Pegunigalsidase alfa
Preclinical and clinical

Prague
November 24, 2017
Disclosure

Research support, travel expenses, honoraria

• Amicus Therapeutics
• Protalix Biotherapeutics
• Sanofi Genzyme
• Shire, Inc.
# Fabry Disease and Currently Available ERTs

## Caused by the loss of function of the lysosomal enzyme α-galactosidase-A (α-Gal-A)

- Occurs in most tissues and cell types of: renal, heart, vascular and nervous system

## This depends on multifactors including mutation type and gender

### Available Treatments

- **agalsidase beta**: Approved in the US & the EU. Administered IV at 1 mg/kg body weight, EOW *

- **agalsidase alfa**: Approved in the EU. Administered IV at 0.2 mg/kg body weight, EOW **

- **Migalastat**: Approved in the EU. Administered orally, 123 mg hard capsules every other day***

## Unmet clinical need

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

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* agalsidase beta USPI; ** agalsidase alfa SMPC; *** Migalastat SMPC
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 resulting in 114 kDa enzyme. Contains additional PEG moieties bound to only one subunit through a lysine residue.

Enzyme maintains its catalytic activity and translocation to the lysosome of target cells.
Pegunigalsidase alfa is designed for:

- Providing continuous presence of enzyme throughout the 2-week dosing interval by means of a stable homodimer without compromising the enzyme activity and internalization to target organ and cells
  - Extended circulatory half life
  - Enhanced stability in plasma and under acidic lysosomal-like conditions
- Potentially providing increase enzyme exposure and enhanced activity to target organs and sustained degradation and prevention of accumulation and re-accumulation of substrate
- PEGylation potentially reduces immunogenicity
Potential Impact on Immunogenicity

**01** Chemical modification has the potential to hamper the antigen presentation of pegunigalsidase alfa by Antigen Presenting Cells (APC)
- Processing
- Recognition by T-helper cells

**02** PEGylation potentially reduces immunogenicity by masking immunogenic epitopes

**03** Potential induction of immune tolerance due to improved stability and prolonging/continuous exposure

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*Adopted from: M. Ing et al., Cell. Immunol. (2015)*
Scientific development and preclinical data

pegunigalsidase alfa
specific characteristics and attributes
Prolonged Stability in Biological Matrices Compared to the other ERTs

Stability in human plasma at 37°C (pH=7.4)

Stability in lysosomal-like conditions (pH=4.6)

pegunigalsidase alfa demonstrates improved stability, implicating for higher potential to deliver an active long-functional enzyme to its site of action.
Extended Circulatory Half Life ($t_{1/2}$) - in Fabry Mice Model

Pharmacokinetic Studies in Fabry Mice Model

Enhanced Delivery & Prolonged Activity in Target Organs of Fabry Mice. Enzyme quantities were assessed by an activity assay.

Increased delivery of the active enzyme to the target organs

Reduced clearance by Liver

$\mu g/ gr \text{ FW} = \text{amount of enzyme [}$\mu g$\text{] per gr of tissue fresh weight [FW], assessed by activity}$
Improved/Reduced Antigenicity

- Antibodies generated by immunization of rabbits with α-Gal-A, recognized pegunigalsidase alfa to a lesser extent than agalsidase alfa or beta.

- Patients who develop antibodies on treatment with agalsidase alfa or beta has the potential to benefit from switching to pegunigalsidase alfa due to a lower recognition of pegunigalsidase alfa by existing antibodies to agalsidase beta and alfa.
Small-fiber neuropathy in Fabry mice - Biomarkers and Functional measures of Neuropathy (hot plate sensitivity testing)

Enzyme injections

- Analysis

2 3 4 5
Age (month)

hot plate test

Iba1 immunohistochemistry

In collaboration with Jinsong Shen, M.D., Ph.D.
Institute of Metabolic Disease, Baylor Research
Protective effect on neuropathy, including peripheral nerves

**Biomarker:**
Change in number of Iba1+ cells in DRG

**Function:**
Change in time to response to heat stimulus following 6 injections EOW

The data support the potential for improved efficacy of pegunigalsidase alfa compared to existing ERTs with regards to a protective effect on peripheral nerves.

