# UNITED STATES
# SECURITIES AND EXCHANGE COMMISSION
**Washington, D.C. 20549**

# FORM 8-K

**CURRENT REPORT**
**Pursuant to Section 13 or 15(d) of the Securities Exchange Act of 1934**

Date of Report (Date of earliest event reported): **March 1, 2016**

# AMICUS THERAPEUTICS, INC.
(Exact name of registrant as specified in its charter)

| **Delaware** | **001-33497** | **71-0869350** |
|---|---|---|
| (State or other Jurisdiction of Incorporation) | (Commission File Number) | (IRS Employer Identification No.) |
| **1 Cedar Brook Drive, Cranbury, NJ** | | **08512** |
| (Address of Principal Executive Offices) | | (Zip Code) |

Registrant's telephone number, including area code: **(609) 662-2000**

(Former name or former address if changed since last report.)

Check the appropriate box below if the Form 8-K filing is intended to simultaneously satisfy the filing obligation of the registrant under any of the following provisions:

o Written communications pursuant to Rule 425 under the Securities Act (17 CFR 230.425)

o Soliciting material pursuant to Rule 14a-12 under the Exchange Act (17 CFR 240.14a-12)

o Pre-commencement communications pursuant to Rule 14d-2(b) under the Exchange Act (17 CFR 240.14d-2(b))

o Pre-commencement communications pursuant to Rule 13e-4(c) under the Exchange Act (17 CFR 240.13e-4(c))

**Item 8.01. Other Events.**

On March 1, 2016, Amicus Therapeutics, Inc. (the "***Company***") issued a press release (the "***Press Release***") indicating that it will be presenting certain data and other information related to its Fabry disease and Pompe disease programs. In particular, the Company will be presenting posters entitled:

- The Validation of Pharmacogenetics in the Identification of Target Fabry Patients for Treatment with Migalastat;
- Phenotype of Fabry Disease in Patients with Mutations Amenable to Migalastat;
- Comparison of Integrated White Blood Cell α-Galactosidase A Activity Exposure Between Every-Other-Day Orally Administered Migalastat and Biweekly Infusions of Agalsidase Beta or Agalsidase Alfa;
- Persistence of Positive Renal and Cardiac Effects of Migalastat in Fabry Patients with Amenable Mutations Following 30 Months of Treatment in the ATTRACT Study;
- Co-Administration of the Pharmacological Chaperone AT2221 with A Proprietary Recombinant Human Acid α-Glucosidase Leads to Greater Plasma Exposure and Substrate Reduction Compared to Alglucosidase Alfa; and
- Six months of Migalastat Treatment Reduces Podocyte Globotriaosylceramide Content in Adult Male Patients with Fabry Disease.

The Press Release and full text of the posters described above are attached hereto as Exhibits 99.1 through 99.7 and are incorporated herein by reference.

**Item 9.01. Financial Statements and Exhibits.**

(d) Exhibits: The Exhibit Index annexed hereto is incorporated herein by reference.

| **Exhibit No.** | **Description** |
|---|---|
| 99.1 | Press Release, dated March 1, 2016. |
| 99.2 | The Validation of Pharmacogenetics in the Identification of Target Fabry Patients for Treatment with Migalastat. |
| 99.3 | Phenotype of Fabry Disease in Patients with Mutations Amenable to Migalastat. |
| 99.4 | Comparison of Integrated White Blood Cell α-Galactosidase A Activity Exposure Between Every-Other-Day Orally Administered Migalastat and Biweekly Infusions of Agalsidase Beta or Agalsidase Alfa. |
| 99.5 | Persistence of Positive Renal and Cardiac Effects of Migalastat in Fabry Patients with Amenable Mutations Following 30 Months of Treatment in the ATTRACT Study. |
| 99.6 | Co-Administration of the Pharmacological Chaperone AT2221 with A Proprietary Recombinant Human Acid α-Glucosidase Leads to Greater Plasma Exposure and Substrate Reduction Compared to Alglucosidase Alfa. |
| 99.7 | Six months of Migalastat Treatment Reduces Podocyte Globotriaosylceramide Content in Adult Male Patients with Fabry Disease. |



**SIGNATURES**

Pursuant to the requirements of the Securities Exchange Act of 1934, the registrant has duly caused this report to be signed on its behalf by the undersigned hereunto duly authorized.

AMICUS THERAPEUTICS, INC.

Date: March 3, 2016

By: /s/ ELLEN S. ROSENBERG

Name: Ellen S. Rosenberg

Title: General Counsel and Corporate Secretary



**EXHIBIT INDEX**

| **Exhibit No.** | **Description** |
|---|---|
| 99.1 | Press Release, dated March 1, 2016. |
| 99.2 | The Validation of Pharmacogenetics in the Identification of Target Fabry Patients for Treatment with Migalastat. |
| 99.3 | Phenotype of Fabry Disease in Patients with Mutations Amenable to Migalastat. |

| | |
|---|---|
| 99.4 | Comparison of Integrated White Blood Cell α-Galactosidase A Activity Exposure Between Every-Other-Day Orally Administered Migalastat and Biweekly Infusions of Agalsidase Beta or Agalsidase Alfa. |
| 99.5 | Persistence of Positive Renal and Cardiac Effects of Migalastat in Fabry Patients with Amenable Mutations Following 30 Months of Treatment in the ATTRACT Study. |
| 99.6 | Co-Administration of the Pharmacological Chaperone AT2221 with A Proprietary Recombinant Human Acid α-Glucosidase Leads to Greater Plasma Exposure and Substrate Reduction Compared to Alglucosidase Alfa. |
| 99.7 | Six months of Migalastat Treatment Reduces Podocyte Globotriaosylceramide Content in Adult Male Patients with Fabry Disease. |

**Amicus Therapeutics Highlights New Phase 3 Fabry Data and Preclinical Pompe Data at WORLD*Symposium*™ *2016***

***New Phase 3 Data for Migalastat for Fabry Disease Demonstrate Persistence of Positive Renal and Cardiac Effects and Substrate Reduction in Important Kidney Cell Type (Podocytes)***

***Preclinical Proof-of-Concept Data Informed Ongoing Clinical Study of Novel Pompe Treatment Paradigm***

**SAN DIEGO, CA and CRANBURY, NJ March 1, 2016** — Amicus Therapeutics (Nasdaq: FOLD), a biotechnology company at the forefront of therapies for rare and orphan diseases, today announced new positive data from both of its Phase 3 studies of the oral small molecule pharmacological chaperone migalastat HCl ("migalastat") for Fabry disease at WORLD*Symposium*™ *2016* in San Diego, California. The Company is also presenting additional proof-of-concept data for its novel product candidate (ATB200/AT2221) for Pompe disease.

John F. Crowley, Chairman and Chief Executive Officer of Amicus Therapeutics, Inc., stated, "We believe that the new histopathology data and longer-term renal and cardiac data at WORLD*Symposium* will further strengthen the totality of our clinical data for migalastat as a potential oral personalized medicine with a novel mechanism of action for Fabry disease. We are also pleased to highlight additional preclinical proof of concept for ATB200/AT2221, our novel product candidate for Pompe disease. Both of these programs represent significant innovations in the field of Lysosomal Storage Disorders, and have the potential to deliver meaningful benefits to patients."

**Data Highlights for Migalastat for Fabry Disease at WORLD*Symposium* 2016**

**Histopathology Data (Podocyte GL-3) from Study 011 (FACETS)**

In an oral presentation and poster(1) from Study 011 (FACETS) in Fabry patients who were naïve to ERT, migalastat demonstrated a consistent and statistically significant reduction in disease substrate (GL-3) in podocytes from baseline to Month 6 (p=0.02). Podocytes play a key role in Fabry nephropathy including proteinuria, and have shown more resistance than other kidney cell types to clear GL-3.

**Renal and Cardiac Function Data at Month 30 from Study 012 (ATTRACT)**

A late-breaking poster(2) demonstrated that the effects of migalastat on kidney function and cardiac function are persistent from the primary treatment period (0-18 months) through the open-label extension phase (19-30 months) in amenable patients who switched from ERT to migalastat in Study 012 (ATTRACT).

- **Kidney function at Month 30**: The annualized change in glomerular filtration rate (GFR) in the migalastat group at month 30 was comparable to the previously reported results for the migalastat and ERT groups through Month 18.
- **Cardiac function at Month 30**: Reductions in left ventricular mass index (LVMi) through month 18 were also demonstrated through month 30, with statistically significant reductions observed in patients who had abnormal cardiac mass (left ventricular hypertrophy, or LVH) at baseline.

| | **Mean Annualized Change in GFR (ml/min/m2/yr) (95% CI) with Migalastat Baseline to Month 30 in Study 012*** |
|---|---|
| **Estimated GFR (eGFR) (CKD-EPI) (n=31)** | -1.7 (-2.6, -0.8) |
| **Measured GFR (mGFR) (n=30)** | -2.75 (-4.8, -0.7) |

*Annualized change in GFR at Month 18 in Study 012: eGFR -1.0 (-3.6, 1.6) for patients on ERT and -0.4 (-2.3, 1.5) for patients on migalastat; mGFR -3.2 (-7.8, 1.3) for patients on ERT and -4.35 (-7.7, -1.1) for patients on migalastat

**Cardiac ECHO Parameters — Change from Baseline to Month 30**

| | **Migalastat (Overall) n=30** | **Migalastat Change (Overall) (Mean, 95% CI) n=28** | **Migalastat (LVH at Baseline) n=11** | **Migalastat Change (LVH at Baseline) (Mean, SD) N=10** |
|---|---|---|---|---|
| Left Ventricular Mass Index (LVMI) ($g/m^2$)** | 94.6 | -3.7<br>(-8.9, +1.3) | 116 | -10.0***<br>(-16.6, -3.3) |

**Normal LVMi: 43-95 (female), 49-115 (male). Change in LVMi at Month 18 in Study 012: ERT group: -2.0 (-11.0, 7.0) for all patients, +4.5 (-20.9, 29.9) for patients with LVH at baseline. Migalastat group: -6.6 (-11.0, -2.1) for all patients, -8.4 (-14.9, -2.0) for all patients with LVH at baseline.
***Statistically significant (95% CI does not overlap zero)

---

The co-primary endpoints in Study 012 assessed the comparability of migalastat to ERT on renal function as measured by eGFR and mGFR at Month 18. Cardiac function (LVMi) was a prespecified secondary endpoint in Study 012.

