MIGALASTAT HCL, AN INVESTIGATIONAL PHARMACOLOGICAL CHAPERONE THERAPY: SCREENING RESULTS FROM FACETS, A PHASE 3 STUDY IN MALE AND FEMALE FABRY PATIENTS

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[2]Conflict of Interest: employees and/or shareholders of Amicus Therapeutics.

Introduction

Fabry Disease

- Fabry disease (FD) is an X-linked lysosomal storage disorder characterized by deficiency of α-galactosidase A (α-Gal A) activity
- Over 600 mutations in the *GLA* gene (encoding α-Gal A) are associated with FD
- Accumulation of globotriaosylceramide (GL-3) isoforms with progressive, multi-organ disease
- GL-3 can be measured in body fluids (plasma, urine), in homogenized tissue, or estimated on histology (stained inclusions)
- There is large variability in clinical phenotypes and clinical progression (chronic, sub-chronic, acute)
- Males and females are both affected
- Enzyme replacement therapy is available (ERT) (agalsidase beta in US; beta & alfa ex-US)
- Migalastat HCl (AT1001; GR181413A) is an investigational pharmacological chaperone for α-Gal A, increase total cellular levels and activity of some mutant forms

FACETS (AT1001-011, NCT00925301)

- A double-blind, randomized, placebo-controlled study to evaluate the efficacy, safety, and pharmacodynamics of migalastat HCl in patients with Fabry disease and migalastat HCl-Responsive *GLA* mutations
- Target randomization: 60 male or female FD patients who are naïve to ERT or have been off ERT for at least 6 months, actually randomized: **67**
- Primary efficacy: Reduction in IC GL-3 deposition evaluated with BLISS method[1] after 6 months double-blind treatment
- Key secondary measures: urinary GL-3, proteinuria, safety and tolerability

Screening Process For FACETS

- Site surveys: prior to screening, sites used available genotype information to enrich for FD patients with responsive α-Gal A mutant forms and who were more likely to have an interest in participating
- Screening, over two months, included:
  - 24 hr urinary GL-3 (> 4 fold upper limit of normal-ULN) using a GLP assay[2]
  - Confirm mutation with *GLA* gene sequencing[3]
  - In vitro response of the mutant α-Gal A to AT1001[4]

[1]Barisoni L, et al. Arch Pathol Lab Med (in press).
[2] Sitaraman S, et al. Presented at SSIEM congress. Geneva, September 2011.
[3] Cincinnati Children Hospital, OH, USA and Eurofins Medigenomix, Germany
[4] Wu X, et al. Hum Mutat 2011;32:965-77

Methods

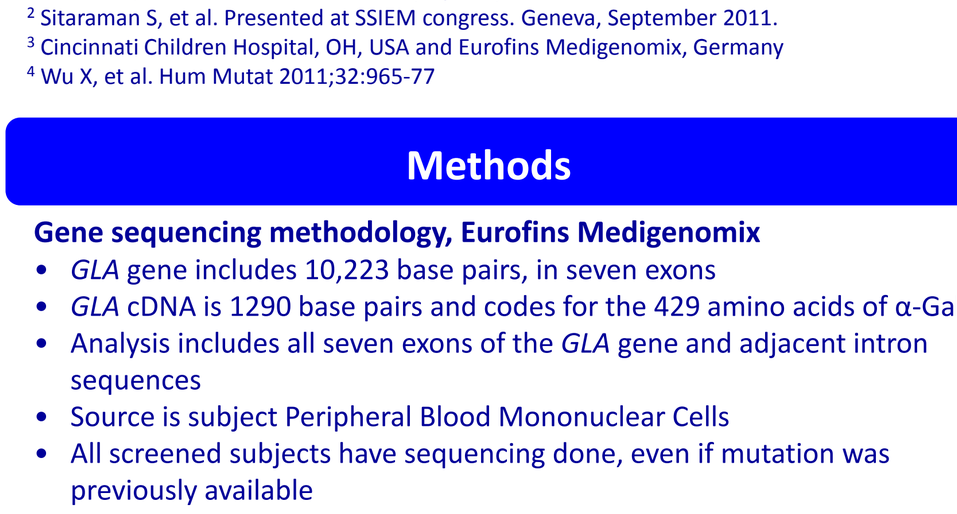
Gene sequencing methodology, Eurofins Medigenomix

- *GLA* gene includes 10,223 base pairs, in seven exons
- *GLA* cDNA is 1290 base pairs and codes for the 429 amino acids of α-Gal A
- Analysis includes all seven exons of the *GLA* gene and adjacent intron sequences
- Source is subject Peripheral Blood Mononuclear Cells
- All screened subjects have sequencing done, even if mutation was previously available

Mutant α-Gal A Responses to AT1001 *in vitro:* A Cell-based Assay

- Created cDNA constructs of every known disease-causing missense or small in-frame ins/del mutation
- α-Gal A mutant forms were transiently expressed in HEK-293 cells
- Cells were incubated ± migalastat HCl for 4-5 days (concentration range: 17 nM to 1 mM)
- α-Gal A levels were measured in cell lysates using a synthetic fluorogenic substrate (4-MU-α-Gal) and by western blot
- Both the magnitude of the increase and EC_{50} values were determined for responsive mutant forms

Representative *In Vitro* Assay Data



Responsive mutant forms, such as R301Q and H225D, show maximal α-Gal A activity (nmol/mg protein/hr) after incubation with migalastat HCl 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.

