The Utility of Pharmacogenetics in the Identification of Fabry Patients Eligible for Treatment with Migalastat

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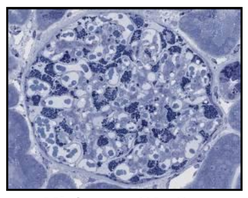
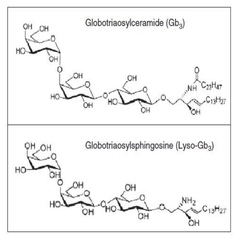
Introduction

Fabry Disease (FD)

- Progressive X-linked lysosomal storage disorder caused by a deficiency in α-galactosidase A
- Estimated FD incidence of approximately 1 in 100,000. Actual prevalence may be higher
- More than 800 disease-causing mutations in *GLA* have been identified; ~60% of these are missense mutations
- Affects males and females; females have a mosaic of healthy and diseased cells
- Globotriaosylceramide (GL-3), a natural substrate of α-Gal A, accumulates and affects multiple organs and organ systems (kidney, heart, brain, gastrointestinal, skin)
- Globotriaosylsphingosine (lyso-Gb_3) is another substrate of α-Gal A that is elevated in plasma of male and female patients with FD



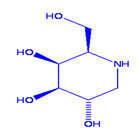
Kidney GL-3



From Auray-Blais et al., 2010

Migalastat for FD:

- Orally administered investigational pharmacological chaperone for patients with amenable mutations
- Increases stability, folding, and cellular trafficking of amenable mutant forms of α-Gal A to lysosomes where the breakdown of substrate can proceed
- Amenable mutant forms of α-Gal A are identified using a GLP-validated HEK-293 cell-based assay (GLP HEK assay)
- 30-50% of patients with FD are estimated to have amenable mutations; the majority of amenable mutations are associated with the classic phenotype of the disease



Migalastat
Deoxygalactonojirimycin
AT1001

Objectives

- To assess the clinical validation of the GLP HEK assay, the mutant α-Gal A responses to migalastat in the assay were compared to Fabry patient pharmacodynamic responses to treatment with migalastat in Phase 2 and 3 clinical studies

Materials & Methods

GLP HEK Assay:

- A bioanalytically validated assay used to individually express FD mutations in human embryonic kidney-293 (HEK) cells and measure increases in mutant α-Gal A activity in response to 10 μM migalastat
- Known FD associated missense, carboxyl-terminal nonsense, small in-frame insertion, deletion, and complex mutant forms of the enzyme qualify for testing in the GLP HEK assay
- Amenable mutant forms are defined as those having a ≥1.2-fold relative increase and ≥3.0% absolute increase in α-Gal A activity
- Patient samples are not required and the approach is applicable to both males and females
- To date, 531 FD mutations have been tested; 224 have met the amenable mutation criteria

Data From Three Phase 2 Studies of Migalastat:

- FAB-CL-201 (NCT00214500), FAB-CL-202 (NCT00283959), FAB-CL-203 (NCT00283933)
- The objectives were to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of migalastat in patients with FD
- All three studies included males only
- Study 201 evaluated different dosages; Studies 202 and 203 evaluated 150 mg migalastat HCl once every other day
- All three studies were open-label, and included initial 12-24-week treatment periods and optional treatment extensions

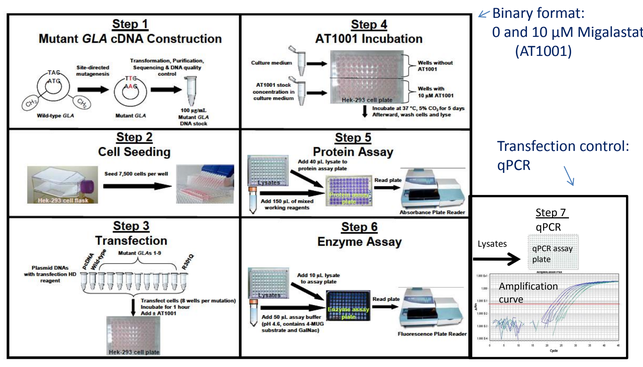
Data From Phase 3 Study AT1001-011 (NCT00925301):

- A double-blind, randomized, placebo-controlled study to evaluate the efficacy, safety, and pharmacodynamics of migalastat HCl in patients with FD and amenable *GLA* mutations
- Key Inclusion Criteria
  - Male or female, diagnosed with FD
  - Amenable *GLA* mutation (during screening the *GLA* mutation was confirmed by gene sequencing; the 'amenable' category was determined by a preliminary HEK-293 cell-based assay)
  - Naïve to enzyme replacement therapy (ERT) or has not received ERT for ≥6 months before screening

