Migalastat Reduces Plasma Globotriaosylsphingosine (Lyso-Gb_3) in Fabry Patients: Results from Phase 3 Clinical Studies

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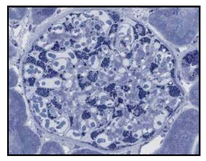
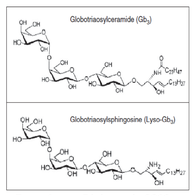
Introduction

Fabry Disease (FD)

- Progressive X-linked lysosomal storage disorder caused by a deficiency in α-galactosidase A
- Estimated FD incidence of 1 in 100,000. Actual incidence 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 males and females living with FD



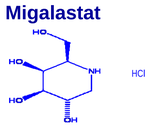
Kidney GL-3



From Auray-Blais et al., 2010

Migalastat for FD:

- Orally administered investigational pharmacological chaperone for patients with amenable mutations
- Facilitates proper 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
- 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

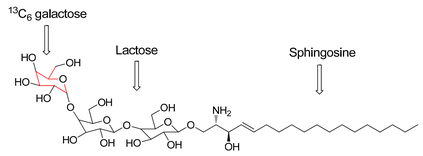


Migalastat

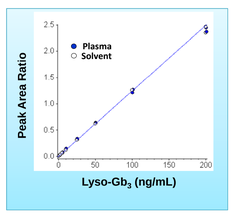
Deoxygalactonojirimycin (DGJ)
AT1001

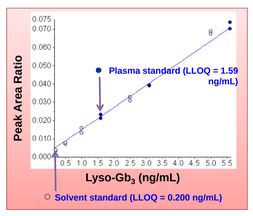
Plasma Lyso-Gb_3 Assay Method

- A liquid chromatography tandem-mass-spectrometry plasma lyso-Gb_3 method using a novel stable isotope-labeled internal standard (IS), $^{13}C_6$-lyso-Gb_3, was developed.



- $^{13}C_6$-lyso-Gb_3 corrects for matrix effects and enables the use of solvent standard calibration curves for lyso-Gb_3 quantification in plasma.

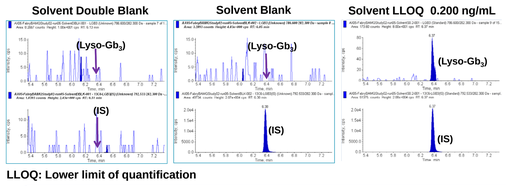




Run Number	Slope		Intercept		R-Squared	
	Solvent	Plasma	Solvent	Plasma	Solvent	Plasma
Core-run 1	0.0126	0.0124	0.00115	0.00235	0.9958	0.9974
Core-run 2	0.0113	0.0127	0.00126	0.00131	0.9874	0.9970
Core-run 3	0.0121	0.0119	0.00108	0.00240	0.9958	0.9964

The table shows <5% relative standard deviation between slopes of solvent and plasma curves

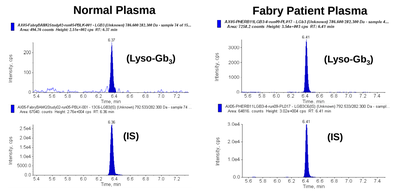
- Representative chromatograms show absence of solvent interference for lyso-Gb_3 and $^{13}C_6$-lyso-Gb_3



LLOQ: Lower limit of quantification

- Assay Procedure:
- Analyte reference standard: lyso-Gb_3 (Matreya)
- IS: $^{13}C_6$-lyso-Gb_3 (synthesized In-house)
- Sample volume: 50 µL K_2 EDTA human plasma
- Sample clean-up: solid-phase extraction (Oasis MCX, Waters)
- Liquid chromatography column: Halo HILIC column (Advanced Materials Technology)
- Liquid chromatography conditions: gradient elution
- Mobile phases:
  - A = Acetonitrile:water/5:95 + 5 mM ammonium formate + 0.5% formic acid
  - B = Acetonitrile:water/95:5 + 5 mM ammonium formate + 0.5% formic acid
- Detection: Positive electrospray ionization and Tandem Mass Spectrometry (AB Sciex 4000 QTRAP)
- Standards prepared in neat solvent (methanol: dimethylsulfoxide/ 1: 1)
- Quality controls prepared in neat solvent and plasma
- Calibration curve: Linear regression with $1/x^2$ weighting
- Quantitation: Peak area ratio of analyte to IS
- Assay range: 0.200 – 200 ng/mL
- Inter-day accuracy and precision:
  - Inter-day accuracy (% difference from nominal) range: -0.7 to -13.4% (solvent QCs) and 1.9 to 4.2% (plasma QCs)
  - Inter-day precision (% coefficient of variation; CV): 5.7 to 16.1% (solvent QCs) and 4.4 to 8.4% (plasma QCs)