**Novel Treatment Paradigm (ATB200/AT2221) for Pompe Disease**

An oral presentation and poster(3) at WORLD*Symposium* describe updated preclinical results that informed the ongoing clinical study ATB200-02 in Pompe patients to investigate a novel treatment paradigm (ATB200/AT2221) that consists of ATB200, a uniquely engineered recombinant human acid alpha-glucosidase (rhGAA) enzyme with an optimized carbohydrate structure to enhance uptake, administered with a pharmacological chaperone (AT2221) to improve activity and stability.

Previously presented preclinical data showed that ATB200 was associated with increased tissue enzyme levels and reduced substrate, which was further improved when co-administered with AT2221. Updated preclinical data at WORLD*Symposium* demonstrated the efficacy of ATB200/AT2221 as a fixed-dose combination:

- AT2221 stabilizes ATB200 *in vitro*, and increases ATB200 exposures;
- Results from dose-range finding *in vivo* studies determined the optimal fixed-dose combination of ATB200/AT2221 to investigate in the ATB200-02 study; and
- The addition of AT2221 further improved glycogen reduction by ATB200 in skeletal muscles, including individual skeletal muscle fibers that are refractory to alglucosidase alfa.

**About Amicus Therapeutics**

Amicus Therapeutics (Nasdaq: FOLD) is a biotechnology company at the forefront of therapies for rare and orphan diseases. The Company has a robust pipeline of advanced therapies for a broad range of human genetic diseases. Amicus' lead programs in development include the small molecule pharmacological chaperone migalastat as a monotherapy for Fabry disease, SD-101 for Epidermolysis Bullosa (EB), as well as novel enzyme replacement therapy (ERT) products for Fabry disease, Pompe disease, and other Lysosomal Storage Disorders.

(1)B. Najafian, **WORLDSymposium 2016**, Six months of Migalastat Treatment Reduces Podocyte Globotriaosylceramide Content in Adult Male Patients with Fabry Disease
(2)D. Bichet, **WORLDSymposium 2016**, Persistence of Positive Renal and Cardiac Effects of Migalastat in Fabry Patients with Amenable Mutations Following 30 Months of Treatment in the ATTRACT Study
(3)R. Khanna, **WORLDSymposium 2016**, Co-Administration of the Pharmacological Chaperone AT2221 with a Proprietary Recombinant Human Acid Alpha-Glucosidase Leads to Greater Plasma Exposure and Substrate Reduction Compared to Alglucosidase Alfa

**Forward-Looking Statements**

This press release contains "forward-looking statements" within the meaning of the Private Securities Litigation Reform Act of 1995 relating to preclinical and clinical development of our product candidates, the timing and reporting of results from preclinical studies and clinical trials, the prospects and timing of the potential regulatory approval of our product candidates, commercialization plans, financing plans, and the projected cash position for the Company. The inclusion of forward-looking statements should not be regarded as a representation by us that any of our plans will be achieved. Any or all of the forward-looking statements in this press release may turn out to be wrong and can be affected by inaccurate assumptions we might make or by known or unknown risks and uncertainties. For example, with respect to statements regarding the goals, progress, timing, and outcomes of discussions with regulatory authorities, and in particular the potential goals, progress, timing, and results of preclinical studies and clinical trials and the expected timing of the EMA's final decision with respect to regulatory approval of migalastat in the European Union, actual results may differ materially from those set forth in this release due to the risks and uncertainties inherent in our business, including, without limitation: the potential that results of clinical or preclinical studies indicate that the product candidates are unsafe or ineffective; the potential that it may be difficult to enroll patients in our clinical trials; the potential that regulatory authorities, including the EMA, may not grant or may delay approval for our product candidates; the potential that we may not be successful in commercializing our product candidates if and when approved; the potential that preclinical and clinical studies could be delayed because we identify serious side effects or other safety issues; and the potential that we will need additional funding to complete all of our studies. Further, the results of earlier preclinical studies and/or clinical trials may not be predictive of future results. With respect to statements regarding projections of the Company's cash position, actual results may differ based on market factors and the Company's ability to execute its operational and budget plans. In addition, all forward-looking statements are subject to other risks detailed in our Annual Report on Form 10-K for the year ended December 31, 2015. You are cautioned not to place undue reliance on these forward-looking statements, which speak only as of the date hereof. All forward-looking statements are qualified in their entirety by this cautionary statement, and we undertake no obligation to revise or update this news release to reflect events or circumstances after the date hereof.

---

CONTACTS:

**Investors/Media:**
Amicus Therapeutics
Sara Pellegrino

Senior Director, Investor Relations
spellegrino@amicusrx.com
(609) 662-5044

**Media:**
Pure Communications
Dan Budwick
dan@purecommunicationsinc.com
(973) 271-6085

FOLD–G

# The Validation of Pharmacogenetics in the Identification of Target Fabry Patients fo Treatment with Migalastat

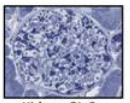
Benjamin ER[1], Della Valle C[1], Wu X[1], Katz E[1], Valenzano KJ[1], Bichet DG[2], Germain DP[3], Giugliani R[4], Hughes DA[5], Schiffmann R[6], Wilcox WR[7], Yu J[1], Ki Barth J[1], Castelli J[1]

[1]*Amicus Therapeutics, Cranbury, NJ, USA;* [2]*Hôpital du Sacré-Coeur, Montréal, Quebéc, H4J1C5, Canada;* [3]*Division of Medical Genetics, University of Versailles, University Paris-Saclay, Montigny, France;* [4]*Medical Genetics Service, HCPA/UFRGS Por Brazil;* [5]*Royal Free Campus, Univ College London, London, UK;* [6]*Baylor Research Institute, Dallas, TX;* [7]*Dept of Human Genetics, Emory Univ, Atlanta, GA, USA*

## 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 Aurey-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 (Migalastat Amenability 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 Migalastat Amenability 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

**Migalastat Amenability Assay (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 Migalastat Amenability 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, 600 FD mutations have been tested; 268 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

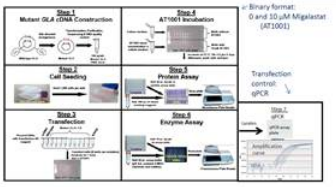
**Data From Phase 3 Study AT1001-011 (NCT00925301):**

- 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

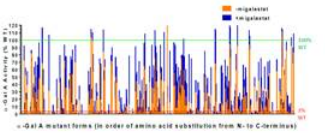
**Data From Phase 3 Study AT1001-012 (NCT01218659):**

- 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

## Migalastat Amenability 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




- The assay data show that 600 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

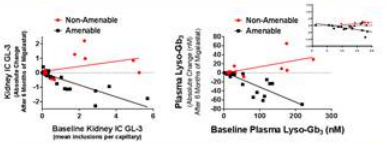
- The mutant α-Gal A responses to migalastat in the Migalastat Amenability Assay and in white blood cells (WBCs) of male Fabry patients orally administered migalastat in clinical studies were compared
- The degree of consistency was evaluated by calculating the sensitivity, specificity, positive predictive 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 |
| All male patients (150 mg QOD) | 1.0 | 0.875 | 0.946 | 1.0 | 51 |

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

- A high degree of consistency between the Migalastat Amenability 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.

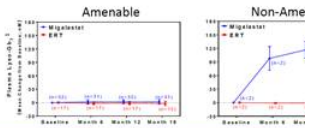
- Male and female kidney interstitial capillary GL-3 (IC GL-3) and plasma lyso-$Gb_3$ absolute changes after six months of treatment were grouped by *GLA* mutation category
- Patients with amenable mutations showed consistent decreases in these substrate levels; larger decreases were observed with increasingly higher baseline values
- In patients with non-amenable mutations, no consistent reductions in lyso-$Gb_3$ were observed

| 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 Migalastat Amenability Assay results to patient substrate responses to migalastat showed high consistency

## Substrate Responses in Study 0

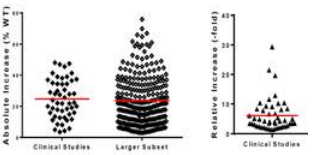



Patients with amenable *GLA* mutations in the Migalastat Amenability Assay. Baseline corrected. Blue dotted line represents a are SD; Least Squares (LS) Mean at Month 18 showed results comparable to the mean (data not shown); Based on subjects w

- In patients with amenable mutations, the plasma lyso-$Gb_3$ levels were c ERT, in both males and females
- In two male subjects with non-amenable mutations, plasma lyso-$Gb_3$ inc ERT as compared to two (1M, 1F) who remained on ERT

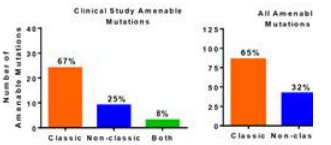
## Phase 2/3 Amenable Mutations Comp

- In total, 51 different amenable mutations were identified in 126 subjects f studies
- This represents 19% of all amenable mutations to date




