Phase 3 Study (FACETS) of Migalastat HCl for Fabry Disease: *Post hoc GLA* Mutation-Based Identification of Subjects Likely to Show a Drug Effect

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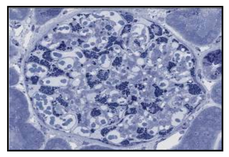
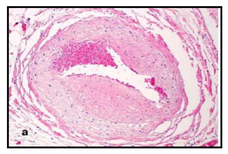
Introduction

Fabry Disease

- X-linked inborn error of metabolism
- Mutations in the *GLA* gene lead to a deficiency in α-galactosidase A (α-Gal A) activity
- More than 600 disease-causing mutations in *GLA* have been identified (~60% missense)
- Affects both males and females; females have mosaic of healthy and diseased cells
- 5,000 – 10,000 patients diagnosed worldwide (likely largely underdiagnosed)
- Globotriaosylceramide (GL-3), the natural substrate of α-Gal A, accumulates and affects multiple tissues (kidney, heart, brain, gastrointestinal system, skin)



Kidney GL-3



Coronary GL-3

Migalastat HCl for Fabry Disease:

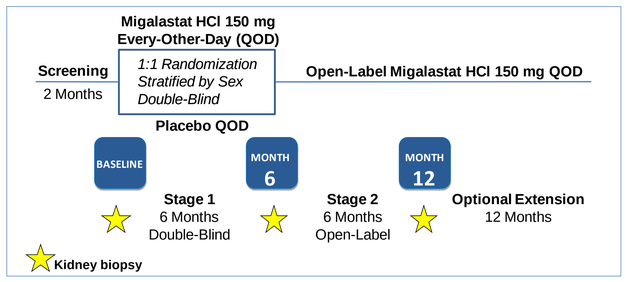
- Migalastat is an orally available investigational pharmacological chaperone
- Designed to selectively and reversibly bind and stabilize endogenous α-Gal A
- Facilitates proper folding and cellular trafficking of some mutant forms of α-Gal A to lysosomes where the breakdown of GL-3 substrate can proceed
- In development for the treatment of Fabry disease in patients who express specific mutant forms of α-Gal as identified using an *in vitro* cell-based assay

Migalastat HCl

Deoxygalactonojirimycin (DGJ)
AT1001

FACETS (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 ("amenable" mutation is explained in later sections of this poster)



- **Key Inclusion and Exclusion Criteria:**
  - Males and females diagnosed with Fabry disease
  - 16 to 74 years old
  - Amenable *GLA* mutation (during screening for FACETS, the *GLA* mutation was confirmed by gene sequencing; the 'amenable' category was determined by the "Clinical Trial HEK-293 cell-based assay" described in later sections of this poster)
  - Naïve to enzyme replacement therapy (ERT) or have 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)

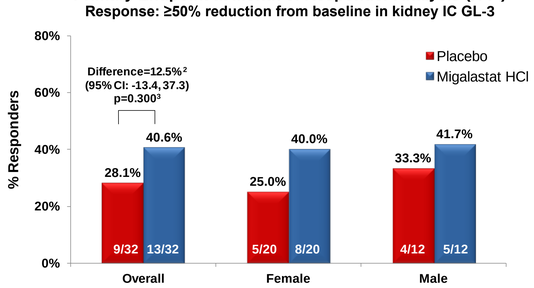
Baseline Characteristics of the Intent-to-treat (ITT) Population

	Placebo n=33	Migalastat HCl n=34	Total n=67
Sex Female n (%) Male n (%)	21 (64) 12 (36)	22 (65) 12 (35)	43 (64) 24 (36)
Age Median (range)	37 (24, 64)	46 (16, 68)	45 (16, 68)
Race White n (%)	33 (100)	32 (94)	65 (97)
Years since diagnosis Mean (SD)	7.1 (7.8)	5.7 (6.8)	6.3 (7.3)
BMI (kg/m²) Mean (SD)	25.5 (4.9)	24.9 (4.3)	25.2 (4.5)
Proteinuria >300 mg/24 h n (%)	13 (39)	9 (26)	22 (33)
eGFR <60 mL/min/1.73 m² n (%)	4 (12)	5 (15)	9 (13)
ACEI/ARB Use: n (%)	13 (39)	6 (18)	19 (28)

- A total of 67 subjects with Fabry disease (43 female and 24 male) were randomized
- During Stage 1, 33 subjects were randomized to placebo and 34 subjects were randomized to migalastat
- The baseline characteristics of the FACETS intent-to-treat population are shown

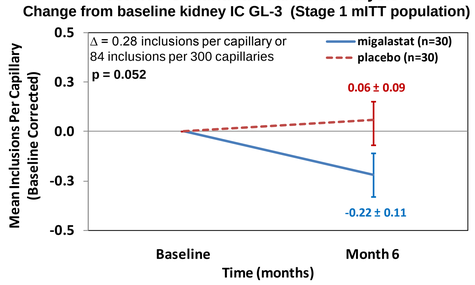
Kidney Interstitial Capillary (IC) GL-3

Primary Endpoint at Month 6: Responder Analysis (ITT[1])
Response: ≥50% reduction from baseline in kidney IC GL-3



