2. SYNOPSIS

Name of Sponsor/Company: Amicus Therapeutics	Individual Study Table Referring to Part of the Dossier
Name of Finished Product: AT1001	Volume:
Name of Active Ingredient: migalastat hydrochloride	Page: *(For National Authority Use Only)*

Title of Study:

A Phase 2, Open-label, Multiple Dose Level, 12-week Study to Evaluate the Safety, Tolerability, and Pharmacodynamics of AT1001 in Female Patients with Fabry Disease

Protocol Number:

FAB-CL-204

Principal Investigators:

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Study Centers:

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Publications (references): none as of the date of this report

Study Period:	**Phase of Development:** 2
Date first patient enrolled: 07 September 2006 Date last patient completed: 09 May 2008	

Objectives:

Primary:

- To evaluate the safety and tolerability of oral AT1001 (50, 100, or 250 mg once every other day) in female patients with Fabry disease

Secondary:

- To gain information about the pharmacokinetics (PK) of 3 dosages of oral AT1001 in female patients with Fabry disease
- To gain information about the pharmacodynamics (PD) of 3 dosages of oral AT1001 in female patients with Fabry disease
- To provide a preliminary assessment of cardiac, renal, and central nervous system function in female patients with Fabry disease

Methodology:

This was a Phase 2, multicenter, open-label trial of AT1001 in female subjects with Fabry disease. The trial included a 12-week treatment phase and an optional 36-week treatment extension. All subjects were stratified by α-Gal A enzyme activity (high or low, as measured in an ex vivo lymphocyte assay) and then randomly assigned to receive one of three AT1001 doses: 50, 150, or 250 mg once every other day (QOD).

Potential subjects were initially screened at Visit 0. Approximately 4 weeks later, additional screening assessments were completed on Days -2 and -1 of the screening/baseline visit. This visit also included Day 1, when subjects initiated treatment with AT1001. During the initial 12-week treatment phase, there were visits at Weeks 2 (Visit 1), 4 (Visit 2), 8 (Visit 3), and 12 (Visit 4). An additional visit occurred at Week 14 (Visit 5). During the optional 36-week treatment extension, there were visits at Weeks 24 (Visit 6), 36 (Visit 7), and 48 (Visit 8).

Number of Subjects (planned and analyzed):

Up to 12 subjects were planned. A total of nine subjects were enrolled and all nine are included in the safety, PK, and PD analysis sets.

Criteria for Eligibility:

A subject was required to meet all of the following inclusion criteria to be considered eligible for study participation:

1. Females between 18 and 65 years of age (inclusive)
2. Heterozygous for Fabry disease
3. Had a confirmed diagnosis of Fabry disease with a documented missense gene mutation (individual or familial)
4. Had enhanceable enzyme activity as defined by meeting one of the following criteria:
   - If residual α-Gal A activity in lymphocytes was less than 1% of normal, then α-Gal A activity after incubation with AT1001 was required to be at least 2% of normal
   - If residual α-Gal A activity in lymphocytes was between 1% of normal and less than 3% of normal, then α-Gal A activity after incubation with AT1001 was required to be at least 2 times the baseline level
   - If residual α-Gal A activity in lymphocytes was between 3% of normal and less than 10% of normal, then α-Gal A activity after incubation with AT1001 was required to be at least 3% of normal higher than the baseline level
   - If residual α-Gal A activity in lymphocytes was greater than 10% of normal, then α-Gal A activity after incubation with AT1001 was required to be at least 1.3 times the baseline level

   Note: This inclusion criterion was altered by protocol amendment 1. Originally, baseline residual α-Gal A activity must have been greater than or equal to 3% of normal, and a 20% or greater increase in activity was required after lymphocytes were incubated with AT1001.
5. Had end organ dysfunction, even minimal, demonstrated by one or more of the following:
   - abnormal electrocardiogram (ECG) or left ventricular hypertrophy(LVH) documented by echocardiogram (ECHO) or cardiac biopsy
   - renal insufficiency documented by common clinical assessments such as creatinine and glomerular filtration rate (GFR) or by renal biopsy

- brain tissue dysfunction as documented by evidence of stroke (clinically or imaging)
- peripheral nervous system dysfunction documented by complaints of intolerance to heat or cold, decreased vibratory sense and proprioception, decreased ability to perspire, or acroparasthesia

6. Previously untreated by enzyme replacement therapy (ERT) or substrate depletion for Fabry disease or were able to stop ERT for at least 18 weeks or up to 13 months safely (in the judgment of the investigator, it must have been considered safe for the subject to stop therapy for the duration of the study).