- This set of amenable mutant forms of α-Gal A (n=51) represented in clin to the larger FD-associated subset that met the amenable mutation crit to migalastat were not significantly different
- The results suggest that the amenable mutant forms evaluated in Phase representative of the larger subset of amenable mutant forms

## Amenable Mutations Grouped by Ph




Dotted line indicates the total number of amenable mutations with phenotype classification; percentage amenable in each phenotype category; mutant forms with unknown phenotype were excluded

- A database of ~800 FD-associated *GLA* mutations was compiled based o
  - Includes all known types of mutations (i.e., missense, small insertions and deletio carboxyl-terminal nonsense mutations, complex mutations, large deletions or inse mutations, splice site mutations)
  - Includes information on whether that mutation has been associated with the class phenotype in the literature
- The results show that a majority , ~65%, of all amenable mutations as w migalastat clinical studies are associated with classic FD

## Conclusions

- The results indicate that the Migalastat Amenability Assay and the amen high predictive value in identifying FD patients who show a pharmacodyr administration of migalastat based on assessment of α-Gal A in WBCs, ki 3 deposition, and plasma lyso-$Gb_3$ concentrations
- The results indicate that the amenable mutations evaluated in the migal studies are representative of the larger subset of amenable mutations
- These results support the clinical validation of the Migalastat Amenabilit identifying the target population for treatment with migalastat: patients mutations
- Approximately 30-50% of patients with FD are estimated to have amenat of amenable mutations are associated with the classic phenotype of the
- As new *GLA* mutations are identified, they can readily be tested in the M to determine amenability to treatment with migalastat

# Phenotype of Fabry Disease in Patients with Mutations Amenable to Migalastat

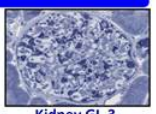
Hughes, D[1], Bichet, DG[2], Germain DP[3], Giugliani R[4], Schiffmann R[5], Wilcox W[6], Castelli J[7], Benjamin E[7], Skuban N[7], and Barth J[7]

[1] University College London, London, UK; [2]Hôpital du Sacré-Coeur, University of Montreal, Canada; [3]Hôpital Raymond Poincaré (AP-HP), University of Versailles – St. Quentin en Yvelines (UVSQ), Garches, France; [4]Medical Genetics Service, HCPA/UFRGS Brazil; [5]Royal Free Campus, University College London, London, UK; [6]Baylor Research Institute, Dallas, TX; [7]Department of Human Genetics, Emory University, Georgia; [8]Amicus Therapeutics, 1 Cedar Brook Drive, Cranbury, NJ, USA

## Introduction

### Fabry Disease

- A devastating X-linked inherited disorder caused by the functional deficiency of lysosomal α-galactosidase A (α-Gal A), with accumulation of glycosphingolipids, including globotriaosylceramide (GL-3), leading to impairment of kidney, heart, brain, and premature death.
- More than 800 disease-causing mutations in *GLA* have been identified (~60% missense).
- Affects males and females; females have mosaic of healthy and diseased cells.

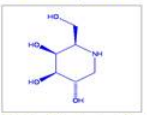


Kidney GL-3

Coronary GL-3

### Migalastat for Fabry Disease

- Binds to α-Gal A, increasing its physical stability, lysosomal trafficking, and cellular activity.
- First-in-class orally administered (QOD) pharmacological chaperone being developed as a targeted medicine for the treatment of Fabry disease in patients with amenable *GLA* mutations.
- Between 30-50% of people with Fabry disease express mutant forms of α-Gal A that are *amenable* to migalastat, based on an *in vitro* GLP-validated Migalastat Amenability Assay.



AT1001; Migalastat HCl; Deoxygalactonojirimycin

## DESIGN of AT1001-011 (FACETS, NCT00925301)

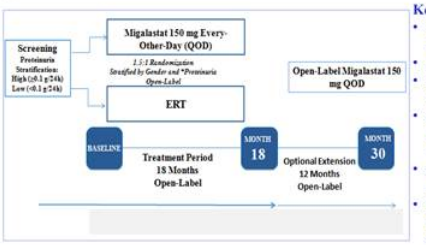
**Study AT1001-011: A Double-Blind, Randomized, Placebo-Controlled Study to Evaluate the Efficacy, Safety and Pharmacodynamics of Migalastat HCl in Patients With Fabry Disease and Amenable *GLA* Mutations**


**Key Inclusion/Exclusion Criteria:**

- Males and females, 16 to 74 years, diagnosed with Fabry disease.
- Amenable *GLA* mutation.
- Naïve to ERT or have not received ERT for ≥6 months before screening.
- eGFR (MDRD) at screening ≥30 ml/min/1.73 m$^2$.
- Urine GL-3 at screening ≥4 times the upper limit of normal (24-hour collection).
- Subjects taking ACE inhibitors, ARBs, or renin inhibitors on a stable dose for at ≥4 weeks before screening.

## DESIGN of AT1001-012 (ATTRACT, NCT01218659)

**Study AT1001-012: A Randomized, Open-Label Study To Compare The Efficacy and Safety Of Migalastat and Enzyme Replacement Therapy (ERT) in Patients With Fabry Disease and Migalastat-Responsive *GLA* Mutations, Who Were Previously Treated With ERT**




**Key Inclusion/Exclusion Criteria:**

- Males and females, 16 to 74 years, diagnosed with Fabry disease.
- Amenable *GLA* mutation
- Initiated treatment with ERT at least 12 months prior to baseline visit.
- Stable ERT dose for 3 months prior to baseline visit and ≥80% of labeled dose.
- eGFR (MDRD) at screening ≥30 ml/min/1.73 m$^2$.
- Subjects taking ACE inhibitors, ARBs, or renin inhibitors on a stable dose for ≥4 weeks before screening.

## Methods

TESTING OF GENOTYPES FOR AMENABILITY:

- 600 Fabry disease-causing mutations were expressed in transfected HEK-293 cells and α-Gal A activity was measured in the presence and absence of 10 μM migalastat.
- Amenable mutant forms were defined by a ≥1.20-fold relative increase and a ≥3.0% wild-type absolute increase in the presence of 10 μM migalastat.
- 268 amenable mutations were identified.

PHENOTYPE :

- Proportions of patients enrolled in Studies 011 and 012 with disease-related involvement of ≥2 organ systems were determined.
- Patient's phenotypes (classical/non-classical) were assessed based on the medical literature definition of genotypes. The classical Fabry phenotype has been used to described patients with early onset, low residual α-Gal A activity (in male patients), elevated plasma lyso-Gb$_3$, and multiple organ-system disease.

## Clinical Phenotypes

- Among mutations characterized in the literature, a majority (64%) of patients in the Phase 3 studies had mutations associated with the classical phenotype.

### Study 011: Amenable mutations of patients and the corresponding clinical phenotype, based on the medical literature

| Amino Acid Change (number of patients with the mutation) | Literature Phenotype | Amino Acid Change | Literature Phenotype |
|---|---|---|---|
| D33G | Unknown | P259R (n=3) | Classical (Ashley, Shabbeer et al. 2001) |
| L36W (n=2) | Unknown | G260A | Classical (Okumiya, Ishii et al. 1995) |
| D55V/Q57L | Unknown | D264Y | Classical (Shabbeer, Yasuda et al. 2006) |
| G85D | Unknown | I270T | Classical (Rias, Gupta et al. 2005) |
| R112H | Non-classical (Eng 1994) | G271S<br>D313Y | Classical (Shabbeer, Yasuda et al. 2006) |
| G144V | Classical (Eng 1994) | | Both (Eng 1993, Froissart, Giffon et al. 2003) |
| A156T (n=3) | Classical (Eng 1994) | M284T (n=2) | Classical (Blanch, Meaney et al. 1996) |
| C174R | Classical (Meng, Zhang et al. 2008) | P293T (n=2) | Classical (Shabbeer, Yasuda et al. 2006) |
| GH83D (n=2) | Classical (Topaloglu, Ashley et al. 1999) | F295C | Unknown |
| M187I | Unknown | L300P | Unknown |
| P205T (n=2) | Classical (Blanch, Meaney et al. 1996) | R301Q (n=3) | Both (Sakuraba, Oshima et al. 1990, Ishii 1992, Germain and Poenaru 1999, Germain, Shabbeer et al. 2002) |
| Y216C (n=3) | Classical (Filoni, Caciotti et al. 2010) | I317T | Classical (Shabbeer, Yasuda et al. 2002) |
| L243F | Classical (Germain, Shabbeer et al. 2002) | D322E (n=2) | Classical (Lee, Heo et al. 2010) |
| D244N | Classical (Eng 1994) | G325R (n=2) | Unknown |
| G258R (n=2) | Unknown | R356W | Classical (Bernstein 1989) |
| I253T (n=4) | Unknown | G373S | Classical (Okumiya, Ishii et al. 1995) |

Abbreviations: ITT=modified intent-to-treat,[*]A female patient had 2 mutations on different chromosomes; categorised as classical based on G271S. | Number of patients with each mutation is 1 unless indicated otherwise

