0.945 between the two readers, indicating a strong agreement among them (**Panel B**). Regression plot of Reader 2 versus Reader 1 scores, each point represents the mean score per biopsy by each reader (x,y) x = Reader 1 value; y = Reader 2 value.

B. Reader 2 vs. Reader 1 - Regression plot

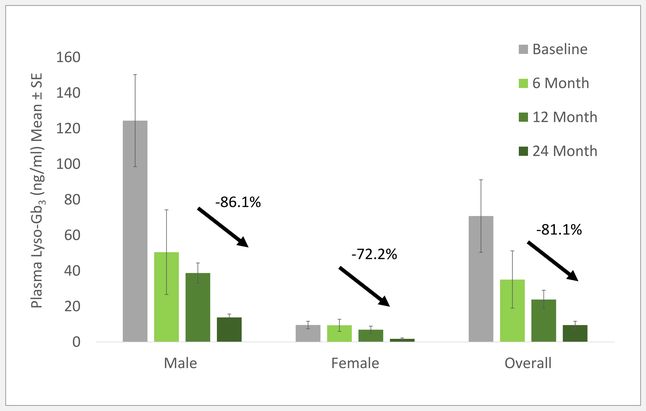


C. Gb_3 Burden in Kidney Biopsies - 6 Months BLISS Results



Overall Gb_3 BLISS score reduction from a mean score of 4.3 at baseline to a mean score of 0.8 after 6 months of pegunigalsidase alfa treatment (67.8% ± 8.9% reduction) was observed (**Panel C**). Male patients exhibited higher baseline levels and higher reduction, however, pronounced reduction was also detected in female patients. Reduction of the BLISS score indicates clearance of Gb_3 deposits in the PTC.

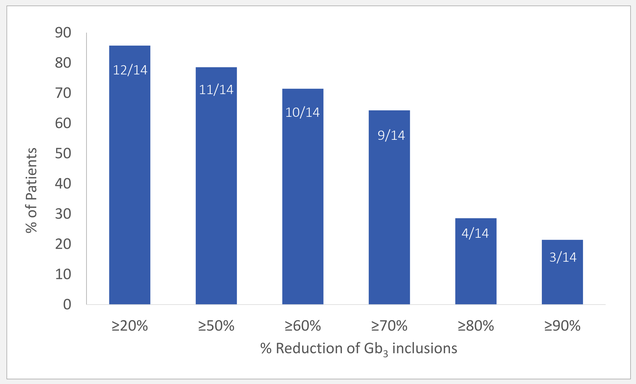
D. Plasma Lyso-Gb_3



Lyso-Gb_3 concentrations were analyzed in plasma obtained from venous blood before treatment initiation, once a month in the first 3 month and every 3 month thereafter. Extraction and quantification of plasma Lyso-Gb_3 was preformed using liquid chromatography tandem mass spectrometry (LC-MS).

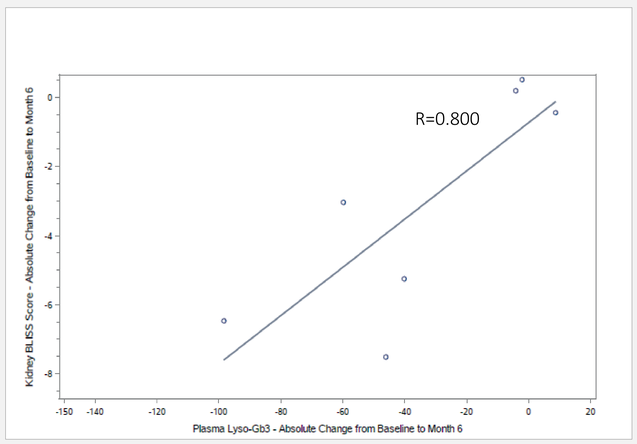
Substantial reduction in plasma Lyso-Gb_3 levels was observed following 6, 12 and 24 months of pegunigalsidase alfa treatment. At baseline, male patents had higher levels of Lyso-Gb_3 than females; nonetheless, both presented significant reduction following the treatment (**Panel D**).

E. Responder analysis – portion of patient achieved reduction in BLISS score (n=14)



The responder analysis evaluates the entire population with available biopsies (n=14), representing the number of patients achieving improvement from baseline to month 6. Improvement in BLISS score was achieved in 85.7% (12 of 14) of the patients with available biopsies. The outcome of ≥ 50% reduction from baseline to month 6 in the average number of Gb_3 inclusions per kidney PTC biopsy samples was demonstrated in 78.6% (11 of 14) of pegunigalsidase alfa treated patients (**Panel E**).

F. Correlation analysis between kidney Gb_3 and plasma Lyso-Gb_3



High correlation was found between the reduction in BLISS score of kidney Gb_3 inclusions and the reduction of plasma Lyso-Gb_3 over 6 months of treatment. This correlation gives additional support to the effectiveness of pegunigalsidase alfa by two independent Fabry related biomarkers (**Panel F**).

OVERALL CONCLUSIONS

- Adult symptomatic, ERT-naive Fabry disease patients enrolled in PB-102-F01/F02 study were evaluated for Gb_3 levels in kidney PTC and for plasma Lyso-Gb_3 concentration.
- Kidney Gb_3 inclusions were assessed by the quantitative BLISS methodology.
- The outcome of ≥ 50% reduction in the average number of Gb_3 inclusions per kidney PTC from baseline to month 6 was demonstrated in 11 of 14 (78.6%) of pegunigalsidase alfa treated patients.
- The overall results demonstrate that pegunigalsidase alfa reach the affected tissue and reduces kidney Gb_3 inclusions burden and Lyso-Gb_3 in the circulation.
- The high correlation found between the two Fabry disease biomarkers, reduction of kidney Gb_3 inclusions and the reduction of plasma Lyso-Gb_3 over 6 months of treatment, are giving additional support to the potential effectiveness of pegunigalsidase alfa in treating Fabry disease.

References:

1. Eng CM, Resnick-Silverman LA, Niehaus DJ, Astrin KH, Desnick RJ. Nature and frequency of mutations in the alpha-galactosidase A gene that cause Fabry disease. Am J Hum Genet. 1993 Dec;53(6):1186-97.
2. Germain DP, Brand E, Burlina A, Cecchi F, Garman SC, Kempf J, Laney DA, Linhart A, Maródi L, Nicholls K, Ortiz A, Pieruzzi F, Shankar SP, Waldek S, Wanner C, Jovanovic A. Phenotypic characteristics of the p.Asn215Ser (p.N215S) GLA mutation in male and female patients with Fabry disease: A multicenter Fabry Registry study. Mol Genet Genomic Med. 2018 Apr 12.
3. Barisoni L, Jennette JC, Colvin R, Sitaraman S, Bragat A, Castelli J, Walker D, Boudes P. Novel quantitative method to evaluate globotriaosylceramide inclusions in renal peritubular capillaries by virtual microscopy in patients with fabry disease. Arch Pathol Lab Med. 2012 Jul;136(7):816-24.

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