   Was willing to undergo two kidney and three skin biopsies
7. Agreed to be sexually abstinent or use an effective method of contraception (greater than 90% effective) when engaging in sexual activity during the course of the study and for a period of 30 days following the completion of the study (for women of childbearing potential)
8. Willing and able to sign an informed consent form

A subject was considered ineligible for study participation if she met any one of the following exclusion criteria:

1. Pregnant or lactating women
2. History of significant disease other than Fabry disease that impairs the subject's ability to participate in the study (e.g., end-stage renal disease; heart disease [per clinical history, documented event, testing, or Class III/IV according to the New York Heart Association classification]; current diagnosis of cancer, except for basal cell carcinoma of the skin; diabetes (unless HbA1c less than or equal to 8); or neurological disease
3. History of organ transplant
4. Serum creatinine greater than 176 μmol/L on Day -2
5. Screening 12-lead ECG demonstrating QTc greater than 450 msec
6. Pacemaker or other contraindication for MRI scanning
7. Taking a medication prohibited by the protocol: Fabrazyme® (agalsidase beta), Replagal™ (agalsidase alfa), Glyset® (miglitol), Zavesca®(miglustat), or any experimental therapy for any indication
8. Participated in a clinical trial in the last 30 days
9. Any other condition which, in the opinion of the investigator, would jeopardize the safety of the subject or impact the validity of the study results

Test product, dose and mode of administration, batch number:

Subjects were randomized to a dose level of 50, 150, or 250 mg AT1001 (two, six, or ten 25-mg capsules, respectively). AT1001 capsules were administered orally, once every other day (QOD). The lot numbers for the AT1001 supplies used in this study were 8901.001, 8901.002, and 8901.003.

Duration of treatment:

For an individual subject, the minimum planned duration of treatment with AT1001 was 12 weeks. With the optional 36-week treatment extension, the duration of treatment was 48 weeks.

Reference therapy, dose and mode of administration, batch number:

Not applicable

Criteria for evaluation:

Pharmacokinetic:

The single- and multiple-dose pharmacokinetics of AT1001 were measured using plasma and urine samples from Day 1, Day 14, and Day 84. From plasma, the PK parameters derived included area under the plasma concentration time curve to the last measurable concentration (AUC_{0-t}), AUC to infinity (AUC_{0-inf}), AUC_{0-t}/AUC_{0-inf}, maximal concentration (C_{max}), time to maximal concentration (t_{max}), apparent elimination rate constant (k_{el}), half-life ($t_{1/2}$), apparent total body clearance (CL/F), $V_{area/F}$ (apparent volume of distribution following oral administration), fluctuation, and accumulation. From urine, the PK parameters derived included cumulative amount excreted (AE_{0-12}) and renal clearance (CL_R).

Pharmacodynamic:

The PD measures were α-Gal A activity in leukocytes, kidney, and skin; globotriaosylceramide (GL-3) in urine, kidney, plasma, and skin; cardiac function assessments (e.g., cardiac magnetic resonance imaging [MRI] and Holter ECG); renal function assessments (e.g., creatinine clearance and estimated glomerular filtration rate [eGFR]); and nervous system function assessments (e.g., cognitive testing). Kidney GL-3 was measured both by LC-MS/MS of GL-3 in tissue homogenate and histological scoring of kidney tissue (e.g., for GL-3 inclusions in renal capillaries).

Safety:

The safety measures were adverse events (AEs), vital signs (blood pressure, heart rate, temperature, and respiratory rate), clinical laboratory measurements (hematology, serum chemistry, and urinalysis), ECGs, ECHOs, physical examinations, and use of concomitant medications.

Statistical methods:

No formal inferential hypothesis testing was performed. Statistical analyses and reporting were performed using Windows SAS version 8.2 or higher. All subjects who received at least one dose of study drug were included in the safety analysis set. All subjects who were in the safety analysis set and who also had at least one non-missing post-baseline PK assessment were included in the PK analysis set. All subjects who were in the safety analysis set and had at least one non-missing post-baseline PD parameter recorded were included in the PD analysis set. Baseline was defined as the last non-missing value before the start of study drug. In an analysis added after the conclusion of the study, the PD measures were also evaluated according to the AT1001-responsiveness of each α-Gal A mutant form in an in vitro assay. In this assay, known mutant forms of α-Gal A were expressed in human embryonic kidney (HEK) cells. Enzyme activity in response to AT1001 was used to categorize each mutant form as AT1001-responsive or AT1001-nonresponsive.