### Study 012: Amenable mutations of patients and the corresponding clinical phenotype, based on the medical literature

| Amino Acid Change (number of patients with the mutation) | Literature Phenotype | Amino Acid Change | Literature Phenotype |
|---|---|---|---|
| M96I | Unknown | G260A | Classical (Okumiya, Ishii et al. 1995) |
| L32P (n=3) | Unknown | Q279E | Non-classical (Ishii 1992) |
| G35R | Non-classical (Davies, Christomanou et al. 1994) | M284T | Classical (Blanch, Meaney et al. 1996) |
| D55V/Q57L | Unknown | M296I | Non-classical (Nakao 1995) |
| G85D (n=4) | Unknown | R301P (n=3) | Classical (Ashley, Shabbeer et al. 2001) |
| A97V | Non-classical (Eng 1997) | R301Q | Both (Sakuraba, Oshima et al. 1990, Ishii 1992, Germain and Poenaru 1999, Germain, Shabbeer et al. 2002) |
| R112G | Unknown | G328A | Classical (Eng 1993) |
| R112H | Non-classical (Eng, Niehaus et al. 1994) | Q312R | Non-classical (Shimotori, Maruyama et al. 2008) |
| A143T (n=3) | Non-classical (Spada, Pagliardini et al. 2006) | D322E (n=4) | Classical (Lee, Heo et al. 2010) |
| A156T (n=6) | Classical (Eng 1994) | R356Q | Non-classical (Chien, Olivova et al. 2011) |
| P205T | Classical (Blanch, Weber et al. 1997) | R363H | Both (Blaydon, Hill et al. 2001, Shabbeer, Yasuda et al. 2002) |
| N215S (n=10) | Non-classical (Dobrovolny, Dvorakova et al. 2005) | L403S | Classical (Shimotori, Maruyama et al. 2008) |
| Y216C | Classical (Filoni, Caciotti et al. 2010) | P409T | Unknown |
| I253S | Unknown | | |

Abbreviations: mITT =modified intent-to-treat

## Patients had Low α-Gal A Activity and Elevated Plasma Lyso-Gb$_3$ Levels

- Low residual α-Gal A activity in male patients and elevated levels of plasma lyso-Gb$_3$ in males and females have been associated with the classical Fabry phenotype (Desnick, Brady et al., 2003; Wilcox, Oliveira et al., 2008; Rombach, Dekker et al., 2010).
- In Study 011, >90% of patients had plasma lyso-Gb$_3$ levels comparable to patients with a classical phenotype (Rombach, Dekker et al., 2010); 91% of males had plasma lyso-Gb$_3$ >51 nM; 94% of females had plasma lyso-Gb$_3$ >1.19 nM.
- 44% of males had baseline α-Gal A Activity <1% of normal, and 87% had baseline activity <3% of normal.
- (Due to previous ERT treatment in patients entering Study 012, enzyme activity and plasma lyso-Gb$_3$ levels were confounded.)

## Patients Had Significant Baseline Dis

| Sex | Fabry Disease in ≥2 Organ Systems | Angio-keratoma or Corneal Whorling | Cardiac Involvement | CNS Involvement | Neuropat Pain |
|---|---|---|---|---|---|
| **Study AT1001-012 (n=57)** | | | | | |
| Males n (%) | 21/24 (88%) | 13/24 (54%) | 16/24 (67%) | 18/24 (75%) | 14/24 (58%) |
| Females n (%) | 29/33 (88%) | 16/33 (48%) | 25/33 (75%) | 12/33 (36%) | 22/33 (67%) |
| **Study AT1001-011 (n=50)** | | | | | |
| Males n (%) | 18/18 (100%) | 12/18 (67%) | 15/18 (83%) | 11/18 (61%) | 13/18 (72%) |
| Females n (%) | 29/32 (91%) | 13/32 (41%) | 11/32 (35%) | 16/32 (50%) | 25/32 (78%) |

Abbreviations: CNS = Central Nervous System; eGFR = estimated glomerular filt hypertrophy; LVMi = left ventricular mass index; TIA = transient ischaemic attac Corneal Whorling based on medical history finding. Cardiac Involvement include medical history), LVH, or conduction abnormality (eg, tachycardia, ST-T segmen history finding or baseline assessment of LVMi. CNS involvement was based on (stroke/TIA, tinnitus/hearing loss). Renal Involvement based on medical history fi mL/min/1.73m$^2$, 24-hr Protein ≥150 mg

- Overall, 91% of patients had Fabry disease involvemer indicating significant disease burden.
- In Study 011, all patients had clinical manifestations, a renal involvement, 52% had cardiac involvement, and involvement.
- In Study 012, all patients had clinical disease manifest patients had renal involvement, 72% had cardiac invol CNS involvement.

## Patients Enrolled in the Migalastat Phase Comparable With Fabry Disease Patients Cu ERT

**Baseline Characteristics in Phase 3 Migalastat Studies Ve**

| | Male Patients | | | | |
|---|---|---|---|---|---|
| | FOS | FR | 011 | 012 | FOS |
| Age at enrolment | 39 | 40 | 40 | 47/44[1)] | 44 |
| Body System involvement (%) | | | | | |
| Dermatologic | 78 | 31 | 67 | 38 | 50 |
| Cardiac | 69 | 13 | 83 | 67 | 65 |
| CNS | 69[2)] | 17[3)] | 61 | 75 | 74[3)] |
| Neuroparesthesias | 76 | 62 | 72 | 58 | 64 |
| Renal | 50 | 17 | 100 | 75 | 50 |
| Gastrointestinal | 55 | 19 | 56 | 58 | 50 |

Abbreviations: CNS=Central Nervous System; FOS=Fabry Outcomes Survey; F ischaemic attack | [1)] Second number reflects age at start of ERT which is more re enrolment into ERT registry; [2)] Combines auditory and TIA/Stroke; [3)] Combines other [Fabry Outcomes Survey (Mehta, Beck et al. 2009); Fabry Registry (Eng, F

## Summary and Conclusio

- The very high proportion of patients with multi-organ s the Phase 3 studies of migalastat (Studies 011 and 012) plasma lyso-Gb$_3$ and low α-Gal A activity in patients n indicate substantial disease burden in this population.
- A majority of patients in the Phase 3 migalastat studies associated with the classical phenotype.
- Patients enrolled in the migalastat Phase 3 studies are c current Fabry disease population being treated with ER Fabry Outcome Survey (Mehta, Beck et al., 2009) and Fletcher et al., 2007; 2014).

## Acknowledgments


- Patients and their families
- Hernan Amartino
- Daniel Bichet
- Drago Bratkovic
- David Finegold
- Gerard Vockley
- Dominique Germain
- Pilar Giraldo
- Majed Dasouki
- Patrick Deegan
- David Dimmock
- Ozlem Goker-Alpan
- Roberto Giugliani
- Eric Hachulla
- Derralynn Hughes
- Charles Jeanette
- Robert Colvin
- Usama Sharaf El Din
- Maryam Banikazemi
- Fatih Ezgu
- Francois Eyskens
- Ulla Feldt-Rasmussen
- Claudio Feliciani
- Ana Jovanovic
- David Koeller
- Nicola Longo
- Robin Lachmann
- Charles Lourenco
- Joel Charrow

# Comparison of Integrated White Blood Cell α-Galactosidase A Activity Exposure Between Every-Other-Day Orally Administered Migalastat and Biweekly Infusions of Agalsidase Beta or Agalsidase Alfa

*F. K. Johnson[1], K. J. Valenzano[1], and J. Castelli[1]*
[1]*Amicus Therapeutics, Cranbury, NJ USA*

## Introduction

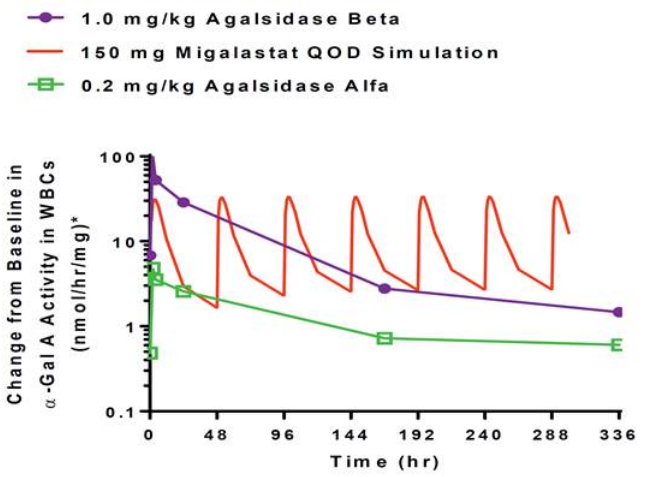
Fabry disease is an x-linked α-galactosidase (α-Gal A) deficiency. It involves progressive globotriaosylcerimide (GL-3) accumulation, which affects multiple organs and organ systems including the kidney and heart. Currently approved treatments include once-every-other-week infusions with enzyme replacement therapies 1 mg/kg agalsidase beta or 0.2 mg/kg agalsidase alfa. Misfolded or unstable α-Gal A is degraded in the endoplasmic reticulum. Migalastat HCl is a low molecular weight iminosugar and is an analogue of the terminal galactose of GL-3 that binds to the active site of α-Gal A. Pre-clinical *in vitro* and *in vivo* studies have demonstrated that migalastat acts as a pharmacological chaperone for α-Gal A, selectively and reversibly binding, with high affinity, to the active site of both wild-type and specific mutant forms of α-Gal A, the genotypes of which are referred to as amenable mutations.[a] In *in vitro* and *in vivo* models bound migalastat stabilizes α-Gal A, slowing its denaturation at neutral pH and body temperature.[b] Migalastat binding stabilizes these mutant forms of α-Gal A in the endoplasmic reticulum facilitating their proper trafficking to lysosomes where dissociation of migalastat allows α-Gal A to reduce GL-3 storage material. In contrast, misfolded and/or unstable α-Gal A is recognized by the endoplasmic reticulum quality control system as aberrant and targeted for degradation, never reaching the lysosome.[c] The PK of migalastat has been well-characterized. Migalastat is dose proportional from 50 to 1250 mg, well absorbed in 3 hours, and has a terminal half-life of approximately 4 hours.[d]

## Data Analysis Methods

The studies included in this data analysis were two Phase 3 studies, AT1001-011 and AT1001-012, and a Phase 2a study, AT1001-0 randomized double-blind, placebo-controlled study, and AT1001-012, a randomized, open-label, comparator study with ERT and migalasta were conducted in Fabry patients with amenable mutations, patients were dosed with migalastat every-other-day, and α-Gal A activity w periodically for up to 24 months. AT1001-013 was an open-label, single dose study in a fixed sequence with ERT alone first, and then co- 150 mg or 450 mg migalastat in male Fabry patients with any mutation. An additional arm with 150 mg migalastat alone was used to cha in Fabry patients, the outcome of which was used in the current analyses. α-Gal A activity in WBCs was measured pre-infusion, and a hours post-start of infusion of agalsidase. The bioanalytical method for determination of α-Gal A activity in WBCs was a fluorescence ass rate of turn-over of an artificial substrate, 4-MUG to 4-MU. Circulating WBCs were selected as an example of a surrogate for tissue up sampling, as well as excellent exposure to both agalsidase and migalastat. Importantly, migalastat-mediated changes in WBC α-Gal A lev similar changes in skin and kidney α-Gal A activity levels.[e] The data analysis methods were comprised of modeling and simulations to p α-Gal A activity following oral administration of 150 mg migalastat every-other-day for 7 doses, 14 days total, and noncompartmental ana WBC α-Gal A activity exposure following single infusions of 1 or 0.2 mg/kg agalsidase beta or alfa, respectively.