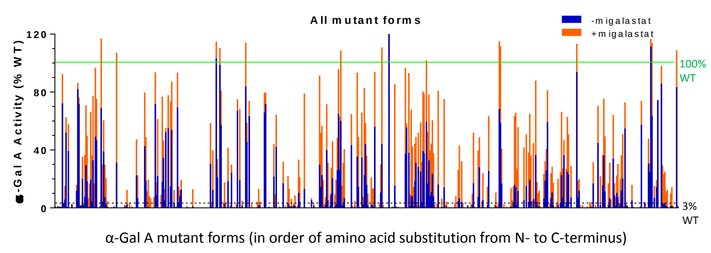
Data From Phase 3 Study AT1001-012 (NCT01218659):

- A randomized, open-label study to compare the efficacy and safety of migalastat HCl and ERT in patients with FD and amenable mutations who were previously treated with ERT
- Key Inclusion Criteria
  - Male or female, diagnosed with FD
  - Amenable *GLA* mutation (during screening the *GLA* mutation was confirmed by gene sequencing; the 'amenable' category was determined by a preliminary HEK-293 cell-based assay)
  - Initiated treatment with ERT at least 12 months prior to the baseline visit

GLP HEK Assay Procedure and Data Overview



- The assay includes: **A**) a thorough and rigorous set of plasmid DNA quality control assessments and storage specifications; **B**) a simple binary design wherein *GLA* transfected HEK-293 cells are incubated in the absence or presence of a single concentration of migalastat (10 μM); **C**) a quantitative real-time PCR (qPCR) transfection efficiency control measurement obtained from every sample; **D**) rigorous and consistent assay acceptance criteria



α-Gal A mutant forms (in order of amino acid substitution from N- to C-terminus)

- The assay data show that 531 tested α-Gal A mutant forms span the entire length of the gene and show a wide range of α-Gal A enzyme activities both at baseline and after incubation with migalastat

Comparison to α-Gal A Responses in Phase 2 and 3

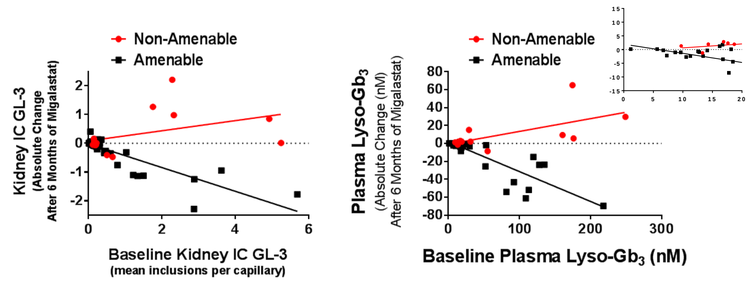
- The mutant α-Gal A responses to migalastat in the GLP HEK assay and in white blood cells (WBCs) of male Fabry patients orally administered migalastat in clinical studies were compared
- The degree of consistency was evaluated by calculating the sensitivity, specificity, positive predictive value, and negative predictive value

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Number of Different Patients
Phase 2 (all doses)	0.9375	1.0	1.0	0.875	23
Phase 2 (150 mg QOD)	1.0	1.0	1.0	1.0	14
AT1001-011 (150 mg QOD)	1.0	0.75	0.875	1.0	22
AT1001-012 (150 mg QOD)	1.0	1.0	1.0	1.0	15

Criteria for a "good" WBC α-Gal A response: ≥2% of normal maximal net increase after oral administration of migalastat. This comparison did not include mutant forms represented in female subjects, because PBMCs derived from females are a mixture of cells that express either wild-type or mutant α-Gal A. Thus the measured α-Gal A activity is dominated by the wild type enzyme, which is responsive to migalastat; hence, neither the baseline activity nor the effect of migalastat on the mutant form can be accurately determined.

- A high degree of consistency between the GLP HEK assay results and the male subject WBC α-Gal A results was obtained

Comparison to Substrate Responses in Study 011



Six months of migalastat refers to the change from baseline to month 6 in subjects randomized to migalastat in Stage 1; it refers to the change from month 6 to month 12 in subjects randomized to placebo in Stage 1.

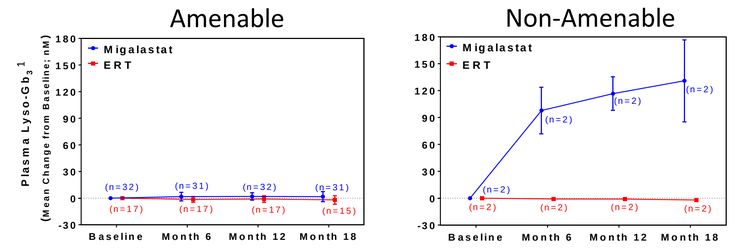
- Male and female kidney interstitial capillary GL-3 (IC GL-3) and plasma lyso-Gb_3 absolute changes after six months of treatment were grouped by *GLA* mutation category
- Patients with amenable mutations showed consistent decreases in these substrate levels; larger decreases were observed with increasingly higher baseline values
- In patients with non-amenable mutations, the regression line for the relationship between baseline plasma lyso-Gb_3 and the post-treatment change reflected increases in substrate with increasingly higher baseline values

Parameter Compared with GLP HEK Assay	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Number of Different Patients
Male Kidney IC GL-3	1.0	1.0	1.0	1.0	18
Male Plasma Lyso-Gb_3	1.0	1.0	1.0	1.0	16
Male and Female Plasma Lyso-Gb_3	0.9286	0.6875	0.8387	0.8462	44

Patients with a kidney IC GL-3 or plasma lyso-Gb_3 absolute change <0.0 after 6 months of treatment were categorized as showing "good" responses, and patients with ≥0.0 were categorized as showing "non/limited" responses; absolute change from baseline in Fabry substrate (i.e., kidney IC GL-3 or plasma lyso-Gb_3) is calculated as the value after 6 months of migalastat treatment minus the value at baseline ; the GLP HEK comparison to male kidney iC GL-3 included only males with a baseline kidney IC GL-3 level ≥0.1.