- Representative chromatograms of lyso-Gb_3 and $^{13}C_6$-lyso-Gb_3 in normal and Fabry patient plasma



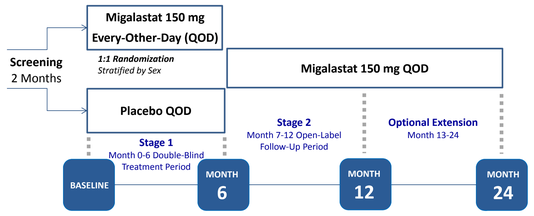
Plasma Lyso-Gb_3 Sample Analysis

- Plasma lyso-Gb_3 levels were measured in samples obtained from Fabry patients enrolled in Phase 3 clinical studies (AT1001-011 and AT1001-012) who signed informed consent
- Plasma lyso-Gb_3 levels were determined at baseline and months 6, 12, and 18/24 (mo 18 in AT1001-012; month 24 in AT1001-011)
- Three samples collected in each visit were analyzed
- Three independent determinations of plasma lyso-Gb_3 were obtained from every sample
- The result from each sample was reported as a mean of the three independent determinations
- The result from each visit was reported as the mean of three samples
- Pre-specified precision criteria were used to trigger additional measurements
- Total number of samples analyzed three times for study AT1001-011*: 531
- Overall mean % CV of three determinations per AT1001-011 sample (n=531 samples)*: 3.88%
- Less than 2% of study samples* required additional measurement
- Plasma lyso-Gb_3 sample analysis was performed blinded and randomized*; reported data were locked prior to unblinding

*Same sample analysis strategy and similar assay performance results for study AT1001-012

AT1001-011 (NCT00925301) Design

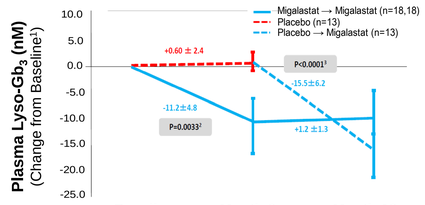
- A Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Efficacy, Safety and Pharmacodynamics of Migalastat HCl in Patients With Fabry Disease and Amenable *GLA* Mutations



- **Key Inclusion and Exclusion Criteria:**
  - Male or female, diagnosed with FD
  - 16 to 74 years old
  - Amenable *GLA* mutation (during screening for AT1001-011, 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
  - Estimated GFR_{MDRD} (eGFR) at screening ≥30 ml/min/1.73 m^2
  - Urine GL-3 at screening ≥4 times the upper limit of normal (24-hour collection)

AT1001-011 Plasma Lyso-Gb_3 Results

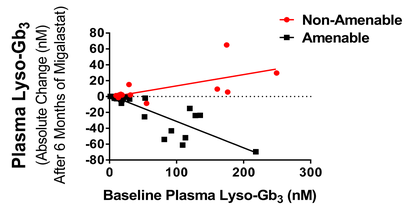
Plasma Lyso-Gb_3 is Reduced in Response to Migalastat



[1]Data points are baseline corrected; represent mean ± SEM change from baseline in plasma lyso-Gb_3 after 6 or 12 months in the study
[2]Analysis of covariance (ANCOVA) comparing migalastat to placebo after 6 months .
[3]ANCOVA analysis of the change from baseline to month 6 compared to the change from month 6 to month 12 in subjects switching from placebo to migalastat.

- Plasma lyso-Gb_3 data are shown for Fabry subjects with an amenable mutation in the final GLP-validated HEK-293 cell-based assay
- A statistically significant reduction in plasma lyso-Gb_3 was observed at month 6 and month 12 following treatment with migalastat in subjects with amenable mutations

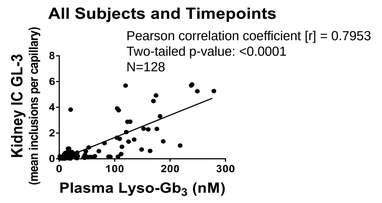
Plasma Lyso-Gb_3 Response Grouped by *GLA* Mutation Category



6 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

- Larger decreases in plasma lyso-Gb_3 were observed with increasingly higher baseline values in subjects with amenable mutations
- Generally, no decreases in plasma lyso-Gb_3 were seen in subjects with non-amenable mutations

Plasma Lyso-Gb_3 Correlation with Kidney Interstitial Capillary (IC) GL-3

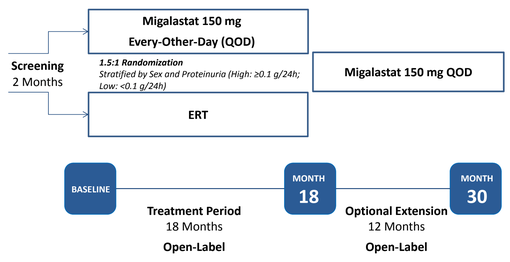


Mean inclusions per capillary indicates that values are the mean of up to four reads per histological slide