## Migalastat administration results in more consistent levels of WBC α-Gal A Activity

### Figure 1. WBC α-Gal A Activity Following 150 mg Migalastat QOD X 7 Doses vs. Single-dose 1 mg/kg Agalsidase beta or 0.2 mg/kg Agalsidase alfa



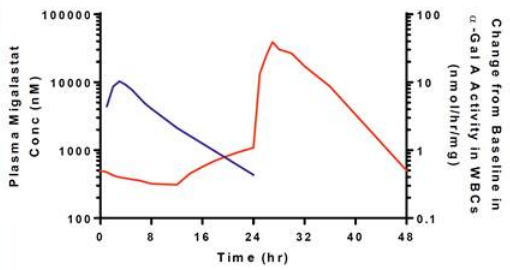

### Table 1. WBC α-Gal A Activity PK Summary

| Treatment (N) | AUC [hr*(nmol/hr/mg)] | $C_{max}$ (nmol/hr/mg) |
|---|---|---|
| 150 mg migalastat HCl (79) | 2969 | 39.9 |
| 1.0 mg/kg agalsidase β (9) | 3091 | 105.9 |
| 0.2 mg/kg agalsidase α (8) | 485 | 4.83 |

As shown in Figure 1, every-other-day dosing with migalastat suggests more consisten α-Gal A activity compared to a 14-day ERT dosing interval. As shown in Table 1, simula migalastat AUC for WBC α-Gal A activity is comparable to that seen following a single mg/kg agalsidase beta and is approximately 6-fold greater than that seen following a 0. agalsidase alfa. Single doses of agalsidase beta resulted in higher $C_{max}$ values (mean rapidly declining α-Gal A activity. The simulated AUC for migalastat represents an atten with more consistent levels of activity as a result of every-other-day dosing over the sa interval. All baseline endogenous levels of activity were subtracted from each time poin estimation of PK parameters. The exposure values presented in the abstract were not corrected.

## Proposed Relationship of Plasma Migalastat to WBC α-Gal A Activity

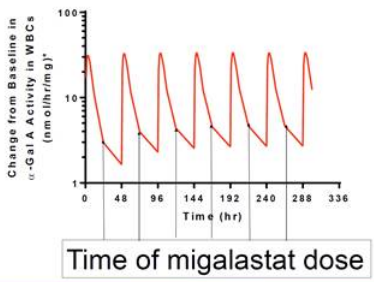
### Figure 2. Plasma Migalastat and WBC α-Gal A Activity vs. Time (14 Days)




Before simulating α-Gal A activity over a 14-day interval, a hypothetical single-dose model was established. Data from 79 Fabry patients were pooled from the 011 and 012 Phase 3 studies. The time of blood sampling for α-Gal A activity determinations were not recorded in either Phase 3 study. Therefore, a hypothetical activity-time curve was constructed using actual WBC α-Gal A activity data from Phase 3 studies in Fabry patients. Each value was baseline-corrected and were used to create the red line plotted on the right Y-axis. The shape of this curve was based upon the pharmacokinetic characterization of α Gal A activity in plasma and WBCs, and the duration of inhibition by migalastat based on *in vitro* data and animal models. The following assumptions were made for assigning time points to WBC activity values: duration of inhibition by migalastat from Time 0 to approximately 24 hours post-dose, a constant or zero-order rate of increase in α-Gal A activity with maximum activity attained by roughly 3 hours, and a biphasic elimination rate consistent with the characterization of α-Gal A activity in plasma and WBCs. Migalastat in plasma from the 013 study in Fabry patients is represented as the blue line plotted on the left Y-axis.

## Multiple-dose Simulation

### Figure 3. WBC α-Gal A Activity Simulation Following 7 Doses with 150 mg Migala




The next step was to fit a 2-compartment mode activity-time curve to obtain volume of distributi rate constants for a multiple-dose simulation. M dose simulation was performed for a total of 14 7 every-other-day oral administrations of 150 m migalastat. An overall AUC was estimated by a noncompartmental analysis on the 14-day mult simulation. The time of migalastat dose adminis indicated by the arrows and followed by an inhi phase lasting approximately 24 hours, and an phase lasting another 24 hours.

## Conclusions Based on Modeling and Simulation and Limitations of the Analysis

**Conclusions:**

- **Following Q14d single-dose infusions with agalsidase beta or alfa to Fabry patients, or 7 QOD oral administrations of 150 mg migalastat HCl to Fabry patients with amenable mutations over 14 days, α-Gal A in WBCs were:**
  - Comparable between agalsidase beta and migalastat, but were 6-fold greater for migalastat compared to agalsidase alfa.
  - More consistent following QOD administration of migalastat, which provided lower $C_{max}$ values and higher $C_{trough}$ values than single infusions of agalsidase which ultimately suggests more consistent cellular α-Gal A activity levels.

**Limitations:**

- **Time of sampling relative to dosing for WBC activity data from Phase 3 studies AT1001-011 and -012 was not recorded**
  - Therefore, a hypothetical activity level-time curve was constructed from actual data
  - Selected activity levels from the combined 011 and 12 data sets were assigned to fit an assumed constant and rapid rate of increase and biphasic elimination rate based upon the characterization of α-Gal A in plasma
- **WBCs are not a disease-relevant tissue for Fabry**
  - However, circulating WBCs were selected as an example of tissue uptake because of ease of sampling, ample exposure to both α-Gal A ERT and migalastat, and association with similar migalastat-mediated changes in α-Gal A activity levels that were observed in skin and kidney tissue
  - WBC α-Gal A activity may be overestimated following agalsidase administration to IgG positive patients who have greater uptake of α-Gal A into WBCs[f]
- **The hypothetical model and simulation presented here represents a mosaic of different amenable mutant forms**
  - Therefore, some individuals may have greater or lesser responses

**Refer**
[a]Ishii, Chang, et al. J Pharmac
[b]Benjamin, Khana, et al. Mol T
[c]Yam, Zuber, et al. Am J Physi
[d]Johnson, Mudd, et al. Clin Ph
[e]Germain, Giugliani, et al. Orp
[f]Linthorst, Hollak, et al. Kidney

Presented at The World Symposium for Lysosomal Disorders Annual Meetir

# Persistence of Positive Renal and Cardiac Effects of Migalastat in Fabry Patients with Amenable Mutations Following 30 Months of Treatment in the ATTRACT Stu

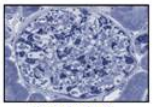
Bichet DG[1], Germain DP[2], Giugliani R[3], Hughes D[4], Schiffmann R[5], Wilcox W[6], Castelli J[7], Cantor E[7], Kirk J[7], Skuban N[7], and Barth J[7] on behalf of the ATTRACT investigators

[1]Hôpital du Sacré-Coeur, University of Montreal, Canada; [2]Hôpital Raymond Poincaré (AP-HP), University of Versailles – St. Quentin en Yvelines (UVSQ), Garches, France; [3]Medical Genetics Service, HCPA/UFRGS Porto Alegre, Brazil; [4]Royal Free Campus, College London, London, UK; [5]Baylor Research Institute, Dallas, TX; [6]Department of Human Genetics, Emory University, Georgia; [7]Amicus Therapeutics, 1 Cedar Brook Drive, Cranbury, NJ, USA

## Introduction

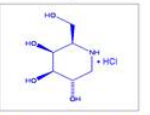
### Fabry Disease

- A devastating X-linked inherited disorder caused by the functional deficiency of lysosomal α-galactosidase A, with accumulation of glycosphingolipids, including globotriaosylceramide (GL-3), leading to impairment of kidney, heart, brain, and premature death.
- More than 800 disease-causing mutations in *GLA* have been identified (~60% missense).
- Affects males and females; females have mosaic of healthy and diseased cells.
- The stabilization or slowing of renal dysfunction and reduction of cardiac complications remain critical medical needs for individuals living with Fabry disease.