- In Study 011, comparisons of GLP HEK assay results to patient substrate responses to migalastat showed high consistency

Substrate Responses in Study 012

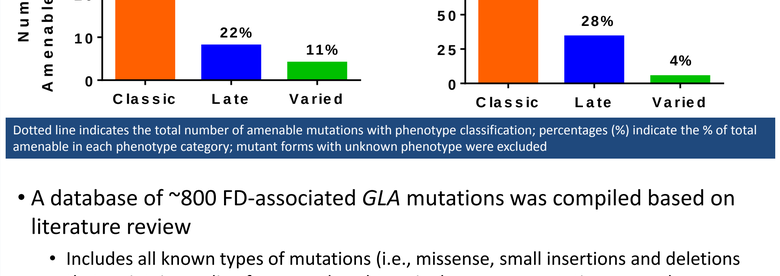


Patients with amenable GLA mutations in GLP-validated HEK assay [1]Baseline corrected. Blue dotted line represents zero; data points represent the mean, Error bars are SD; Least Squares (LS) Mean at Month 18 showed results comparable to the mean (data not shown); Based on subjects with available samples for this analysis

- In patients with amenable mutations, the plasma lyso-Gb_3 levels were comparable to those seen with ERT, in both males and females
- In two male subjects with non-amenable mutations, plasma lyso-Gb_3 increased following switch from ERT as compared to two (1M, 1F) who remained on ERT

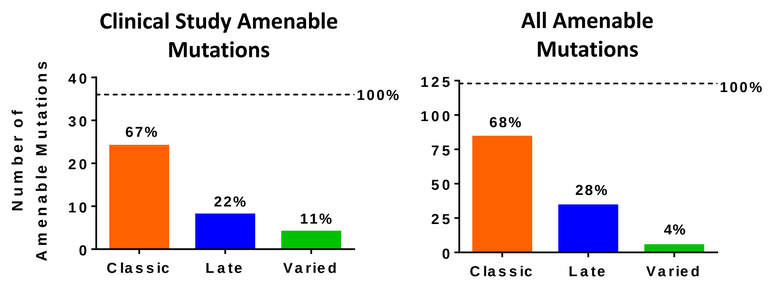
Phase 2/3 Amenable Mutations Compared to All

- In total, 51 different amenable mutations were identified in 126 subjects from Phase 2 and 3 clinical studies
- This represents 23% of all amenable mutations to date



- This set of amenable mutant forms of α-Gal A (n=51) represented in clinical trials were compared to the larger FD-associated subset that met the amenable mutation criteria (n=224); the responses to migalastat were not significantly different
- The results suggest that the amenable mutant forms evaluated in Phase 2 and 3 clinical studies are representative of the larger subset of amenable mutant forms

Amenable Mutations Grouped by Phenotype



Dotted line indicates the total number of amenable mutations with phenotype classification; percentages (%) indicate the % of total amenable in each phenotype category; mutant forms with unknown phenotype were excluded

- A database of ~800 FD-associated *GLA* mutations was compiled based on literature review
  - Includes all known types of mutations (i.e., missense, small insertions and deletions that maintain reading frame, carboxyl-terminal nonsense mutations, complex mutations, large deletions or insertions, truncations, frameshift mutations, splice site mutations)
  - Includes information on whether that mutation has been associated with the classic and/or late-onset (variant) phenotype in the literature
- The results show that a majority , ~68%, of all amenable mutations as well as those represented in migalastat clinical studies are associated with classic FD

Conclusions

- The results indicate that the GLP HEK assay and the amenable mutation criteria have high predictive value in identifying FD patients who show a pharmacodynamic response to oral administration of migalastat based on assessment of α-Gal A in WBCs, kidney interstitial capillary GL-3 deposition, and plasma lyso-Gb_3 concentrations
- The results indicate that the amenable mutations evaluated in the migalastat Phase 2 and 3 clinical studies are representative of the larger subset of amenable mutations
- These results support the clinical validation of the GLP HEK assay and its utility in identifying the target population for treatment with migalastat: patients with FD who have amenable mutations
- Approximately 30-50% of patients with FD are estimated to have amenable mutations; the majority of amenable mutations are associated with the classic phenotype of the disease
- As new *GLA* mutations are identified, they can readily be tested in the GLP HEK assay to determine amenability to treatment with migalastat