- Plasma lyso-Gb_3 and kidney IC GL-3 from all subjects at all time points were significantly correlated (p<0.0001)
- These two datasets from all subjects at only the baseline time point were also significantly correlated (p<0.0001; figure not shown)

AT1001-012 (NCT01218659) Design

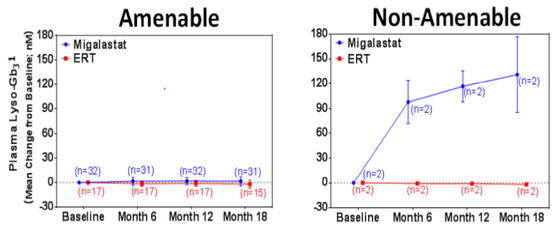
- A Randomized, Open-Label Study to Compare the Efficacy and Safety of AT1001 and Enzyme Replacement Therapy (ERT) in Patients with Fabry Disease and AT1001-Responsive *GLA* Mutations Who Were Previously Treated with ERT



- **Key Inclusion and Exclusion Criteria:**
  - Male or female, diagnosed with FD
  - 16 to 74 years old
  - Amenable *GLA* mutation (during screening for AT1001-012, 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
  - Dose level and regimen of ERT stable for the 3 months prior to the baseline visit and ≥80% of the labeled dose
  - GFR ≥30 ml/min/1.73 m^2
  - Subjects taking ACEs or ARBs must be on a stable dose for a minimum of 4 weeks before the screening visit

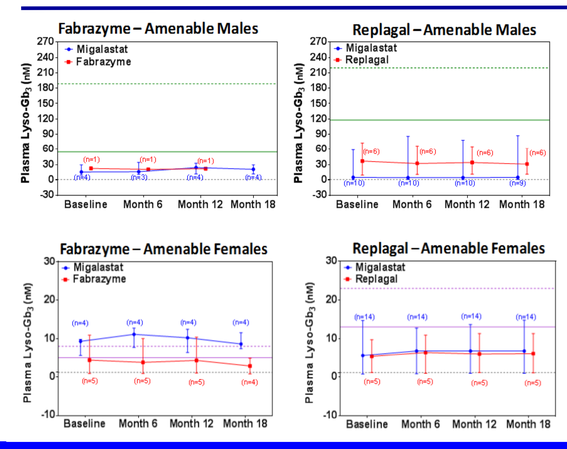
AT1001-012 Plasma Lyso-Gb_3 Results

Plasma Lyso-Gb_3 Remains Low and Stable Following the Switch from ERT to Migalastat



- Plasma lyso-Gb_3 data are shown for subjects grouped by their *GLA* mutation category in the final GLP-validated HEK-293 cell-based assay
- Plasma lyso-Gb_3 remained low and stable in male and female patients with amenable mutations who continued on ERT, and those who switched from ERT to migalastat
- In two male patients with non-amenable mutations, plasma lyso-Gb_3 increased following switch from ERT to migalastat as compared to two patients (1M, 1F) who remained on ERT

Figure legend:
- [1]Baseline corrected. Blue dotted line represents zero; data points represent the mean, error bars are standard deviation; 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 subjects with amenable mutations, plasma lyso-Gb_3 levels were further grouped by prior ERT treatment (i.e., Fabrazyme or Replagal) and gender
- Lyso-Gb_3 levels remained low and stable for up to 18-months in both males and females following their switch from ERT (Fabrazyme or Replagal) to oral migalastat

Figure legend:
- Data points are median values, error bars represent the range
- Blue dotted line represents the upper range of the normal reference range, i.e., 1.19 nM (normal control plasma lyso-Gb_3 range: 0.374-1.19 nM (n=46))
- Dotted and solid lines (green for males and magenta for females) represent the baseline and median values after 1 year of ERT (Fabrazyme or Replagal) treatment , respectively, for a cohort of classic males and females (van Breemen et al. 2011)

Conclusions

- An LC-MS/MS method was analytically validated (non-GLP) at Amicus Therapeutics for the quantitation of lyso-Gb_3 in human plasma and was used to measure plasma lyso-Gb_3 levels in Fabry patients enrolled in Phase 3 clinical studies (AT1001-011 and AT1001-012).
- The results of Study AT1001-011 demonstrate that migalastat reduces plasma lyso-Gb_3 during the first six months of treatment, which is maintained over time, in subjects with amenable mutations.
- The results of Study AT1001-012 demonstrate that plasma lyso-Gb_3 levels remained low and stable for up to 18 months in both males and females with amenable mutations following their switch from ERT (Fabrazyme or Replagal) to oral migalastat.