Kidney GL-3

Coronary GL-3



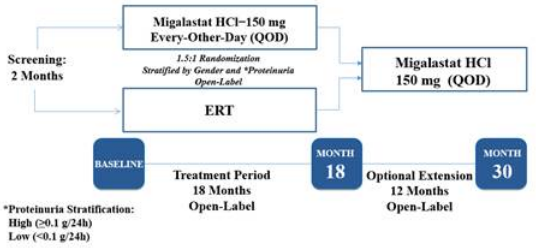
AT1001; Migalastat HCl; Deoxygalactonojirimycin

### Migalastat for Fabry Disease

- First-in-class orally administered (QOD) pharmacological chaperone being developed as a targeted medicine for the treatment of Fabry disease in patients with amenable *GLA* mutations.
- Between 30-50% of people with Fabry disease express mutant forms of α-galactosidase A that are *amenable* to migalastat, based on an *in vitro* GLP-validated Migalastat Amenability Assay.
- In patients with Fabry disease, migalastat binds and stabilizes the amenable mutant forms of the enzyme in the endoplasmic reticulum throughout the body and restores trafficking to lysosomes where the enzyme can catabolize accumulated glycosphingolipids.
- As an oral small molecule treatment, migalastat therapy is unlikely to exhibit the limitations of ERT, which include infusion-associated reactions, formation of antibodies to the exogenous protein, and the significant burden that biweekly infusions place on patients and their families.

## DESIGN of AT1001-012 (ATTRACT, NCT01218659)

**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**




Randomized patients were 16-74 years of age and had:

- A genetically confirmed diagnosis of Fabry disease.
- Initiated ERT ≥12 months before the baseline visit and a stable dose (>80% of the labeled does) for 3 months prior to the baseline visit.
- A responsive *GLA* mutation based on a preliminary cell-based assay.
- Estimated glomerular filtration rate ($eGFR_{MDRD}$) ≥30ml/min/1.73m$^2$.
- Patients taking angiotensin converting enzyme inhibitors, angiotensin receptor blockers had to be on a stable dose for ≥4-weeks before the screening visit.

## Methods

RENAL

- $eGFR_{CKD-Epi}$ was assessed at intervals of 2-3 weeks until month 24 and again at month 30.
- $mGFR_{Iohexol}$ was assessed at baseline and months 6, 12, 18, and 30.
- The long-term effect was assessed by calculating the annualized rates of change for each patient using the slope of the linear regression between the observed values and the assessment times.

ECHOCARDIOLOGY

- Left ventricular mass index (LVMi) collected by Echo using 2D or M-mode every 6-12 months through blinded, centralized evaluation (Cardiocore, Rockville, MD).
- The long-term effect was assessed by calculating the change from baseline to the last available timepoint and the 95% confidence interval for each patient.

## Baseline Characteristics

| Intent-to-Treat Population | Migalastat Arm (n=36) | ERT Arm (n=21) |
|---|---|---|
| Sex<br>Female n (%)<br>Male n (%) | <br>20 (56)<br>16 (44) | <br>12 (57)<br>9 (43) |
| Median Age (range) | 54 (18, 70) | 48 (18, 72) |
| Years since diagnosis Mean (SD) | 10 (12) | 13 (12) |
| $eGFR_{CKD-EPI}$* (mL/min/1.73 m$^2$) Mean (SD) | 89.6 (22) | 95.8 (19) |
| $mGFR_{Iohexol}$ (mL/min/1.73 m$^2$) Mean (SD) | 82.4 (18) | 83.6 (24) |
| 24-hr Urine Protein (mg) Mean (SD) | 260 (532) | 417 (735) |
| ACEi/ARB /RI Use: n (%) | 16 (44) | 11 (52) |
| Amenable based on Migalastat Amenability Assay: n (%) | 34 (94) | 19 (90) |

## Baseline Disease Severity

| Sex | Fabry Disease in ≥2 Organ Systems | Angio-keratoma or Corneal Whorling | Cardiac | CNS | Neu pat Pa |
|---|---|---|---|---|---|
| Males n (%) | 21/24 (88%) | 13/24 (54%) | 16/24 (67%) | 18/24 (75%) | 14/ (58 |
| Females n (%) | 29/33 (88%) | 16/33 (48%) | 25/33 (75%) | 12/33 (36%) | 22/ (67 |

Abbreviations: CNS = Central Nervous System; eGFR = estimated glomerular f ventricular hypertrophy; LVMi = left ventricular mass index; TIA = transient isc Angiokeratoma or Corneal Whorling based on medical history finding. Cardiac cardiac event (based on medical history), LVH, or conduction abnormality (eg, abnormality) based on medical history finding or baseline assessment of LVMi. medical history findings (stroke/TIA, tinnitus/hearing loss). Renal Involvement baseline eGFR <90 mL/min/1.73m$^2$, 24-hr Protein ≥150 mg. |

- All Study 012 patients with amenable mutations had clinical manifestations of Fabry and were eligible for treatment based on
- The age at enrollment/start of ERT treatment and the percentages of patients with involvement of different organ systems in St with those for patients reported in the Fabry Outcomes Survey (Mehta, Ricci et al. 2004) and the Fabry Registry (Eng, Fletche
- These findings indicate that Study 012 patients are comparable with the current Fabry population being treated with ERT, as re

## 30-Month Renal Results

| | | Parameter | |
|---|---|---|---|
| Parameter | Statistic | $eGFR_{CKD-EPI}$ | $mGFR_{Iohexol}$ |
| Annualized Rate of Change (mL/min/1.73 m$^2$) | | | |
| Baseline – Month 30 | n | 31 | 30 |
| | Mean | -1.718 | -2.746 |
| | SD | 2.5501 | 5.5318 |
| | 95% CI | (-2.653, -0.782) | (-4.812, -0.681) |
| | Median | -1.934 | -3.190 |

- The 30-month analyses include patients with amenable mutations (based on the Migalastat Amenability Assay) and baseline/post-baseline measures of eGFR and mGFR (renal analyses) or LVMi (ECHO analyses).

## 30-Month LVMi Res

| Parameter | Statistic | |
|---|---|---|
| LVMi (g/m$^2$) | | |
| Baseline | n | |
| | Median | |
| | Mean | |
| | SD | |
| OLE Period Month 30 | | |
| Actual | n | |
| | Median | |
| | Mean | |
| | SD | |
| Change from Baseline | n | |
| | Median | |
| | Mean | |
| | SD | |
| | 95% CI | (- |

## Summary of 30-Month Study Renal and LVMi Findings

- 31 male/female patients with amenable mutations who were randomized to the migalastat group completed the 18-month randomized period and entered the 12-month open-label extension . 49 patients with amenable mutations received ≥1 dose of migalastat during the combined 30 months.
- The annualized rates of change in $eGFR_{CKD-EPI}$ and $mGFR_{Iohexol}$ for migalastat (see Table above) are comparable to those previously reported in patients receiving ERT for 18 months: -1.0 (-3.6, 1.6) and -3.2 (-7.8, 1.3), respectively.
- For patients receiving ERT, previously reported 18-month changes in LVMi were -2.0 (-11.0, 7.0) for all patients and +4.5 (-20.9, 29.9) for patients with baseline LVH.
- For renal function, in patients switched from ERT, the effect of migalastat is persistent, with similar results observed over 18 and 30 months of treatment.
- For LVMi, the reduction in patients switched from ERT to migalastat is also persistent with similar results observed over 18 and 30 months of migalastat treatment.
- In patients with LVH at baseline, the reduction to month 30 for migalastat was statistically significant based on the 95% CIs.

## Safety (ITT Patient

- The 51 patients in the safety population – amen mutations– had a mean duration of migalastat
- Only 1 SAE was assessed as possibly related t investigator: proteinuria. This occurred during history of proteinuria during pregnancy.
- Migalastat was generally safe and well tolerated based on adverse event, laboratory, and physical exam data.

Patie Stud

## Conclusions

- The GFR stabilization and reduction in LVMi demonstrated with migalastat treatment are clinically relevant.
- Based on the literature, annualized rates of decline in GFR in ERT-treated Fabry patients are -2.2 to -2.9 ml/min/m$^2$.
- In patients switched from ERT to migalastat, the annualized rates of change (95% CI) in $eGFR_{CKD-EPI}$ and $mGFR_{Iohexol}$ at month 30 were: -1.0 (-3.6, 1.6) and -3.2 (-7.8, 1.3), respectively.
- LVH is the greatest risk factor for cardiac events in Fabry disease (Patel, Cecchi et al. 2011), and any reduction in LVH has been shown to have a positive impact on cardiovascular morbidity and mortality in hypertensive heart disease (Pokharel and Bella 2013).
- Migalastat reduced cardiac mass in all 012 patients following 30-month treatment and, most importantly, in patients with LVH (cardiac hypertrophy), the reduction was statistically significant.
- The effects of migalastat on GFR and LVMi observed following 18 months persist over 30 months.
- The results suggest that migalastat is a promising first-in-class oral chaperone treatment for male and female patients with amenable mutations.

