# Reduction of globotriaosylceramide inclusions in renal peritubular capillaries in patients with Fabry disease following treatment with pegunigalsidase alfa

Laura Barisoni[1], J. Charles Jennette[2], Myrl D. Holida[3], Ozlem Goker-Alpan[4], Gustavo Maegawa[5], Pilar Giraldo[6], Derlis E. Gonzalez[7], Ahmad M. Tuffaha[8], Sari Alon[9], Einat Brill-Almon[9], Raul Chertkoff[9], Derralynn Hughes[10]

[1]Duke University School of Medicine, Durham, NC, United States; [2]University of North Carolina System, Chapel Hill, NC, United States; [3]The University of Iowa Health Care, Iowa City, IA, United States; [4]Lysosomal Disorders Research and Treatment Unit, Fairfax, VA, United States; [5]University of Florida, Gainesville, FL, United States; [6]Hospital de Dia Quiron, Zaragoza, Spain; [7]Instituto Privado de Hematología e Investigación Clínica, Asunción, Paraguay; [8]University of Kansas Health System, Kansas City, KS, United States; [9]Protalix Biotherapeutics, Carmiel, Israel; [10]Royal Free London NHS Foundation Trust, London, United Kingdom

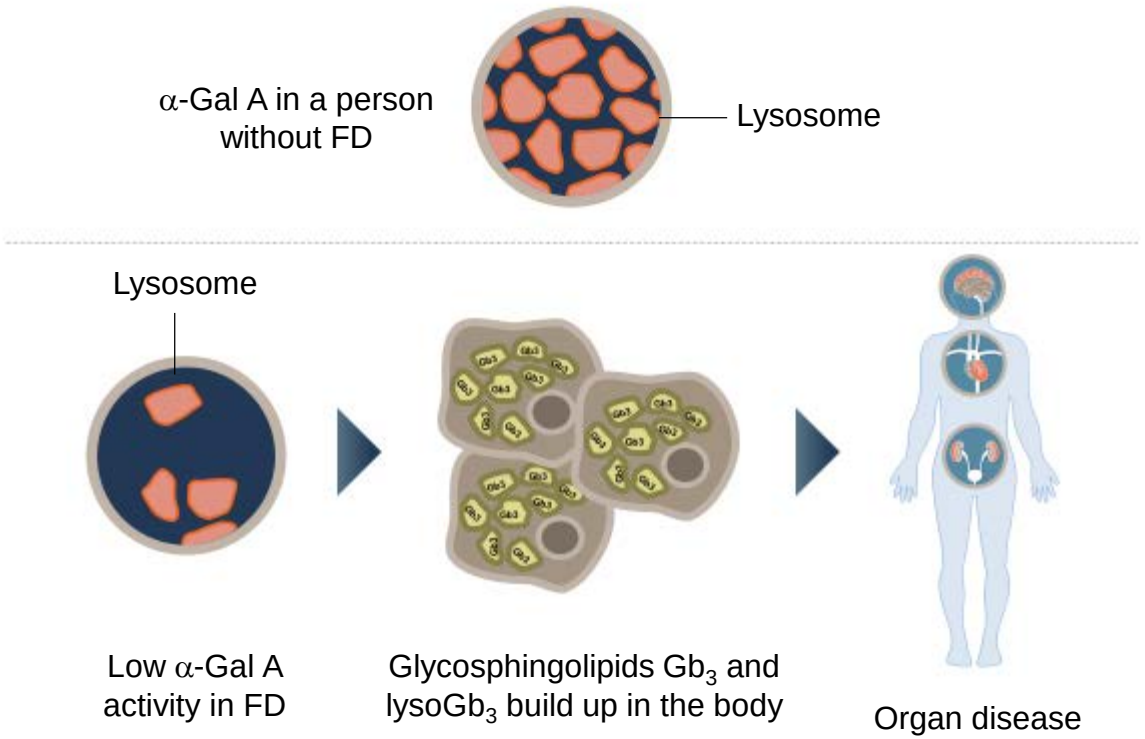
# Disclosures and study sponsorship statement