[1]Subjects with baseline biopsy result but are missing the month 6 biopsy result are counted as failure
[2]Difference=migalastat HCl minus placebo in % responders
[3]P-value based on exact Cochran-Mantel-Haenszel test stratified by gender

***Post Hoc* Continuous Variable Analysis**
Change from baseline kidney IC GL-3 (Stage 1 mITT population)

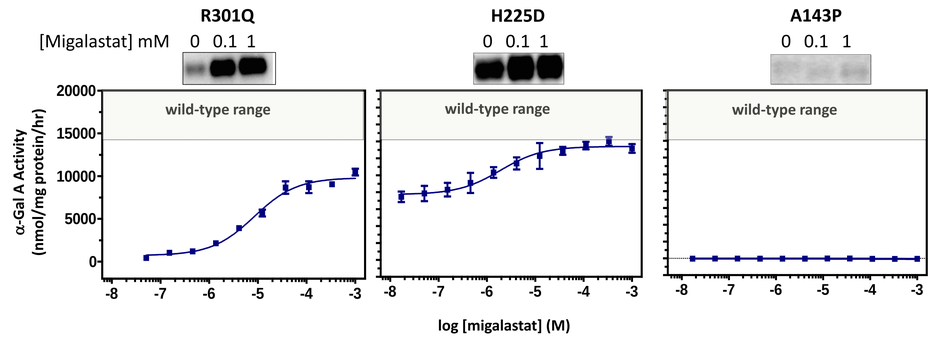


Data points represent the mean ± standard error (SEM) change from baseline in the mean number of GL-3 inclusions per capillary after 6 months of treatment with migalastat or placebo. The p-value corresponding to the least-square mean difference between migalastat and placebo is displayed.

- The primary endpoint of this study was the percent change from baseline in kidney IC GL-3 inclusions (responder analysis, 50% reduction threshold), which was not met
- A *post hoc* analysis of the change from baseline (difference) in kidney IC GL-3 inclusions was conducted using an ANCOVA model with covariate adjustment for the baseline value and treatment-by-baseline interaction applied to all subjects in the mITT population (n=30 per group)
- The analysis of the change from baseline in kidney IC GL-3 will be implemented prospectively for Stage 2

Clinical Trial HEK Cell Assay Used as Entry Criteria in FACETS

- Created cDNA constructs of 531 known disease-causing missense or small in-frame ins/del mutation. The corresponding α-Gal A mutant forms were transiently expressed in HEK-293 cells. Cells were incubated ± AT1001 (17 nM to 1 mM) for 4 to 5 days. After, α-Gal A levels were measured in cell lysates using a synthetic fluorogenic substrate (4-MU-α-Gal) or by western blot.
- 'Amenable mutation' criteria: ≥1.2-fold relative increase and ≥3.0% of wild-type (WT) absolute increase after 10 μM migalastat incubation; criteria were developed based on comparison of the mutant α-Gal A responses determined in the clinical trial HEK assay to male Phase 2 subject peripheral blood mononuclear cell α-Gal A responses after oral administration of migalastat.



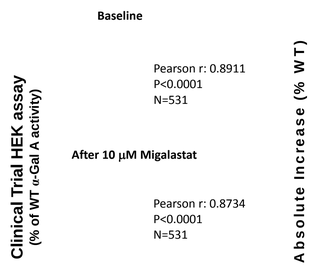
Mutant forms, such as R301Q and H225D, show maximal α-Gal A activity (nmol/mg protein/hr) after incubation with migalastat that is significantly greater than the activity at baseline (two-tailed, paired t-test; *p* value ≤ 0.05). A representative non-responsive mutant form, A143P, is also shown. Data points represent the mean ± SEM of quadruplicate determinations.

Comparison of Clinical Trial and GLP HEK-293 Cell-based Assays

- The Clinical Trial HEK-293 cell-based assay was transferred to a CRO to create a bioanalytically validated version of the assay in compliance with current regulatory guidance and relevant GLP regulations. The "GLP HEK Assay" is similar to the Clinical Trial HEK assay, but includes modifications to increase the level of quality control, rigor, precision, and consistency. Testing of 531 mutant forms was completed prior to the availability of FACETS Stage 1 data.

Clinical Trial and GLP HEK Assays: Key Differences		
	Clinical Trial HEK	**GLP HEK**
Number of Migalastat Concentrations Tested	**1** (0 and 10 μM migalastat)	**11** (0 and 11-point migalastat concentration-response; range: 17 nM to 1 mM migalastat)
Lower Limit of Detectable α-Gal A Activity	**0** (nmol/mg/hr[1])	**142** (nmol/mg/hr)
Mean Wild-type α-Gal A Activity	**In low range assay[2]: 13,489** **In high range assay[3]: 37,149** (nmol/mg/hr)	**In single range assay: 33,772** (nmol/mg/hr)
Transfection Control	**Wild-type α-Gal A activity tested in parallel**	**qRT-PCR[4] of the C-terminal *GLA* cDNA-pcDNA6 junction in every sample**