# Co-Administration of the Pharmacological Chaperone AT2221 with A Proprietary Recombinant Human Acid α-Glucosidase Leads to Greater Plasma Expos Reduction Compared to Alglucosidase Alfa

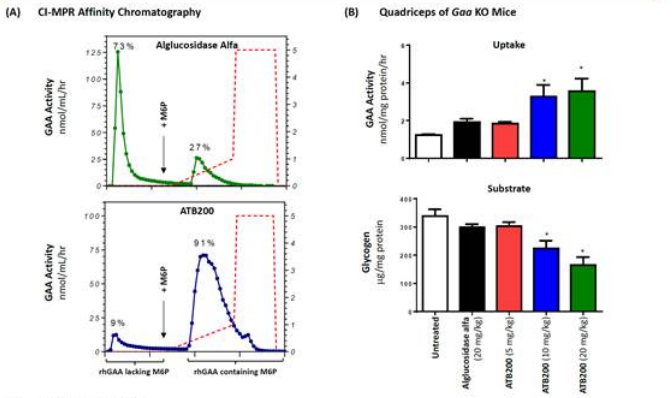
Khanna R, Xu S, Hilliard D, Lun Y, Schilling A, Soska R, Nair A, Chang K, Feng J, Frascella M, Garcia A, Pendino K, Johnson FK, Benjamin ER, Gotschall R, Do H, and Valenzano KJ

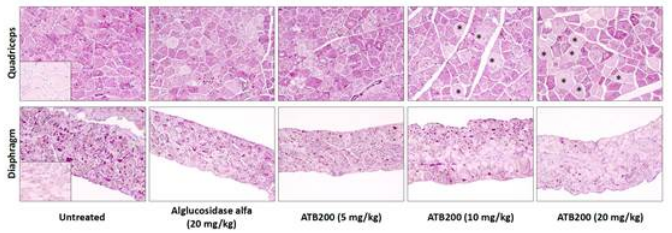
Amicus Therapeutics, 1 Cedar Brook Drive, Cranbury, NJ 08512, USA

## Introduction

Pompe disease is an inherited lysosomal storage disorder that results from a deficiency in acid alpha-glucosidase (GAA) activity, and is characterized by progressive accumulation of lysosomal glycogen in cardiac and skeletal muscles. Enzyme replac recombinant human GAA (rhGAA) is the only approved treatment available for Pompe disease. While rhGAA provides some clinical benefits, the infused enzyme shows insufficient uptake into key disease-relevant muscles, which is likely due to sub-optimal lev (M6P), a carbohydrate that binds cation-independent M6P receptors (CI-MPR) at the cell surface resulting in enzyme internalization and lysosomal targeting. In order to increase the targeting efficiency of ERT, we have developed a proprietary mammalian cell that yields a novel form of rhGAA (designated as ATB200) with a significantly higher M6P content compared to the alglucosidase alfa. In this study, we have examined the effects of ATB200 on tissue exposure and substrate reduction with and without the pharmacological chaperone (PC) AT2221.

## 1. ATB200 Has a Higher M6P Content and Results in Better Tissue Uptake and Greater Glycogen Reduction *in vivo* Compared with Alglucosidase Alfa



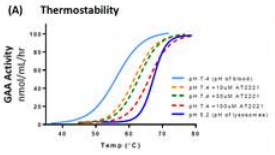




(A) Alglucosidase alfa or ATB200 was loaded onto a CI-MPR column. Only enzyme that contained M6P was retained and then eluted from the column using free M6P of increasing concentration (dotted line in red). Both unbound (flow-thru) and bound/eluted fractions were collected and assayed for GAA activity. The majority of ATB200 (91%) was bound compared to alglucosidase alfa (27%), suggesting that ATB200 has a higher M6P content, which is key to the efficient endocytosis and lysosomal targeting of rhGAA.

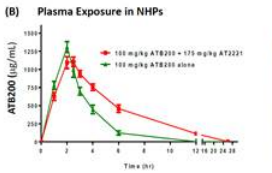
(B) Twelve-week-old male *Gaa* KO were administered 2 bi-weekly bolus intravenous (IV) injections of alglucosidase alfa (20 mg/kg) or ATB200 (5-20 mg/kg) via tail vein (n=6-7 per group). Quadriceps were collected 14 days post the last dose and measured for GAA activity and glycogen levels. ATB200 shows dose-dependent increases in uptake and substrate reduction. Importantly, 5 mg/kg ATB200 is comparable to 20 mg/kg alglucosidase alfa, whereas 20 mg/kg ATB200 is significantly better than alglucosidase alfa, indicating improved potency of ATB200. Bars represent mean ± SEM. * p<0.05 vs. alglucosidase alfa in 2-sided t-test.

(C) Paraffin sections of quadriceps and diaphragm from study described in panel B were also examined for glycogen accumulation by Periodic acid-Schiff's reagent (PAS), which stains glycogen magenta. Consistent with the biochemical measurements, 20 mg/kg ATB200 appeared more effective in glycogen reduction compared to 20 mg/kg alglucosidase alfa in both tissues. Images of age-matched wild-type (WT) animal are shown in the insets. Each image is representative of 6-7 animals per group. Magnification is 200x.

## 2. The Pharmacological Chaperone AT2221 Increases the Stability and Exposure of ATB200




| Test Condition | Tm (°C) |
|---|---|
| pH 7.4 | 56.2 |
| pH 7.4 + 10 µm AT2221 | 61.6 |
| pH 7.4 + 30 µm AT2221 | 62.9 |
| pH 7.4 + 100 µm AT2221 | 66.0 |
| pH 5.2 | 67.3 |

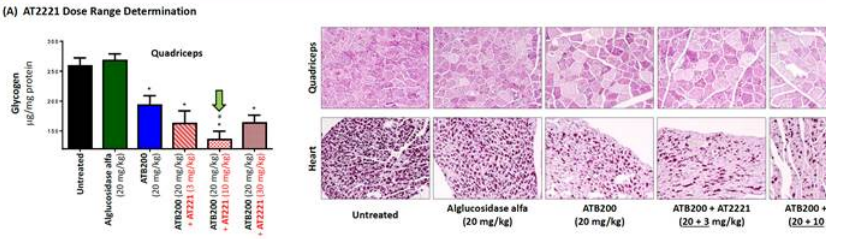



| PK Measurement | ATB200 | ATB200 + AT2221 |
|---|---|---|
| **AUC** (µg · hr/mL) | 3647 | 6801 |
| **$T_{\frac{1}{2}}$** (hr) | 1.2 | 3.2 |
| **Cmax** (µg/mL) | 1155 | 1105 |
| **Tmax** (hr) | 2 | 2.5 |

(A) The stability of ATB200 in acidic or neutral pH buffers was evaluated in a thermostability assay using SYPRO Orange. AT2221 stabilizes ATB200 at pH 7.4 in a concentration-dependent manner, to approaching the level seen at pH 5.2, a condition that mimics the acidic environment of the lysosome, as demonstrated by a nearly 10°C increase in the melting temperature ($T_m$) of ATB200.

(B) Cynomolgus monkeys (2-3 years of age) were administered a single 2-hour IV infusion of 100 mg/kg ATB200 alone or with oral administration of 175 mg/kg AT2221 30 minutes earlier. Plasma samples were collected over the following 24 hours and GAA activity was determined. Co-administration resulted in an approximate 2-fold increase in ATB200 exposure (AUC) and half-life ($T_{\frac{1}{2}}$), compared to administration of ATB200 alone. Each time point represents the mean ± SEM of 8 NHPs (4 males and 4 females)/group.

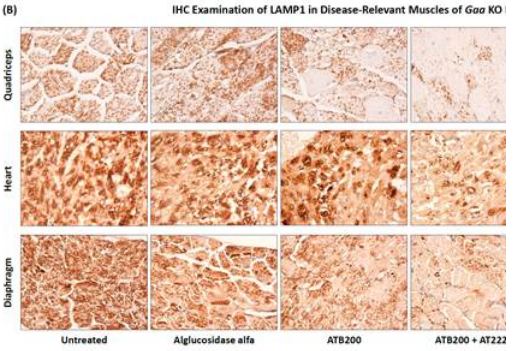
## 3. AT2221 Co-administration Leads to Greater ATB200-mediated Glycogen Reduction and Reduces Lysosor Disease-relevant Muscles of *Gaa* KO Mice

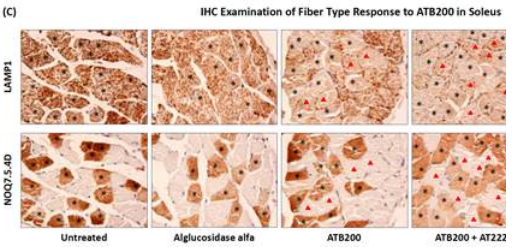



(A) Twelve-week-old male *Gaa* KO mice were administered a total of 2 bi-weekly bolus IV injections of 20 mg/kg alglucosidase alfa or ATB200. In addition, ATB200 v doses of AT2221 (3-30 mg/kg). Glycogen levels were determined in quadriceps collected 14 days post-last dose. (**Left**) While ATB200 alone resulted in greater alglucosidase alfa, its efficacy is further improved by co-administration, mostly significantly with 10 mg/kg AT2221 (green arrow). Bars represent Mean ± SEM alglucosidase alfa; # p<0.05 vs. ATB200 alone in 2-sided t-test. (**Right**) PAS staining also showed the lowest glycogen level in quadriceps with the co-administration of ATB200 (upper panels). Co-administration of 10 mg/kg AT22221 also led to marked further reduction in glycogen compared to ATB200 alone in heart (bottom panels shown). Magnification = 200x.

(B) Subsequently, the effect of co-administration of 20 mg/kg ATB200 + 10 mg/kg AT2221 was compared with 20 mg/kg alglucosidase alfa or ATB200 alone in another 2-biweekly-administration study in male *Gaa* KO mice of twelve-weeks of age. IHC examination of lysosome marker LAMP1 in quadriceps revealed a substantial up-regulation of LAMP1 in fibers of untreated animals (top panel), which is indicative of lysosomal proliferation, a hallmark of Pompe disease. Unlike alglucosidase alfa, ATB200 alone leads to a marked decrease in LAMP1 signal, whose level was lowered further still with the co-administration of AT2221, approaching that seen in WT tissues. The change in LAMP1 level closely follows the change in glycogen level in quadriceps, and is repeated in additional tissues, such as heart, diaphragm, and soleus. Magnification = 400x.