By-subject data listings are provided for all subjects in the safety analysis set. Safety data were summarized by treatment and study visit. Continuous variables were summarized by presenting the number of subjects, mean, median, standard deviation, and range. Categorical variables were summarized by presenting the frequency and percentage of subjects in each category. Change from baseline was presented as appropriate. Treatment-emergent adverse events (TEAEs) were summarized by system organ class (SOC) and preferred term. The number of subjects reporting each TEAE and the number of TEAEs reported were summarized. Shift tables summarizing changes from normal to abnormal ranges were provided for serum chemistry, hematology, and urinalysis. Potentially clinically significant (PCS) changes in vital signs and ECGs were summarized at each time point. The PK analyses are described in the appended PK report.

SUMMARY:

This study enrolled nine adult females with Fabry disease, with an age range of 36 to 62 years. At study entry, all subjects had documented end-organ dysfunction: all nine subjects had cardiac abnormalities; three subjects had proteinuria; and seven subjects had neurological abnormalities (predominately peripheral nervous system dysfunction), with two subjects reporting previous transient ischemic attacks. Two subjects were randomized to 50 mg AT1001 QOD, four subjects were randomized to 150 mg AT1001 QOD, and three subjects were randomized to 250 mg AT1001 QOD. All nine subjects completed the initial 12-week treatment period and the 36-week extension period.

Pharmacokinetic Results:

The PK profiling of AT1001 indicated that oral doses of 50, 150, or 250 mg AT1001 were rapidly absorbed. Median t_{max} values in plasma ranged from 2 to 3.5 hours. Following repeat oral administration of AT1001 QOD, AT1001 plasma exposures (AUC_{0-10} and C_{max}) did not deviate significantly from dose proportionality across the dose range studied. No appreciable accumulation of AT1001 was observed over 84 days of QOD oral dosing. After 150 mg AT1001, 34 to 60% of the dosed AT1001 was recovered unchanged in urine within 10 hours of dosing. After 250 mg AT1001, 30 to 39% of the dosed AT1001 was recovered unchanged in urine within 10 hours of dosing.

Pharmacodynamic Results:

The PD outcome measures included both biochemical (α-Gal A activity and GL-3 levels in multiple tissues) and functional measures (including cardiac, renal, and neurological). At the beginning of the study, subjects had been stratified by lymphocyte α-Gal A activity. However, as this stratification did not account for females' mosaic expression of both wild type and mutant forms of α-Gal A, the sex-independent HEK-cell based assay was used to later categorize subjects based on the AT1001-responsiveness of each subject's individual α-Gal A mutant form. This retrospective categorization was then considered in the interpretation of the PD results.

With AT1001 treatment, α-Gal A activity in leukocytes and in kidney and skin tissue tended to increase at all dose levels.

In the eight females with missense mutations in GLA, baseline leukocyte α-Gal A activity was relatively high (29.0 to 114.1% of the activity measured in healthy adult males). The lowest baseline leukocyte α-Gal A activity (14.8%) was seen in the one subject with a deletion mutation. By Week 24, all subjects demonstrated an increase relative to their baseline leukocyte α-Gal A activity. At Week 48, eight of the nine subjects maintained this increase.

In kidney tissue, α-Gal A activity increases were seen at Week 12 for eight of the nine subjects.

In skin tissue, α-Gal A activity increases were seen at Week 12 for all nine subjects.

With AT1001 treatment, urine and kidney GL-3 tended to decrease at all dose levels.

Most subjects (eight of nine) had elevated urine GL-3 at baseline (up to 12-fold higher than the upper limit of normal). A decrease was seen by Week 48 for seven of the nine subjects. The two subjects (both in the 150 mg group) who did not have a decrease in urine GL-3 at Week 48 both have α-Gal A mutant forms that are AT1001-nonresponsive in the in vitro HEK assay. The earliest and most consistent declines in urine GL-3 were seen in those subjects who were on the 150 or 250 mg AT1001 dose and who also have an α-Gal A mutant form that is AT1001-responsive in the in vitro HEK assay.

