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-galactosidase in Europe).
- New targets for treating Fabry disease include ERT, kinase inhibitors, and pharmacological chaperones.

Methods

Screening Process For FACETS
- Site surveys: prior to screening, sites used available genotypic 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 for urinary GL-3 (> 4 fold upper limit of normal-ULN) using a GLP assay.
  - Confirmation with GLA gene sequencing
  - In vitro response of the mutant α-Gal A to AT1001.

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 with 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 EC50 values were determined for responsive mutant forms.

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.

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).
- 60/68 (88%) amino acid changes were in domain 1.
- 7/66 (10%) of amino acid changes were in domain 2.
- 49/68 (72%) were non-conservative amino acid substitutions.
- 19/68 (28%) were conservative.
- 56/68 (82%) mutations were considered amenable.
- 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.
- More than one mutation seen at a common amino acid position (e.g., 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 z 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.
- 120 patients in 13 countries were randomized to receive migalastat HCl for 4-5 days (concentration range: 17 nM to 1 mM).
- 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.