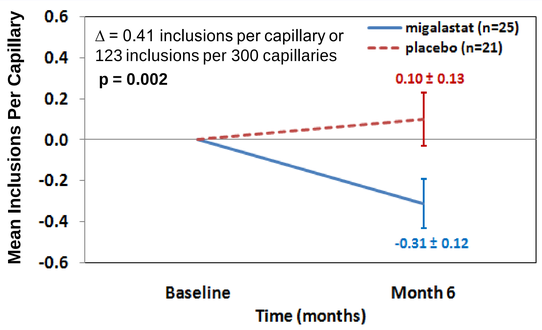
[1]nmoles of free 4-MU generated/milligram protein/hour. [2]mutant forms tested using 10 μL transfected cell lysate in the enzyme assay. [3]mutant forms with baselines of at least 10% WT α-Gal A activity that failed to meet the amenable mutation criteria were re-assayed using transfected cell lysate further diluted by 10 to 50-fold prior to enzyme assay; re-assay results were reported. [4]quantitative real-time polymerase chain reaction.



Pearson correlation coefficients [r] and two-tailed p-values are shown; values of zero were imputed for α-Gal A activity below the limit of detection

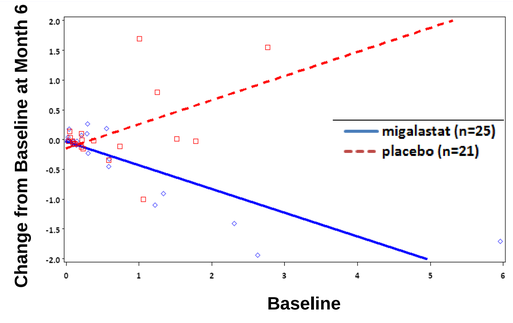
- The baseline % WT α-Gal A activity and the % WT α-Gal A activity after incubation with 10 μM migalastat were significantly correlated between the Clinical Trial and GLP HEK-293 cell-based assays.
- After applying the 'amenable mutation' criteria of ≥3.0% of WT absolute increase and ≥1.20-fold relative increase after incubation with 10 μM migalastat, comparison of the Clinical Trial HEK and GLP HEK data shows that 475 of 531 (89%) of mutations maintained the same category, but 56 of 531 (11%) mutations changed categories.
  - 31 of 232 (13%) amenable in the Clinical Trial HEK Assay changed to non-amenable in GLP HEK assay
  - 25 of 299 (8%) non-amenable in the Clinical Trial HEK Assay changed to amenable in the GLP HEK assay
- 8 of 40 (20%) mutations represented in 15 of 67 (22%) subjects randomized in the FACETS switched from "amenable" in the Clinical Trial HEK assay to "non-amenable" in the GLP HEK assay (one GLP HEK non-amenable mutation, R342Q, was represented in 8 subjects, 7 GLP HEK non-amenable mutations were uniquely represented in 7 subjects)
- Mutations in study 011 change from 'amenable' to 'non-amenable' for 15 of 67 randomized subjects
- The mutations represented in 52 of 67 subjects (78%) remain 'amenable'

Post Hoc Kidney Ic GL-3 Analysis: Change from Baseline (Stage 1 mITT, Excluding GLP HEK Non-amenable)



- ANCOVA model on change from baseline excluding 15 subjects with GLP HEK non-amenable mutations
- GLP HEK amenable subjects only showed a statistically significant (p=0.002) decrease in kidney IC GL-3 inclusions with migalastat treatment compared to placebo
- GLP HEK assay subset analyses will be implemented prospectively for Stage 2

Kidney IC GL-3: Impact of Baseline (Stage 1 mITT, Excluding GLP HEK Non-amenable)



- Change from baseline to month 6 in the mean number of GL-3 inclusions per interstitial capillary from placebo- and migalastat-treated subjects were plotted as a function of the baseline value
- The results show that many subjects had relatively low kidney IC GL-3 values at baseline
- In migalastat-treated subjects, larger decreases in kidney IC GL-3 were observed with increasingly higher baseline values

Safety in Stage 1

Adverse event	Placebo n=33	Migalastat HCl n=34
Any event	91%	91%
Headache	21%	35%
Fatigue	12%	12%
Nausea	9%	12%
Nasopharyngitis	6%	15%
Paresthesia	12%	9%

- No withdrawals due to AEs
- No deaths
- No SAEs deemed by investigators to be treatment related during Stage 1
- No subjects met mandatory stopping criteria*

*(serum creatinine ≥1.3x baseline, cardiac ejection fraction ≤0.75x baseline, cerebrovascular event with sequelae

Conclusions

- The analysis of the change from baseline in kidney IC GL-3 demonstrates a measurable drug effect during the first 6 months of treatment with migalastat
- The effect of migalastat is more pronounced in subjects with GLP HEK amenable mutations and higher baseline kidney IC GL-3
- The change from baseline in kidney IC GL-3 and GLP HEK assay subset analyses will be implemented prospectively for Stage 2
- Complete Stage 2 (month 12) and open-label extension study (month 24) data are expected in the first half of 2014

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