(C) Moreover, the fiber type response to ATB200 was investigated by IHC with LAMP1 antibody (top) and a type I (slow twitch) fiber-specific antibody NOQ7.5.4D (bottom) on adjacent sections of soleus, which has a relative equal representation of both type I and type II (fast twitch) fibers. ATB200 alone is much more effective than alglucosidase alfa, as indicated by the normalization of LAMP1 levels in most type I fibers and, significantly, a fraction of type II fibers as well, contrary to their reported resistance to alglucosidase alfa. With co-administration, a reversal of lysosomal proliferation was achieved in the majority of muscle fibers, regardless of fiber type. This result is consistent with the observed superiority of ATB200 + AT2221 compared to alglucosidase alfa in quadriceps and diaphragm (B), tissues with a predominant type II fiber content. Asterisks mark all the type I fibers in a section, while the red triangles highlight the type II fibers with significantly reduced LAMP1 signals. Magnification = 400x.




## Summary and Conclusions

- We have developed a novel rhGAA, ATB200, with a significantly higher M6P content compared to alglucosidase alfa enzyme uptake and glycogen reduction in disease-relevant tissues of *Gaa* KO mice, likely due to the improved e targeting of the exogenous recombinant enzyme mediated by the binding of M6P to its receptor CI-MPR.
- More importantly, we showed that co-administration with the optimized pharmacological chaperone AT2221 leads to t efficacy of ATB200, possibly via binding and stabilizing ATB200 in the blood, keeping the enzyme in a properly folde accessible for tissue uptake and lysosomal delivery. As a result, AT2221 improves the exposures of ATB200, broade achieves significantly greater glycogen reduction in disease-relevant cell types/tissues that have responded poorly t as type II skeletal muscle fibers and skeletal muscles with a higher content of type II fibers.
- Taken together, these preclinical data highlight the efficacy of our proprietary rhGAA, ATB200, in mice when combin chaperone using our proprietary CHART platform, and thus warrant further investigation.

# Six months of Migalastat Treatment Reduces Podocyte Globotriaosylce Content in Adult Male Patients with Fabry Disease

Najafian B. [1], Sokolovskiy A. [1], Barth J. [2], Castelli J.[2], Williams H.[2], Mauer

1.University of Washington, Seattle, WA; 2. Amicus Therapeutics; 3. University of Minnesota, Minneap

## Background

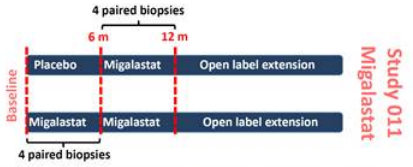
- Deficiency of α-galactosidase-A in Fabry disease leads to accumulation of globotriaosylceramide (GL-3) inclusions in cells, causing organ damage. Progressive kidney failure is a major complication of Fabry disease.
- Podocytes are terminally differentiated cells with limited regeneration capacity. Recent studies suggest a key role for podocytes in Fabry nephropathy. In young Fabry patients, we showed that podocyte GL-3 accumulation occurs early, is progressive with age, and is associated with podocyte injury and proteinuria (Najafian et al. Kidney Int 2011). Reducing podocyte GL-3 burden may reduce progression of Fabry nephropathy. However, podocyte are far more resistant than other kidney cells to clear GL-3 following enzyme replacement therapy.
- Migalastat (MIG) is an investigational pharmacologocal chaperone that stabilizes "amenable" mutant α-gal-A and enhances its trafficking to lysosome. MIG reduced peritubular capillary endothelial cell GL-3 in 6 months (study 011).

### Hypothesis

MIG reduces GL-3 inclusion content in podocytes in patients with Fabry disease with amenable mutations.

## Materials and Methods

- 8 paired biopsies (baseline and 6 months post-migalastat) from male patients with "amenable" GLA mutations




| No | Sex | Age | GLA Mutation | eGFR | UPr-24 (mg) | ACR (g/g) |
|---|---|---|---|---|---|---|
| 1 | M | 25 | D55V/Q57L | 114 | 198 | 6 |
| 2* | M | 33 | D244N | 115 | 349 | 14 |
| 3 | M | 34 | Y216C | 119 | 400 | 16 |
| 4 | M | 35 | G144V | 105 | 240 | 1 |
| 5 | M | 45 | L243F | 102 | 161 | 2 |
| 6 | M | 45 | P259R | 105 | 335 | 7 |
| 7* | M | 45 | P259R | 86 | 1909 | 119 |
| 8 | M | 45 | A156T | 74 | 247 | 4 |
| 9 | M | 52 | D33G | 82 | 367 | 9 |
| 10* | M | 56 | R301Q | 56 | 2351 | 126 |
| 11 | M | 60 | D322E | 41 | 918 | 34 |

* Asterisk indicates patients with no paired biopsies after 6 months migalastat (e.g. only BL and M6 on placebo)
**Data includes all amenable male patients with ICF and paired assessable biopsies

Biopsy structural parameters by electron microscopic stereology

- Vv(Inc/PC): Fraction of podocyte cytoplasm occupied by GL-3 inclusions
- V(PC): Average podocyte volume
- V(Inc/PC): Average volume of GL-3 inclusions per podocyte

Other Parameters

- Age, eGFR, 24 hr urine protein, albumin/creatinine ratio, protein/creatinine ratio, plasma lyso Gb3, and peritubular capillary inclusion score (BLISS)

## Results

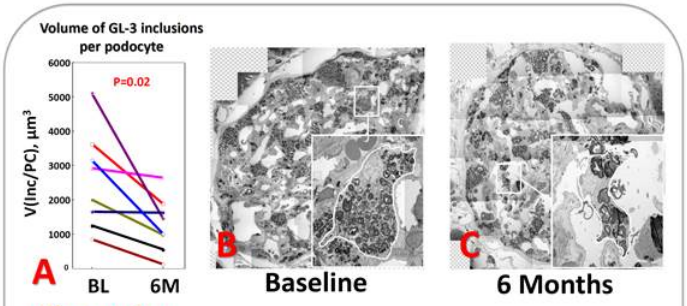



**Figure 1**. (A) Volume of GL-3 inclusions per podocyte was reduced from baseline (BL) to 6 months (6M) post-treatment. (B) A glomerulus from a Fabry patient at baseline; and (C) 6 months after migalastat.

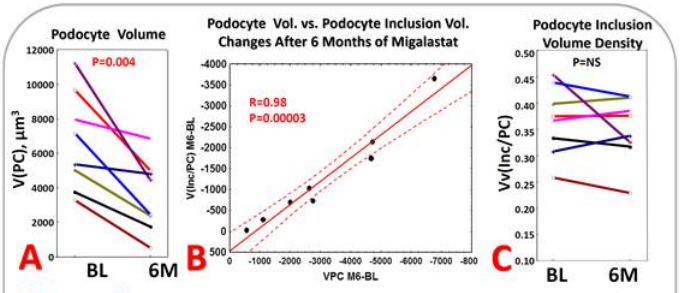



**Figure 2**. (A and B) GL-3 reduction in podocytes was closely paralleled by a reduction in podocyte volume. (C) Volume fraction of GL-3 inclusions in podocytes did not change during the 6 months migalastat treatment.

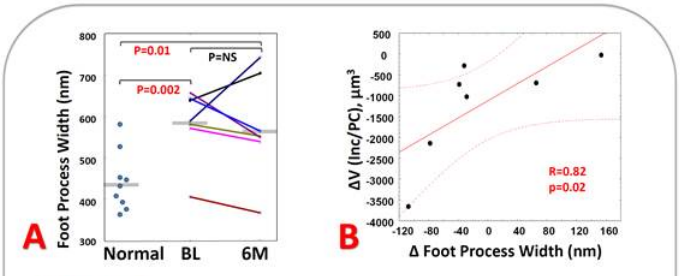



**Figure 3**. (A) Average foot process width in Fabry patients before or after 6 months treatment with migalastat was greater than values from 9 healthy control subjects . Foot process width was reduced in 5/7 and increased in 2/7 cases after 6 months MIG, but the change was not statistically significant. (B) The magnitude of foot process width reduction correlated with the magnitude of reduction in GL-3 inclusion volume in podocytes. Likewise, the magnitude of foot process width reduction correlated with the magnitude of reduction in GL-3 inclusion volume density in podocytes (R=0.82, p=0.02) and reduction in podocyte size (R=0.089, p=0.007).

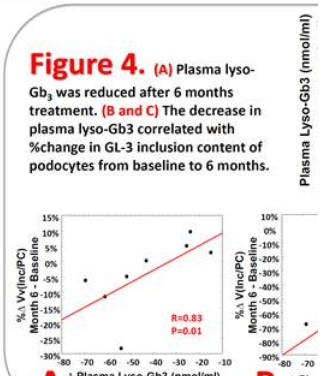



**Figure 4.** (A) Plasma lyso-$Gb_3$ was reduced after 6 months treatment. (B and C) The decrease in plasma lyso-Gb3 correlated with %change in GL-3 inclusion content of podocytes from baseline to 6 months.

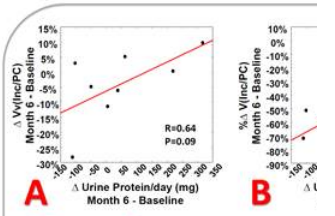



**Figure 5.** (A and B) There were stati associations between 24-hr urine protein and inclusion content of podocytes.

No statistically significant changes in eGFR, al proteinuria over 6 months treatment.

## Conclusio

- In patients with Fabry disease and "amena migalastat treatment led to a reduction in months. This reduction correlated with pr podocyte volume, leading to no significant fraction in podocytes.
- The observed direct relationship between process width and GL-3 content in podocyt of migalastat treatment is suggestive of re
- It will be crucial to confirm these findings in la if with longer treatment duration, podocytes migalastat treatment.
- Future studies are needed to confirm if migala ameliorate podocyte loss.
- This study shows that sensitive quantitative st assess treatment efficacy in much shorter time than scoring methods.

*This study was sponsored by a from Amicus Therap*