In collaboration with Jinsong Shen, M.D., Ph.D. Institute of Metabolic Disease, Baylor Research
Clinical Experience in studies PB-102-F01/F02/F03 with Fabry patients. 2 years Interim report
**Main Inclusion Criteria:**
- Symptomatic Fabry patients
- ERT naïve or patients who are off ERT in the last 6 months; negative IgG anti PRX-102 antibody
- eGFR ≥ 60 mL/min/1.73m²

**Main Exclusion Criteria:**
- Chronic kidney disease stages 3-5
- Severe myocardial fibrosis by MRI
- Pregnant or nursing
- Known allergies to ERT

**Adult Fabry Patients**

<table>
<thead>
<tr>
<th>Three dose groups:</th>
<th>Intravenously, every 2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 mg/kg</td>
<td></td>
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<tr>
<td>1 mg/kg</td>
<td></td>
</tr>
<tr>
<td>2 mg/kg</td>
<td></td>
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</tbody>
</table>

**Phase I/II, Open Label, Dose Ranging**

**General Design**

<table>
<thead>
<tr>
<th>Study Identifier</th>
<th>Duration</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB-102-F01</td>
<td>3 months</td>
<td>n=16</td>
</tr>
<tr>
<td>PB-102-F02</td>
<td>9 months</td>
<td>n=16</td>
</tr>
<tr>
<td>PB-102-F03</td>
<td>Up to 60 months</td>
<td>n=11</td>
</tr>
</tbody>
</table>

**24M Interim report**

**Reasons for early discontinuation:**
- Female—pregnancy
- Female—planned pregnancy
- Male—Patient declined to further participate in a clinical study
- 2 pts (a mother and son) withdrew consent due occupational constrains
## Demographics & Baseline Enzymatic Activity

<table>
<thead>
<tr>
<th></th>
<th>0.2 mg/kg</th>
<th>1 mg/kg</th>
<th>2 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean age (years) ± SD (range)</strong></td>
<td>30.0 ± 10.8 (21-50)</td>
<td>34 ± 9.7 (17.5-52.5)</td>
<td>40.6 ± 9.5 (21-54)</td>
</tr>
<tr>
<td><strong>Male : Female</strong></td>
<td>4:2</td>
<td>6:2</td>
<td>1:3</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>African American</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Mean Enzymatic Activity</strong></th>
<th>0.2 mg/kg (males=4, females=2)</th>
<th>1 mg/kg (males*=6, females=2)</th>
<th>2 mg/kg (males=1, females=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>In leucocytes (range)</td>
<td>Males: 3.15 (1.6-5)</td>
<td>Males: 2.67 (0-7.8)</td>
<td>Male 0.56</td>
</tr>
<tr>
<td>(normal 33-134 nmol/hr/mg prt.)</td>
<td>Females: 27.5 (15-40)</td>
<td>Females: 69.5 (67-72)</td>
<td>Females: 42.66 (33-53)</td>
</tr>
<tr>
<td>In plasma (range)</td>
<td>Males: 0.22 (0-0.4)</td>
<td>Males: 0.28 (0.05-0.44)</td>
<td>Male: 0.4</td>
</tr>
<tr>
<td>(normal 4-21.9 nmol/hr/ml)</td>
<td>Females: 3.15 (2-4.3)</td>
<td>Female: 6.8 (5.8-7.8)</td>
<td>Females: 4.80 (2.52-7.8)</td>
</tr>
</tbody>
</table>

* one subject discontinued due to AE; one subject discontinued due to non compliance
Pharmacokinetics - Available Enzyme Throughout 2-Week Interval

PK sampling was done throughout the 1Y treatment.