- LB and CJC are currently acting as consultants for Sangamo, and LB is also a consultant for Vertex
- MH has been or is currently involved in clinical trials with Sanofi-Genzyme, Sangamo, AVROBIO, Protalix and Idorsia (no direct funding is received for these trials as they are institution directed)
- PG has been involved in pre-marketing studies with Genzyme, Protalix, and Idorsia, and has received grants from Sanofi-Genzyme and Takeda; all financial amounts for these activities have been deposited into the Spanish Foundation for the Study and Treatment of Gaucher Disease (FEETEG) to contribute to the development of research in lysosomal storage disorders
- DG has been involved in clinical trials with Takeda and Protalix
- AT has received consulting fees for advisory board meetings from Chiesi and Sanofi
- DH has received speaker honoraria and consulting fees for advisory boards from Protalix, Takeda, Sanofi, Freeline, and Sangamo administered through University College London consultants, and used in part to support research in lysosomal storage disorders
- The remaining authors have no financial conflicts of interest to disclose
- Protalix Biotherapeutics sponsored this study. Medical writing and editorial support were provided by Kelsey M Hodge-Hanson, PhD, Oxford PharmaGenesis Inc., Newtown, PA, USA, and this support was funded by Chiesi USA, Inc.

# Introduction

- **Fabry disease** (FD) is a rare genetic disorder characterized by reduced α-galactosidase A (α-Gal A) enzyme activity, resulting in accumulation of glycosphingolipids such as **globotriaosylceramide ($Gb_3$)** in cells of various organs, including renal cells[1,2]
- **$Gb_3$ clearance** from renal peritubular capillaries (**PTCs**) has been used as a surrogate endpoint in trials of approved FD therapies[3,4]
- **Pegunigalsidase alfa** is a novel, PEGylated, α-Gal A enzyme in development for the treatment of patients with FD, which offers enhanced pharmacokinetics compared with current treatments[1,5]

## Glycosphingolipid accumulation in FD leads to organ dysfuntion[1,2]




α-Gal A, α-galactosidase A; FD, Fabry disease; $Gb_3$, globotriaosylceramide; $lysoGb_3$, globotriaosylsphingosine; PTC, peritubular capillary.

1. Schiffman R, et al. *J Inherit Metab Dis.* 2019;42:534-544. 2. Germain DP. *Orphanet J Rare Dis.* 2010;5:30. 3. Germain DP, et al. *N Engl J Med*. 2016;375:545-555. 4. Eng CM, et al. *N Engl J Med*. 2001;345:9-16.
5. Kizhner T, et al. *Mol Genet Metab*. 2015;114:259-267.

# Study design

**Objective:** To quantify the reduced burden of $Gb_3$ inclusions in PTCs in patients with FD participating in a 12-month phase 1/2 trial (NCT01678898) of pegunigalsidase alfa (recombinant α-Gal A) enzyme replacement therapy

## Patient population enrolled in the clinical trial

- 18 patients received pegunigalsidase alfa (safety population)
  - 16 completed the 12-month treatment period (efficacy population)
  - 14 had pre- and post–treatment biopsy (8 males and 6 females)
  - 13 were included in the final analysis (1 male was excluded due to minimal renal manifestation at baseline)

