Analysis of the baseline characteristics of Fabry disease patients screened for the pegunigalsidase alfa Phase III BALANCE study

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Abstract

Enzyme replacement therapy (ERT) may lead to formation of anti-drug antibodies (ADA) in "classical" patients with Fabry disease (FD). Recent studies provided evidence that neutralizing antibodies (nAb) may interfere with ERT efficacy. Higher doses of ERT have been recently shown to overcome the activity inhibition caused by ADAs (Lenders et al. 2018, https://doi.org/10.1016/j.jaci.2017.12.1001).

Pegunigalsidase alfa, a novel ERT treatment for Fabry patients, had demonstrated improved plasma exposure and increased delivery of the active enzyme to the target organs (kidney and heart) compared to commercially available ERT in Fabry mouse model. In ERT-naive Fabry patients a low incidence of ADA with transient and reversible impact on pegunigalsidase alfa pharmacokinetics (PK) with no or minimal effect on pharmacodynamics (PD) was observed (Hughes et al. LDN 2017).

BALANCE (PB-102-F20; NCT02795676) is a double blind head-to-head study comparing the effect of pegunigalsidase alfa to agalsidase beta (1mg/kg every 2 weeks) on renal function. The screening strategy selected FD patients with progressive loss of kidney function despite long-term ERT (1-16 years). The current analysis describes the baseline characteristics of FD patients screened for this study.

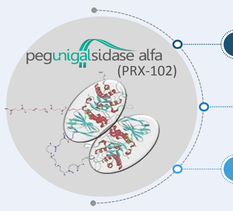
Among male patients currently screened for the study, 55% were positive for both binding and neutralizing ADA to agalsidase beta, with *in-vitro* mean enzyme activity inhibition of ~80% and IgG titers of 60 to 127,933. One female was ADA-positive (binding ADA only). *In-vitro* evaluation of the cross-reactivity of the nAb-positive samples toward pegunigalsidase alfa showed a lower mean enzyme activity inhibition of ~64%, resulting in a greater amount of effective (non-inhibited) enzyme of ~40% vs ~20%.

The lower enzyme activity inhibition combined with ~40-fold longer plasma half-life of pegunigalsidase alfa compared to agalsidase beta (~80 h vs ~2h) are expected to result in a greater amount of effective enzyme available in the circulation of patients switching from a long-standing treatment with agalsidase beta to pegunigalsidase alfa.

Further evaluation of ADA status and renal function of this cohort indicates that 64% (14/22) of the ADA-positive FD male patients also have significant proteinuria (UPCR≥ 500 mg/gr).

The unique characteristics of pegunigalsidase alfa, encompassing an improved pharmacokinetic profile, lower immunogenicity, and low cross reactivity to pre-existing anti-agalsidase beta nAb, may result in higher levels of effective enzyme to reach target organs, with the potential to improve long-term clinical outcomes and attenuation of renal function deterioration in FD patients.

Pegunigalsidase alfa: PEGylated, Chemically Modified α-Gal-A Enzyme



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

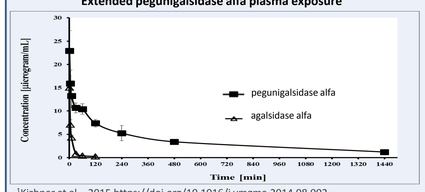
Shown extended half-life and stability in plasma as well as lysosomal-like conditions, while reaching target organs and subcellular lysosomes

PEG moieties are masking some enzyme epitopes which could be recognized by the immune system → Potentially reducing the immune response to the enzyme plus reduced cross reactivity to pre- existing ADA

Prolonged Exposure in Fabry Mice Compared to the other ERTs[1]

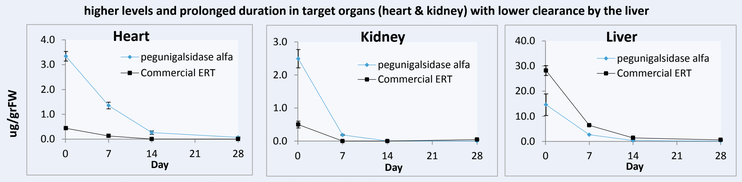
Plasma levels

Extended pegunigalsidase alfa plasma exposure



Tissue levels

higher levels and prolonged duration in target organs (heart & kidney) with lower clearance by the liver



µg/gr FW = amount of active enzyme [µg] per gr of tissue fresh weight [FW]

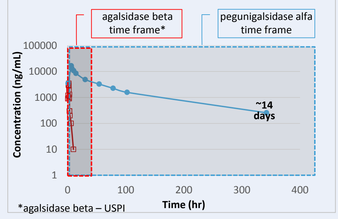
[1]Kizhner et al., 2015 https://doi.org/10.1016/j.ymgme.2014.08.002

pegunigalsidase alfa demonstrates an improved stability, implicating for higher potential to deliver an active long-functional enzyme to its site of action