Plasma pegunigalsidase alfa concentration vs. time

- $T_\frac{1}{2}$
- $C_{\text{max}}$
- $\text{AUC}_{0-\infty}$

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Graph showing concentration vs. time for different doses (0.2mg/kg, 1mg/kg, 2mg/kg).

- $T_\frac{1}{2}$ values for each dose.
- $C_{\text{max}}$ values for each dose.
- $\text{AUC}_{0-\infty}$ values for each dose.
Substantially greater enzyme exposure than current ERTs*;**

**T½: Approx. 80 hours

AUC (0-∞): >35 fold from current ERTs (Both drugs at 1mg/kg)

- pegunigalsidase alfa (1mg/Kg)
- agalsidase beta (1mg/Kg)
- agalsidase alfa (0.2mg/Kg)

*agalsidase beta – USPI; ** agalsidase alfa –SMPC; AUC units: ng•hr/mL; 1ng•hr/mL = 0.06 µg•min/mL
Immunogenicity Evaluation – Current Clinical Experience

Low incidence of treatment induced Anti Drug Antibodies (ADA)

Anti-Drug Antibodies (ADA) multi-tier assessment *
* With accordance to the current regulatory guidelines, using sensitive and validated methods

- Screening
- Confirmatory
- Confirmed positive samples further characterized for:
  - Titer
  - Neutralizing activity

Results
- Serum samples were drawn once a month for 4 first months and then once every 2 months
- 123/144 samples (16 patients) tested negative for ADA

Low incidence of ADA:
3/16 patients → 19%
- 2 in the 0.2mg/kg
- 1 in the 1mg/kg
- None in the 2mg/kg

Low titers
Max titer 4633

Neutralizing ADA:
- 2 of 3 positive patients had neutralizing activity in some samples

Immune tolerization
- All 3 ADA positive patients turned to be negative for ADA after 12M of treatment
Reduced Immunogenicity combined with longer enzyme coverage
No effect on continues Biomarkers reduction in ADA positive (including Nab) pts.

**ADA+; IgG Titers**

Subject 15-F106; 0.2mg/kg

Subject 17-F105; 0.2mg/kg

Subject 07-F113; 1mg/kg

**PK profiles**

**Biomarker**

Lyso-Gb3 (ng/mL)
Efficacy
Reduction of Gb3 in Kidney Peritubular Capillaries
Quantitative BLISS Score

- >300 PTCs were scored for Gb3 inclusions in each biopsy
- Slides underwent digital imaging before scoring
- Images were distributed in a random and blinded manner for annotation by 1 pathologist, and subsequent scoring by 2 other pathologists

<table>
<thead>
<tr>
<th>% change ±SE</th>
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</thead>
<tbody>
<tr>
<td>All Patients (n=13)</td>
</tr>
<tr>
<td>Classic Patients (n=8)</td>
</tr>
</tbody>
</table>
Continuous Reduction of Biomarkers – 24 months
Plasma Gb3 and Lyso-Gb3 (N=11)
Continuous Stability of Renal Function- 24 months (N=11)