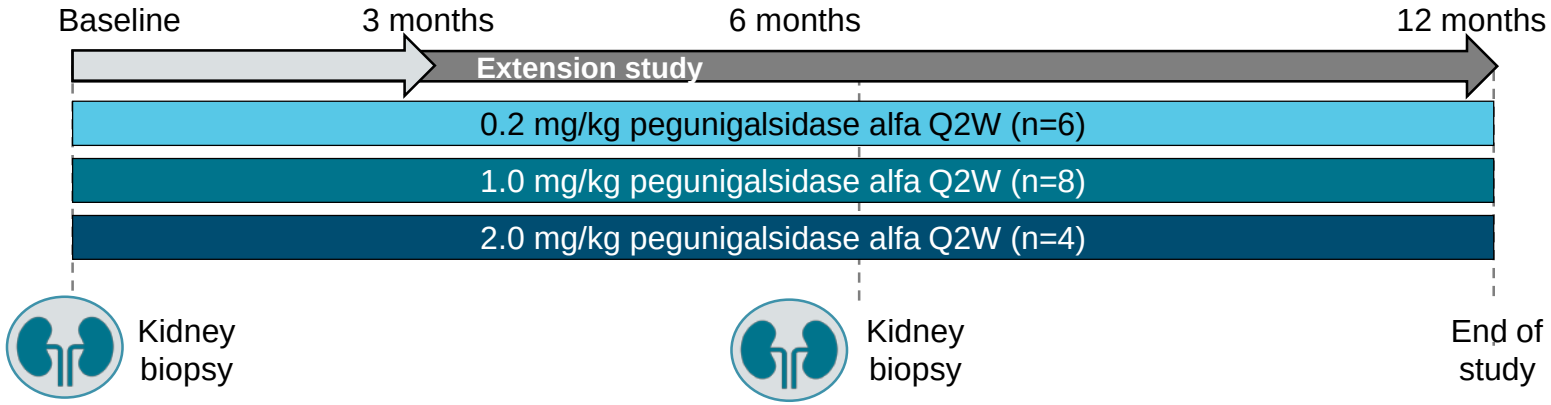
## Inclusion criteria

- Adults with symptomatic FD
  - α-Gal A below the normal laboratory range
- Either naive to treatment or had not received a currently available ERT in the 6 months before enrollment
- eGFR ≥60 mL/min/1.73m$^2$
- No history of CKD (stages 3–5) or renal transplantation

### Renal biopsy protocol

2 renal biopsies per study subject were taken for comparison at:

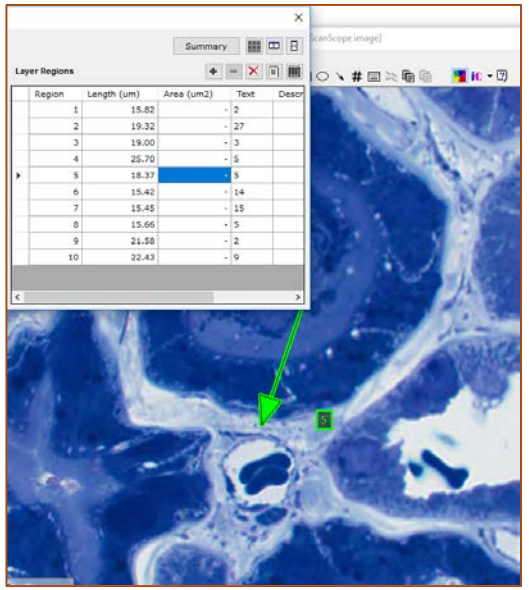
- Baseline
- 6 months

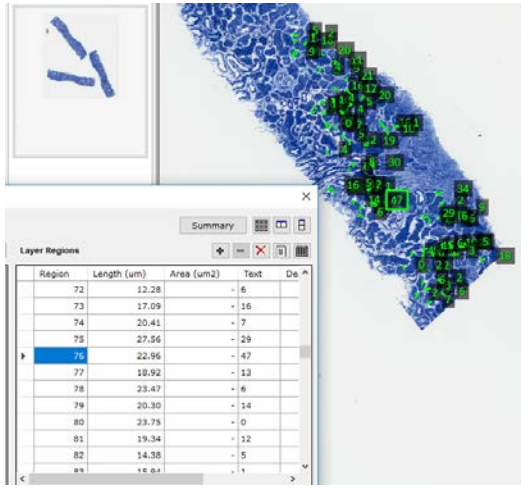



α-Gal A, α-galactosidase A; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; FD, Fabry disease; $Gb_3$, globotriaosylceramide; PTC, peritubular capillaries; Q2W, every 2 weeks.
Schiffmann, et al. 2019. *J Inherit Metab Dis.* 42;534-544.