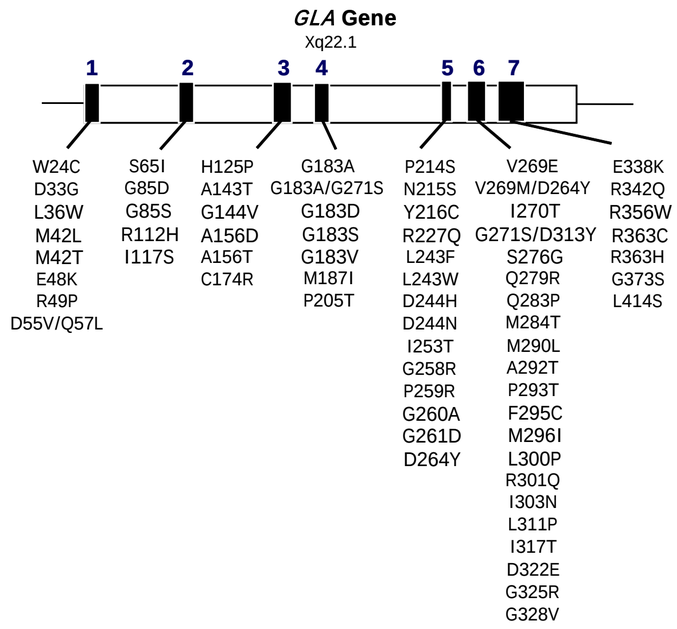
Screening Results As of December 15th, 2011

Subjects' Mutation Status

- **180** individuals with FD have been screened (**60** males and **120** females), the mean (SD) age was **41** (**14**) years
- **154/180 (86%)** subjects carried a missense mutation
  - **4 of the 154** were not genotype confirmed
- **11/180** had other types of mutations
- **10/180** had no *GLA* mutation upon sequencing confirmation
- **5/180** had no genotyping results available
- Of the **154** subjects with a missense mutation:
  - **1/154** (**0.6%**) had a mutation in the precursor peptide of α-Gal A
  - **135/154** (**87.7%**) resulted in an amino acid change in domain 1 of α-Gal A
  - **18/154** (**11.7%**) resulted in an amino acid change in domain 2
- Of the 154 subjects with a missense mutation:
  - **136/154** (**88%**) carried an amenable mutation and were eligible on this criteria
  - **18/154** (**12%**) had a mutation that was considered not amenable
- Mutations in multiple subjects: **N215S** (**N=17**), **R342Q** (**N=8**), **R112H** (**N=7**), **R301Q** (**N=7**), **H125P** (**N=5**)
- Among the 67 randomized patients, 40 different mutations are represented, of which 7 mutations were also represented in Phase 2 clinical studies

FACETS Sites and Countries

Types of Missense Mutations



- **68** unique missense mutations were confirmed
- **15** mutations were, to our knowledge, not previously described in the literature
- **1/68** (**2%**) amino acid changes was in the precursor peptide (1-31 aa) (W24C)
- **60/68** (**88%**) amino acid changes were in domain 1 (32-330 aa)
- **7/68** (**10%**) of amino acid changes were in domain 2 (331-429 aa)
- **49/68** (**72%**) were non-conservative amino acid substitutions; **19/68** (**28%**) were conservative
- **56/68** (**82%**) mutations were considered amenable and **12/68** (**18%**) were not
- The **4** most frequent mutations (R112H, N215S, R301Q and R342Q) were all considered amenable
- **1** mutation was in the active domain site (R227Q). It was not considered amenable
- More than one mutation seen at **8** amino acid positions (e.g. **4** substitutions at G183)
- **4** double mutations

Genotype-Phenotype Correlation

- Limited by the number of individuals with the same mutation
- However, the association between urinary GL-3 level and mutations present in the individual was explored
- **17** subjects with N215S: none had urinary GL-3 that was ≥ 4-fold ULN (consistent with the previously described FD 'cardiac variant' phenotype)
- **7** subjects with R301Q: 4 (1 male, 3 females) had urinary GL-3 ≥ 4-fold ULN
- **8** subjects with R342Q: all (4 males, 4 females) had urinary GL-3 ≥ 4-fold ULN (classic FD phenotype in the literature)

Conclusions

- **The majority of male and female FD subjects screened for FACETS carry missense mutations**
- **Of these subjects, 88% were considered to have amenable mutations and were potentially eligible for the FACETS phase 3 study**
- **A total of 180 patients in 16 countries signed an informed consent to participate into FACETS and were screened**
- **Of ALL subjects screened with the enriched screening process, 75% were considered to have amenable mutations and were potentially eligible for the FACETS phase 3 study**
- **New missense mutations were discovered (15/68)**
- **The most frequent missense mutations were R112H, N215S, R301Q and R342Q**
- **N215S (cardiac variant) was not associated with urinary GL-3 excretion**
- **R342Q was associated with abnormally high levels of urinary GL-3**