Individual eGFR\textsubscript{CKD-EPI} absolute values

<table>
<thead>
<tr>
<th>PID</th>
<th>Sex</th>
<th>eGFR at Base line</th>
<th>eGFR at 12M</th>
<th>eGFR at 24M</th>
</tr>
</thead>
<tbody>
<tr>
<td>51-F102</td>
<td>F</td>
<td>113</td>
<td>116.8</td>
<td>121.6</td>
</tr>
<tr>
<td>12-F103</td>
<td>M</td>
<td>127.5</td>
<td>135.8</td>
<td>121.6</td>
</tr>
<tr>
<td>15-F106</td>
<td>M</td>
<td>112.4</td>
<td>122.6</td>
<td>112.1</td>
</tr>
<tr>
<td>26-F104</td>
<td>M</td>
<td>82.4</td>
<td>75.1</td>
<td>78.8</td>
</tr>
<tr>
<td>17-F105</td>
<td>M</td>
<td>131</td>
<td>130.9</td>
<td>130.3</td>
</tr>
<tr>
<td>07-F113</td>
<td>M</td>
<td>102</td>
<td>85.9</td>
<td>99.9</td>
</tr>
<tr>
<td>09-F108</td>
<td>M</td>
<td>114.3</td>
<td>117.7</td>
<td>108.1</td>
</tr>
<tr>
<td>15-F116</td>
<td>F</td>
<td>120.5</td>
<td>119.7</td>
<td>122.6</td>
</tr>
<tr>
<td>15-F117</td>
<td>F</td>
<td>88.2</td>
<td>81.1</td>
<td>92.4</td>
</tr>
<tr>
<td>17-F118</td>
<td>F</td>
<td>77.7</td>
<td>68.3</td>
<td>74.4</td>
</tr>
<tr>
<td>09-F119</td>
<td>M</td>
<td>119.2</td>
<td>117.2</td>
<td>117.5</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>108.02</td>
<td>106.46</td>
<td>107.20</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>18.10</td>
<td>23.98</td>
<td>18.63</td>
</tr>
</tbody>
</table>
Improvement in Gastrointestinal Symptoms- 24 months (N=11)

Scoring legends:
- Severity of abdominal pain: 0 (no pain) to 4 (very severe pain)
- Frequency of abdominal pain: 0 (never) to 4 (daily) at least once a day
- Frequency of diarrhea: 0 (never) to 4 (daily) at least once a day
Mainz Severity Score Index (MSSI)- 24 months (N=11)
Stable Cardiac Parameters (by MRI) - 24 months (N=11)
Mean LVM, LVMI and EF

• No cardiac fibrosis developed throughout the 24 months of treatment

### Normal Ranges (MRI)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVM (g)</td>
<td>85-181</td>
<td>66-115</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>46-84</td>
<td>37-67</td>
</tr>
<tr>
<td>EF (%)</td>
<td>55-74</td>
<td>54-74</td>
</tr>
</tbody>
</table>
Safety
Safety in 906 Infusions (=34.8 patient years)

100% (295)
Total AEs in 11 patients completing 2 years of treatment

98.6% (291) AEs mild and moderate
- 2 Severe treatment related, 1 pt. (Migraine, Headache)
- 2 Severe not treatment related in 2 other pts. (Renal Hematoma, Pain in Extremity)

1.4% (4) Severe

0.3% (1) Serious AEs
- Kidney hemorrhage post biopsy, not treatment related*

14.6% (43) Related, possibly related
6 patients

85.4% (252) Not related, unlikely related
11 patients

*28 year old male, pre treatment renal hematoma post kidney biopsy- Not related.
9 patients experienced 70 Adverse Events at the day of the infusion

22/70 (31.4%) Adverse Events were reported as treatment related

All recovered without sequelae

Infusion Treatment Related Adverse Events:
- Abdominal pain (7)
- Nausea (2)
- Chest discomfort (2)
- Infusion related reaction (2)
- Chest Pain (1)
- Diarrhea (1)
- Oedema (1)
- Oedema peripheral (1)
- Dizziness (1)
- Nervousness (1)
- Paranasal sinus hypersecretion (1)
- Sneezing (1)
- Pruritus (1)
**Overall Conclusions- Interim 24 month**

**Pegunigalsidase alfa** – PEGylated covalently-linked recombinant alpha-GAL-A enzyme, stable homodimer, produced in plant cells