# BLISS protocol for kidney biopsy assessment

- **Tissue Processing:**
  - Renal biopsies were fixed in glutaraldehyde and embedded in plastic
  - 1 micron thick sections were stained with toluidine blue
  - Glass slides stained with toluidine blue were transformed into whole slide images (WSI)
- **Scoring Process:**
  - **Case distribution for annotation:** Digital renal biopsies were randomly distributed across 3 pathologists. Each pathologist served as an annotator for 1/3 of the biopsies and as a reviewer for the other 2/3 on a rotation basis
  - **Annotation:** Each annotating pathologist digitally annotated 300 PTC/biopsy with a unique number recorded in a digital scoring sheet linked to the image
  - **Case distribution for scoring:** the WSI and scoring sheet containing the list of enumerated PTCs were duplicated and distributed to the 2 scoring pathologists
  - Scoring for $Gb_3$ inclusions was performed by applying BLISS:
    - The BLISS capillary score (# $Gb_3$ inclusions/PTC) was recorded in the electronic scoring sheet linked to the image and containing the list of the enumerated PTCs
    - The BLISS biopsy score was calculated as an average of $Gb_3$ inclusions per PTC




BLISS, Barisoni Lipid Inclusions Scoring System; $Gb_3$, globotriaosylceramide; PTC, peritubular capillary; WSI, whole slide image.
Barisoni L, et al. *Arch Pathol Lab Med.* 2012;136:816-824.