Pharmacokinetics & Pharmacodynamics - Fabry Disease Patients

Pharmacokinetics

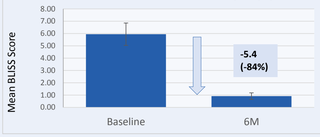
Plasma pegunigalsidase alfa concentration vs. time



*agalsidase beta – USPI

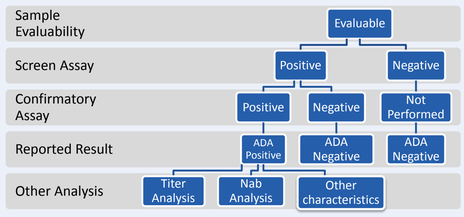
Pharmacodynamics

Reduction in Gb3 inclusions - Kidney Biopsy



Phase 1/2 studies with adult ERT-naïve Fabry disease patients (Hughes et al. LDN 2017):
Full 14 days enzyme coverage in plasma resulting with enzyme translocation and clear reduction of Gb3 in the kidney

ADA testing approach



Main Guidelines - Immunogenicity

- EMA guideline EMEA/CHMP/BMWP/14327/2006 Rev. 1 'Guideline on Immunogenicity Assessment of Biotechnology-Derived Therapeutic Proteins' ; September **2015**
- 'Guidance for Industry Assay Development for Immunogenicity Testing of Therapeutic Proteins'. Draft Guidance. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), April **2016**
- 'Guidance for Industry Immunogenicity Assessment for Therapeutic Protein Products', Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Aug **2014**

ADA positivity and relationship to ERT clearance in ERT-naïve Fabry patients[1]

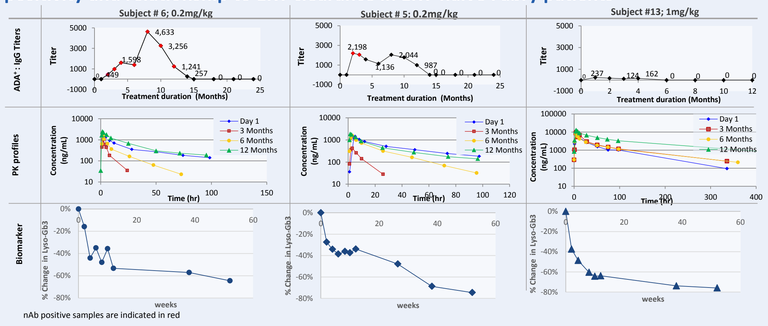
Low incidence of ADA with low titers:

- ADA positives: 19%
- neutralizing ADA positive: 12% (2 patients)
- Max titer 4633

Immune tolerization
All ADA positive patients turned to be negative for ADA after 12M of treatment

Impact on PK and PD
Transient and reversible effect on PK
None or minimal effect on PD

[1]Schiffmann, New Horizons in FD Conference .2017



nAb positive samples are indicated in red

The impact of ADA positivity on pegunigalsidase alfa plasma clearance was transient and reversible following reduction of titers and ADA tolarization

BALANCE study screening strategy

- Adult Fabry patients (> 18 y.o.)
- Impaired renal function
- Linear negative slope of eGFR of ≥ 2 mL/min/1.73 m²/year based on at least 3 serum creatinine values over approximately one year (range of 9 to 18 months including the value obtained at the screening visit)
- Treated with agalsidase beta for approximately 1 year and on a stable dose for at least 6 months
- No more than 50% of treated patients will be female
- ADA status is not an eligibility criteria

Methods & Results

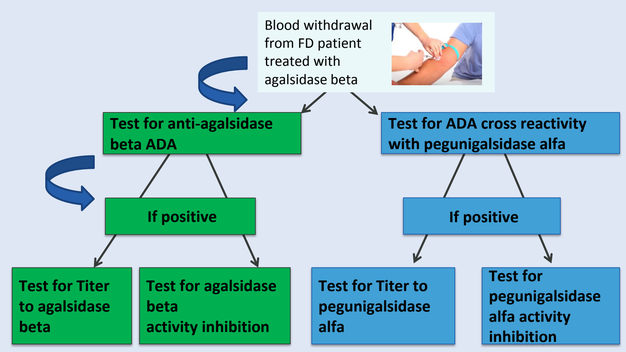
Antibodies Assessment Approach

for Samples of FD patient previously treated with anti-agalsidase beta

ADA testing approach:

- Same sample is tested for presence of ADA to either drugs in a multi-tiered approach
- Same amount of either enzyme (drug) is used at any step and method
- Assays having similar sensitivities
- Assays essentially differ only by the test article (i.e. agalsidase beta *Vs.* pegunigalsidase alfa)