In the histological analyses for kidney GL-3, categorical scoring of GL-3 levels in multiple cell types showed that the most affected cells were podocytes and distal tubular cells. For podocytes, there were no trends for decreased GL-3 scores with AT1001 treatment. For distal tubular cells, four subjects had GL-3 deposition evident at baseline; by Week 48, all four of these subjects showed a decrease in their GL-3 score for this cell type. For interstitial capillaries, initial categorical analyses were unable to detect GL-3 deposition at baseline for eight of the nine subjects. However, when using the fully quantitative Barisoni method, interstitial capillary GL-3 was detected at baseline for all subjects. Then, a decrease in interstitial capillary GL-3 was seen for six of the nine subjects between baseline to their last available biopsy (Week 48 or, if not available, Week 12). Of the five subjects with an α-Gal A mutant form that is AT1001-responsive in the in vitro HEK assay, four subjects had a decrease in the average interstitial capillary GL-3 inclusions from baseline to their last available biopsy; one subject had no change. Of the four subjects with an α-Gal A mutant form that is AT1001 non-responsive in the in vitro HEK assay, two subjects had a decrease from baseline in GL-3 inclusions and two subjects had no change.

Plasma GL-3 levels were low at baseline and remained low throughout the study.

For the functional measures (cardiac, renal, and neurological assessments), after 48 weeks of treatment the abnormalities present at baseline either improved, remained stable, or had non-clinically significant changes.

All nine subjects in this study had cardiac abnormalities at baseline. Bradycardia was common and no improvement was seen with AT1001 treatment. LVH was the most frequently indicated cardiac abnormality, present in five of the nine subjects. Three of these subjects had MRI, ECG, and/or ECHO results that indicated improvement in their LVH after treatment with AT1001. Additionally, one subject had an improvement in left ventricle ejection fraction (LVEF). Three of these four subjects have a mutant α-Gal A form that is AT1001-responsive in the HEK assay.

All subjects had reasonably good renal function at baseline (eGFRs greater than 60 mL/min/1.73m^2). This level of renal function was preserved during treatment with AT1001.

For the neurological assessments there were no significant deficits present at baseline, and any treatment effect is thus difficult to distinguish.

For the pharmacodynamic results overall, when considered by whether each subjects' mutant α-Gal A form is or is not AT1001-responsive in the in vitro HEK assay, the HEK assay did tend to be predictive of a beneficial response to AT1001 treatment.

Safety Results:

No deaths and no SAEs related to treatment occurred during the study. There were two SAEs, one during screening (cardiac tamponade) and one during treatment (muscular chest pain). Both were assessed as unrelated to treatment.

No subject had an interruption or reduction in study drug dosing due to an AE or discontinued study drug due to an AE.

All treatment-related TEAEs were mild or moderate in severity and all but two were reported as unlikely to be related to study drug. All subjects reported at least one TEAE. A total of 85 TEAEs were reported, of which 25 were considered treatment-related. Of these 25 treatment-related TEAEs, none of the relationships to study drug were reported as definite, 1 was reported as probably related, 1

was reported as possibly related, and 23 were reported as unlikely to be related to study drug. All of the TEAEs were mild or moderate in severity.

There were no trends in laboratory results or vital signs. The majority of out-of-range values were considered non-clinically significant. AT1001 was safe and well tolerated; no treatment-limiting toxicities were identified.

CONCLUSIONS:

The primary objective of the study was to evaluate the safety and tolerability of AT1001 in female subjects with Fabry disease. The secondary objectives were to gain information about the PK and PD effects of AT1001.

Treatment with 50, 150, or 250 mg AT1001 QOD for 48 weeks was safe and well tolerated. No treatment-limiting toxicities were identified.

At all doses, AT1001 increased α-Gal A activity. Increased leukocyte α-Gal A activity was seen in all subjects by Week 24. At Week 48, eight of the nine subjects maintained this increase. Most subjects had elevated urine GL-3 at baseline (up to 12-fold higher than the upper limit of normal) and a decrease was seen by Week 48 for seven of the nine subjects. When using the fully quantitative Barisoni method for histological analyses of interstitial capillary GL-3, a decrease in interstitial capillary GL-3 was seen for six of the nine subjects from baseline to their last available biopsy (Week 48 or, if not available, Week 12).

The earliest and most consistent declines in urine GL-3 were seen in the three subjects who were on the 150 or 250 mg AT1001 dose and whose α-Gal A mutant form is AT1001-responsive in the in vitro HEK assay. Four of the five subjects with an α-Gal A mutant form that is AT1001-responsive in the in vitro HEK assay had a decrease in their average interstitial capillary GL-3 inclusions from baseline to their last available biopsy. The subject without a decrease was on the 50 mg AT1001 dose. Further, three of the four subjects who received 150 mg AT1001 had cardiac results indicating improvement.

Increases in α-Gal A activity, reductions in urine and tissue GL-3, and improvement in cardiac function suggests that the efficacy and safety of AT1001 should be further investigated in females with Fabry disease.

Date of the report: 22 April 2011