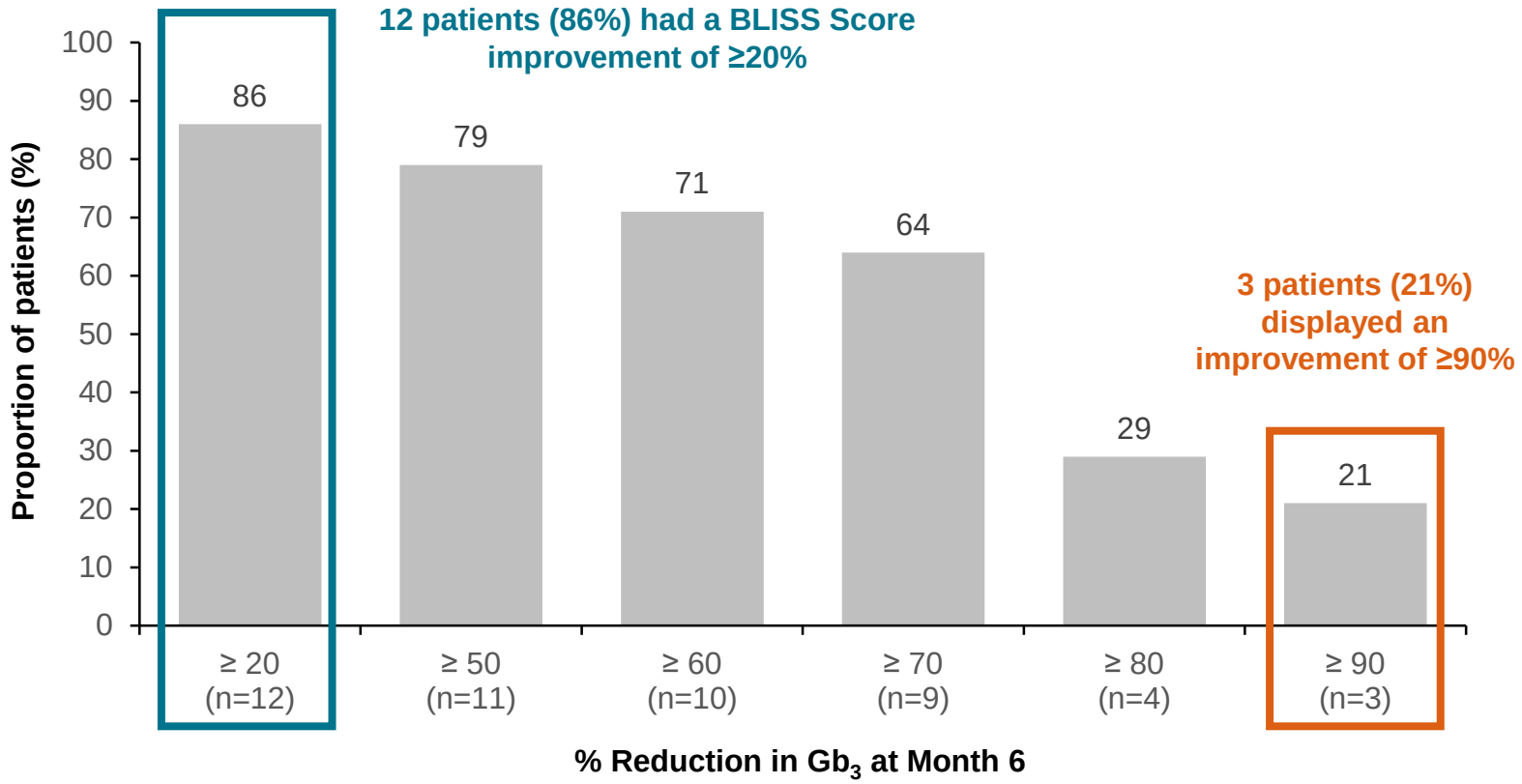
# Patient baseline characteristics

| Characteristic | Dose Group | | |
|---|---|---|---|
| | 0.2 mg/kg n=6 | 1.0 mg/kg n=8 | 2.0 mg/kg n=4 |
| **Age (years), mean ± SD** | 30.0 ± 10.8 | 33.5 ± 11.7 | 40.0 ± 16.5 |
| Median (min–max) | 26.0 (21–50) | 30.0 (17–52) | 43.0 (20–54) |
| **Sex, n** | | | |
| Male | 4 | 6 | 1 |
| Female | 2 | 2 | 3 |
| **Disease phenotype, n** | | | |
| Classic | 5 | 6 | 1 |
| Non-classic | 1 | 2 | 3 |
| **α-Gal A activity, mean (min–max)[a]** | | | |
| *Leukocytes (nmol/hr/mg protein)* | | | |
| Male | 3.2 (1.6–5.0) | 2.7 (0.0–7.8) | 0.6 |
| Female | 27.5 (15–40) | 69.5 (67–72) | 42.7 (33–53) |
| *Plasma (nmol/hr/mL)* | | | |
| Male | 0.2 (0.0–0.4) | 0.3 (0.1–0.4) | 0.4 |
| Female | 3.2 (2.0–4.3) | 6.8 (5.8–7.8) | 4.8 (2.5–7.8) |

Adapted from Schiffmann R, et al. *J Inherit Metab Dis* 2019;42:534-544 with permission under CC BY 4.0.
[a]Normal range: leukocytes, 33–134 nmol/hr/mg protein; plasma, 4–21.9 nmol/hr/mL.
α-Gal A, alpha galactosidase A; max, maximum; min, minimum; SD, standard deviation.