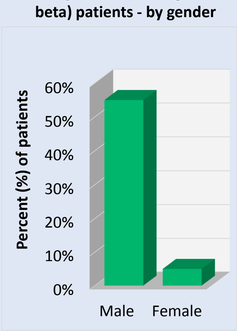
Characterization of Positive Samples



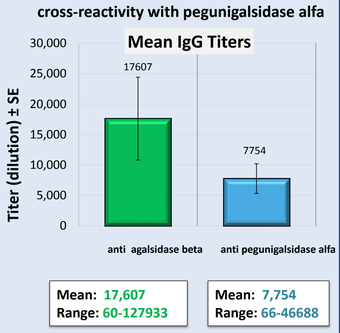
Antibody Assessment: % of ADA-positive & Cross Reactivity Comparison

- The majority of the males screened to the study were ADA-Positive, most females are ADA-negative
- Cross reaction with pegunigalsidase alfa: Less then 100% with lower titers

% ADA-positive (anti- agalsidase beta) patients - by gender



ADA titers to agalsidase beta and their cross-reactivity with pegunigalsidase alfa



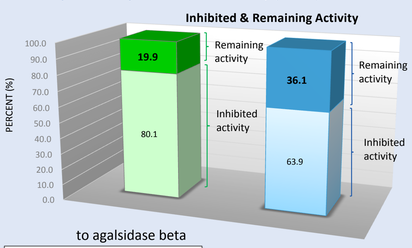
Mean: 17,607
Range: 60-127933

Mean: 7,754
Range: 66-46688

Assessment of ADA Neutralizing Activity

for samples of FD patients previously treated with ERT, (n=60)

- pegunigalsidase alfa enzyme activity is substantially less inhibited by anti-algalsidase beta neutralizing antibodies (nAb)
- Approximately 50% higher remaining activity: ~36% with pegunigalsidase alfa vs. ~20% remaining activity with agalsidase beta



to agalsidase beta

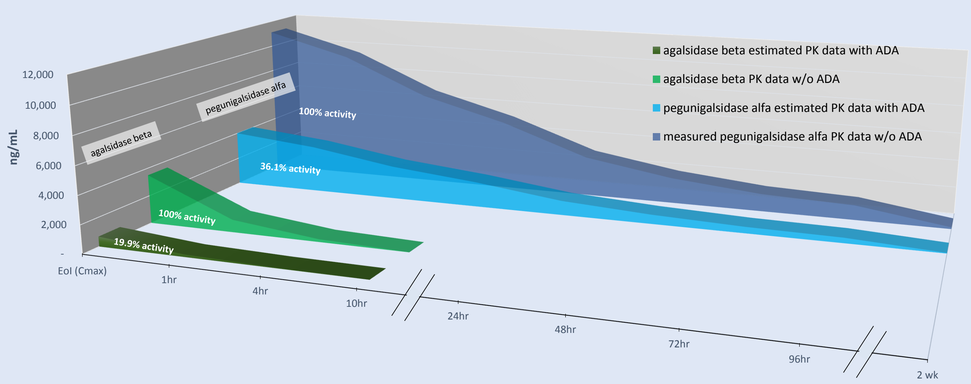
% inhibition:
Mean: **80.1%**
Range: 38.4-95.37%
Remaining activity: 19.9%

to pegunigalsidase alfa

% inhibition:
Mean: **63.9%**
Range: 38.8-88.8%
Remaining activity: 36.1%

- Drug plasma concentrations of agalsidase beta and pegunigalsidase alfa were plotted based on public domain available PK data to generate the curves representing the levels without ADA ("w/o ADA").
- A simulation of the predicted remaining active enzyme concentrations using the % inhibition observed as part of PB-102-F20 screening process per ERT were used to generate the "with ADA" curves.
- **Conclusion**: The simulation predicts a remaining higher level of active (non-inhibited) pegunigalsidase alfa enzyme in the presence of ADA throughout the 2 weeks IV intervals.

ADA status and its potential effect on drug availability - Simulation



Summary & Conclusions

- Naïve Fabry patients treated with pegunigalsidase alfa had low incidence of treatment-induced anti drug antibodies with transient and reversible impact on PK . All ADA positive patients turned to be ADA negative after 12M of treatment and remain negative throughout the 4 year follow up period
- Pegunigalsidase alfa have substantially improved PK profile together with lower immunogenicity compared to agalsidase beta, suggesting a unique potential to overcome the compromising effect of ADA on treatment outcome
- Treatment with pegunigalsidase alfa could potentially result in higher levels of non-inhibited, available active enzyme to reach target organs, with potential to improve long-term clinical outcomes in FD patients due to the longer half-life and a substantially low cross-reactivity of pre-existing anti-agalsidase beta antibodies toward pegunigalsidase alfa
- As the effectiveness of ERT in FD may be compromised by serum-mediated inhibitory antibodies (nAb), additional analysis in a larger number of patients might characterize at which extent antibodies generation impacts on clinical efficacy of the currently available ERT in FD, and the potential beneficial effect of pegunigalsidase alfa

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