<table>
<thead>
<tr>
<th><strong>PK:</strong> pegunigalsidase has a longer half-life and a substantially higher AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ Available enzyme throughout 2-week infusion intervals</td>
</tr>
<tr>
<td>▪ Markedly extended circulatory half-life compared with other ERTs</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Safety:</strong> Pegunigalsidase is well tolerated</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ Majority of adverse events – mild and moderate in severity</td>
</tr>
<tr>
<td>▪ Low incidence of treatment induced ADA with reversible &amp; transient effect on PK</td>
</tr>
<tr>
<td>▪ ADA response was transient and tolerization was observed</td>
</tr>
<tr>
<td>▪ ADA positivity had no observed impact on safety and efficacy</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Efficacy:</strong> Demonstrated effectiveness, in various disease endpoints including:</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ Stable kidney and cardiac function</td>
</tr>
<tr>
<td>▪ Reduction of Gb3 inclusions in kidney peritubular endothelial cells</td>
</tr>
<tr>
<td>▪ Continuous reduction of plasma Gb3 and Lyso-Gb3</td>
</tr>
<tr>
<td>▪ Improvement in Gastrointestinal Symptoms- parameters</td>
</tr>
</tbody>
</table>
Special thanks to:

- The Patients and their families
- Phase I/II Investigators:
  - Derralynn Hughes
  - Pilar Giraldo
  - Derlis Gonzales
  - Myrl Holida
  - Simeon Boyd
  - Mohamed Atta
  - Derralynn Hughes
  - Kathy Nicholls
  - Ahmad Tuffaha
  - Ozlem Goker-Alpan
  - Gustavo Maegawa

- Martha R. Charney for PK analysis and projections
- Study site clinical teams and co-authors
EU Positive opinion for orphan drug designation (ODD) for pegunigalsidase alfa – Obtained- November 2017

- ODD was based on medically plausible evidence that pegunigalsidase alfa will provide a significant benefit over existing approved therapies in the European Union

- Significant benefit of pegunigalsidase alfa with a clinically relevant advantage was based on data compared to other authorized treatments in the EU:

  Non clinical
  - Reduced accumulation of toxic metabolites (Gb3) in relevant tissues
  - Reduced peripheral neuropathy

  Clinical
  - Stabilization of kidney function
  - Reduced immunogenicity
Following FDA & EMA discussions Phase 3 Program initiated
Data supporting design of on-going pegunigalsidase alfa Phase 3 program

Based on **In-vitro** data
- Improved drug stability:
  - stability in plasma
  - stability in Lysosomal-like conditions
- Reduced antigenicity

Based on **Preclinical** data
- Improved PK in animals:
  - Longer half-life
- Enhanced biodistribution
- Protective effect on neuropathy

Based on Phase 1/2 **Clinical** Data
- Improved PK in humans:
  - Drug availability for the 2 week interval
- Immunogenicity:
  - Transient ADA and reversible impact on PK
  - Induced tolerization
- Efficacy:
  - Stabilization of renal & cardiac parameters
  - Reduction in biomarkers
On Going Phase 3 Studies

**balance**
- A randomized, double blind, active control study
- Evaluate the safety and efficacy of *pegunigalsidase alfa* compared to agalsidase beta in patients with FD previously treated with agalsidase beta with rapidly declining renal function
- 2 years treatment duration
- Extension study will be offered to patients at the end of the study

**bridge**
- An open label switch over study from agalsidase alfa
- Assess the safety and efficacy of *pegunigalsidase alfa* patients with FD treated with agalsidase alfa for at least 2 years
- 1 year treatment duration
- Extension study will be offered to patients at the end of the study

**bright**
- An open label, switch over study from agalsidase alfa and beta
- Assess the safety, efficacy and PK of *pegunigalsidase alfa* 2 mg/kg IV administrated every 4 Weeks in FD patients currently treated with ERT
- 1 year treatment duration
- Extension study will be offered to patients at the end of the study

ClinicalTrials.gov Identifier:NCT02795676  
ClinicalTrials.gov Identifier:NCT03018730  
ClinicalTrials.gov Identifier:NCT03180840
Thank You