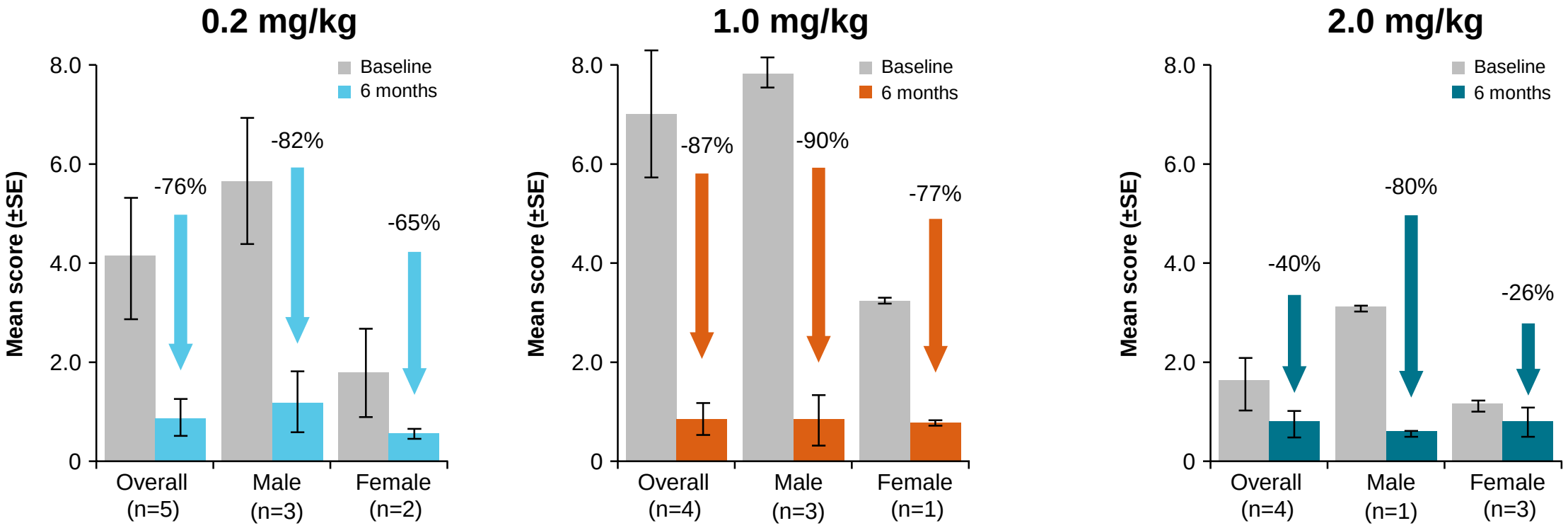
# Responder analysis of BLISS score change from baseline following 6 months of treatment with pegunigalsidase alfa

- A responder analysis (n=14) included patients with an evaluable kidney biopsy after 6 months of treatment with pegunigalsidase alfa




BLISS, Barisoni Lipid Inclusions Scoring System; $Gb_3$, globotriaosylceramide.

# Change from baseline in BLISS score after 6 months of treatment with pegunigalsidase alfa

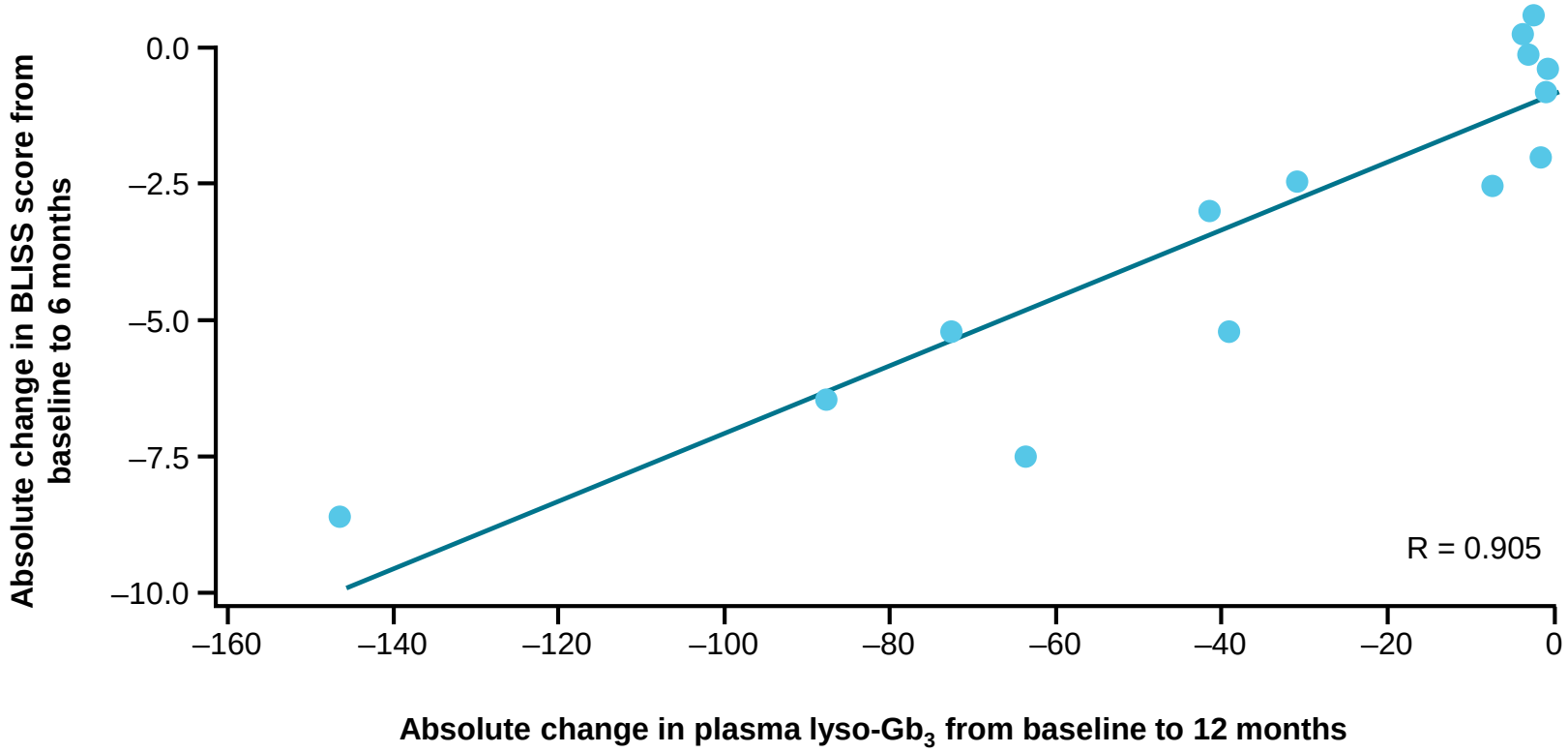



- Overall, there was a reduction in mean BLISS score from baseline to 6 months in all pegunigalsidase alfa dose groups among patients with an evaluable biopsy[a]
  - Reduction in BLISS score was evident in both male and female patients
  - The magnitude of reduction was numerically greater in males (n=7; 85%) than females (n=6; 48%)

[a]Patients with evaluable biopsy data, excluding 1 patient with *GLA* variant p.N215S (n=13).
BLISS, Barisoni Lipid Inclusions Scoring System; $Gb_3$, globotriaosylceramide; $lysoGb_3$, globotriaosylsphingosine; SE, standard error.

# Correlation between $Gb_3$ inclusions and plasma $lysoGb_3$

- The reduction in $Gb_3$ inclusions at 6 months was correlated with a reduction in plasma $lysoGb_3$ at 12 months (R=0.905 [n=14])




BLISS, Barisoni Lipid Inclusions Scoring System; $lysoGb_3$, globotriaosylsphingosine.

# Conclusions and acknowledgements

- Results from this phase 1/2 study demonstrate that pegunigalsidase alfa reaches affected tissue and effectively reduces the number of $Gb_3$ inclusions in renal PTCs at 6 months in adults with FD
- The high correlation between reduction in $Gb_3$ inclusions and plasma lyso-$Gb_3$ reinforces the overall results of pegunigalsidase alfa efficacy, and confirms the BLISS methodology as a sound approach for evaluating pegunigalsidase alfa efficacy

**Acknowledgements:**
The investigators would like to thank the patients and their families for their participation in this study and Robert B Colvin for his contributions to the overall study. Protalix Biotherapeutics was responsible for the design of the study with input from the investigators, monitoring of the study, and collection and analysis of the results.
Statistical analysis was conducted by Target Health Inc.

FD, Fabry disease; $Gb_3$, globotriaosylceramide; lysoGb$_3$, globotriaosylsphingosine; PTC, peritubular capillary.